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PART 1: Screening of Thirty-one Medicinal Plant Species Against Herpes Simplex Virus, Acetone and Methanol Extracts from the Root Tissue of *Kalanchoe pinnata* Interferes with HSV Types 1 and 2 DNA replication and Early and Late Gene Expression Preventing the Spread of HSV *in vitro*. PART 2: Professional Development Curriculum: Integrating Molecular Biology and Microbiology into the Existing Secondary Biology Curricula

Mary Ruth Greer
Brigham Young University

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Methanol Extracts from the Root Tissue of *Kalanchoe pinnata* Interferes with HSV

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Part 2
Professional Development Curriculum: Integrating

Molecular Biology and Microbiology into the

Existing Secondary Biology Curricula

Mary Ruth Jaeger Greer

A dissertation submitted to the faculty of
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Doctor of Philosophy

F. Brent Johnson
Richard A. Robison
John S. Gardner
Rex G. Cates
Kim L. O'Neill

Department of Microbiology and Molecular Biology

Brigham Young University

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ABSTRACT

Part 1

Screening of Thirty One Medicinal Plant Species Against Herpes Simplex Virus: Acetone and Methanol Extracts from the Root Tissue of *Kalanchoe pinnata* Interferes with HSV Types 1 and 2 DNA Replication and Early and Late Gene Expression Preventing the Spread of HSV *in vitro*

Part 2

Professional Development Curriculum: Integrating Molecular Biology and Microbiology into the Existing Secondary Biology Curricula

Mary Ruth Jaeger Greer

Department of Microbiology and Molecular Biology

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PART 1: Thirty-one medicinal plant species from Hawaii, Morocco, and the Sonoran Desert, USA have been shown in past studies to be highly inhibitory to pathogenic bacteria, fungi, and certain cancer cell lines. However, none were tested for antiviral activity. Acetone and methanol extracts from these species were bio-assayed for antiviral activity against herpes simplex virus types 1 and 2 (HSV 1 and HSV 2) and for cytotoxicity to the Vero C1008 cell line. Extracts from these species were tested *in vitro* for antiviral activity using an immunoperoxidase mini-plaque

reduction assay to detect viral structural protein synthesis. Sulforhodamine B and neutral red assays were used to qualitatively and quantitatively assess the cytotoxicity of extracts to C1008 cells, and to compute a 50% cytotoxic concentration (CC_{50}) using a dose response curve. Eight of the 31 plant species assayed showed significant antiviral activity against herpes simplex virus types 1 and 2. The acetone extract of *Kalanchoe pinnata* Pers. (Crassulaceae) produced the most promising results with an IC_{50} of 0.025 mg/ml and a CC_{50} of 1.25 mg/ml yielding a therapeutic index of 50. Additionally, this extract reduced plaque numbers to zero or near zero at a concentration of 0.1 mg/ml when added 30 min before and up to 8 h post infection. Further tests were performed on the *K. pinnata* extract in pursuit of the mechanisms of observed antiviral properties. Quantitative PCR was used to determine HSV susceptibility to the acetone extract. Antiviral mechanisms were investigated by measuring the reduction of viral DNA at different time points post infection and by measuring the reduction of viral RNA transcripts for five specific genes: alpha gene UL54, beta genes UL23 and UL30, and gamma genes US4 and UL17. Examination of transcript number found a significant decrease in viral DNA replication and early and late gene transcription when infected cells were exposed to *K. pinnata* suggesting post entry events were blocked by extract.

PART 2: The professional development curriculum was written for the Alpine School District and will offer teachers the opportunity to develop and enhance skills for effective science teaching emphasizing molecular biology and microbiology disciplines. The course begins with four assumptions about the nature of secondary science in-service. First, the understandings and abilities required to be a masterful teacher of science are not static. Second, science content increases and changes, and a teacher's understanding in science must keep pace. Third, knowledge about the process of learning is continually developing, requiring teachers to stay

informed. And fourth, we live in a changing society that deeply influences events in schools; social changes affect students as they come to school and affect what they need to carry away with them. While the main intent of this course is to improve the knowledge base for secondary life science teachers in the microbiology and molecular biology disciplines, it is expected that teachers will return to their own classroom and use the materials and ideas they have acquired from this course. Included in the concepts stressed are: 1) the historical routes of molecular biology, (2) biotechnology and its influence in society, (3) the relationship between viruses and evolution, order and organization, (4) the immune system and examples that stress structure and function, change and constancy, and (5) the personal and social impact of pathogens. Teachers are introduced to new information in virology, molecular biology and immunology using case studies, practicing scientific inquiry, and recognizing the unifying themes of biology in microbiology and molecular biology.

Keywords: antiherpetic, curriculum, immunology, microbiology, molecular, professional, quantitative PCR, virology

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Background

Throughout the world, crude extracts are used by millions of people for their primary source of medicine. It is essential to identify those extracts that can be developed for modern medical application (Fabricant & Farnsworth, 2001; Farnsworth, 1988; Feeny, 1976). Also it is necessary to understand the molecular mechanisms involved when a specific plant extract exhibits antimicrobial activity. Two chapters of the dissertation are devoted to this study. Chapter two is concerned with the investigation of 61 plant extracts and their antimicrobial effects including antiherpetic effects in Vero C1008 cells. Chapter three is concerned with specific antiviral mechanisms that may be at work in the herpes simplex virus types 1 and 2 (HSV 1 and HSV 2) infected cell, treated with the acetone extract form the root tissue of *Kalanchoe pinnata*. This work began in the Natural Product Research Laboratory at Brigham Young University which continues to identify plants extracts that may suggest positive uses for animals and humans. Many of these plants had not been investigated in depth, nor had the mechanisms of antiviral activity been determined. Specifically my interests were to investigate an extract and the mechanisms of action of that extract against the HSV 1 and HSV 2 infection.

The World Health Organization estimates that today 80% of the people in developing countries rely on traditional medicine for their primary health care needs and about 85% of the traditional medicine involves the use of plant extracts. Plant extracts have long sparked the interest of scientists and nonscientist alike, largely because of their secondary metabolites. The production of secondary metabolites requires energy otherwise used for reproduction and growth therefore plants will not typically produce these compounds without specific environmental

pressures. The pathways of many unique secondary metabolic systems are derived from primary metabolism with a variety of enzymes that play roles as signaling molecules and function in processes as diverse as UV protection, defense against herbivores and pathogens, and recruiting pollinators and seed dispersers (Coley, Bryant & Chappin, 1985). This suggests that many of these compounds can be found only in plants growing in their natural habitat and may not be reproduced in a greenhouse.

Kalanchoe pinnata: history and medicinal uses

Greeks, Native Americans, Chinese, and Africans have treated many afflictions using plants and plant extracts (Igwe & Akunyili, 2005). One such plant is *Kalanchoe pinnata* Linn (Crassulaceae), a fleshly shrub that is about 60–120 cm high and branched from the base with opposite, simple or trifoliate petiolate leaves. Although this perennial herb probably originated from Madagascar, *K. pinnata* abounds in tropical regions of the world, usually growing widely in hot and humid areas, around dwelling places, along roadsides, and in abandoned farms and fields. In Nigeria and some other West African countries, the fleshy leaves of *K. pinnata* are frequently used as an herbal remedy for an array of human disorders, including hypertension, diabetes mellitus, bruises, wounds, boils, abscesses, insect bites, arthritis, rheumatism, joint pains, headaches, and body pains (Ojewole, 2002). Also, the leaves are used for inguinal lymphadenitis and ear diseases (Adjanohoun, Ahiyi, Ake-Assi, Dramane, Elewude, Fadoju, Gbile, Goudote, Johnson, Keita, Morakinyo, Ojewole, Olatunji & Sofowora, 1991). There is extensive documentation on the use of leaves from *K. pinnata* as an ethnomedicine for the treatment of earache, burns, ulcers, insect bites, diarrhea and lithiasis (Agoho, 1974; Chopra, Nayar & Chopra, 1980; Ofokansi, Esimone & Anele, 2005; Ojewole, 2002; Ojewole, 2005). It is commonly used in traditional medicine as a painkiller, particularly in cases of menstrual pains

and breast abscesses. For the treatment of earache, the juice from the boiled leaves is squeezed into the affected ear and for the treatment of breast abscess; the crushed leaves are mixed with kaolin or china clay and applied externally to the entire breast. In the management of menstrual pain, kaolin mixture of the whole plant extract is applied to the waist and umbilical region. The plant has been reported to be used in the treatment of headache, as a diuretic, and also for shaving (Dalziel & Hutchinston, 1958). It is applied to burns, and when rubbed on bodies of febrile children the material has an antipyretic effect (Dalziel, 1956).

Secondary metabolites in Kalanchoe pinnata tissue

A number of investigators have shown that a host of secondary plant metabolites found in *K. pinnata* possess medicinal properties in experimental animal models (Adzu, Amos, Kapu & Gamaniel, 2003; Akah & Okafor, 1992; Dongmo, Kamanyi, Dzikouk, Nkeh, Tan, Nguelefack, Nole, Bopelet & Wagner, 2003; Li, Lin, Myers & Leach, 2003; Liu, 1995; Marles & Farnsworth, 1995; Ojewole, 2002; Price, Johnson & Fenwick, 1987; Simon, Najid, Chulia, Delage & Rigaud, 1992; Singh, Singh, Bani, Gupta & Banerjee, 1992; Sugishta, Sakae & Yukio, 1982; Taesotiku, Panthong, Kanjanapothi, Verpoorte & Scheffer, 2003). The phenolic compounds in *K. pinnata* include a group of secondary metabolites known as flavonoids and tannins. Many of which are known to be anti-microbial agents. Ofakansi et al. (2005) report that *K. pinnata* is effective in the treatment of typhoid fever and other bacterial infections, particularly those caused by *Staphylococcus aureus, Echerichia coli, Bacillus subtilis, Pseudomonas aeruginosa, Klebsiella aerogenes, Klebsiella pneunoniae* and *Salmonella typhi. K. pinnata* is useful in treating the placenta and navel of newborn babies because of its healing properties and aid in preventing infections. Okwu and Josiah, (2006) report that flavonoids are potent water-soluble antioxidants and free radical scavengers and aid in preventing oxidative cell damage and have strong

anticancer activity (Del-Rio, Obdululio, Casfillo, Marin & Ortuno, 1997; Okwu & Josiah, 2006; Okwu & Okwu, 2004; Salah, Miller, Pagange, Tijburg, Bolwell, Rice & Evans, 1995). Flavonoids in the intestinal tract lower the risk of heart disease, and as antioxidants, provide anti-inflammatory activity. Tannins have stringent properties and hasten the healing of wounds and inflamed mucous membranes. Additionally, hydrolyzable and condensed tannins have been tested for anti-HSV activity (Fukuchi, Sagagami, Okuda, Hatano, Tanuma, Kitajima, Inoue, Inoue, Ichikawa, Nonoyama & Kono, 1989; Takechi, Tanaka, Takehara, Nonaka & Nishioka, 1985). Tannic acid was found to inhibit virus adsorption by the cells. However, few studies have elucidated clearly the antiviral mode of tannins. These authors showed that hydrolysable leaf tannins of *K. pinnata* (a) were able to inhibit directly HSV attachment to and penetration into cells, (b) affected the late event(s) of HSV 2 infection, and (3) exhibited viral inactivation activity at high concentrations.

The high terpenoid-based saponin in *K. pinnata* justifies the use of extracts to stop bleeding and in treating wounds. Saponins are known to precipitate and coagulate red blood cells. Some characteristics of saponins include formation of foams in aqueous solutions, hemolytic activity, cholesterol binding properties and bitterness (Okwu & Okwu, 2004; Sodipo, Akiniyi & Ogunbamosu, 2002). Physiologically active alkaloid compounds have been isolated from *K. pinnata.* Synthetic derivatives are used as medicinal agents for analgesic, antispasmodic and bactericidal effects (Okwu & Okwu, 2004; Stary, 1998)

Use of Kalanchoe pinnata as an anti-HSV agent

Virucidal activity of *K. pinnata* was evaluated by Carlucci et al. (Carlucci, Ciancia, Matulewicz, Cerezo & Damonte, 1999). Using virus suspension containing 2 x 10^7 PFU HSV 2 was mixed with or without multiple leaf extract concentrations of *K. pinnata* for 6 h at room

temperature. It was concluded that antiviral activity of *K. pinnata* was not related to its virucidal ability. Cheng et al. (Cheng, Lin & Lin, 2002) confirmed that *K. pinnata* continued to exhibit antiviral activity even added 12 h after infection. These observations suggest that *K. pinnata* affects the late event(s) of HSV 2 infection. Other studies on the mode of action have shown that *K. pinnata* inhibits the attachment and penetration of HSV 2 into cells. HSV attachment is mediated by glycoprotein C (gC), which interacts with cell sulfate proteoglycans carrying heparan sulphate carbohydrate (Spear, Shieh, Herold, WuDunn & Koshy, 1992). The stability of attachment between viruses to cells is dependent on the presence of glycoprotein D (gD) (Rajcani & Vojvodova, 1998). Fusion of the membrane between virion envelope and plasma membrane of the target cell requires glycoproteins D, B, H and L, or in a combination of all of them (Roizman & Spears, 1996; Spear, 1993; Spear et al., 1992). According to their results on viral attachment and penetration assays, *K. pinnata* possibly affects the attachment and penetration of viruses into cells through the disturbance of viral glycoproteins.

Aside from studies showing that some root extracts of *K. pinnata* are used to treat coughs and burns (Iwu, 1982) there is limited research into the antimicrobial properties and mechanisms involved in the antiviral properties of root tissue of *K. pinnata*. As suggested by Cassady and Whitley (1997), future anti-herpesviruses agents may target enzymes or viral factors essential for infection or inhibiting other steps in the viral infection cycle such as viral entry, protein synthesis or capsid assembly.

Herpes simplex virus

Herpes simplex virus types 1 and 2 (HSV 1 and HSV 2) are members of the alphaherpesvirus subfamily Herpesviridae. The life cycle of HSV is extremely complex and there are numerous opportunities for the development of a novel antiviral therapy. Successful infection by HSV

requires the virus particles to attach to and penetrate into cells: uncoat the viral DNA and deposit it into the nucleus: transcribe genes sequentially to produce immediate early (α), early (β) and late (γ) mRNAs: synthesize regulatory proteins, DNA replication enzymes and structural viral proteins from these mRNAs: replicate its DNA genome, assemble new capsids and virions, and release these particles from the infected cell (Roizman & Spears, 1996). The multiple steps to HSV encapsidation and egress from cells are targets of interest for antivirals to prevent the spread of HSV in target cells. Acute and recurrent HSV infections are distributed worldwide and cause a wide range of diseases including gingivostomatitis, keratoconjunctivitis, genital tract infections, encephalitis, and infection of neonates and immunocompromised patients (Whitley, 2002; Whitley, Kimberlin & Roizman, 1998). After the primary infection, HSV often persists in the neurons of nerve ganglia virus types are neurotropic and may spread to the brain during primary or recurrent infections (Igwe & Akunyili, 2005) . HSV 1 is the most usual cause of sporadic encephalitis, except in neonates, with a mortality rate of 70-75% and severe permanent sequelae in survivors (Griffin, 1991). Over the last two decades the number of immunocompromised patients has increased dramatically. This is the consequence of aggressive chemotherapy regimens, expanding organ transplantation and the rising incidence of human immunodeficiency virus infection (Levin, 1993). Reactivation of latent HSV, which is very common during the deficiency of immunity, causes recurrent herpetic infection. The most common treatments for herpetic infections include acyclovir (ACV), valaciclovir, famciclovir and cidafovir (Cassady & Whitley, 1997). Some alpha herpes viruses have become resistant to ACV and other nucleoside analogues thus the efficacy of these drugs is limited (Englund, Zimmerman, Swierkosz, Goodman, Scholl & Balfour, 1990). With the rise of immunocompromised patients and emergence of ACV-resistant herpesviruses, new medications,

especially novel antiviral agents, are needed for continuous effective treatment of associated diseases.

The Purpose and Problem

The main purpose of this study was to screen a large collection of plant species for general antiviral properties and test the inhibitory extracts for levels of cytotoxicity search for the antiviral molecular mechanisms of the most inhibitory but least cytotoxic extract. The plant eventually chosen for in-depth examination was *K. pinnata.* Eventually, quantitative polymerase chain reaction assays (q-PCR) were used to assess whether or not the acetone extract of the root tissue extract from *K.* pinnata reduced gene transcription and DNA replication of the virus itself.

In chapter two the problem begins with the search for antiherpetic extracts of plant tissues obtained from ethnobotanical collections from Morocco and the U.S.A. Extracts were prepared and tested and the more promising of the extracts were subjected to further bioassays. This research began with two extracts (acetone and methanol) made from thirty-one plant species from Morocco, the Sonoran Desert and Hawaii. These extracts were screened for general cell toxicity using a sulphorhodamine assay and for activity against infectious microbes and cancer cells, in vitro. Using the same extracts, I screened for antiviral activity against HSV 1 and HSV 2. These viruses were chosen because viral resistance toward anti-HSV agents is increasing among HSV infected patients and the need for new effective compounds against HSV exists (Coen, 1996; Cohen, 2004). Along with the increasing number of immunocompromised patients and the prolonged administration of antiviral agents, the problem of drug-resistance among herpes viruses is not expected to fade. While nucleoside analogues such as acyclovir have been used successfully to treat herpetic infections, resistant strains and drug toxic side effects

encourage research for new compounds, either synthetic or natural, to improve the current antiviral arsenal (Cordell, Beecher & Pezzuto, 1991; Cordell & Colvard, 2005).

After initial antiviral screening extracts exhibiting significant plaque reduction were subjected to a further quantitative measure of cell toxicity using a neutral red assay. To confirm earlier results and check for reproducibility, repetitive mini-plaque assays using alternative experimental designs: adding the extract at different times (pre and post-infection) and at multiple concentrations post infection the few extracts showing more than 50% antiviral inhibition were tested for cytotoxicity to Vero C1008 cells in vitro.

Changes in cell morphology were continually observed and noted using light microscopy. These changes included the formation of plaques (holes in the monolayer due to cell death) and syncytia (masses of cytoplasm containing several nuclei enclosed within a single plasma membrane and commonly found in HSV infected cell culture). Through all these tests, the list of plant extracts was eventually narrowed to one specific plant extract from the root tissues of *K. pinnata*. Activity of this plant became apparent early on in the initial screening of antimicrobial activity and was pursued aggressively in search of the mechanisms of action on HSV 1 and HSV 2 infections in vitro. The plant root tissues collected for this study came from the islands of Maui and Kauai, Hawaii. It's remarkable ability to nearly eliminate HSV's capacity to form syncytia or plaques in C1008 cell cultures led me to further investigate the mechanisms involved.

Chapter three of this dissertation expands to an investigation of the mechanism(s) involved in the observed antiviral behavior of *K. pinnata,* and ends with results obtained from q-PCR experiments to detect areas of molecular influence *K. pinnata* has on HSV's reproduction, gene expression and spread in host cells.

Research Objectives

1. Define the relationship between the antiherpetic effects of the acetone extract of *K. pinnata* root tissue and the molecular mechanism of action in vitro.
2. Pursue a path in discovery of how the extract interferes with HSV replication in the host cell in vitro.

Research Hypotheses

Hypothesis 1: *K. pinnata* extract inhibits HSV DNA replication.

Hypothesis 2: *K. pinnata* extract inhibits immediate early transcription of one or more HSV genes.

Hypothesis 3: *K. pinnata* reduces the production of one or more early (β) or late (γ) gene transcripts responsible for the production of scaffolding proteins, capsid production, DNA encapsidation or other stage of HSV replication causing the significant reduction in the spread of the virus in vitro.

Limitations of the Study

A broad screening method to initially identify antimicrobial compounds from ethnobotanical plants should eventually produce useful drugs, but it is contingent on the availability of adequate funding and appropriate predictable bioassay systems. It is reasonable to say that many ethnobotanical plants, kept out of the cultivation and genetic manipulation arena, will contain chemicals that protect the plant from natural predators and environmental pressures that drive natural selection. These plants are good choices to study, for their distinct niche and ability to survive. All plant tissues were taken from reliable and consistent sources. Due to changes in environmental pressures at collection sites for *K. pinnata* tissues, there may be some variable compound profiles over the six years of study but overall, results of the assays were similar. A

purification process for the crude extract would yield more reliable results and could reduce the concentration dose at which the final results were obtained.

Significance of the Study

The significance of the laboratory research was that valuable information derived from these studies will contribute to the body of pharmaceutical knowledge about the less understood ethnobotanical medicinal plants in certain parts of the world. It is important to identify medicinal plant species, preserve their natural habitat, and record the history of their uses. It is also essential that scientists examine medicinal plants against their asserted claims and if found valid in the laboratory, investigate the molecular mechanisms contributing to the cellular and pathogen responses. Not only are these plants of significance to many people in less industrialized societies, but if these plants are truly valuable for the health of human populations, ecological reasons for protecting and maintaining the plant habitats are that much more compelling (Heinrich, 2001).

Professional Development Curriculum

Background

Chapter four departs from the laboratory research component of the dissertation and addresses the need for a well-designed professional development curriculum for secondary biology teachers needing current knowledge and laboratory experiences in the microbiology and molecular biology disciplines. The 2007 Trend in International Mathematics and Science Study offered bad news for the U.S. science education community. The TIMSS results, released in December 2008, showed statistically no change from the 1995 scores in (National Center Educational Statistics, 2003). TIMSS measures student performance in science content and cognitive skills of knowing, applying and reasoning. In 2007, Singapore, Chinese Taipei, Japan,

Hong Kong, Russia and England had higher average scores than the United States in science. Factors behind this disappointment include the No Child Left Behind (NCLB) Act, resulting in less time, resources and focus on science education, including the lack of pre-service preparation and in-service professional development for science education. Nearly 75% of the teachers questioned said they needed substantial professional development to deepen their own science content knowledge (Slater, 2008). In response to the poor results of TIMSS, the education community has seen a surge of public interest in the training and subject matter competency of secondary science teachers. This interest has resulted in congressional hearings and high-profile publications such as *Rising Above the Gathering Storm: Energizing and Employing America for a Brighter Economic Future* (Jacobs, Martin & Otieno, 2008). Politicians, business leaders, teachers and administrators of higher education have taken notice of the crucial link between the training of qualified secondary teachers and the production of a well-prepared college student population and workforce. Large sums of money have been awarded on the basis that partnership with organizations such as the Math-Science Partnership (MSP) and the National Science Foundation will result in a higher quality teaching force and better prepared teachers and students (National Science Foundation, 2009).

A high level of expertise should be expected from our science teachers. Real expertise comes from working in the field. Most experts have what may be called –econtributory expertise"—sufficient knowledge and understanding to make contributions (Collins, 2007). This level of expertise can be attained only by spending time interacting with other contributory experts in the field. Most established secondary science teachers do not have –econtributory expertise". And if they do not have opportunities to update their skills and knowledge, they are unable to maintain the level of knowledge once they go into the classroom full time. Even among newer life science

teachers, the course requirements are slim in the molecular and microbiology departments (Table 1, Chapter 4). Therefore, development of a molecular/microbiology in-service course for life science teachers, involving current curriculum, inquiry activities and discussion about the epistemology of science is critical to the continuing education and competence of secondary biology teachers. Continued education in this field will enable the ~~"n~~on-expert" scientist such as the secondary life science teacher to acquire science knowledge attained through inquiry, practice and integration of microbiology and molecular concepts resulting in more confident and prepared teachers and thus the students they educate.

The current reform effort in science education requires a substantive change in how science is taught and an equally substantive change in professional development practices (Arzi & White, 2007). Teacher preparation courses and in-service activities in methods of teaching science frequently emphasize technical skills rather than decision making, theory and reasoning. If reform is to be accomplished, professional development must include experiences that engage teachers in active learning, expands their content knowledge and models ~~"b~~est" science teaching practices as described in the teaching standards of the NSTA (National Committee on Science Education Standards and Assessment, 1996). Professional development courses must answer serious questions about science education today such as what does a science teacher need to know, what does it mean to know a lot or a little, what is a sound science foundation and in-depth understanding. It is assumed that teachers of science will continue to study science throughout their careers. Most teachers of science have a broad base of scientific knowledge extensive enough to understand the nature of scientific inquiry, its central role in science, and have some skills in scientific inquiry. Teachers understand the fundamental facts and concepts in major science disciplines. Fewer make conceptual connections within and across science

disciplines. Beyond the foundation in content standards and objectives, how much more science a teacher needs to know is an issue of breadth versus depth. Breadth implies a focus on the basic ideas of science and is central to teaching science at all grade levels. Depth refers to knowing and understanding the basic ideas within a science discipline and the supporting experimental and theoretical knowledge. This professional development course focuses on depth in the biological areas of virology, molecular biology, and immunology learned using methods of inquiry and investigation.

Traditionally, conventional biology is taught in a very linear style, separating microbiology and molecular biology concepts into separate chapters. As a result, students leave with a fragmented opinion of what are a dynamic, socially, politically, and ethically relevant areas of biology that impact their lives. Moreover, specific areas of microbiology: virology, molecular biology and immunology, can be used to teach almost every theme and overarching concept in biology if one is shown the relationships and connections among them. My course uses current application scenarios for inquiry activities and discussion points concerning science, society and biotechnology. Aspects of microbiology or molecular biology are discussed and expanded within every level of the course. Components of this curriculum are meant to be used with confidence by teachers who have completed this in-service course. It is prepared so teachers can take the materials and pedagogy and immediately use it in their own classroom.

The Purpose

The purpose for designing a professional development course for secondary biology teachers is in two parts. First, I wanted to demonstrate my acquired knowledge and experience in molecular and microbiology disciplines by designing a curriculum that integrates the specific areas of virology, molecular biology, and immunology into the secondary biology curriculum. I

see such a course is necessary for the continued education of life science teachers who most likely did not receive courses in these areas, or who have long ago completed their formal education and need to update their knowledge and skills in these areas. Second, having participated in a graduate microbiology and molecular biology program, and as an experienced and active high school biology teacher, I wanted to establish a curriculum within my own classroom that integrates these disciplines with the concepts I teach in order to satisfy the state's established core standards and objectives.

Objectives of the Professional Development Curriculum

1. Design an in-service curriculum that will contain important, current and necessary knowledge in the areas of virology, molecular biology and immunology.
2. Increase teacher knowledge base and interest in these specific areas.
3. Create a course where teachers can apply this knowledge to current social and political events and improve the application standard in their biology curriculum.

This professional development curriculum began with four assumptions about the nature of secondary science in-service. First, professional development for a science teacher should be a continuous, lifelong process. The understanding and abilities required to be a masterful teacher of science are not static. Second, science content increases and changes, and a teacher's understanding in science must keep pace. Third, knowledge about the process of learning is continually developing, requiring teachers to stay informed. And fourth, we live in a changing society that deeply influences events in schools; social changes affect students as they come to school and affect what they need to carry away with them. Science teachers must be involved in the acquisition of current, accurate and relevant scientific information as well as development

and refinement of new approaches to teaching, assessment, and curriculum (National Center Educational Statistics, 2003).

In this course science learning experiences for teachers will involve teachers actively investigating phenomena that can be studied scientifically, interpreting results, making sense of findings consistent with currently accepted scientific understanding, and address issues, events, problems, or topics significant in science. They will be exposed to scientific literature, media, and technological resources that can expand their science knowledge and their ability to access further knowledge. An important aspect of this course is to allow teachers to work in the lab with experienced graduate students from local universities. Teachers are given the opportunity to be like a real scientist, asking questions, developing hypotheses, researching background information and writing an experimental design. This experience will change the way science teachers think about and prepare laboratory experiences for their own students.

The significance of the professional development curriculum will be to teachers and ultimately to their students. Microbiology and molecular biology concepts are written into this course which includes case studies, inquiry activities and organizational skills, without sacrificing content to meet state standards and core objectives. By fostering independent thinking and encouraging students to actively work towards understanding the material, teachers prepare students to approach the material rationally and accurately. The content and skills taught in this curriculum will help biology teachers help their student to better understand the global perspective of science, be successful in college biology classes and enhance their success in later life and providing a pathway for lifelong learning.

Summary

This dissertation is divided into four chapters. Chapter one is the dissertation introduction. The research topics were briefly described. The first objective discussed concerned the identification of a plant extract whose antiherpetic effects and low cytoxicity was worthy of additional investigation into the mechanisms of action. This part of the study included molecular examination of potential mechanisms involved in the observed antiviral properties of *K. pinnata.* The second objective discussed the need for a professional development course aimed to secondary biology teachers; to update their molecular and microbiology knowledge and practice scientific inquiry while integrating these disciplines into the standard core curriculum.

Chapter two is a scientific paper, prepared for Pharmaceutical Biology, a peer reviewed journal published by Informa Pharmaceutical Science. This journal accepts manuscripts describing the discovery, methods for discovery, description, analysis characterization, and production/isolation of biologically-active chemicals or other substances utilized in systems of traditional medicine. My paper was written and submitted to the journal October 30, 2009 for request of publication and was accepted for publication on November 7, 2009, as is. In this paper I report screening sixty-two extracts from thirty-one plant species for cytotoxic activity against the Vero C1008 cell sub-line and for antiviral activity in cells infected with HSV types 1 and 2. Initial tests for cytotoxicity and antiherpes activity were performed; both sulforhodamine B assays and neutral red assays to detect general cytotoxic effects and immunoperoxidase mini-plaque reduction assays to detect viral structural protein synthesis. Of the thirty-one species tested only the acetone and methanol extracts of *K. pinnata* were acceptably non-cytotoxic to host cells while showing significant inhibitory effects on HSV 1 and HSV 2. Results of this

study provided a scientific rationale for further study into potential mechanisms of HSV inhibition in vitro by the root extracts of *K. pinnata.*

Chapter three is a scientific paper formatted to conform to Antiviral Research, a peer reviewed multidisciplinary journal of antiviral agents, natural host defense mechanisms and interferons and antiviral vaccines, published by the International Society for Antiviral Research. My work examines possible mechanisms by which a crude acetone extract from root tissue of *K. pinnata* inhibits HSV 1 and HSV 2 infection in cultured C1008 cells. The efficacies of combinations of extract concentrations that inhibit the formation of plaques in relation to their effects on cell viability were examined. Antiviral mechanisms were investigated using real time PCR: measuring template number of HSV DNA at different time points post infection and by measuring transcript number of HSV RNA for four specific genes: beta genes UL23 and UL30, and gamma genes UL17 and US4 when infected C1008 cells were exposed to the extract. An examination of transcript number found a significant decrease in these genes. Reduced viral DNA replication as well as reduced RNA transcription of three viral genes suggested that multiple modes of herpes virus inhibition by *K. pinnata* were present and that this extract contains multiple anti-HSV compounds that interfere with the normal viral life cycle process.

Finally, chapter four of this dissertation diverts attention to meeting the educational needs of the professional high school biology teacher; specifically in the areas of virology, molecular biology and immunology. Chapter four is divided into two sections, A and B, for the trainer of the professional development course and the teacher who is attending the course, respectively. Each section is divided into four units: 1) Virology, 2) Molecular Biology, 3) Immunology and, 4) Pathogenesis and Society, and each unit consists of specific segments. In Section A, the segments are entitled 1) Learning Objectives, 2) Essential Prior Knowledge, 3) Trainer

Preparation Notes, which include misconceptions that should be addressed with the class and anticipated results 4) Essential Questions, 5) Core Concepts, to be introduce and explained if necessary, 6) Anticipatory Set, 7) Comprehension and Application Activities, to promote inquiry, integration and discussion, 8) Higher Order Thinking Skills, 9) Interactive Notebook/Journal Writing, and 10) Assessment, to monitor the progress and understandings of the participants. Included in the lesson materials will be suggested organizational tools the trainer should model for the teachers. Cooperative learning activities are provided where possible so that teachers will see how the concepts can be presented and practiced in an inquiry based environment. Section B is subdivided into four units consisting of four segments each. These segments are entitled: 1) Worthy of Note, which may be copied and given to students to help the teacher modify his existing ideas based on new information, 2) Moral/ Ethical/ Reasoning/ Dilemma Scenarios, to strengthen the relevance of the lesson and emphasize the relationship between society and technology, 3) Divergent and Creative Thinking Activity, to emphasize the process of learning and promote higher order thinking skills in the classroom, and 4) Laboratory Component, to allow the teachers to become more comfortable and knowledgeable about the new technology used in a microbiology and molecular biology laboratory and stress the inquiry aspects of learning.

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Chapter Two - Activity of acetone and methanol extracts from thirty one medicinal plant species against herpes simplex virus types 1 and 2

Mary R. Jaeger Greer[1, 2], Rex G. Cates[1], F. Brent Johnson[2], Levon Ohai[3]

[1]Natural Products Laboratory, Department of Biology, Brigham Young University, Provo, Utah, USA; [2]Department of Microbiology and Molecular Biology, Brigham Young University, Provo, Utah, USA; [3]University of Hawaii, Oahu, Hawaii, USA

Running Head: Activity of medicinal plants against HSV 1 and 2

Address for correspondence: Rex G. Cates, Natural Products Laboratory, Department of Biology, Brigham Young University, 425 WIDB, Provo, UT 84602, USA. Fax: (801) 422-0090; E-mail: rex_cates@byu.edu

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Abstract

Context: Thirty-one medicinal plant species from Hawaii, Morocco, and the Sonoran Desert, USA have been shown in past studies to be highly inhibitory to pathogenic bacteria, fungi, and certain cancer cell lines. However, none were tested for antiviral activity.

Objective: Acetone and methanol extracts from these species were bio-assayed for antiviral activity against herpes simplex virus types 1 and 2, and for cytotoxicity to the Vero C1008 cell line.

Materials and methods: Extracts from these species were tested *in vitro* for antiviral activity using an immunoperoxidase mini-plaque reduction assay to detect viral structural protein synthesis. A 50% inhibitory concentration (IC_{50}) was computed. Sulforhodamine B and neutral red assays were used to qualitatively and quantitatively assess the cytotoxicity of extracts to C1008 cells, and to compute a 50% cytotoxic concentration (CC_{50}) using a dose response curve.

Results: Eight of the 31 plant species assayed showed significant antiviral activity against HSV 1 and HSV 2 viruses. The acetone extract of *Kalanchoe pinnata* Pers. (Crassulaceae) produced an IC_{50} of 0.025 mg/ml and a CC_{50} of 1.25 mg/ml yielding a therapeutic index of 50. Additionally, this extract reduced plaque numbers to zero or near zero at a concentration of 0.1 mg/ml when added 30 min before or 30 min after virus infection.

Discussion and conclusion: The mechanism of inhibition against HSV 1 and HSV 2 viruses is now being investigated, along with fractionation of the acetone extract in search of the active compound or compounds.

Introduction

Natural products play an important role in the discovery of leads for the development of drugs to treat human diseases (Newman & Cragg, 2007). Over two-thousand compounds extracted from higher plants are used in a medical setting throughout the world, and at least forty-six percent of these have not been used in the United States (Fabricant & Farnsworth, 2001). Knowledge about the medicinal use of these species may be held individually, tribally, or documented in well established tomes (Newman et al., 2008). But often scientific information is lacking as to whether these plants contain anti-carcinogenic, antibiotic, antifungal, and antiviral properties (Cordell et al., 1991).

Researchers in the Natural Products Laboratory, Brigham Young University have collected and tested 157 medicinal plant species for activity against pathogenic microbial diseases, cancer cell lines, and for cytotoxicity (Donaldson et al., 2004; Donaldson & Cates, 2005). From these, 31 plant species (one Hawaiian plant, 15 from Morocco, 15 from North America) were selected that had inhibition levels at 70% or greater against one or more of the above organisms or cancer cell lines. However, none of these species have been tested for activity against human herpes simplex virus types 1 or 2 (HSV 1, HSV 2).

HSV 1 and HSV 2 are common human pathogens which cause several symptoms such as vesiculo-ulcerative lesions at mucocutaneous junctions, gingivostomatitis, keratitis, and encephalitis (Arduino & Porter, 2007). In immunocompetent hosts these clinical symptoms are often benign, but in immunocompromised patients they become severe, may be progressive, and require more time for healing (Whitley, 2002). Life threatening herpes infections may be transmitted to neonates during delivery where a wide spectrum of clinical manifestations can occur including encephalitis. HSV may account for 10% to 20% of all viral encephalitis

infections in the United States (Tang et al., 1999). Many medicinal plants may have a variety of chemical constituents known to inhibit the replication cycle of various types of DNA or RNA viruses (Schang, 2006), including herpes simplex. As a consequence attention has been given to the development of antiviral agents from traditional plant medicines (Jassim & Naji, 2003).

Our objective was to determine if the selected 31 plant species would demonstrate significant activity against HSV 1 and HSV 2 viruses *in vitro*. Acetone and methanol extracts from these species were bio-assayed for activity against these viruses using the immunoperoxidase mini-plaque assay. Cytotoxicity was determined using a neutral red assay. A therapeutic index (TI), defined as the ratio of the cytotoxic concentration in 50% of the cell monolayers (CC_{50}) to the inhibiting concentration of the extract that showed 50% antiviral effects on the cell monolayers (IC_{50}) (Burns, 1999), was computed for the four most inhibitory species.

Materials and Methods

Plant tissue collection

Medicinal plant species were collected in Hawaii (2002and 2004), in Morocco (2002 and 2004), and in the Sonoran Desert, USA, 2000 - 2004 (Donaldson et al., 2005; Donaldson & Cates, 2004). Samples from the USA were labeled and placed in plastic bags, frozen in coolers containing dry ice, and then shipped to the Natural Products Laboratory, Brigham Young University. These were stored in a -80°C ultralow until analyzed. Samples from Morocco were air dried, ground to a fine powder using a Wiley Mill, and stored in labeled sample bags in boxes. Species analyzed in this study along with their collection number and traditional medicinal plant use are found in Table 1.

Tissue extraction and drug preparation

Three grams of air dried leaves, stems, or roots, or five grams of frozen tissue, were ground using a mortar and pestle in liquid nitrogen in 15 ml hexane and then filtered through cheese cloth and VWR grade 415 filter paper. The filtrate was collected in vials. This process was repeated twice using 10 ml and 5 ml, respectively. These were combined into one pre-weighed vial and taken to dryness using nitrogen gas. The remaining plant material was extracted in acetone using the same protocol as above. The 15, 10, and 5 ml aliquots were combined into a pre-weighed vial and dried using nitrogen gas. The remaining plant material was extracted with 70% methanol following the same protocol as above, combined into a pre-weighed vial, and dried using nitrogen gas.

Distilled water was added to the dried acetone or methanol extract vial to make an 8 mg/ml concentration. Each vial was vortexed and sonicated to homogenize the extract, filtered through 0.2 µm Minisart filter (Sartoirus Stedim Biotech, France), placed into 1.5 ml Ependorf tubes, and stored at -80°C until assayed.

Cell culture and viruses

African green monkey kidney cells (ATCC CRL-1586, sub-line C1008) were maintained at 37°C and 5% CO_2 in Dulbecco modified eagle medium (DMEM, Sigma Chemical Company, St. Louis, Mo.) supplemented with 5% cosmic calf serum (HyClone Laboratories, Logan, Utah), 10 µM HEPES buffer, and 50 µg/ml gentamicin (Sigma-Aldrich, Inc. St. Louis, MO). Stocks of HSV 1 (McIntyre strain) and HSV 2 (strain 333) (Jensen & Johnson, 1994) which were stored at -80°C were used to determine the activity of the plant extracts.

Mini-plaque reduction assay

C1008 cell monolayers were used to determine if any of the plant extracts inhibited HSV 1 or HSV 2 infection *in vitro* (Table 2). Cells were seeded into 24-well plates, and when cell monolayers were 90% confluent the extract was introduced to each well. Three concentrations were used, and each concentration was replicated three times. The old media was removed and to the appropriate well was added 1 ml DMEM containing 6.25, 12.5, or 25 µl of plant extract resulting in concentrations of 0.05, 0.1, and 0.2 mg/ml, respectively. Control wells contained C1008 cells growing in l ml of medium without extracts and were randomly located. After 30 min 0.1 ml virus suspension was added to each well for each concentration at a multiplicity of infection of one (MOI=1) and incubated at 30˚C and 5% CO_2 for 30 h (Taniguchi & Yoshino, 1964).

A concern was that the plant extracts might prevent entry or destroy the virus particles. Consequently, C1008 cells were seeded into 24-well plates, incubated to 95% confluency, and then HSV 1 or HSV 2 were introduced to the monolayers 30 min before the extract was added. Viruses were added by removing the old media and adding 0.1 ml of virus suspension at MOI=1 to test wells; 0.1 ml media was added to the control wells. Plates were incubated at 37°C and 5% CO_2 for 30 min. Then 1.1 ml of media with extract in one of three concentrations (0.05, 0.1, and 0.2 mg/ml) were added and plates were incubated for 30 h.

Microscopic observations and plaque counts to measure extract inhibition

In vitro HSV 1 and HSV 2 infections and plant extracts that induced cytotoxicity might cause observable changes in C1008 cell monolayers. Consequently, microscopic observations were recorded to determine if HSV infections and/or plant extracts might adversely affect cell growth, and therefore indicate cytotoxicity. Plaques due to virus infection appeared as holes in the

monolayer due to cell death and syncytia formation. In the case of *K. pinnata* only foci (individual stained cells indicating the presence of virus structural proteins) were observed, and these were counted to determine percent inhibition for this species. Observable cellular changes due to adding plant extracts were minimal, but rounding up of cells and changes in cell membrane shape was noted for extracts (especially at 0.2 mg/ml) (Table 2). However, none of the changes influenced quantification of the effects of viruses on cell monolayers.

Immunoperoxidase staining to determine plaque number in cell monolayers

Following Luker et al. (1991) all wells were stained using an immunoperoxidase staining protocol to detect plaque and syncytia numbers or presence of foci. Each well was separated into quadrates and plaques were counted manually using an inverted phase contrast microscope (Nikon Eclipse TS100). Overlapping plaques were deemed individual when lobes were apparent (Zielinska et al., 2005). Plaque number was calculated as a mean of three replicates, and growth inhibition of infected cells was given as a percentage of the control (Table 2). If the established cell monolayer appeared disrupted, detached, or absent from the well after the extract was added and just before staining, the extract was assumed to be toxic to the C1008 cells. This was noted as "tx" in Table 2. The IC_{50} (50% inhibitory concentration of viral effect) was determined from the dose response curve (Table 3). Positive controls used in the IC_{50} determination were infected cell monolayers without extract.

Neutral red assay for cytotoxicity of active extracts

The four extracts confirmed to be inhibitory to HSV 1 and 2 by the mini-plaque assay were tested for cytotoxicity to C1008 cells using a neutral red (NR) assay (Cytotox NR Kit, Xenometrix, Switzerland). The assay is based on the ability of viable cells to incorporate and bind neutral red within lysosomes (Motohashi et al., 2003). C1008 cells were treated with serial

dilutions of each plant extract (0.0125, 0.025, 0.05, 0.1, 0.2, 0.4, 0.8 and 1.6 mg/ml), and the concentrations were replicated three times. After 72 h cell viability was measured using a spectrometer (Fusion α-HT Universal Microplate Analyzer, Packard Instruments, Meriden, CT) with a 540 nm filter and a 690 nm reference filter. Results were determined from the dose-response curves and a CC_{50} (50% cytotoxic concentration) was reported (Table 3) (Rajbhandari et al., 2007).

Virucidal assay

Antiviral screening assays must be viewed cautiously when apparent antiviral activity is observed until a determination is made that the extract is not destroying or disrupting the virus particle. To determine if the acetone extract of *K. pinnata* interfered with virus particle structure, the extract was permitted to incubate with the virus prior to exposure to the cell monolayer. The acetone extract of *K .pinnata* (0.2 mg/ml) was added to the thawed HSV 2 virus stock of 8×10^7 pfu/ml (plaque forming units per ml) and incubated at 37°C for 30 min. Six, 10-fold serial dilutions of virus stock (10^{-1} to 10^{-6}) with and without extract were added to cell monolayers in 24-well plates and incubated at 37ºC and 5% CO_2 for 36 h. Staining followed that of the mini-plaque assay described above. Plaque numbers were counted as described for the mini-plaque assay. The concentration of virus, in plaque forming units (pfu as a measure of the number of particles capable of forming plaques per unit of volume), was calculated for infected wells with and without extract exposure.

Data Analysis

Cytotoxicity and mini-plaque assays were performed in triplicate in two independent experiments. The CC_{50} and IC_{50} values were calculated and a therapeutic index (TI, selective index; defined as CC_{50} /IC_{50}), was reported for the four most promising extracts (Table 3).

Statistical comparisons were made using a one-sample t statistic (significant differences at $P<0.05$).

Results

Inhibition of extracts against HSV 1 and HSV 2 using the mini-plaque reduction assay

Eight of the 31 plant species assayed showed significant ($P<0.05$) antiviral activity against one or both of these viruses at one or more concentrations when compared to the control (Table 2). Also, percent inhibition was not significantly different when cells were infected with viruses before or after the addition of the extracts. Consequently, the protocol of adding the virus before the extract was added was chosen for subsequent experiments because this modeled the natural order of infection.

Species were selected for further tests based on an extract being at least 60% inhibitory to HSV 1 or HSV 2 infection and showing no obvious cytotoxicity at any concentration based on microscopic observations (Table 2). This group included the acetone extracts of *Atractylis macrophylla* Desf. (Asteraceae) and *Hymenoclea salsola* Torr. & A. Gray (Asteraceae), and the methanol and acetone extracts of *K. pinnata* (Table 2). The other extracts showing significant inhibition (*Clematis cirrhosa* L. (Ranunculaceae), *Gnaphalium chilense* Spreng. (Asteraceae), *Lithospermum officinale* L. (Boraginaceae), *Psilostrophe cooperi* Greene (Asteraceae), and *Tetraclinis articulate* Mast. (Cupressaceae) were not pursued due to cytotoxicity (indicated by "tx" in Table 2).

Cytotoxicity of extracts on C1008 cells

Upon initial contact with any of the four extracts at 0.2 mg/ml, changes in the shape of some cell membranes were observed. Since these observations may indicate cytotoxic effects, cytotoxicity of these four extracts was determined using the neutral red assay. Based on CC_{50} values,

K. pinnata was two to five times less toxic than the extracts of *A. macrophylla* and *H. salsola* (Table 3). The CC_{50} for the acetone and methanol extracts of *K. pinnata* were 1.25 ± 0.123 mg/ml (mean ± se) and 0.95 ± 0.15 mg/ml, respectively. This indicated that the acetone-derived extract was less toxic to the C1008 cells than the methanol extract. Additionally, *K. pinnata* extracts had a 50% cytotoxic concentration up to 50 times higher than the concentration necessary for 50% virus inhibition as indicated by the TI value (Table 3).

Virucidal activity of the acetone extract of *K. pinnata*

The virucidal assay for the acetone extract of *K. pinnata* showed that titers of surviving virus were 8.2×10^7 pfu/ml for the control culture and 8.3×10^7 pfu/ml for the extract-exposed cultures. These data indicated that the acetone extract was not directly interfering with the virus particles before their attachment and entry into the C1008 cells.

Discussion

For all eight of the 31 species that showed antiviral activity, the acetone fractions showed greater inhibition than the methanol extracts (Table 2). If the acetone extract was toxic to the cell monolayer, the methanol extract from the same species displayed similar toxicity regardless of whether the virus was added before or after the extract. In addition virucidal assays indicated that extracts were not inhibiting entry of the virus or destroying virus particles. Extracts of the remaining 23 species did not protect C1008 cells from HSV induced cytopathic effects or they were toxic to the cells. Of the eight species that originally showed activity for at least one of the extracts, five (*C. cirrhosa, G. chilense, L. officinale, P. cooperii* and *T. articulata*) showed high cytotoxicity at extract concentrations less than 0.1 mg/ml. These observations, and previous research identifying specific organic compounds responsible for the cytotoxicity of some of the species, lowered their priority for further testing (Herz et al., 1970).

Previous studies identified specific compounds in *H. salsola* and their adverse effects on cellular activities (Torrance et al., 2006). The neutral red assay in this study confirmed the low CC_{50} (0.28 mg/ml) for *H. salsola* indicating that a low concentration of extract was cytotoxic to the cell monolayer. Similarly, the acetone extract of *A. macrophylla* was found to be cytotoxic to C1008 cells at a low concentration (0.25 mg/ml). Alternatively, the acetone extract (CC_{50} of 1.25 mg/ml) and the methanol extract (CC_{50} of 0.95 mg/ml) of *K. pinnata* showed a higher CC_{50} indicating a higher concentration of extract was needed to bring about a similar level of cytotoxicity. Furthermore, *K. pinnata* TI values of 50 (acetone extract for both HSV 1 and HSV 2) and 19 (methanol extract for HSV 1) indicated significant antiviral activity at lower cytotoxic concentrations.

Conclusion

Of the thirty-one species tested only the acetone and methanol extracts of *K. pinnata* were not cytotoxic to host cells. The acetone extract from the roots of *K. pinnata* revealed plaque numbers near zero at concentrations less than 0.1 mg/ml. Due to significant antiviral activity against HSV 1 and HSV 2 and low cytotoxicity to C1008 cells *in vitro,* the acetone extract was selected for additional studies. Studies underway include fractionation of the extract in search of an active compound or compounds, and investigation of the molecular mechanisms involved in the antiviral properties of the acetone extract of *K. pinnata.*

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Declaration of Interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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Table 1. Plant species*, family, collection number, and their medicinal use by indigenous peoples.

Genus/Species(Family)/ Collection Number	Known Uses in Indigenous Cultures
Acacia constricta Benth. (Fabaceae) (JD67)	Diarrhea, stomach problems[a] , conjunctivitis, dysentery, disinfectant, diaper rash[c]
Acacia farnesiana L. Wild. (Fabaceae) (JD30)	Aphrodisiac, astringent, demulcent, emetic, stimulant, cancer, carbuncles, cholera, conjunctivitis, convulsions, delirium, dysentery, dyspepsia, epilepsy, headache, inflammation, insanity, nausea, ophthalmic, parturition, rabies, rinderpest, snake bite, sore, spasms[a]
Aristolochia watsonii Wooton & Standl. (Aristolochiaceae) (JD32)	Stimulates immune system, antibacterial[i]
Atractylis macrophylla Desf. (Asteraceae) (A3)	None reported
Berberis hispanica Boiss. & Reut. (Berberidaceae) (B6)	Cough, gastrointestinal disturbance, mouth and skin ailments[a]
Cistus creticus Sibth. & Sm. (Cistaceae) (C10)	Sclerosis (uterus)[a]
Cistus ladaniferus Gouan ex Steud. (Cistaceae) (C8)	Astringent, fumigant, hemostat, nervine, hernia, tumor (anal)[a]
Cistus salviifolius Boiss. (Cistaceae) (C9)	Bronchitis, hemorrhages[a]
Clematis cirrhosa L. (Ranunculaceae) (C12)	Antirheumatic, analgesic, vesicatory, relieve rheumatic or joint pain[k]
Epilobium canum (Greene) P.H.Raven (Onagraceae) (JD27)	Infected sores, disinfectant, febrifuge, fever, kidney troubles, urinary problems, anti-hemorrhagic, cathartic, tuberculosis, syphilis, sores[b]; gynecological wash[f]
Gnaphalium chilense Spreng. (Asteraceae) (JD52)	Skin ailments[a]
Hymenoclea salsola Torr. & A.Gray (Asteraceae) (JD16)	Rash, rheumatism, swelling, lung, trachea[a]

Hyptis emoryi Torr. (Lamiaceae) (JD45)	Asthma, cold, dyspnea, earache, toothache[a]; hemorrhages[g]
Inula viscosa L. Aiton (Asteraceae) (I16)	Malaria[a]
Isocoma tenuisecta Greene (Asteraceae) (JD18)	Antimicrobial[l]
Juniperis oxycedrus (Cupressaceae) (C6)	Cancer, eczema, leprosy, psoriasis, sores, toothache[a]
Kalanchoe pinnata Pers. (Crassulaceae) (RC17)	Asthma, chest cold, fever, headache, mouth sores[a]; antileishmania[d]
Lithospermum officinale L. (Boraginaceae) (RC1)	Contraceptive, anodyne, diuretic, litholytic, sedative[a]
Plumbago europaea L. (Plumbaginaceae) (P28)	Cancer, itch, scabies, swelling, toothache, poison[a]
Prosopis juliflora DC. (Fabaceae) (JD53)	Burns, diarrhea, dysentery, eye ailments, flu, gastrointestinal disturbances, head colds, hoarseness, itch, measles, mouth ailments, pink eye, skin ailments, stomach ache[a]; internal antimicrobial, diarrhea, conjunctivitis[c]
Psilostrophe cooperi Greene. (Asteraceae) (P4)	Antimicrobial[l]
Ruta chalepensis Wall. (Rutaceae) (R31)	Cough, earaches, stomach pain, paralysis[a]
Salvia columbariae Benth. (Lamiaceae) (JD39)	Eye ailments[a] ; disinfectant[b] ; eye medicine[b]
Saponaria glutinosa M.Bieb. (Caryophyllaceae) (A6)	Eczema itch, cough, bronchitis, respiratory tract[e]
Sarcostemma hirtellum (Vail) R.Holm (Asclepiadaceae) (RC3)	Fruits used by Tohono O'Odham people[m]
Satureja calamintha Scheele (Lamiaceae) (D5)	Cancer, sclerosis (spleen)[a]

Tetraclinis articulata Mast. (Cupressaceae) (D2)	Carcinoma, diarrhea, gout, piles, rheumatism[a]
Teucrium polium Decne. Ex Capers (Lamiaceae) (D4)	Abscesses, inflammation, piles, tumor[a]
Tribulus terrestris L. (Zygophyllaceae) (JD62)	Cancer, cough, dysuria, gonorrhea, rheumatism[b;] ceremonial medicine (Navajo)[h] ; diuretic, kidney stones (Yemen)[i]
Verbena gooddingii Briq. (Verbenaceae) (JD51)	Common cold[b]
Zinnia acerosa A.Gray (Asteraceae) (JD8)	Diarrhea[a]

*Sources: [a]Johnson 1999, [b]Moerman 1989, [c,i]Moore 1989, [d]Muzitano et al., 2006, [e]Chopra et al. 1980, [f]Moroyoqui, personal communication, [g]Bean and Saubel 1972, [h]Ghazanfar 1994, [i]Hocking 1956, [j]Ohai, personal communication, [k] Palmese et al. 2003, [l]Hoffman et al. 1993, [m]Epple 1995

Table 2. Percent inhibition by methanol and acetone plant extracts of plaque formation of HSV-1 and HSV 2 as measured by the immunoperoxidase assay.

Genus/ species	HSV 1								HSV 2							
	Acetone				Methanol				Acetone				Methanol			
	.025†	0.05	0.1	0.2	0.025	0.05	0.1	0.2	0.025	0.05	0.1	0.2	0.025	0.05	0.1	0.2
A. constricta	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A. farnesiana	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A. watsonii	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.macrophylla	10	21	69	87	-	-	-	-	33	56	63	64	-	-	-	-
B. hispanica	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C. creticus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C.ladaniferus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C. salviifolius	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C. cirrhosa	10	52	64	tx	-	-	-	-	10	44	50	tx	-	-	-	-
E. canum	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
G. chilense	-	-	23	35	-	-	-	-	-	-	-	-	-	-	-	-
H. salsola	10	22	45	92	-	-	10	-	10	31	89	87	-	-	-	-
H. emoryi	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
I. viscosa	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
I. tenuisecta	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
J. oxycedrus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
K. pinnata	55	66	76	95	45	52	81	93	65	85	96	98	22	41	44	62
L. officinale	22	42	62	tx	-	-	-	-	31	40	63	tx	-	-	-	-
P. europaea	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
P. juliflora	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
P. cooperi	46	tx	tx	tx	-	-	tx	tx	24	tx	tx	tx	-	-	tx	tx

R. chalepensis	-	-		-	-	-	-	-	-	-	-	-	-	-	-	-
S.columbariae	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S.glutinosa	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S. hirtellum	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S. calamintha	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
T. articulata	10	12	32	tx	-	-	-	-	54	83	tx	tx	-	-	-	-
T. polium	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
T. terrestris	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
V. gooddingii	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Z. acerosa	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

*****Values are mean of triplicate readings; (-), no inhibition, "tx" = cytotoxic
[†]mg/ml

Table 3. Cytotoxic concentration (CC_{50}), inhibition concentrations (IC_{50}), and therapeutic index (TI) values for HSV 1 and HSV 2 infected C1008 cells monolayers in the presence of four plant extracts.

Virus	Plant	*A.macrophylla*	*H. salsola*	*K. pinnata*	
	Extract	Acetone	Acetone	Acetone	Methanol
	CC_{50}	0.25mg/ml	0.28mg/ml	1.25mg/ml	0.95mg/ml
HSV1	IC_{50}	0.1mg/ml	0.1mg/ml	0.025mg/ml	0.05mg/ml
	TI	2.5	2.8	50	19
HSV2	IC_{50}	0.05mg/ml	0.1mg/ml	0.025mg/ml	0.2mg/ml
	TI	5.0	2.8	50	4.75

Chapter Three - Kalanchoe pinnata root extract interferes with HSV types 1 and 2 DNA replication and early and late gene transcription.

Mary R. Jaeger Greer[a, b], Levon Ohai[c], Brian D. Poole[b], Rex G. Cates[a], F. Brent Johnson[b,*]

[a]Natural Products Laboratory, Department of Biology, Brigham Young University, Provo, Utah

[b]Department of Microbiology and Molecular Biology, Brigham Young University, Provo, Utah

[c]University of Hawaii, Oahu, Hawaii, USA

*Corresponding author. (801) 422-2331
E-mail address: brent_johnson@byu.edu

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Abstract

Crude polar extracts of the root tissue from the Hawaiian grown *Kalanchoe pinnata* were tested against herpes simplex virus types 1 and 2 (HSV 1 and HSV 2). Cytotoxicity, antigen expression and quantitative polymerase chain reaction (PCR) assays tested antiviral activity and searched for antiviral molecular mechanisms. Control virus infected cultures manifested large multi-cellular plaques and syncytia. In contrast, cultures exposed to acetone and methanol extracts (100 µg/ml) of *K. pinnata* showed sparse numbers of infected cells appearing as single, isolated cells at 30 min before and up to 8 h after infection suggesting the extract inhibited late stages of the virus life cycle. Real-time PCR was used to determine HSV susceptibility to the acetone extract. Antiviral mechanisms were investigated by measuring the reduction of viral DNA at different time points post infection and by measuring the reduction of viral RNA transcripts for five specific genes: alpha gene UL54, beta genes UL23 and UL30, and gamma genes US4 and UL17. Examination of transcript number found a significant decrease in viral DNA replication and early and late gene transcription when infected cells were exposed to *K. pinnata* suggesting post entry events were blocked by one or more extract compounds.

Introduction

Herpes simplex virus

Herpes simplex virus types 1 and 2 (HSV 1 and HSV 2) are members of the alphaherpesvirus subfamily Herpesviridae. The life cycle of HSV is extremely complex and there are numerous opportunities for the development of a novel antiviral therapy. Successful infection by HSV requires the virus particles to attach to and penetrate into cells: uncoat their DNA and deposit it into the nucleus: transcribe its genes sequentially to produce immediate early (α), early (β) and late (γ) mRNAs: synthesize regulatory proteins, DNA replication enzymes and structural viral proteins from these mRNAs: replicate its DNA genome, assemble new capsids and virions, and release these particles from the infected cell (Roizman, 1996). The multiple steps to HSV encapsidation and egress from cells are targets of interest for anti-virals to prevent the spread of HSV in target cells.

Acute and recurrent HSV infections are distributed worldwide and cause a wide range of diseases including gingivostomatitis, keratoconjunctivitis, genital tract infections, encephalitis, and infection of neonates and immunocompromised patients (Whitley, 2002). After the primary infection, HSV often persists in the neurons of nerve ganglia (Baringer & Swoveland, 1973). Both virus types are neurotropic and may spread to the brain during primary or recurrent infections. HSV 1 is the most usual cause of sporadic encephalitis, except in neonates, with a mortality rate of 70-75% and severe permanent sequelae in survivors (Griffin, 1991).

Over the last two decades the number of immunocompromised patients has increased dramatically. This is the consequence of aggressive chemotherapy regimens, expanding organ transplantation and the rising incidence of human immunodeficiency virus (HIV) infection (Levin, 1993). Reactivation of latent HSV, which is very common during the deficiency of immunity, causes recurrent herpetic infection. The most common forms of treatment for herpetic

infections include acyclovir (ACV), valaciclovir, famciclovir and cidafovir (Cassady & Whitley, 1997). Some alpha herpes viruses have become resistant to ACV and other nucleoside analogues thus the efficacy of these drugs is limited (Englund et al., 1990). With the rise of immunocompromised patients and emergence of ACV-resistant herpesviruses, new medications, especially novel antiviral agents, are needed for continuous effective treatment of associated diseases.

Kalanchoe pinnata

For several centuries, indigenous peoples of the world have cured physical afflictions using plants and plant extracts (Igwe & Akunyili, 2005). One such plant is *Kalanchoe pinnatum* Linn (Crassulaceae), a succulent shrub that is about 60–120 cm high and branched from the base with opposite, simple or trifoliate petiolate leaves. Although this perennial herb probably originated from Madagascar, its species abound all over tropical regions of the world, usually growing widely in hot and humid areas, around dwelling places, along roadsides, and in abandoned farms and fields.

Aside from studies showing that some root extracts of *K. pinnata* are used to treat coughs and burns (Iwu, 1982) there is limited research into the antiviral properties of root tissue of *K. pinnata* . As suggested by Cassady and Whitley (1997), future anti-herpesviruses agents may target enzymes or viral factors essential for infection or inhibiting other steps in the viral infection cycle, such as viral entry, protein synthesis or capsid assembly.

In this study, acetone and methanol extracts of root tissue of *K. pinnata* were tested against HSV-1 and HSV-2 as well as cytotoxicity to C1008 cell monolayers in vitro. The aim of the present study was to evaluate the antiherpetic activities of the acetone extract in mammalian cells employing a quantitative polymerase chain reaction (q-PCR) assay designed to locate some HSV

genes with reduced expression as a result of the extract's presence, thereby elucidating, in part, mechanisms of antiviral activity by *K. pinnata.*

Materials and Methods

2.1 Plant tissue collection

K. pinnata root tissue was collected in Hawaii by Rex Cates and Levon Ohai in 2005. Excess dirt was washed from the tissue, allowed to dry in the shade and then put in labeled plastic bags, placed in coolers containing dry ice, and shipped to the Natural Products Laboratory, Brigham Young University. These samples were stored in a -80°C ultralow freezer until analyzed (Blamires, 2002).

2.2 Tissue Extraction and Drug Production

Five grams of frozen root tissue from *K. pinnata* collected from Kauai and Maui, Hawaii was ground using a mortar and pestle in liquid nitrogen in 15 ml hexane and filtered through cheese cloth and VWR grade 415 filter paper. This was repeated with 10 and 5 ml of hexane, respectively, removing non-polar terpenoid compounds from the tissue sample. The process was repeated again with acetone using 15, 10 and 5 ml respectively. These acetone collections were combined into one pre-weighed vial and taken to dryness using nitrogen gas. Distilled water was added to the dried extract in each vial to make an 8 mg/ml concentration. Each vial was vortexed and sonicated to homogenize the extract: dH_2O, filtered through 0.2 µm Minisart filter (Sartoirus Stedim Biotech, France), placed into 1.5 ml Ependorf tubes, and stored at -80°C until assayed. Using the remaining tissue, the process of grinding, filtering, collecting and drying was repeated using 70% methanol. Again the dried extract was reconstituted with dH_2O in a pre-weighted vial to make 8 mg/ml concentration.

2.3 Virus stocks and cell lines

The HSV 1 (McIntyre strain) and HSV 2 (strain 333) (Jensen & Johnson, 1994) isolates were used for all experiments. African green monkey kidney cells (ATCC CRL-1586, sub-line C1008) were maintained in Dulbecco modified eagle medium (DMEM, Sigma Chemical Company, St. Louis, Mo.) supplemented with 5% cosmic calf serum (HyClone Laboratories, Logan, Utah), 10 µM HEPES buffer, and 50 µg Gentamycin per ml media at 37°C.

2.4. Cytotoxicity

2.4.1. Microscopic observations

The inverted phase contrast microscope (Nikon Eclipse TS100) allowed observation to help determine the extract's effect on non-infected cell monolayers (Fig. 1). Expected observed changes due to virus infection of C1008 cells included the formation of plaques, syncytia, and foci (Fig. 2).

2.4.2. Neutral red assay for cytotoxicity of active extracts

The effect of *K. pinnata* extracts was evaluated to ensure that they showed no cytotoxic effect on cell viability at concentrations which blocked HSV infection. Therefore, a neutral red (NR) assay (Cytotox NR Kit, Xenometrix, Switzerland) was applied. Neutral red is a vital stain used to quantify viable cell numbers. The assay is based on the ability of viable cells to incorporate and bind neutral red within lysosomes and Golgi apparatus (Motohashi et al., 2003). To carry out this test, C1008 cells were treated with serial dilutions of each extract (25, 50, 100, 200, 400, 800 and 1600 µg/ml), replicated three times. After 72 h the supernatant was removed and twice washed with sterile phosphate buffer saline (PBS) after which 1 ml of 50 µg/ml NR in PBS was added. The cultures were incubated at 37˚C in an atmosphere of 5% CO_2 for 90 min. After washing the cells twice again NR was eluted with an aqueous solution containing 50% ethanol plus 1% acetic

acid for 10 min at room temperature. Spectrometry data were collected using a Fusion α-HT Universal Microplate Analyzer (Packard Instruments, Meriden, CT) with a 540 nm filter and a 690 nm reference filter. Results were reported as 50% cytotoxic concentration (CC_{50}) and were determined from dose-response curves (Rajbhandari et al., 2007) (Table 2).

2.4.3. Five day proliferation assay

Since other unrelated compounds have been observed to arrest cell proliferation without affecting an existing cell monolayer, the issue of cytotoxic potential was addressed more rigorously by analyzing the effects of the extracts on Vero cell proliferation in the absence of contact inhibition (Boulware et al., 2001). Vero cells were seeded into 6-well plates at a density of 5×10^4 cells per well, which was sufficiently low to allow them to proliferate in the absence of contact inhibition for six days. On the following day the media were removed from the 6-well plates containing the overnight culture of cells. Each well was given a total of 3 ml fresh media with enough extract to represent 2-fold serial dilutions (0.10 to 3.2mg/ml). The plates were incubated for 5 days and then stained with crystal violet. All assays were performed in duplicate and the maximum nontoxic concentration (MNTC) was determined to be the highest concentration at which no alteration in cellular proliferation could be detected by inspection of cell density following crystal violet staining (Table 2).

2.5. Virucidal activity of K. pinnata

To confirm that the acetone extract of *K. pinnata* was not disabling or disrupting the virus particles directly, extracts at 200 µg/ml concentration were added to HSV 2 virus stock, a titer of 8×10^7 pfu/ml and incubated at 37°C for 30 min. Six 10-fold serial dilutions (10^{-1} to 10^{-6}) were made from each supernatant sample and added to C1008 confluent monolayers in 6-well tissue culture plates in triplicate and 24-well plates. After virus adsorption for 1 h, the

Vero monolayers in the six well plates were overlaid with complete minimal essential medium containing 5% FBS and 1% methylcellulose to restrict dissemination of progeny virions and the plates were incubated at 37°C for 5 days. Monolayers were fixed with 37% formaldehyde and stained with Gram's crystal violet. Plaques were counted by visual inspection to determine the number of PFU per milliliter of supernatant. Cells in the 24-well plates were not overlaid but instead allowed to incubate at 37°C for 24 hours before being fixed and subjected to the immunoperoxidase staining protocol explained in section 2.6.2.below. Stained foci were counted as infected cells.

2.6 Antiherpetic assays

2.6.1. HSV antigen expression

In order to determine if the acetone extract of *K. pinnata* inhibited HSV 1 or HSV 2 infection, C1008 cells were seeded into 24-well micro-titer plates. *K. pinnata* acetone extract was introduced to wells in three concentrations (25, 50, 100, and 200 μg/ml) when the cell monolayers were 90% confluent. One ml of medium without extract was placed into randomly placed control wells containing cells only. After 30 min 0.01 ml virus suspension was added to each well, in triplicate, at a multiplicity of infection (MOI=1) and incubated at 37˚C for 30 h in 5% CO_2 atmosphere.

To determine if the extract was inhibiting entry of the virus or destroying the virus particle, each virus type was introduced to the monolayer 30 min prior to the extracts. C1008 cells were seeded into 24-well micro-titer plates and incubated to 90% confluence. The old media were removed and 0.1 ml of virus suspension (MOI=1) was added to test wells and 0.1 ml of media to the control wells. Plates were incubated at 37˚C for 30 min in a 5% CO_2 atmosphere after which

1.1 ml of media with extract in 50, 100, and 200 µg/ml concentrations was added to the test wells and incubated for 30 h.

2.6.2. Immunoperoxidase staining to determine plaque number in cell monolayers

All wells were stained using an immunoperoxidase staining protocol to detect viral structural protein synthesis following Luker (Luker et al., 1991). In short, cell monolayers were fixed for 10 min in FAA fixative (5% formalin, 5% glacial acetic acid, 80% ethyl alcohol in distilled water), followed by 30 min incubations (39˚C) in each of the three reagents. The first reagent was a primary HSV antibody (Accurate Chemical and Scientific Corp., Westbury, N.Y.), the second was a protein A-peroxidase conjugate (Zymed Laboratories, Inc., South San Francisco, Calif.) and third was a 4-chloro-naphthol- horseradish peroxidase substrate-chromogen reagent. The cells were washed with distilled water between incubations in each reagent. Following staining the monolayers were observed for antigen-containing cells and cytopathic effects by bright-field microscopy using an inverted phase contrast microscope (Nikon Eclipse TS100). Mean values of triplicates expressed in plaque number were calculated and the growth inhibition of infected cells was given as percentage of control values (Table 1).

Results were reported as 50% inhibitory concentration (IC_{50}) and were determined by comparison with positive control wells (Table 2). Positive controls used in the IC_{50} determination were infected cell monolayers without extract.

2.6.3. Time of addition studies

In order to investigate *K. pinnata*'s effect on various stages of the viral life cycle, an experiment varying the time of extract addition was performed. The acetone extract of *K. pinnata* was added at the interval of 30 min pre-infection and 0, 2, 4, 6, 10 and 12 h post-HSV-2 infection. Virus yield was determined by plaque forming assay. The antiviral activity of the

extract was evaluated at various time periods up to 18 h using a modified procedure of Boulware (Boulware et al., 2001). Vero cells were seeded into 25 cm^2 culture flasks at a density 2 x 10^6 cells/flask and incubated at 37°C for 24 h. Cell monolayers were infected with 2 x 10^5 pfu HSV- 2 per flask. One hundred microgram per milliliter of either extract was added into the flasks 30 min pre-infection with HSV 2, concurrent with HSV 2 infection (0 h) or at intervals of 2, 4, 6, 8, 10, and 12 h post-infection. After 18 h post infection, cells were collected and viruses were released from cells by freeze-thawing twice. Cell pellets were removed by centrifugation at 3000 rpm for 10 min. The supernatants were divided into small quantity and stored at -80°C until use. A virus titer of each supernatant was determined by a plaque assay (Abou-Karam & Shier, 1990).

2.7 In vitro HSV 1 and HSV 2 DNA replication assay

2.7.1 Cell preparation for DNA replication assay:

In preparation for the replication assay C1008 cells were seeded at 2 x 10^6 cells per 25 cm^2 flasks, and the next day, after reaching 100% confluency, they were infected with 2x10^5 PFU per flask of either type of HSV. The acetone extract of *K. pinnata* was added at a final concentration of 100 µg/ml 30 min post infection and collected at seven time points: 2, 4, 7, 13, 15, 18, and 22 h for HSV-1 and 1.5, 5, 8, 11, 15, 18, and 24 h for HSV-2. Control flasks included flasks infected with HSV but not subjected to *K. pinnata* extract and flasks containing only C1008 cells. The experiment was done in triplicate and collected at the same time points. Collection was accomplished by removing the growth media, rinsing the monolayer with trypsin twice, and allowing less than 0.2 ml trypsin to remain in the flask to lift the cells from the flask. PBS was used to collect the cells and placed in 1.5 microcentrifuge tubes. The cells were spun down at

10,000 g for 1 min and the residual liquid removed with a pipette. The cell pellet was placed in a -20°C freezer until DNA extraction.

2.7.2. DNA extraction

PureLink Genomic DNA Mini Kit (Invitrogen Life Technologies, Carlsbad, CA) was used to extract total DNA from each cell collection. Cells were resuspended in 200 µl PBS, proteinase and RNase added, along with the lysis/binding buffer, incubated at 55°C for 10 min and lysed with 100% ethanol. The purity and amount of DNA was determined on a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE) and used immediately for PCR or kept frozen at -80°C until further analysis.

2.7.3. Quantitative PCR

To assess the amount of viral DNA replication at multiple time points, a quantitative real-time PCR (q-PCR) assay was performed, in duplicate wells. Quantitative PCR was performed under the following conditions: 50°C for 2 min, 95°C for 2 min, 45 cycles of 95°C for 30 s and 60°C for 30 s, with a 500 nM concentration of each primer set. To prepare total DNA for analysis, 3 µg of total DNA from each time point was added to SYBR green real-time PCR master mix (Toyobo) according to the manufacturer's instructions and was analyzed on an Applied Biosystems sequence detection system. Four different primer sets were used (Table 3). Amplifications were carried out for 45 cycles and a cycle threshold (CT) value was determined by automated threshold analysis using the Applied Biosystems software for each time point under each situation (C1008 cells alone, HSV 1 or HSV 2 infected C1008 cells and HSV 1 or HSV 2 infected C1008 cells with 100 µg/ml of the *K. pinnata* extract). Dissociation curves were recorded after each run.

2.8. In vitro transcriptional activity assay

2.8.1. RNA extraction

Cells were prepared similar to those for DNA extraction, section 2.7.1 except there were four collection time points (6, 12, 19, and 25 h). To quantify the inhibitory effect of the extract on HSV infection, the intracellular HSV RNA content of extract-treated and untreated cells was quantified by real-time PCR. Total RNA was extracted from C1008, C1008/HSV 1 or HSV 2 infected cells and C1008/HSV 1 or HSV 2 infected cells treated with the acetone extract of *K. pinnata* at 100 µg/ml using the RNeasy Mini Kit (Qiagen, Valencia, CA). RNA was recovered from the spin columns in a final elution volume of about 40 µl. The purity and amount of RNA was determined on a nanodrop spectrophotometer and purified RNA was stored at -80°C until first strand cDNA was synthesized.

2.8.3. cDNA synthesis

Reverse transcription of RNA to single-stranded cDNA was performed by using the SuperScript III protocol according to the manufacturer's instructions (Invitrogen Life Technologies, Carlsbad, CA). RNA, 10 mM deoxynucloside triphosphates and 50 µm oligo (dT) were incubated for 5 min at 65°C to denature the secondary structure of the RNA. First strand cDNA synthesis was carried out in 20 µl reaction mixtures with 1X reverse transcriptase buffer, 40 U/µl of an RNA inhibitor (RNase OUT), and 200 U/µl SuperScript III. The reaction mixture was incubated at 50°C for 50 min. The reaction mixture was inactivated by heating to 85°C for 5 min followed by RNase H digestion at 37°C for 20 min. The purity and amount of cDNA was determined on a nanodrop spectrophotometer and used immediately for q-PCR or kept frozen at -80°C until further analysis.

2.8.4. cDNA amplification using Q-PCR

Because microscopic observations noted a lack of syncytia or plaque formation in HSV infected extract exposed monolayers with occasional stained single cells, it was suspected that the extract was interfering with later stages of virion development, such as encapsidation and egress processes. To detect specific transcripts, forward and reverse primers were used and PCR was performed under the same conditions as for DNA replication tests. Primer sets for both HSV types were designed to amplify portions of alpha, beta and gamma genes, specifically UL54, UL23, UL30, US4 and U17 viral genes (Table 3). UL 54 is an immediate early (α) gene responsible for reduction of cellular transcription. UL23 and UL30 are early (β) transcription genes encoding thymidine kinase and viral DNA polymerase, respectively. US4 and UL17 are late (γ) transcription genes. US4 codes for glycoprotein G (gG), dispensable for virus growth in culture but necessary for *in vivo* viral envelope development (Brown & MacLean, 1998). The UL17 gene is vital for DNA encapsidation, participating in the cleavage of viral DNA concatemers as the genome enters the procapsid.

2.9 Statistical Analysis

Data sets from q-PCR were analyzed with a general linear model using the SAS system for Windows 9 (SAS Institute Inc., Cary NC, USA). A log transformation was used to normalize the data and directly model the ratios of transcript quantification. The analysis of variance (ANOVA) allowed analysis with unequal variances for all gene targets. Statistical significance was set at $P = 0.05$.

Results

3.1. Cytotoxicity of extract on C1008 cells

3.1.1 Microscopic, qualitative assessments

The possibility existed that extracts might induce cytotoxicity thereby causing observable changes in cell morphology. It was also important to identify types of C1008 monolayer changes due to HSV infection *in vitro.* Non-infected cells in the presence of the acetone extract of *K. pinnata* exhibited normal growth and cell morphology compared to untreated control cultures (Fig. 1). In some cases cellular changes were observed due to the addition of an extract at the higher concentration, (>1600µg/ml), such as the rounding up of cells and changes in the cell membrane. Normal plaques and syncytia were prevalent in both HSV 1 and HSV 2 infected cell monolayers without the extract (Fig. 2). In a few cases single cells showed the presence of the HSV antigen but there were no syncytia or apparent spread from the originally infected cells. There was little microscopic observational difference between the infected monolayers with 50, 100 and 200 µg/ml extract concentrations for either HSV type.

3.1.2. Quantitative assessment of cytotoxicity

Cytotoxicity of the acetone and methanol extracts from the root tissues of *K. pinnata* on the Vero C1008 cells were reported as a CC_{50} of 1.25 and 0.95 mg/ml, respectively, using the neutral red assay. Along with the five day proliferation assay, an MNTC of 1.3 and 1.0 mg/ml respectively, it seemed clear that C1008 cells were fully capable of growth and reproduction well above the necessary concentrations of either extract to bring about viral inhibition (Table 1). The compounds in the acetone extract were effective against clinical isolates of HSV 1 and HSV 2 infections *in vitro* while being non-toxic to 50% of the C1008 cell monolayers at a concentration fifty times greater than the 50% inhibitory concentration.

3.2. Virucidal activity of K. pinnata

The virucidal assay for the acetone extract of *K. pinnata* showed that titers of surviving virus were 8.2 x 10^7 pfu/ml for the control culture and 8.3 x 10^7 pfu/ml for the extract-exposed cultures. These data indicated that the acetone extract was not directly virucidal or interfering with the virus particles before their attachment and entry into the C1008 cells.

3.3. Effect of K. pinnata on viral infectivity pre and post infection

The acetone extract of *K. pinnata* displayed at least 50% inhibition at the lowest tested concentration (25µg/ml) when added to C1008 cell monolayers 30 min post infection (Table 1). In the time of addition study to assess antiviral activity as a function of time of extract introduction, delaying the time of exposure to the acetone extract of *K. pinnata* up to 8 h failed to significantly decrease its anti-HSV 2 activity. In other words, *K. pinnata* remained active at inhibiting the virus even when added 8 h post infection. Adding the extract 10 h after infection yielded plaque formation similar to the positive control wells. This result suggested that the acetone extract of *K. pinnata* affected the later stages (>8 h) of HSV 2 infection.

Though both the acetone and methanol extracts produced antiviral effects (Table 1), the acetone extract was chosen for further experiments. HSV 1 and HSV 2 infected cells in the presence of the acetone extract of *K. pinnata* rarely exhibited any more than a few infected single cells and no virus proliferation was observed (Fig. 2). Syncytia or plaque formation were not observed in any of the infected wells inoculated with the acetone extract at 50 µg/ml, even if the extract was added to the cells 8 h post infection. Although results generated from plaque reduction assays showed that *K. pinnata* was an HSV inhibitor, these were unable to provide any information on the mode of action of this compound. However these results demonstrated that *K. pinnata* may act as an antiherpetic extract with a mechanism of action on later stages of HSV

replication cycle, such as viral egress or capsid maturation processes. Therefore, a series of experiments was carried out to determine the stage at which the acetone extract of *K. pinnata* affected the life cycle of both HSV 1 and HSV 2.

3.4 Effects of K. pinnata on HSV DNA replication

Quantitative PCR analyses were performed to assess viral DNA synthesis. DNA replication was diminished an average (all three primer sets) of 21 fold at t=22 h (P= 0.0026) when HSV 1 infected C1008 cells were exposed to 100 µg/ml of acetone extract. There was almost a 50 fold decrease in DNA replication at t=22 h (P≤0.001) when HSV 2 infected C1008 cells were exposed to 100 µg/ml of acetone extract (Fig. 3). Regardless of which primer set was used to measure the amount of viral DNA present in the infected cells with and without the extract present, the results were significantly similar.

3.5 Effects of K. pinnata on HSV gene expression

Of the five gene targets, the alpha gene UL54 showed the least significant fold decrease in transcript number in HSV 2 infected cells treated with the acetone extract of *K. pinnata.* There was between a 14 and 18 fold decrease in UL54 gene transcription at 15 and 21 h post infection for HSV-2 and no significant decrease for HSV 1 (Fig. 4).

When the acetone extract of *K. pinnata* was present in HSV 1 and HSV 2 infected cell monolayers, the beta gene UL23 transcripts were significantly reduced with a 14 and up to 23 fold decrease in expression at 15 h post infection and beyond (Fig. 5). Assays for the beta gene UL30 transcripts showed greatest maximum fold decrease in transcription at 12 and 19 h post HSV 2 infection by nearly 120 times, though there was also significant reduction in UL30 transcripts for HSV 1 by nearly 20 times (Fig. 6).

The late gene, US4, showed a 55 and nearly 300 times decrease in transcript number, at 19 h post infection in extract treated HSV 1 and HSV 2 infected cells respectively. In extract treated HSV 1 infected cells, the gamma gene UL17 experienced a 544 fold decrease at 19 h post infection and in extract treated HSV 2 infected cells a 292 fold decrease at 12 h post infection (Fig. 7).

Discussion

In the present study we have evaluated the efficacies of several extract concentrations to inhibit the formation of herpes virus plaques in relation to extract effects on cell viability. We have examined the mechanisms by which an acetone extract from root tissue of *K. pinnata* inactivates HSV ability to replicate in cultured C1008 cells. Immunoperoxidase staining of viral proteins and q-PCR assays indicated that HSV 1 and HSV 2-infected C1008 cells treated with the acetone extract of root tissue from *K. pinnata* may be susceptible at various stages of HSV replication; after viral entry but during viral DNA replication and especially during genome encapsidation and envelope protein synthesis. The observation that 0.1 mg/ml represented a concentration where replication inhibition and mRNA expression were reduced up to 500 times in some cases confirmed that the acetone extract could specifically inhibit HSV replication and transcription with little detectable consequences to the host cell.

Microscopically observed lack of syncytia and plaque formation in the presence of the acetone extract may be explained by the dramatic reduction in transcripts from both beta and gamma genes, UL23, UL30, UL17 and US4. Because the fold decrease in transcript number for the alpha gene UL54 and the beta gene UL23 were a fraction of that for the other three genes, it is suspected that they were not reduced due to the presence of the extract but rather as a result of the limited new virus particles and spread beyond the initial infection because new virus egress

and further cell infection *in vitro* begins near this time (Aquilar et al., 2005) and reduced DNA replication. The absence of a significant reduction in UL54 transcription previous to 15 h post infection may indicate that compounds in the acetone extract of *K. pinnata* do not interfere with immediate early transcription. This would explain why, when the extract was added 8 h post infection thus allowing the virus to attach, enter, uncoat and begin immediate early transcription, there was little to no new virion production or egress from initial infected cells.

The γ gene, US4, responsible for glycoprotein G and found in the viral envelope responsible for cell-to-cell spread of new virion particles experienced impressive reduction at 19 h post infection with a 55 fold decrease in transcription in HSV 1 and 288 fold decrease in transcription in HSV 2 in the presence of the acetone extract of *K. pinnata.* It would be beneficial to examine the level of US4 transcription after 12 h but before 19 h post infection to assess if there was a significant reduction in transcript number prior to a second infection cycle. Transcription of the γ gene UL17, whose protein product is required for cleavage of viral concatemeric DNA into unit-length genomes (Salmon et al., 1998, Taus et al., 1998) and is conserved in all subfamilies of *Herpesviridae* (Klupp et al., 2005) also experienced significant reduced expression in extract treated HSV 1 and HSV 2 infected cells (Fig. 7). Along with UL25 proteins, UL17 proteins are necessary for stable encapsidation of the DNA genome (Snow, 2001). It is suggested that the mechanism responsible for the antiviral activity observed in this study is due to interference with at least these 2 strategic γ gene transcripts, explaining why little to no infected cells are found in C1008 monolayers treated with the acetone extract of *K. pinnata* up to 8 h post HSV 1 or HSV 2 infection.

Based on previously documented antinociceptive, anti-inflammatory, antimicrobial properties of *K. pinnata* and present results of antiviral activity, we believe one or more of the root extract

compounds in *K. pinnata* represent promising molecules for future anti-HSV drug design. It is known that there are high quantities of flavonoids, saponins and alkaloids in *K. pinnata* leaf tissues and that phenolic compounds and tannins are found in trace amounts (Okwu & Josiah, 2006). Due to the lack of literature on the pharmacological actions of root tissue extracts of *K. pinnata*, it is suggested that the acetone and methanol extracts be carefully fractionated and a quantitative determination of the phytochemical constituents be assessed. The common compounds in the acetone and methanol root extracts may explain the mutual antiviral activity observed from each extract. The variability between the acetone and methanol extracts' toxicity to C1008 cells may result from distinctive compounds found in each and, if removed from the extracts, may result in a higher therapeutic index for each and allow a more accurate dosage for HSV-inhibitory observations.

Our proposed hypothesis for the mechanism of HSV inhibition is that at least two γ genes, US4 and UL17, whose expression results in cell to cell spread and DNA encapsidation is affected by the presence of polar extracts from the root tissues of *K. pinnata.* The significant reduction in transcription of at least five viral genes at some point in the HSV replication cycle suggests there are multiple compounds working alone or synergistically to reduce HSV replication and spread, multiple gene transcripts have a common transcription control, or a common early product is blocked.

The chemical constituents of root tissue from *K. pinnata* associated with the reduction of specific viral mRNA transcripts and reduction of HSV cytopathic effects is still not clear. These findings encourage additional studies, such as the delineation of compounds in the roots of *K. pinnata* and repeating the assays using the individual components of the crude extract and the examination of antiviral activity against other Herpes family viruses.

Acknowledgments

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Table 1
***Percent inhibition of plaque and syncytia formation from HSV-1 and HSV-2 infection due to methanol and acetone extracts of *K. pinnata*: 4 different concentrations (†µg/ml) as measured by an immunoperoxidase mini-plaque assay.**

		HSV 1								HSV 2							
		Acetone				Methanol				Acetone				Methanol			
	†25	50	100	200	25	50	100	200	25	50	100	200	25	50	100	200	
K.pinnata	*55	66	76	95	45	52	81	93	65	85	96	98	22	41	44	62	

Table 2
Results of neutral red assay for cytoxicity (CC_{50}) and mini-plaque assays for percent inhibition (IC_{50}) with resulting TI values and MNTC values for acetone and methanol extracts of *K. pinnata*.

		K. pinnata	
		Acetone	Methanol
	CC_{50}	1.25 mg/ml	0.95 mg/ml
	MNTC	1.3 mg/ml	1.0 mg/ml
HSV1	**IC_{50}**	0.025 mg/ml	0.05 mg/ml
	TI	**50**	**19**
HSV2	**IC_{50}**	0.025 mg/ml	0.2 mg/ml
	TI	**50**	**4.75**

Table 3: Primer Sets

Gene Name	FUNCTION/PROTEIN	PRIMER SEQUENCE (5'-3')[a]
For mRNA expression assays		
HSV 1 US4	cell to cell spread/envelope glycoprotein G	F CGTCATCCACACCTTATCGTTT
		R GGACCAACCGCCACACA
HSV 2 US4		F CTACCACGCGTCGCTTTTG
		R ATAAACGTGCGGCCCGTAA
HSV 1 UL17	DNA encapsidation, capsid transport, DNA packaging	F TTTACTCTGCTCGCGTTTACTA
		R GGCTTAACAATCGTCCAATCG
HSV 2 UL17		F ACCGCTCCGAAGCCTTTC
		R TTTCAACAACTGGGCGAAGTC
HSV 1 UL30	DNA replication/DNA polymerase catalytic subunit	F GGCGTGCGTGACATTCAAG
		R CCTTTTTAACAGACTCTCGGTATC
HSV 2 UL30		F GGGTGATCGGCGAGTATTGT
		R GTGCGGCAGAAACTTGAAGAA
HSV 1 UL23	Nucleotide metabolisms/thymidine kinase	F CAGTAGCGTGGGCATTTTCTG
		R CTCGCCGGCAACAAAAAG
HSV 2 UL23		F CCCGATATGAGGAGCCAAAA
		R ACCAGCGCCCAGATAACAAT
HSV 1 UL54	Gene regulation, RNA metabolism and transport/multifunctional expression regulator	F CGGGCCTGATCGAAATCC
		R CGCAGACACGACTCGAACA
HSV 2 UL54		F CGCCAGGAAAATCTCATCGA
		R TGGTGAATGCACATCTTGCA
For DNA replication assays		
HSV 1 UL12	DNA processing/deoxyribonuclease	F AAGTGGCTGTCGAGCACGTA
		R CCCGTCACCCGAACTTTAAG
HSV 1 UL17	DNA encapsidation, capsid transport, DNA packaging	F TTTACTCTGCTCGCGTTTACTA
		R GGCTTAACAATCGTCCAATCG
HSV 2 UL17		F ACCGCTCCGAAGCCTTTC
		R TTTCAACAACTGGGCGAAGTC
HSV 1 UL13	Protein phosphorylation/ PK family	F GGTGTGAAAGTTGGCTGTGGTT
		R CAAGTCCCCCCGGATGTT
HSV 2 UL13		F TGCGTGCGCTGGTAATTTATA
		R CTTCGCAATCCCAAGATTCG
HSV 2 UL15	Terminase protein, processing and packaging of DNA	F ACCGCTCCGAAGCCTTTC
		R TTTCAACAACTGGGCGAAGTC
Housekeeping Primers for all q-PCR		
Chlorocebus aethiops African Green Monkey	Vero β-actin	F GCGCGGCTACAGCTTCA
		R CTTAATGTCACGCACGATTTCC
NCBI reference Sequence NC_001806.1 and NC_001798.1		

[a] Sequence of forward (F) and reverse (R) primers in 5' to 3' orientation

Fig. 1. Cytotoxicity of *K. pinnata* acetone extract at a concentration of 100 µg/ml, a) confluent, uninfected C1008 cells, b) confluent, uninfected C1008 cells (100x).

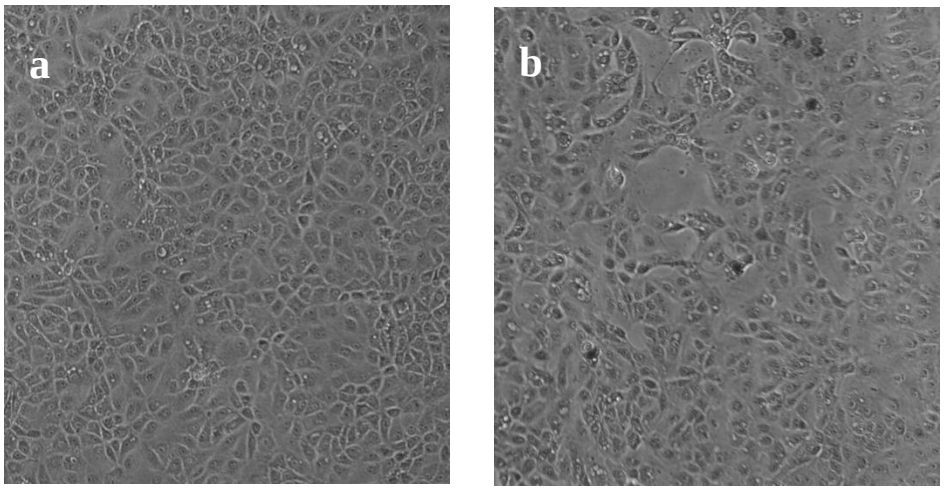


Fig. 2. Reduction in plaque, syncytia and infected cells due to *K. pinnata* acetone extract at a concentration of 0.1 mg/ml, a) HSV-1 infected C1008 cells, b) HSV-2 infected C1008 cells, c) HSV-1 infected C1008 cells with 100 µg/ml *K. pinnata* acetone extract, d) HSV-2 infected C1008 cells with 100 µg/ml *K. pinnata* acetone extract (100x).

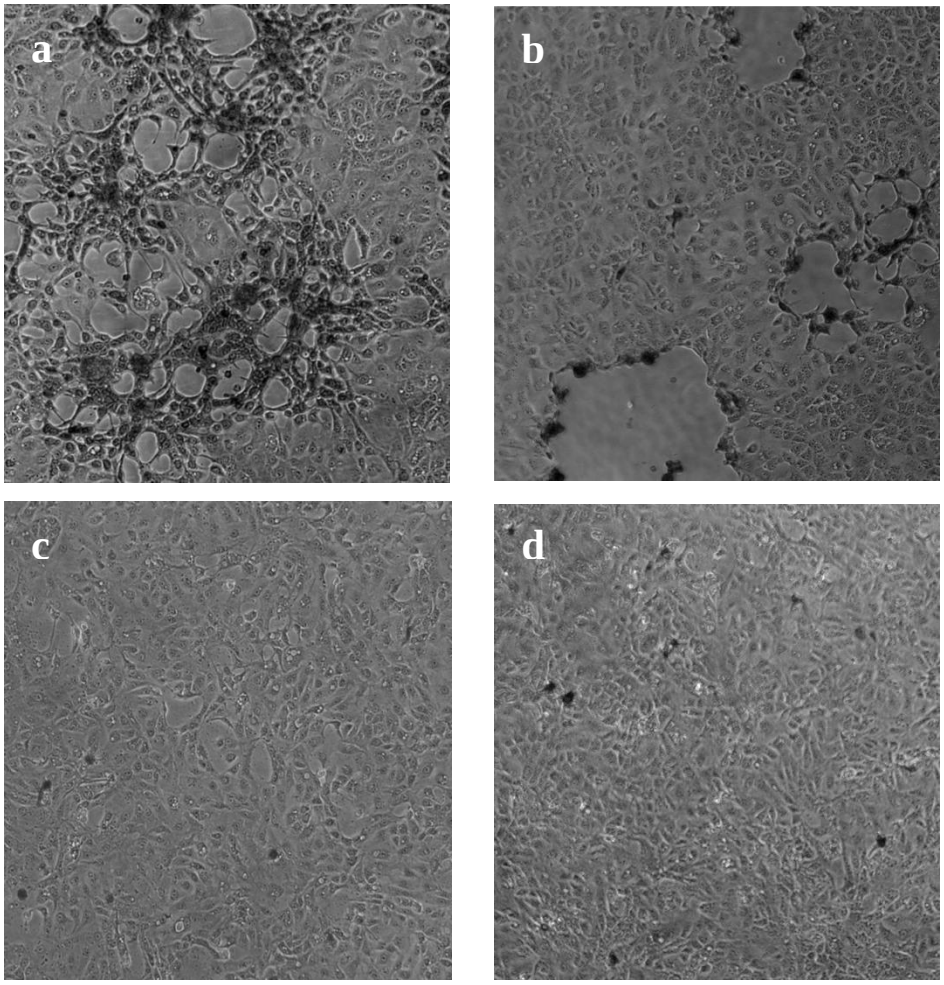


Fig. 3. Effects of *K. pinnata* on three different gene targets to assess difference in HSV DNA replication *in vitro* using Q-PCR. Data represents mean ± S.E.M. of three independent experiments. *P≤ 0.05, P≤0.001

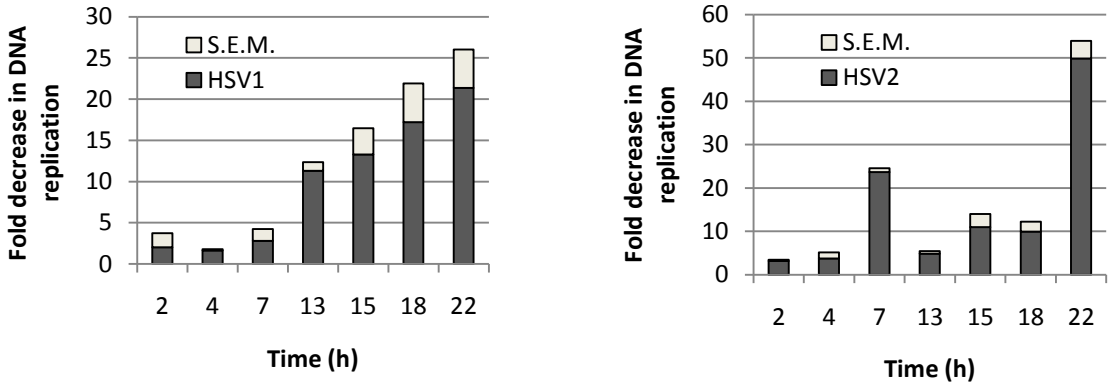


Fig. 4. Effects of *K. pinnata* on HSV types 1 and 2 alpha gene UL54 mRNA expression. *P≤ 0.05

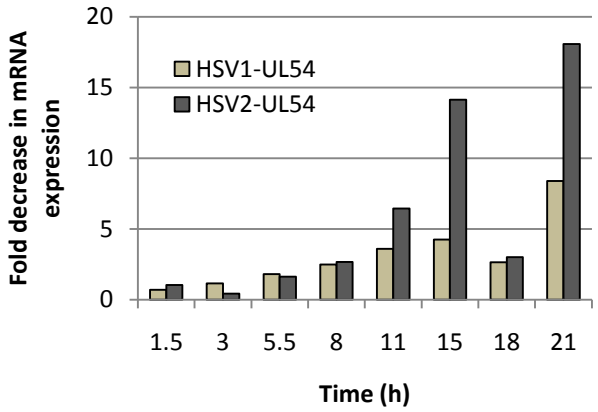


Fig. 5. Effects of *K. pinnata* on HSV types 1 and 2 beta gene UL23 mRNA expression. *P≤ 0.05

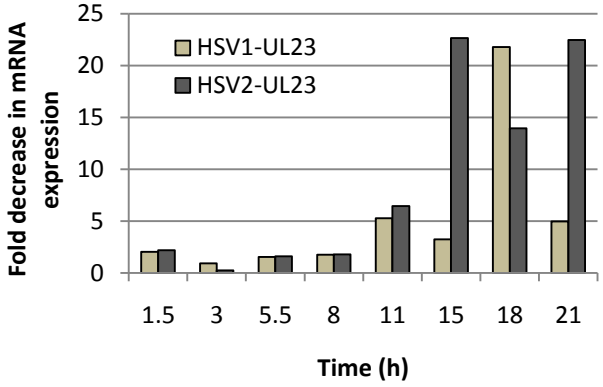


Fig. 6. Effects of *K. pinnata* on HSV types 1 and 2 beta gene UL30 mRNA expression. Data represents mean ± S.E.M. of three independent experiments. *P≤0.05, **P≤0.001

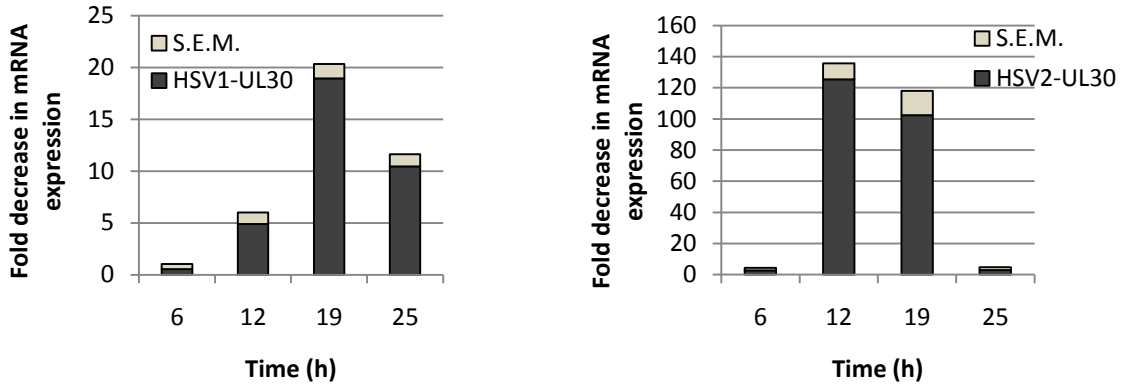
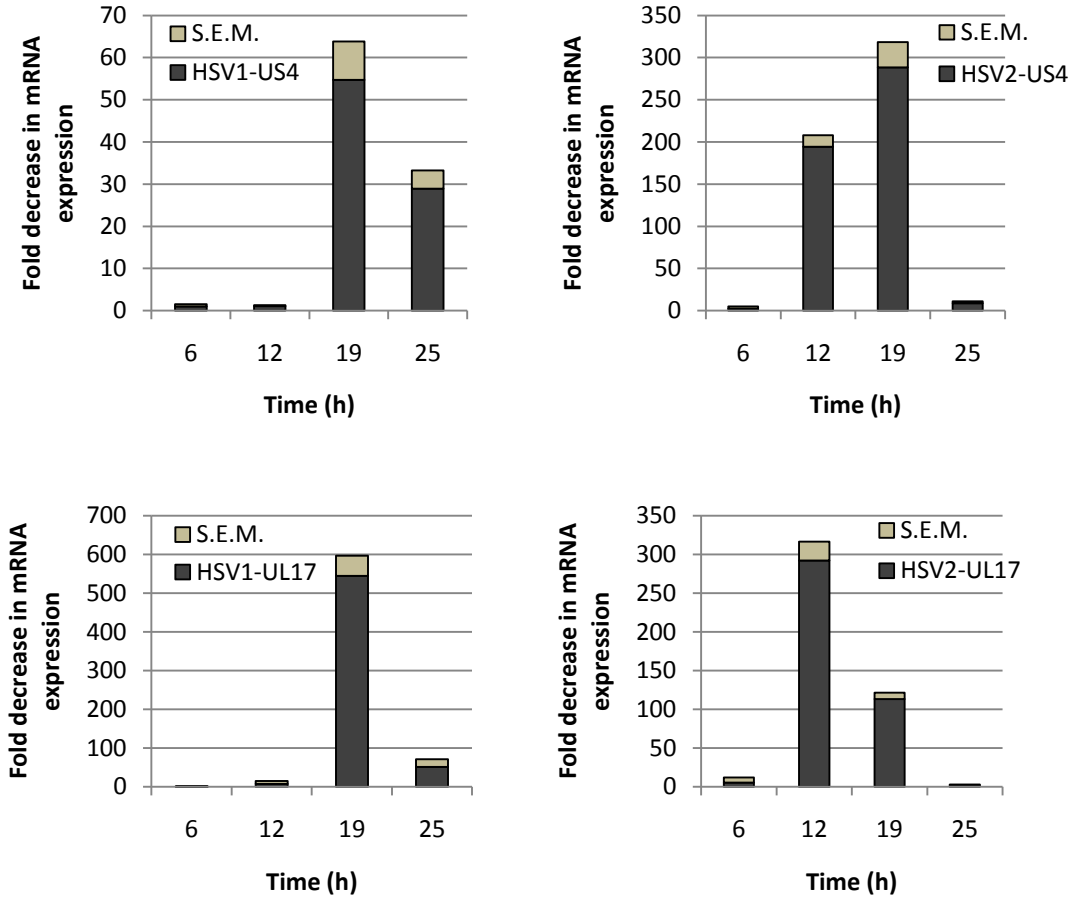


Fig. 7. Effects of *K. pinnata* on HSV types 1 and 2 gamma genes, US4 and UL17, mRNA expression. Data represents mean ± S.E.M. of three independent experiments. *P≤0.05, **P≤0.001



Chapter Four - Professional development curriculum: Integrating molecular biology and microbiology into the existing secondary biology curricula.

Mary R. Jaeger Greer

Brigham Young University, Provo, UT

"Those who know, do.
Those who understand, teach"

Aristotle, Greek critic, philosopher, physicist and zoologist (384-322 BC)

Keywords: high school biology, immunology, in-service, integrated, molecular, professional development, virology

Contents

Overview

Effective science teaching is more than having knowledge of science content and some teaching strategies. Skilled science educators tailor learning situations to the needs of individuals and groups and integrate unique understandings and abilities into their curriculum. This special knowledge, called "pedagogical content knowledge," distinguishes the science knowledge of teachers from that of scientists (National Science Foundation, 2009). It is one element that defines a professional teacher of science. Research demonstrates that teachers' background knowledge and the methods they use to teach science have a very strong impact on how much their students learn and on their attitudes about science (Elfring, 2002). One of the best ways to ensure the quality of undergraduates in the biological sciences is to have them arrive at the university with solid knowledge and skills. For this to happen, students must be taught by teachers who are well informed and current in their content and teaching methods. The most important resource to a science student is a professional science teacher (Llewellyn, 2005).

To effect a positive impact on students, teachers must know the content they teach, understand the nature of learning, and use a range of teaching strategies to accomplish the primary job of a teacher: to promote learning. It follows that teachers should be dedicated learners; to stay current in the science disciplines they teach, and continue to learn new skills, technology and information in their field. Teachers do not leave pre-service programs with a complete understanding of all the science they will need in their teaching careers, and they need to continue to clarify and deepen their understanding of the science content that is part of their teaching responsibility. Since biology in general and molecular and microbiology in particular are so dynamic in scope and content, it is imperative that teachers have an opportunity to continue learning; made clear by the observation that tomorrow's students will have markedly

different needs from today's students. Even today's employers require employees who can frame problems and design their own tasks, think critically, and work together (Krajcik *et al.*, 2007). This professional development course offers teachers and their school district an opportunity to develop and enhance these needed skills for effective science teaching emphasizing the molecular biology and microbiology disciplines.

The Rationale

The 2007 Trend in International Mathematics and Science Study offered bad news for the U.S. science education community. The TIMSS results, released in December 2008, showed statistically no change from the 1995 scores in science (National Center for Education Statistics, 2008). TIMSS measures student performance in science topics/content and cognitive skills of knowing, applying and reasoning. In 2007, Singapore, Chinese Taipei, Japan, Hong Kong, Russia and England had higher average scores than the United States in science. Factors behind this disappointment include the No Child Left Behind (NCLB) Act, resulting in less time, resources and focus on science education, including the lack of pre-service preparation and in-service professional development for science education. Nearly 75% of the teachers questioned said they needed substantial professional development to deepen their own science content knowledge (Slater, 2008). In response to the poor results of TIMSS, the education community has seen a surge of public interest in the training and subject matter competency of secondary science teachers. This interest has resulted in congressional hearings and high-profile publications such as *Rising Above the Gathering Storm: Energizing and Employing America for a Brighter Economic Future* (Jacobs *et al.*, 2008a). Politicians, business leaders, and higher education have taken notice of the crucial link between the training of qualified secondary teachers and the production of a well-prepared college student population and workforce. Large

sums of money have been awarded on the basis that partnership with organizations such as the Math-Science Partnership (MSP) and the National Science Foundation will result in a higher quality teaching force and better prepared teachers and students (National Science Foundation, 2009).

A high level of expertise should be expected from our science teachers. Real expertise comes from working in the field. Most experts have what may be called ―contributory expertise"—sufficient knowledge and understanding to make contributions (Collins, 2007). This level of expertise can be attained only by spending time interacting with other contributory experts in the field. Most established secondary science teachers do not have ―contributory expertise". And if they do not have opportunities to update their skills and knowledge, they are unable to maintain the level of knowledge once they go into the classroom full time. Even among newer life science teachers, the course requirements are slim in the molecular and microbiology departments (Table 1). Therefore, development of a molecular/microbiology in-service course for life science teachers, involving current curriculum, inquiry activities and discussion about the epistemology of science is critical to the continuing education and competence of secondary biology teachers. Continued education in this field will enable the ―non-expert" scientist such as the secondary life science teacher to acquire science knowledge, not just articulated in a text, but attained through inquiry, practice and integration, resulting in more confident and prepared teachers and thus the students they educate. In the past two decades, programs with similar goals have been introduced, primarily by universities. The American Association of Immunologists (Cobb, 2009) Summer Research Fellowships for Middle and High School Teachers program provides summer research support for teachers to work in the laboratories of AAI members for a four-week period coupled with one to two weeks of curriculum development. The John H. Wallace High School

Teachers Program, in which science teachers work with "mentor" scientists at immunology laboratories is designed for a four to six week workshop where teachers participate in research and devise lab exercises to take back to the classroom. The program's goal was to engage teachers who may not have had experience in a research lab setting (American Association of Immunologists, 2005).

The current reform effort in science education requires a substantive change in how science is taught and an equally substantive change in professional development practices (Arzi and White, 2007). Teacher-preparation courses and in-service activities in methods of teaching science frequently emphasize technical skills rather than decision making, theory, and reasoning. If reform is to be accomplished, professional development must include experiences that engage practicing teachers in active learning, expands their content knowledge and models "best" science teaching practices as described in the teaching standards of the NSTA. Professional development courses must answer serious questions about science education today such as what does a science teacher need to know, what does it mean to know a lot or a little, what is a sound science foundation and in-depth understanding? It is assumed that teachers of science will continue to study science throughout their careers. Most teachers of science have a broad base of scientific knowledge extensive enough to understand the nature of scientific inquiry, its central role in science, and have some skills in scientific inquiry. Teachers understand the fundamental facts and concepts in major science disciplines. Fewer make conceptual connections within and across science disciplines. Beyond the foundation in content standards and objectives, how much more science a teacher needs to know is an issue of breadth versus depth to be debated. Breadth implies a focus on the basic ideas of science and is central to teaching science at all grade levels. Depth refers to knowing and understanding the basic ideas within a science discipline and the

supporting experimental and theoretical knowledge. This professional development course focuses on depth in the biological areas of virology, molecular biology, and immunology while learning through the perspectives and methods of inquiry. The curriculum presented here is quite different. It is designed by an experienced high school biology teacher who has currently completed a graduate degree in the microbiology and molecular biology disciplines and who is returning to the secondary classroom. It is designed to be taught by and for life science high school teachers; taking materials and pedagogy learned in this course and immediately implementing it into their own classroom. Inquiry experiences will focus on what materials the teacher has available in their high school laboratory.

This professional development curriculum began with four assumptions about the nature of secondary science in-service. First professional development for a science teacher should be a continuous, lifelong process. The understanding and abilities required to be a masterful teacher of science are not static. Second, science content increases and changes, and a teacher's understanding in science must keep pace. Third, knowledge about the process of learning is continually developing, requiring teachers to stay informed. And fourth, we live in a changing society that deeply influences events in schools; social changes affect students as they come to school and affect what they need to carry away with them. Science teachers must be involved in the acquisition of current, accurate and relevant scientific information as well as development and refinement of new approaches to teaching, assessment, and curriculum (National Center Educational Statistics, 2003).

In this course science learning experiences for teachers will involve teachers actively investigating phenomena that can be studied scientifically, interpreting results, making sense of findings consistent with currently accepted scientific understanding, and address issues, events,

problems, or topics significant in science. They will be exposed to scientific literature, media, and technological resources that can expand their science knowledge and their ability to access further knowledge. An important aspect of this course is to allow teachers to work in the lab with experienced graduate students from local universities. Teachers are given the opportunity to be like a real scientist, asking questions, developing hypotheses, researching background information and writing an experimental design. This experience will change the way science teachers think about and prepare laboratory experiences for their own students.

Goals

There are three primary goals of this in-service course curriculum. First, it must be integrative in nature. The lesson information and inquiry activities offered must not add to the already overload of biological information a life science teacher must include in one year's instruction. This course uses the disciplines of virology, molecular biology, and immunology to meet many of the standards and objectives in the science core of the state. This course supports the trainer with specific examples. Second, the course must include time for teachers to make connections between what they already know and new information and concepts through inquiry. Thirds, the curriculum must allow teachers to be creative and versatile in their lessons by modeling for them a variety of pedagogical techniques (best practices) for teaching high school students. Emphasis is placed on the role of teacher as a coach and facilitator.

While the main intent of this course is to improve the knowledge base for secondary life science teachers in the microbiology and molecular biology disciplines, one would expect that teachers will return to their own classroom and use the materials and ideas they have acquired from this course. As a result, this course is written with the teacher and their students in mind. The ongoing information explosion in biology makes these goals challenging. Just as science is

built on curiosity and a sense of wonder, this curriculum is not intended to be a stagnant or deteriorate but will require constant modification over time.

Primary emphasis will be on developing an understanding of concepts rather than on memorizing terms and technical details. Specifically, included in the concepts stressed are: 1) the historical routes of molecular biology, (2) biotechnology and its influence in society, (3) the relationship between viruses and evolution, order and organization, (4) the immune system and examples that stress structure and function, change and constancy, and (5) the personal and social impact of pathogens. Teachers in this course will be given opportunities to experience scientific inquiry, recognition of unifying themes that integrate the major topics of biology, application of biological knowledge, and critical thinking about environmental and social concerns.

Biology teachers will be encouraged to maintain an open and questioning mind, to pose their own questions about historical events and discoveries in microbiology and molecular biology fields. They will be instructed on methods that integrate virology, molecular biology and immunology into the general biology curriculum: to develop connections between these emphasized disciplines and the dynamic biological activities of the present day. Personal relevance will be a focal point. This will allow teachers to see current application of what they are teaching and, in turn, help their students value science. Teachers will create their own interactive notebook/journal in preparation to teach their students how to improve writing skills in science.

Science instruction should cultivate and build on the teachers' present knowledge and understandings. After attending the in-service, teachers will be able to write lessons that will integrate specific examples of molecular and microbiology disciplines into their current

biological units as well as increase the number of investigative activities and discussion sessions relevant to current science issues.

Research Design and Questions

I am interested in epistemic practices: posing questions and developing theories about phenomena, learning to make representations, and learning by inquiry, reflecting the reality of studying the nature and practice of science. To design a curriculum that will contain important, current and necessary knowledge in the areas of molecular biology, immunology virology and general microbiology in an integrative approach requires consideration of the following research questions:

- If teacher education curriculum materials mediate integrating concepts in biology and inquiry practices, is there an increase in knowledge base and interest in the specific areas of molecular and microbiology from teachers in training?
- For what level of scientific expertise should the curriculum aim?
- With newly acquired knowledge in molecular and microbiology, will teachers take it and implement it into their lessons and discussions in the classroom?
- Will new and experienced teachers demonstrate this new information by improving inquiry exercises, case study discussion, questioning and problem solving, integration of concepts, drawing relevance to their students' lives, and add more depth to their lectures?
- Will students increase in knowledge, application and interest in the molecular and microbiology areas of science as assessed informally? (See appendix: Possible Assessment Products)

The Audience, the Time, the Place, and the Funding

This course is designed for current secondary biology teachers who have not participated in a detailed lecture and/or laboratory courses in microbiology, molecular biology, virology and immunology or who have not taken these science courses at a college in the past 3 years. This course will be held two weeks in the summer for a total of 80 hours and may offer college credit to the attendees. The course will meet at the biotechnology lab located at Mountain View High School in Orem, UT. This lab is equipped with necessary materials that allow teachers to acquire hands-on application experiences. Funding for this course will be provided by grants from NSF and the Alpine School District. The term —teacher" refers to the beginning and continuing high school biology teacher participating in this course of study. The term —trainer" refers to the instructor for this teacher education science course.

Key Components of the Course: Inquiry, Integration, and Application

Teacher learning is analogous to student learning: learning to teach science requires that the teacher articulate questions, pursue answers to those questions, interpret information gathered, propose applications, and fit the new learning into the larger picture of science teaching. This course includes opportunities and modeling exercises that allow teachers to question, interpret, apply and integrate new science concepts and information into classroom lessons, without adding to the overload of information science teachers feel compelled to cover each year. Learning is also a developmental process that takes time and is hard work. As does any professional, teachers of science will stumble, wrestle, and ponder while realizing that failure is a natural part of developing new skills and understanding. However, effective teachers know how to access research-based resources and, when faced with a learning need, pursue new knowledge and skills that are based on research or effective practice (National Research Council, 1996). The content

emphasized will include basic and more current information in the areas of virology and bacteriology, molecular biology, and immunology. Differing degrees and forms of expertise represented in the participants were kept in mind in the design process. An important goal of this course was to deepen and enrich understanding and ability as well as impart current science content knowledge and include a range of strategies for teachers to use as they instruct their own students.

Inquiry

Reform in science education has highlighted that science consists of ~~"h~~abits of mind" that rely on modes of thinking and reasoning to make sense of phenomena in the world Epistemic practices (including inquiry) imply asking questions, collecting data, making descriptions of observations, finding patterns in the data, and developing scientific reasoning. An important stage of inquiry and of students' learning is the oral and written discourse that focuses the attention on how students know what they know and how their knowledge connects to larger ideas, other domains, and the world beyond the classroom. Teachers directly support and guide this discourse in two ways: they require students to record their work, teaching the necessary skills as appropriate, and they promote many different forms of communication (for example, spoken, written, pictorial, graphic, mathematical, and electronic). Teachers are models for the students they teach. A teacher who engages in inquiry with students models the skills needed for inquiry. Students engaged in inquiry understand the nature of science as well as procuring a more in-depth knowledge of key concepts in biology. Tai and colleagues found that students participating in high school courses examining fewer concepts in more depth as well as working meaningful problems and engaging in inquiry activities performed much better than students whose teachers sped through more concepts in a textbook-centered, teacher-driven class (Tai *et*

al., 2009). More and more university science courses are inquiry based and using active learning strategies (Knight and Wood, 2005; Udovic *et al.*, 2002). Students engaged in inquiry experiences are more likely to be drawn to careers in science. As a result, these students complete college science courses in greater numbers than students in non-inquiry based courses (Gibson and Chase, 2002). Teachers who exhibit enthusiasm and interest and who speak to the power and beauty of scientific understanding instill in their students some of those same attitudes toward science. This course asks the in-service teacher to participate in questioning, describing observations, finding patterns in data, and developing scientific reasoning as they learn about the specific disciplines in this course and practice laboratory techniques specific to those disciplines.

Integration

Although the literature on integrated science shows that there is a lack of any consensus on the definition and conceptualization of 'integration', it is obvious that integrated science is consistent with the aim of raising the level of scientific literacy of all students in primary and secondary schools (National Committee on Science Education Standards and Assessment, 1996). The initial motivation for the design of this curriculum was to create an integrated molecular and microbiology curriculum that secondary life science teachers could learn, practice and share with their students in a compressive but timely manner. The course does not cover all topics that are in the science curriculum. However, it does provide a background in virology and bacteriology, molecular biology, and immunology that can integrate into almost any unit of a high school biology course. By emphasizing depth rather than breadth, this course seeks to empower teachers rather than intimidate them with a collection of isolated and forgettable facts.

Application

College professors want students who are problem solvers and are able to ―think their way out of a brown paper bag.‖ Merely memorizing information does not adequately prepare students for the rigors of college where deciphering, interpreting, and reasoning is critical to success in science courses. High school teachers allowing discussion of real-life scenarios that focus on biological concepts provide the opportunity for greater understanding in their students. These scenarios allow students the opportunity to apply their knowledge of concepts in a practical, thoughtful manner. For example, when high school students are confronted with a problem about an oil spill in the ocean and are encouraged to use both ecological and microbiology concepts, (such as carrying capacity, biotic and abiotic composition of ocean biome, bacterium and protozoan populations, etc.) they must work with these concepts first-hand rather than learn them passively. During their initial biology course in college, the students recall these scenarios to assist them in deepening their knowledge of the concepts. If students do not actively work with relevant issues in the high school, then the college courses can be a frustrating experience because the concepts learned in high school are one-dimensional. This course will include activities that require the use of problem solving skills, application discussions, inquiry activities, and integrating themes in biology.

Biotechnology Skills

Safe use of lab materials, set up and organization, and responsible storage and disposal of reagents are important components of any science teacher's ―lab skills‖ tool box. This course moves beyond the typical laboratory skills into discussions about research laboratories and how data are presented and results interpreted in the molecular and microbiology disciplines. Ideas about the purpose and uses for buffers, measuring small volumes using micropipettes,

configuring molar concentrations, % mass/volume, cell culturing, contamination, cytopathic effects, and working with bacteria and viruses will be discussed. Teachers will have the opportunity to perform a concentration assay and various forms of chromatography. They will be introduced to the study of proteins and nucleic acids and the tools used in the research laboratory. The use of monoclonal antibodies, determination of protein concentration, understanding the differences between precision, accuracy and reproducibility, DNA and RNA-extraction, reverse transcription of RNA, PCR technology and gel electrophoresis, the use of primers and how they work, an introduction to flow cytometry technology and analysis of data are included in discussion and demonstration. Though most teachers will not have means to provide equipment and materials in their own schools, teachers having multiple experiences with the molecular tools and microbiology techniques can go back to the classroom lab with confidence that they can demonstrate and explain what students are reading about in the case studies as well as current biology textbooks.

Organizational Tools and Resources

High school science teachers using teacher-directed instruction involving lecture, with a few labs scattered throughout the year. To substantiate their methods, teachers claim it is necessary because ―student need to be able to be good note-takers in college.” But these methods provide a cursory view of the content where students learn for an exam but forget soon after. When notes are taken, the students need to work with the notes, and personalize them with sketches, charts, schematics and summary statements (Einstein *et al.*, 1985). Merely taking notes without synthesizing the ideas involves little thought and consideration. In this course, teachers practice concept mapping and use of an interactive notebook to adequately connect scientific concepts in a meaningful way. Using concept maps during high school facilitates students’ understanding of

concepts and their relationship to one another. Concept maps also provide a useful skill for working with concepts as a study skill once in college. The more high school teachers can provide ways for students to study content in a meaningful manner, the more prepared students will be to handle the challenges of college. Concept maps are vital for integration of themes and concepts, and are included in this curriculum.

Other skills to be addressed are: how to teach students how to keep a laboratory notebook – the right hand rule, interactive notebook, organization, documentation, etc., laboratory organization and logistical problems managing a lab with twenty-eight plus 15 to 18 year-old students, how to mediate case study discussions, and how to use the inquiry methods as a means of delivering new information to students. This concern will be addressed in the course.

Reading science for understanding is critical and those high school teachers who have their students read actual scientific papers and science articles in magazines such as *Discover, Skeptical Inquirer*, and National Geographic provide opportunities to engage in the scientific literature (Sadler and Tai, 2009). Having students examine and critically evaluate science based websites also aids in developing scientific literacy. Being able to summarize key ideas presented in the readings assists students in developing their own understanding of concepts. Courses in science should allow teachers to develop understanding of the logical reasoning that is demonstrated in research papers and how a specific piece of research adds to the accumulated knowledge of science (Windschitl *et al.*, 2008). Courses should support teachers in using a variety of technological tools, such as computerized databases and specialized laboratory tools. This in-service course includes a book list and websites to be used as resources for teachers and their students, along with a compilation of case studies relative to the course materials. Teachers

choosing to use these materials will offer students the opportunity to read for understanding and experience, in a small way, the –science" of experimentation and discovery.

Teaming with the Local University Graduate Students

This curriculum proposes a cooperative project with the local university. The proposed project combines laboratory research and educational experiences of university graduate students with the teaching experience of 9^{th}-12^{th} grade science teachers to create a new life science teaching synergy. Graduate students will work on location with students and their teachers, sharing their experience with research practices and graduate science education. They will be role models for young people as they direct and organize projects with students, share their knowledge of current research and life science issues and be a facilitator and mentor to students and teachers. Workshops, forums and collaborative meetings will be organized for team building and information exchange necessary to yield an improved science curriculum and a growing interest in science among young students (Spillane, 2004). This in-service course includes an opportunity for biology graduate students (American Association of Immunologists) and high school teachers to network together, plan time during the school year when they can collaborate on lesson planning and instruction by leveraging the research experience of the fellows. As modern role models, fellows and teachers can provide classroom and laboratory learning experiences that will instill a robust understanding of science's dynamic role within modern society. Fellows can recognize the role their research plays in shaping future 11^{th} and 12^{th} grade science education and inspire students to explore and pursue careers in science.

Assessment of the Professional Development Course

In evaluating the success of teacher development programs, valid and scalable measures of teaching practice are needed. The Science Lesson Plan Analysis Instrument (Jacobs *et al.*, 2008)

for quantitative evaluation of teacher-generated multiday lesson plans was developed by Jacobs (2008) as a complement to surveys and classroom observation, and demonstrates its use in two pilot studies. The SLPAI was used formatively to measure the teaching practices of incoming program cohorts and tailor program instruction. It was also used to track changes in teaching practice and pedagogical knowledge of participants over time, providing summative evidence of program effectiveness. It is suggested that this instrument be used to evaluate this in-service curriculum as well as the individual teachers' lessons they develop as a result of this course. SLPAI consists of four major subscales: Alignment with Endorsed Practices (AEP), Lesson Design and Implementation-Cognitive and Metacognitive Issues (CMI), Lesson Design and Implementation-Sociocultural and Affective Issues (SCAI), and Portrayal and Uses of the Practices of Science (PUPS) (Jacobs *et al.*, 2008).

Components and Arrangement of the Curriculum

Dividing science teaching into separate components oversimplifies a complex process. This integrative approach to the teaching and learning of biological concepts found in the microbiology and molecular biology disciplines includes subcomponents for optimal structure, organization, balance and presentation to teachers in the course that will ultimately be used as models in their own classrooms. Conceptual and procedural schemes unify science disciplines and provide students and teachers with powerful ideas to help them understand the natural world (National Science Foundation, 2009). Since underlying principles are emphasized in respectable science organization such as the American Association for the Advancement of Science, National Committee on Science Education Standards and Assessment, Utah State Office of Education, Advance Placement College Board, and National Science Teachers Association, a set of overarching themes are included and provide focus for the individual concepts in the

designated disciplines of the course. As the course is presented, these unifying concepts will be emphasized. They include 1) Systems, Order and Organization, 2) Change, Constancy and Measurement, 3) Evidence, Models and Explanation, 4) Evolution and Equilibrium, and 5) Form Fits Function

This curriculum includes two major sections, A and B, for the trainer of the professional development course and the teacher who is attending the course, respectively. Each section is divided into four units and each unit consists of specific segments. The four units are entitled: 1) Virology, 2) Molecular Biology, 3) Immunology and, 4) Pathogenesis and Society. Any of the materials may be shared, copied and altered to meet the needs of the participants in the course.

Section A begins with the course objectives, a time table for the two week course, and a pre-assessment to be given on the first day of class. Section A is then subdivided into four units consisting of up to ten segments each. These segments are labeled 1) Learning Objectives, 2) Essential Prior Knowledge, 3) Trainer Preparation Notes, which include misconceptions that should be addressed with the class and anticipated results 4) Essential Questions, 5) Core Concepts, to be introduce and explained if necessary, 6) Anticipatory Set, 7) Comprehension and Application Activities, to promote inquiry, integration and discussion, 8) Higher Order Thinking Skills, 9) Interactive Notebook/Journal Writing, and 10) Assessment, to monitor the progress and understandings of the participants. Included in the lesson materials will be suggested organizational tools the trainer should model for the teachers. Teachers must be given opportunities to share what they do in their classroom as well. Levels of rigor should be discussed wherever possible. Cooperative learning activities are provided wherever it is possible so that teachers will see how the concepts can be presented and practiced in an inquiry based environment.

Section B is subdivided into four units consisting of four segments each. These segments are entitled: 1) Worthy of Note, which may be copied and given to students to help the teacher modify his existing ideas based on new information, 2) Moral/ Ethical/ Reasoning/ Dilemma Scenarios, to strengthen the relevance of the lesson and emphasize the relationship between society and technology (Loucks-Horsley *et al.*, 2003), 3) Divergent and Creative Thinking Activity, to emphasize the process of learning (Wei, 2009), and promote higher order thinking skills in the classroom and, 4) Laboratory Component, to allow the teachers to become more comfortable and knowledgeable about the new technology used in a microbiology and molecular biology laboratory and stress the inquiry aspects of learning.

Section A: Trainer Materials

Course Objectives

The National Science Education Standards envisioned change throughout the system. The science content standards encompass the following changes in emphases and were kept in mind as this course was developed (National Science Foundation, 2009).

LESS EMPHASIS ON	**MORE EMPHASIS ON**
Knowing scientific facts and information	**Understanding scientific concepts and developing abilities of inquiry**
Studying subject matter disciplines (physical, life, earth sciences) for their own sake	**Learning subject matter disciplines in the context of inquiry, technology, science in personal and social perspectives, and history and nature of science**
Separating science knowledge and science process	**Integrating all aspects of science content**
Covering many science topics	**Studying a few fundamental science concepts**
Implementing inquiry as a set of processes	**Implementing inquiry as instructional strategies, abilities, and ideas to be learned**
Activities that demonstrate and verify science content	**Activities that investigate and analyze science questions**
Investigations confined to one class period	**Investigations over extended periods of time**
Process skills out of context	**Process skills in context**
Emphasis on individual process skills such as observation or inference	**Using multiple process skills—manipulation, cognitive, procedural**

Getting an answer	**Using evidence and strategies for developing or revising an explanation**
Science as exploration and experiment	**Science as argument and explanation**
Providing answers to questions about science content	**Communicating science explanations**
Individuals and groups of students analyzing and synthesizing data without defending a conclusion	**Groups of students often analyzing and synthesizing data after defending conclusions**
Doing few investigations in order to leave time to cover large amounts of content	**Doing more investigations in order to develop understanding, ability, values of inquiry and knowledge of science content**
Concluding inquiries with the result of the experiment	**Applying the results of experiments to scientific arguments and explanations**
Management of materials and equipment	**Management of ideas and information**
Private communication of student ideas and conclusions to teacher	**Public communication of student ideas and work to classmates**

10-Day Time Table

Day 1

- General Molecular Biology – Learning from the virus world
- Historical perspective - Science as a Process
- The Central Dogma of Molecular Biology: Overview of Replication, Transcription and Translation
- Expression of genetic information,
- Transcriptional control of expression
- Posttranscriptional control of expression

Day 2

- General Virology
- Model Viruses: Herpes (DNA) and Influenza (RNA)
- Components of a virus – Relationship of Structure and Function
- Capsid symmetry, variations, relationship to genome size and diversity
- Enveloped viruses – how having an envelope affects characteristics
- Complex virus structures – antireceptors, matrix proteins
- Genome diversity
- Single stranded and double stranded
- RNA and DNA
- Overview of viral replication, replication cycle, replication patterns
- Protein – nucleic acid interactions and genome packaging

Day 3

- General Immunology – Systems, Order and Organization
- How to use bacteria and viruses to teach immunology
- Immune response to virus infection in host cells
- Immune response to bacterial infection in host cells
- Apoptosis

- Interferons – Regulation and Communication

Day 4

- Evolution and Diversity
- The course of virus infections
- Virus vectors
- The ecology of viruses and their hosts
- Zoonosis

Day 5

- Evolution
- Virus evasion of host immune system
- Virus-host interactions
- Pathogenesis
- Cellular injury
- Viruses and immunodeficiency
- Viruses and cancer
- Interesting and relative diseases – relative issues to students.

Day 6

- Past, present and future of a molecular biology lab.
- Basic principles
- Culture media and cell culturing.
- Microscopy
- Good practices in the micro lab
- Practice in a micro and molecular lab
- Equipping a microbiology lab.
- What is practical to do in a high school laboratory classroom?
- Experiments and activities you can do in your lab

Day 7

- Application and Comprehension Activities – Science as a Process
- Why use case studies?
- How to develop them, what are the components?
- Bioterrorism – Science, Technology and Society
- New and emerging viruses
- Vaccinations – Past, present and future
- Viruses for gene delivery
- Viruses to destroy other viruses

- Viruses and nanotechnology

Day 8

- Writing a Case Study for your classroom
- Why use case studies? Science as a Process
- How to develop them, what are the components?
- Writing a Case Study for your classroom using examples in **Technology and Society**
- How to assess the learning outcomes when using case studies.
- How to assess your case study before giving it to students.
- Bioterrorism – Science, Technology and Society
- New and emerging viruses
- Vaccinations – Past, present and future
- Viruses for gene delivery
- Viruses to destroy other viruses
- Viruses and nanotechnology

Day 9

- Wrap it Up – So, What Does the Secondary Life Science Teachers Need to Know About Micro and Molecular Biology in the Year 2010 and Beyond?
- ~~"D~~Don't give me more, tell me what to replace!"
- Review of all the THEMES in this course
- Compiling and evaluating a personal course binder, to be used in your classroom (part of your grade)

Day 10

- Sharing case studies by presentation (each teacher will need to prepare enough copies for all the other teachers in the class as part of your grade)
- Personal evaluation of your case study (part of participant's grade)
- Final Exam
- Course evaluation

Promoting Higher Order Thinking Skills

To allow in-service teachers to learn as much as possible in the ten day period about virology, molecular biology and immunology while modeling good pedagogical techniques to scientific inquiry, the following 18 methods of presenting and discussing these concepts should be used with specific examples and identified as such for teachers (A working definition of the method is indicated in parentheses). A list of alternative assessment products that encourage higher order thinking skills can be found in Appendix: Possible Assessment Products.

1. **PARADOXES:** (Common notion not necessarily true in fact. Self-contradictory statement or observation.
2. **ATTRIBUTES:** Inherent properties. Conventional symbols or identities. Ascribing qualities
3. **ANALOGIES:** Situations of likeness. Similarities between things. Comparing one thing to another.
4. **DISCREPANCIES:** Gaps of limitations in knowledge. Missing links in information. What is not known.
5. **PROVOCATIVE QUESTIONS:** Inquiry to bring forth meaning. Incite knowledge exploration. Summons to discovering new knowledge.
6. **EXAMPLES OF CHANGE:** Demonstrate the dynamics of things. Provide opportunities for making alterations, modifications, or substitutions.
7. **EXAMPLES OF HABIT:** Effects of habit-bound thinking. Building sensitivity against rigidity in ideas and well-tried ways.
8. **ORGANIZED RANDOM SEARCH:** Use familiar structure to go at random to build another structure. An example from which new approaches occur at random.
9. **SKILLS OF SEARCH:** Search for ways something has been done before (historical search). Search for the current status of something (descriptive search). Set up an experimental situation and search for what happens (experimental search).
10. **TOLERANCE FOR AMBIGUITY:** Provide situations which puzzle, intrigue, or challenge thinking. Pose open-ended situations which do not force closure.
11. **INTUITIVE EXPRESSION:** Feel about things through all the senses. Skill of expressing emotion. Be sensitive to inward hunches or nudges.
12. **ADJUSTMENT TO DEVELOPMENT:** Learn from mistakes or failures. Develop from rather than adjust to something. Develop many options or possibilities.

13. **STUDY CREATIVE PEOPLE AND PROCESS:** Analyze traits of eminently creative people. Study processes which lead to problem solving, invention, incubation, and insight.

14. **EVALUATE SITUATIONS:** Decide upon possibilities by their consequences and implications.

15. **CREATIVE READING SKILL:** Develop a mind-set for using information that is read. Learn the skill of generating ideas by reading.

16. **CREATIVE LISTENING SKILL:** Learn the skill of generating ideas by listening. Listen for information allowing one thing to lead to another.

17. **CREATIVE WRITING SKILL**: Learn the skill of communicating ideas in writing. Learn the skill of generating ideas through writing.

18. **VISUALIZATION SKILL:** Express ideas in visual forms. Illustrate thoughts and feelings. Describe experiences through illustrations.

First Day Pre-Assessment

NOTE TO TRAINER: It is important that you review the unifying themes in biology and connect with the content of this curriculum. To assess the teachers' knowledge and discover the level of understanding, the following activity should be used at the beginning of the course. Enlarge the form below and provide one for each teacher. Ask the teachers to work in small groups and discuss specific biological concepts and examples, writing them in boxes that would be relevant to the branch of microbiology listed in the left column and overarching biological theme along the top row. The ideas should be recorded, presented in the large group, in preparation for further instruction. This activity will allow the trainer to assess the current understandings of virology, immunology and molecular biology and may be used as both a pre and post assessment for the course.

	Systems, Order, and Organization	**Change, Constancy and Measurement**	**Evidence, Models and Explanation**	**Evolution and Equilibrium**	**Form Fits Function**
Virology					
Molecular Biology					
Immunology					
Pathogenesis and Society					

Unit 1: Virology

Learning Objectives:

- A virus is an infectious organism that reproduces within the cells of an infected host.
- A virus is not alive until it enters the cells of a living plant or animal.
- A virus contains genetic information wrapped in a protein coat.
- Viruses can be useful as well as harmful.
- A virus that mutates ensures its own survival by making itself unrecognizable to immune systems and vaccines.
- Even viruses engineered for useful purposes can be harmful if unchecked.

Essential Prior Knowledge:

It is not expected that teachers will be very familiar with the study of viruses with the exception of the typical bacteriophage that is introduced in the high school text book. This unit will begin with the basics and move as far as possible within the time constraint of three day.

Trainer Preparation Notes:

The following basics should be explained, discussed and informally assessed the first day of training.

- Basic Virology - Components of a virus – **Structure for Function**
- Capsid symmetry, variations, relationship to genome size and diversity
- Enveloped viruses – how having an envelope affects characteristics
- Complex virus structures – antireceptors, matrix proteins
- Genome diversity
- Single stranded and double stranded
- RNA and DNA

- Overview of viral replication, replication cycle, replication patterns
- Protein – nucleic acid interactions and genome packaging.

Essential Questions:

- How have microscopes changed the way we picture microbes?
- What kinds of organisms do scientists study with a microscope?
- How do scientists study and use viruses in the laboratory?
- How do viruses replicate in host cells?
- How do microbes evolve, where did they come from?

Core Concepts:

The Nature of Viruses

- Size, comparison of structures
- Genomes, size, structure, modification at the ends of virus genomes
- Virus proteins
- Virus capsids and envelopes
- Discussion of living or nonliving
- Replication models
- Transcription and translation models
- Characteristics of the proteins and nucleic acids of viruses and the interactions between these molecules and molecules of host cells.

Some viruses are useful

- Phage typing of bacteria
- Vaccines

- Sources of enzymes such as reverse transcriptase from retroviruses and RNA polymerases from phages
- Pesticides, such as baculovirus and myxoma virus
- Gene vectors for protein production
- Gene vectors for treatment of genetic disease

Virus studies contribute to knowledge of molecular biology, cell biology and cancer

- Hershey and Chase, 1952, T2 and *E. coli*
- First enhancers characterized in genes of simian virus 40
- First transcription factor to be characterized, T antigen of SV40
- First nuclear localization signal of protein identified in the T antigen of SV40
- Introns discovered during studies of adenovirus transcription
- Role of the cap structure at the 5' end of mRNA discovered in studies with vaccinia virus and reovirus
- First internal ribosomal entry site discovered in the RNA of poliovirus
- First RNA pseudoknot discovered in genome of turnip yellow mosaic virus.

Virus attachment and entry into cells

- Cell receptors and co-receptors
- Virus attachment sites
- Enveloped vs. naked viruses
- Intracellular transport
- Genome uncoating

Virus transcription, translation and transport

- Intro to transcription, translation and transport

- The four main categories of virus genomes and their distinct modes of transcription
- Transcription – A Comparison with eukaryotic cells (promoters, enhancers, transcription factors, transcriptase, capping transcripts, polyadenylation of transcripts, splicing)
- Translation – A comparison with eukaryotic cells (ORF, initiation and translation, bicistronic mRNAs, co and post translational modifications
- Transport within and between cells

Virus genome replication

- Virus enzymes and cell enzymes (DdDp, RdRp, RdDp)
- Locations of virus genome replication
- Initiation of genome replication
- Types of genome replication (dsRNA, ssRNA, dsDNA, ssDNA, reverse transcription

Virus assembly and exit from cells

- Nucleocapsid assembly
- Formation of viral membrane proteins and virus envelope
- Exiting the host cell – immune responses can be picked up here

Origins and evolution of viruses

- Possible virus origins
- New viruses may evolve as a result of viruses infecting new host species (HIV)
- Mechanisms of virus evolution (mutations, recombination, reassortment, acquisition of cell genes)
- Co-evolution of viruses and their hosts

Anticipatory Set:

Upon course acceptance, to be taught as a professional development course, this portion of the curriculum will be completed.

Comprehension and Application Activities:

Case Study: A Case Study Involving Influenza and the Influenza Vaccine by John Bennett, Carroll College. This case study presents a discussion about the benefits of the influenza vaccine between Mary, a nursing student, and her coworker, Karen. Karen is not convinced by Mary's arguments in favor of vaccination, and she counters with several common rationalizations for not getting the vaccine. Students **work in small groups** to evaluate the arguments for and against vaccination from the perspective of each woman. In addressing the questions associated with the case, students learn about the general biology of viral infections, treatment of infections, and immunity.

Interactive Notebook/Journal Write:

The success of some viruses lies in their ability to evolve within the host. Such a virus evades the host's defenses by rapidly mutating and producing many altered progeny viruses before the body can mount an attack. Thus, the viruses present late in infection differ from those that initially infected the body. Discuss this as an example of evolution in microcosm. Which viral lineages tend to survive and how do they survive?

Assessment:

Upon course acceptance, to be taught as a professional development course, this portion of the curriculum will be completed.

Resources:

- *Virology: Principles and Applications* by John Carter and Venetia Saunders, Wiley Publisher, 2007 ISBN:978-0-470-02387.
- *How Pathogenic Viruses Work* by Lauren Sompayrac, Jones and Bartlett Publishers, 2002 ISBN: 0-7637-2082-8.
- http://www.biologyteacher.uconn.edu/teachers.html - From DNA to Organism: A Study in DNA Function for the High School Biology Classroom.

Unit 2: Molecular Biology/Microbiology

Learning Objectives:

- To understand the structure of proteins, their purification and properties and introduces the teachers to enzyme kinetics.
- Discuss the practical aspects of using important tools of biochemistry such as gel electrophoresis, ways to purify proteins, the use of radioisotopes and techniques for studying cell membranes.
- To understand the fundamentals of prokaryotic biology that lie at the heart of modern molecular techniques.
- To describe the prokaryotic structure and function including bacterial growth, differences between Gram-positive and Gram-negative bacteria, mechanisms of action of antibiotics and antibiotic resistance.
- To apply the concepts of gene regulation and protein synthesis including positive and negative regulation of transcription to specific examples: the lac, arabinos, and tryp operons.
- To discuss gene transfer in bacteria including conjugation in Gram-positive and Gram-negative bacteria.
- To explain transformation including natural competence in bacteria and artificial competence (i.e. technological transfer systems).
- To explain transduction including specialized vs. general transduction, bacteriophage genetics.
- Restriction and modification systems including different types of restriction enzymes (class I, II and III).

- In all cases emphasis will be on both the basic science behind these phenomena, and how they are exploited in modern research strategies.

Essential Prior Knowledge:

- Historical perspective - **Science as a Process**
- Central Dogma of Molecular Biology - Overview of Replication, Transcription and Translation
- Expression of genetic information,
- Transcriptional control of expression
- Posttranscriptional control of expression

Trainer Preparation Notes/ Essential Questions/Core Concepts – Two possible lessons

1. This first lesson will meet *Standard II: Students will understand that all organisms are composed of one or more cells that are made of molecules, come from preexisting cells, and perform life functions* (from the Utah State Department of Education).

Somatic cell cloning – how it is done, why it is done, who does it.

What information will be discussed as a result of teaching about cloning?

- Nucleus and components
- Mammalian egg structure
- Somatic vs. germ cells
- Cell membrane structure
- Chromatin structure, DNA wrapping, cellular controls to RNA transcription
- Hormonal regulation

WHY? Real life examples help students' awareness, interest and understanding.

2. This second lesson to meet *Standard IV: Students will understand that genetic information coded in DNA is passed from parents to offspring by sexual and asexual reproduction.*

Chromosome Smears – how it is done, why it is done, who does it.

What information will be discussed as a result of doing chromosome smears?

- Cell and particularly nuclear structure
- Cell cycle, particularly components of mitosis
- DNA structure, packaging, different formations
- Genes on DNA, location, markers
- Karyotyping
- Genetic Counseling

WHY? Doing this activity is fun, we photograph the smears and print them, students do a karyotyping activity.

Other Core Concepts:

Maintenance of the Genome

- DNA as genetic material – evidence
- Chromosomes, chromatin and nucleosome
- DNA mutability, proofreading and repair
- DNA replication, base pairing, models of, assisting proteins, recombination
- DNA replication in prokaryotes
- DNA structural model, scientific process of building
- Ends of the DNA molecules
- RNA structure
- Site-Specific recombination and transposition of DNA

Expression of the Genome

- Mechanisms of Transcription
- RNA splicing
- Translation
- The Genetic Code

Regulation

- Gene regulation in Prokaryotes
- Gene regulation in Eukaryotes
- Gene regulation during Development
- Comparative genomics and evolution of animal diversity

Molecular Methods

- Nucleic acids – electrophoresis, restriction enzymes, DNA hybridization, DNA cloning, plasmid vectors, DNA libraries, PCR, Forensics
- Proteins – purification, column chromatography, affinity chromatography, Polyacrylamide gels, antibody use for visualization, proteomics

Why do Scientist use these Model Organisms?

- Bacteriophage
- Bacteria
- *Saccharomyces cervisiae*
- *Caenorhabditis elegans*
- *Drosophila melanogaster*
- *Mus musculus*

Transforming Bacterial Host Cells

- Transformation is a process through which bacteria can take up DNA from outside themselves. Its most famous use was the Griffith experiment that demonstrated the heritability of traits even from dead bacteria. (review this experiment)
- Discuss how to transform specially prepared competent *E. coli*. The *E. coli* will then replicate the plasmid for us, expressing a protein from a gene inserted into the plasmid. Bacteria that take up the plasmid are selected using the ampicillin resistance gene that is also on the

plasmid. Only ligated plasmids will function in the bacteria and allow for antibiotic resistance.

- Discuss the two primary methods of getting cells to take up plasmids. Chemically competent cells will take them up after heat shock. Electroporation can also be used to punch tiny holes in the bacterial cell wall and drive in the DNA.

Bacteriology – Overview of a Few Representative Pathogens

For each of these, discuss their life cycle, general mechanisms for evasion of the immune system, spread, symptoms and treatments

- *Staphylococcus*
- *Streptococcus pneumonia*
- *Yersinia pestis*
- Yeast - *Candida albicans*
- Molds - *Aspergillus*
- Intestinal protozoa – *Giardia, Cryptospiridium parvum, Entamoeba histolytica*
- Malaria - *Plasmodium falciparum*
- *Leishmania – Tropicana, brasiliensis, Donavani*
- Gut helminthes – Ascaris lumbricoides

Anticipatory Set:

Read article: "The Ivory Trail". The illegal slaughter of African elephants for ivory is now worse than it was at its peak in the 1980s. New forensic tools based on DNA analysis can help stop the cartels behind this bloody trade (Scientific American, July 2009). After reading the article, discuss how molecular biology tools are at work in a variety of settings.

Case Study: Introduce and discuss the following case study, "Do You Really Know What You're Eating? A Case Study on Genetically Modified Foods" by Wayne Shew, Birmingham-Southern College, and Mary Celeste Reese, Mississippi State University. Starting from a fictional "news" report about an apparent allergic reaction to a taco tainted by genetically modified corn, students consider some of the techniques and procedures used in modern molecular genetics and microbiology as well as some of the issues associated with genetically modified organisms (GMOs). Originally designed for role-play and PowerPoint assignments, suggestions for a shortened version are also provided. Suitable for a general microbiology course, the case could also be used in an introductory molecular biology course with appropriate modifications. Various levels of coverage of the topic of recombinant DNA are possible.

Comprehension and Application Activities:

Case Study: Tazswana's Story: How Alternative mRNA Splicing Leads to Genetic Disease and Cure by S. Catherine Silver Key, North Carolina Central University. While students easily grasp the concepts of gene mutation, their effect on protein function, and their association with disease states, the concept of RNA processing is often foreign and less easily understood. In this directed case study, students read about a little girl with β-thalassemia, a life-threatening disease. Through a series of increasingly complex activities, they learn how alternative pre-mRNA processing (splicing) has caused her disease and how gene therapy that targets the process may provide a cure.

Case Study: "SNPs and snails and puppy dog tails, and that's what people are made of ..." A Case Study on Genome Privacy by Debby Walser-Kuntz, Sarah Deel, and Susan Singer, Carleton College. In this case on genome privacy, students work together to research one of six assigned lobbying groups' views in this area and then present their groups' position before a

mock meeting of a U.S. House of Representatives Subcommittee as they consider voting on the Genetic Information Nondiscrimination Act. In working through the case, students learn about single nucleotide polymorphisms (SNPs), common molecular biology techniques, and current legislation governing genome privacy. The case was developed for use in an introductory biology course entitled ―Genes, Evolution, and Development."

Case Study: Chimpanzee Droppings Lead Scientists to Evolutionary Discovery by Erica F. Kosal, North Carolina Wesleyan College. This case study focuses on the research of Dr. Beatrice Hahn, who investigates DNA sequences in chimpanzee droppings in order to explore the origins of the human immunodeficiency virus (HIV). Students first consider the types of data that can be gained through collecting chimpanzee feces and studying the behavior of these animals. Students then apply this information to learn more about microevolution when they compare DNA sequences. Finally, students learn about ELISA tests and consider the role of basic and applied science.

Interactive Notebook/Journal Write:

1. A biologist inserts a gene from a human liver cell into the chromosome of a bacterium. The bacterium then transcribes and translates this gene. The protein produced is useless and is found to contain many more amino acids than does the protein made by the eukarytotic cell. Explain why.
2. A plant biologist observed a peculiar pattern when a tropical shrub was attacked by caterpillars. After a caterpillar ate a leaf, it would skip over nearby leaves and attack a leaf some distance away. The researcher found that when a leaf was eaten, nearby leaves started making a chemical that deterred caterpillars. The biologist suspected that a damaged leaf sent out a chemical that signaled other leaves. How could the researcher test this hypothesis?

Assessment:

Upon course acceptance, to be taught as a professional development course, this portion of the curriculum will be completed.

Resources:

- *Medical Microbiology and Infection at a Glance* by Stephen Gillespie and Kathleen Bamford, Blackwell Publishing, 2007 ISBN: 1-4051-5255-9
- *The Microbiology Coloring Book* by I. Edward Alcamo and Lawrence M. Elson, Harper Collins College Publishers, 1996 ICBN:0-06-500941-X

Unit 3: Immunology

Learning Objectives:

Upon course acceptance, to be taught as a professional development course, this portion of the curriculum will be completed.

Essential Prior Knowledge:

This unit is intended to be taught to high school biology teachers, who will already have a very good understanding of the following: basic cell structure, prokaryote and eukaryote cell organization, organelles of eukaryotic cell, cytoskeleton role and components, extracellular components and connections, eukaryotic cell membrane structure, transport, basics of cell-signaling, basic definitions of innate and adaptive, humoral and cell-mediated immunity, reason behind blood types and familiar with diseases from bacteria, viral and fungal pathogens. The trainer will be able to assess the breadth and depth of the teachers' understandings of these subjects and immunology from the anticipatory set activity.

Trainer Preparation Notes:

The following is an overview of basic concepts that will need to be discussed in class followed by narrative that may be used in the presentation. A typical high school level biology textbook should be used for teachers to read, add to and begin drawing connections from the immunological concepts to other biological concepts found throughout the chapters of the text.

- General features of innate immunity: phagocytosis, target cell lysis, inflammation
- General features of adaptive immunity: specificity, division of labor, memory, diversity, tolerance.
- Interplay between the innate and adaptive responses
- Cellular Components of the immune system

- Leukocyte communication
- The complement
- Antigen recognition and receptors
- Immune cell surface and signaling molecules
- Closer look at T cells- development, sub-populations, memory, receptors
- Closer look at B cells and their development, differentiation, expansion and memory
- Antibodies – structure and function
- A closer look at Natural Killer cells
- A closer look at dendritic cells
- Apoptosis
- The Prokaryote – general structures and their functions, the immune defenses, the host response, model organisms (*Staphylococcus, Pseudomonas)*
- Fungi and ectoparasites – general structures and their functions, the immune defenses, the host response, model organisms (*Chalmydia, Rickettsia*)
- Viruses – general structures and their functions, the immune defenses, the host response, model organisms (Herpesviruses, Influenza) [More in-depth material is found in Unit 2: Molecular Biology and Virology]
- What happens when thing go wrong in the immune system?
- Technology in research and medicine- Hemagllutination, ELISA, Immunoblotting, Immunofluorescence, and Flow cytometry

Essential Questions/Core Concepts –A possible lesson that meets *Standard I: Students will understand that living organisms interact with one another and their environment.*

Case Study: "Boy with fever and rash"

DISCUSSION POINTS: Symptoms, diagnosis (PCR – detects DNA of the spirochete), infecting organism, transmission of disease (arthropod), other diseases with same mode of transmission, host response to infecting organism, organism's response to host defense system, environmental conditions, treating the disease – how and why is it necessary?

In early June a 15-year old boy comes to your practice with his mother. He had been fine until about five days ago when he developed a fever. He has a stiff neck and a rash on his back. He and his family live in Connecticut near the New York State border. His mother reports that he was playing in the woods with some friends recently.

Begin with this story:

Cows, Beer, and Beyond

—Gradually, through a series of discoveries over hundreds of years, scientists have learned about the way the immune system works and how to safely harness its protective power. One of the most significant advances in immunology—the creation of the first vaccine, in 1796—resulted from the effort to protect people against smallpox, a disfiguring viral scourge throughout history (and one that has recently risen again as a bioterrorism threat). Over the centuries, people around the world tried to protect themselves against smallpox by exposing themselves to the actual disease (a method known technically as inoculation). An early Chinese emperor, for example, inhaled dried smallpox scabs in an effort to inoculate himself. George Washington inoculated his troops against smallpox during the Revolutionary War by introducing the virus into the skin, then keeping troops in isolation until they were no longer contagious. But these early inoculation efforts, which involved exposure to the

unaltered—and therefore quite virulent—smallpox virus itself, killed some people and made others extremely ill.

The world's first vaccine, developed for smallpox, relied on a similar strategy but provided a much safer alternative. The road to discovery began in the late 1700s, when Edward Jenner, a country doctor, sought to explain why milkmaids and other farmhands exposed to cowpox, a relatively mild disease, did not come down with smallpox. To test the theory that cowpox exposure somehow bolstered a person's defenses against smallpox, Jenner took pus from a milkmaid infected with cowpox and injected it into a healthy eight-year-old boy. Jenner then exposed the child to smallpox, but the child never succumbed. After several additional experiments, Jenner proved that it was possible to prevent an infectious disease with a vaccine—a word derived from the Latin word *vacca*, or cow—derived from a substance that was similar but not identical to the real culprit.

Although Jenner's discovery represented a significant advance, it took many more years before scientists began to understand how diseases occur and to recognize clues that, in the late nineteenth and early twentieth centuries would reveal why vaccines confer protection"(The Dana Foundation, 2008).

Historical Perspective

- **Edward JENNER 1749-1823,** credited as a pioneer of smallpox vaccine and sometimes referred to as the "Father of Immunology".
- **Ignaz Philipp SEMMELWEIS, 1818-1865**, Also known as the "savior of mothers" because he discovered that the incidence of childbed fever could be drastically cut by use of hand washing standards in obstetrical clinics.

- **Louis PASTEUR 1822-1895,** the germ theory of disease.
- **Robert KOCH 1843-1910,** Isolated *Bacillus anthracis, Tuberculosis bacillus,* and *Vibrio cholera* for his development of Koch's Postulates, awarded the Nobel Prize in Physiology or Medicine for his tuberculosis findings.
- **Eli METCHNIKOFF 1845-1916,** Russian zoologist and microbiologist who received (with Paul Ehrlich) the 1908 Nobel Prize for Physiology or Medicine for his discovery in animals of amoeba-like cells that engulf foreign bodies such as bacteria—a phenomenon known as phagocytosis and a fundamental part of the immune response.
- **Paul EHRLICH 1854-1915**, theory that the germicidal capability of a molecule depended on its structure, particularly its side-chains, which could bind to the disease-causing organism (among many other things pertaining to staining and a most successful product of this quest, Salvarsan—dihydroxydiaminoarsenobenzenedihydrochloride—and Neosalvarsan, the most effective drugs for treating syphilis until the advent of antibiotics in the 1940s.
- **Theobold SMITH 1859-1934**, pioneering epidemiologist and pathologist and is widely-considered to be America's first internationally-significant medical research scientist
- **Clemens Von PIRQUET 1874-1929**, in 1906 he noticed that patients who had previously received injections of horse serum or smallpox vaccine had quicker, more severe reactions to a second injection. He, along with Bela Schick, coined the word allergy (from the Greek allos meaning "other" and ergon meaning "reaction") to describe this hypersensitivity reaction.
- **John Berdon Sanderson HALDANE 1892 – 1964**, Host-Pathogen interaction, one of the most important factors in evolution.

Immunology Basics

- General mechanisms of innate and adaptive immune system

- Recognition and receptors – the keys to immunity
- Evolution of immune mechanisms
- Cells involved in immunity, the hematopoietic system

Innate immunity

- Receptors of the innate system
- Complement
- Acute inflammation
- Phagocytic cells and phagocytosis

Adaptive immunity

- The molecular basis – immunoglobulins, MHC, T-cell receptor, antibody structure and function
- The cellular basis – lymphocytes, lymphoid organs
- The adaptive response – antigen processing and presentation, antibody response, antigen-antibody interaction and complexes, cell-mediated immune response
- Regulation – tolerance, cytokine network
- V(D)J recombination – molecular biology: genes can change in somatic cells!

Potential useful immunity

- Antimicrobial immunity – the general scheme
- Immunity to viruses (herpesviruses, influenza, mumps and measles, polio, hepatitis, retroviruses)
- Immunity to bacteria (*streptococcus, staphylococcus, mycobacteria, salmonella, tetanus*)
- Immunity to fungi (*Candida albicans, Cryptococcus, Aspergillus)*
- Immunity to protozoa (malaria, *Leishmania, Entamoeba histolytica, Giardia)*

Undesirable effects of immunity

- Harmful immunity – overview
- Allergies and anaphylaxis
- Immune complexes, complement and disease – rheumatoid arthritis
- Chronic and cell-mediated inflammation - granulomas, Crohn's Disease, Chronic Granulomatous Disease
- Autoimmunity – Diabetes, Rheumatoid arthritis, Systemic lupus
- Immunodeficiency – SCID, DiGeorge syndrome, Agammaglobulinemia
- HIV and AIDS
- Vaccinations and immuno-stimulation – passive, active
- Antibacterial agents
- Anticancer agents being investigated

Outcomes of infection

- Abortive, latent to persistent, productive to persistent or apoptosis
- Innate immunity response**,** Adaptive immunity

Anticipatory Set:

In a high school biology class, students are circulating the room, test tubes of clear liquid in hand. At a nod from their teacher, they exchange the contents of their test tubes with as many other students as they wish. Three minutes later, when the teacher stops them, she reveals that while all the test tubes looked identical, one originally contained an "infectious agent." Distributing a chemical that signals the presence of the agent by turning dark, she invites the students to test the liquids now in their test tubes. With a unanimous shudder of dismay, they watch more than half of their test tubes test "positive."The "infectious agent" is "fake"—diluted

horseradish—but the underlying scientific principles are incontrovertibly real, as is the ensuing discussion on HIV and other infectious diseases. (From the Third Faculty of Medicine: Charles University, http://www.lf3.cuni.cz/en/studium/magisterske/studijni-programy/i-cyklus/1-rocnik/modul-1b/CCBGMI21.html).

Comprehension and Application Activities:

Materials:

♦ 4 groups of students

♦ 4 index cards

♦ 4 transparencies and markers

Procedure:

1. The class will be divided up into four groups, with one person being responsible for writing, and one person responsible for speaking.

2. One index card will be distributed to each group that describes the scenario that they are responsible for working on.

3. The job of each group is to place themselves in a particular scenario and determine what course of action they would take.

4. All possible options must be explored and written down, and actions must be justified. After the groups have discussed their scenario and plan, the writer should write down the scenario they were given and describe how they dealt with it.

5. Each of the four index cards will have one scenario.

Scenario I: You are home alone and it is 12:30 at night. Your parents are out of town and are not expected back until the following day. You wake up from a deep sleep when you hear a loud noise outside in the street. What are your options and which one would you be most likely to

take? The sound is nonspecific, and could generate many different responses. The main point that this should be used to illustrate is that the immune system has primary and secondary defenses as well as specific and nonspecific defenses. This loud noise in the street could be a potential danger (such as somebody who sitting next to you in class who is visibly sick), but, at this point it probably will generate no significant response from the person in the house (your immune system).

Scenario II: You are home alone and it is 12:30 at night. Your parents are out of town and are not expected back until the following day. You are awakened from a sound sleep when you hear a loud noise downstairs. What are your options and which one would you be most likely to take? The sound is once again nonspecific, however, it would most likely generate a different response from the one generated from scenario I. The possible responses are many and analogously could be applied to a nonspecific secondary immune response. The potential danger is in the house (body), and the person who was sleeping in the house could respond to this situation in several different ways. The immune system has phagocytes and macrophages, which are leukocytes that can provide nonspecific secondary defense against pathogens.

Scenario III: You are home alone and it is 12:30 at night. Your parents are out of town and are not expected back until the following day. You wake up from a sound sleep when you hear a window break downstairs. What are your options and which one would you most likely take? This situation could generate several different responses, and these all should be explored and and developed. This scenario, however, could be used to develop an understanding for an inflammatory response generated from a foreign object penetrating the surface of the skin. The broken window (Enfield) could cause the person sleeping (histamines) to call the police (phagocytes) who will respond and remove the potential threat (pathogen/bacteria).

Scenario IV: You are home alone and it is 12:30 at night. Your parents are out of town and are not expected back until the following day. You wake from a sound sleep when you hear somebody downstairs. What are your options and which one would you take? This situation could also generate several different responses; however, these all can be used to generate an understanding for specific secondary defense mechanisms in the immune system. The role of pathogens, antigens, b lymphocytes, antibodies, t lymphocytes, and helper and suppressor t lymphocytes should be developed and explained.

6. After each group has had approximately 10-15 minutes to work on the activity, each group is asked to explain their scenario and the actions they decided to take as a result.

Input:

- The class will be introduced to the new unit on immunology and asked to share what they know about the immune system with the rest of the class. What does our immune system do?
- The general ideas will be written down on the chalkboard as a word splash, and these concepts will be related back to the stranger in the house scenario in order to define their functions.
- What are pathogens and how do they relate to diseases? Discuss Koch's Postulates, viruses,
- bacteria, protists, and fungi. How are pathogens transmitted? What are the means of detection that the immune system utilizes and how can these factors be related back to the stranger in the house scenario? How do antigens, antibodies, and B and T lymphocytes relate to this system?

Closure: What is the difference between humoral and cell-mediated immunity and how do they conceptually relate to the stranger in the house scenario?

Interactive Notebook/Journal Write:

Case Study: Immunological Malfunction? by Karen A. Pinco, Westfield State College, MA

This problem-based case study was developed to complement the study of the immune system and to emphasize the crosstalk that occurs at the cellular level between B and T cells for proper immune system function. In reading the story of a young couple trying to understand the cause of their infant boy's constant bacterial infections, students will review the different classes of antibodies, their specific functions, and how they arise through isotope switching.

Case Study: The Case of Baby Joe: Chronic Infections in an Infant by Kristen L.W. Walton, University of North Carolina–Chapel Hill

This interrupted case study follows the declining health of an infant who suffers from recurrent infections and finally is diagnosed with severe combined immunodeficiency (SCID). The case was developed for use in an undergraduate upper-level immunology course to supplement discussion of B and T cell development and the generation of antibody diversity. It could also be modified for use in a genetics class, with emphasis on the molecular aspects of RAG-mediated recombination and the inheritance patterns of the disease.

Assessment:

The post assessment may be the use of the questions below or creating a formal assessment with multiple choice and essay questions. For this unit to be used with teachers, the concept grid/ themes/topics grid should be used. Teachers complete the table, placing immunological concepts they are comfortable discussing in their classroom. This assessment will not only be useful for a personal evaluation of their understanding but useful for their curriculum planning in their own classroom.

- How would a macrophage deficiency likely affect a person's immune defenses?

- How do allergy medications work?
- In myasthenia gravis, antibodies bind to and block acetylcholine receptors at neuromuscular junctions, preventing muscle contractions. Explain the type of altered immunity that occurs.
- Severely burned patients generally must receive numerous skin grafts. What is the advantage of using skin from an unburned part of a patient's own body?
- What cells and functions would be deficient in a child born without a thymus?
- What is the major difference in the types of antigens bound by B cell receptors and T cell receptors?
- One of the reasons for the success of invertebrates, which make up more than 90% of living animal species, is their effective defense against microbes. Describe some mechanisms by which invertebrates combat such invaders and discuss how thieve mechanisms comprise an evolutionary adaptation that is retained in the vertebrate immune system.

Resources:

- *Immunology at a Glance* by J.H.L Playfair and B.M. Chain, series of at a glance, 9th edition
- Wiley-Blackwell, Oxford, 2009, 109 pages, ICBN: 978-1-4051-8052-8
- *Kuby Immunology* by Thomas J Kindt, Richard A Goldsby, and Barbara A Osborne, W H Freeman and Company, New York, 2007, 6th edition, 574 pages
- *Immunity: The Immune Response in Infectious and Inflammatory Disease*, by Anthony L DeFranco, Richard M Locksley, and Miranda Robertson
- Series called: Primers in Biology, New Science Press Ltd. London, 2007, 387 pages
- *Primer to Immune Response* by Tak W Mak and Mary E Saunders
- Academic Press, San Diego, 2008, 436 pages

Unit 4: Pathogenesis and Society

Learning Objectives:

Upon course acceptance, to be taught as a professional development course, this portion of the curriculum will be completed.

Essential Prior Knowledge:

Upon course acceptance, to be taught as a professional development course, this portion of the curriculum will be completed.

Trainer Preparation Notes:

This unit focuses on the strategies employed by pathogenic bacteria, fungi, and viruses to subvert the human immune defenses and cause disease. Representative examples of intracellular bacterial pathogens, extracellular bacteria pathogens, fungal pathogens, and viral pathogens are discussed in detail. Other topics include epidemiology and disease, innate defenses and normal flora, host/parasite interactions, and bacterial virulence factors.

Essential Questions:

- To appreciate the relationship between microbes, the immune system, and disease outcomes.
- To understand how the immune system functions in a specific and non-specific way, to defend the host against infections by bacteria, fungi and viruses.
- To recognize the structural components of microbes (bacteria, fungi and viruses) and how these impact the pathogenesis of disease.
- To know the common microorganisms associated with specific clinical diseases and what factors are involved in pathogenesis.
- To appreciate the role of vaccines in disease prevention.

- To appreciate the role of the clinical laboratory in diagnosis and management of infectious diseases.
- To develop the ability to correlate the clinical picture with laboratory information to establish a diagnosis.

Core Concepts:

Emerging viruses

- New host species (Bunyaviruses, Paramyxoviruses)
- Viruses in new areas (West Nile virus)
- Viruses in new hosts species in new areas (Ebola, Monkeypox)
- New viruses (SARS, new strains of Influenza)
- Recently discovered viruses (human metapneumovirus)
- Re-emerging viruses (measles, mumps)
- Bioterrorism

Viruses and cancer (cover at least two of these)

- Kaposi's sarcoma (Kaposi's sarcoma associated herpesvirus)
- Hepatocellular carcinoma (Hep B and Hep C)
- Adult T cell leukemia (HTLV-1)
- Burkitt's lymphoma
- Nasopharyngeal carcinoma (Epstein-Barr virus)
- Anogenital carcinomas (HPV)
- B-cell leukemia/lymphoma
- Sarcoma (retroviruses)

Anticipatory Set:

Discussion Point: The Vaccine Search Goes On. The unfinished quest for an AIDS vaccine has become a search for new approaches to the problem (Scientific American, Nov 2008).

Discussion Point: Can HIV Be Cured? Eliminating HIV from the body would require flushing the virus out of its hiding places and preventing those reservoirs from being refilled. A tall order but perhaps not impossible (Scientific American, Nov 2008).

Comprehension and Application Activities:

Case Study: Between the Living and the Dead by Kari Mergenhagen, University at Buffalo

As Jen pores over her introductory biology textbook, she falls asleep and enters a nightmarish world in which bacteria and viruses dwarf human beings. This engaging case explores the differences between viruses and bacteria while teaching about the basic components and ~~–li~~fe" cycle of a T-even bacteriophage. The case includes a follow-up assignment in which students explore the risks and potential benefits of using bacteriophage to control bacterial disease.

Interactive Notebook/Journal Write:

Case Study: The Unfortunate Nurse: A Case Study of Dengue Fever and Social Policy by Karen M. Aguirre, Coastal Carolina University, Conway, SC

Based on an actual incident in which dengue virus was transmitted by an accidental needle stick, this case study introduces students to ~~–e~~merging pathogens" and other concepts in parasitology, immunology, epidemiology, public policy, and science writing. Students also read a primary paper and learn about two modern techniques widely used in medical and research settings (i.e., EIA and Taqman RT-PCR).

Assessment:

Upon course acceptance, to be taught as a professional development course, this portion of the curriculum will be completed.

Resources:

- Lewin, B. (2008) *Genes IX,* (Lewin, 2008).
- Watson, J. D., Baker, T. A., Bell, S. P., Gann, A., Levine, M. & Losick, R. (2004) *Molecular Biology of the Gene,* (San Francisco, Benjamin Cummings).

The Take-Away for Teachers

Teachers will leave the course with a compilation of relevant reading materials for students, case studies, lists of websites, problem solving questions, inquiry activities and internet research activities. They will also be aware of sources to check out materials for lab activities or facilities that provide ~~"in~~-house" experiences for high school biology classes. Each participant will be responsible for locating a possible grant proposal from which they may begin building their own classroom with micro and molecular materials to more fully allow the teacher to transfer what they learn from this course into their classroom.

Evaluation of the Course

Evaluation of this course will include the teachers' ability to use the information, case studies and activities in their own classroom. This will be done in the following manner:

Each teacher will:

- Prepare at least one comprehension/application activity over the two week period that will be discussed in a group setting, re-worked and improved, if necessary, and shared with the other teachers in the course, each teacher leaving the course with all the case studies developed.

- At the end of the school year following participation in this course, the participant (teacher) will write a final paper that includes quantitative and qualitative documentation concerning what they did in their class as a direct result of the summer in-service.

Summary

High school teachers play a vital role in the preparation of students for college science courses. Through inquiry, content integration, epistemological pedagogy techniques, relevant case studies, augmenting organizational tools and resources, and fostering interdependence among professional scientists and educators, students most assuredly will be more successful in science courses and approach science careers as a possibility. In this course, teachers are encouraged to use examples from microbiology and molecular biology disciplines as they discuss case studies, introduce inquiry activities and teach organizational skills, without sacrificing content to meet state standards and core objectives. By fostering independent thinking and encouraging students to actively work towards understanding the material, teachers prepare students to approach the material rationally. Not only will these skills help our students be successful in college, but will enhance their success in later life as well as provide the pathway for lifelong learning.

Section B: Teacher Materials

Unit 1: Virology

Worthy of Note:

A virus is a non-cellular particle that consists minimally of protein or nucleic acid (DNA or RNA). Except for a few cases, viruses are not surrounded by a membrane. In order to survive, it must replicate inside another cell, such as a bacterium or a plant and animal cell.
Depending on their host species, viruses are distinguished between plant viruses multiplying almost exclusively within plant cells, bacterial viruses (bacteriophages) that depend on living bacteria, and animal viruses. For the sake of discussion I will refer to animal viruses as "human viruses."

Human viruses cannot grow in foods. Since viruses are very host-specific, a human virus will rarely multiply even in foods that are still alive (like oysters). However, they can persist for a long time.

The cell walls of plants are tough and plant viruses have no specific mechanism for entering the host cell. Plant viruses depend therefore on a mechanical breach (injury) of the integrity of a cell wall to directly introduce a virus particle into a cell or on transmission *via* invertebrates (insects, nematodes, etc.). While bacterial and fungal diseases are common in sprouts, plant virus diseases of sprouts are relatively rare. Infection is scarcely strong enough to kill the plant.

Food Safety Implication

- Although human viruses don't grow on food, food serves as a transportation device to get viruses from one host to another. Once the contaminated food is eaten, a virus can multiply in living cells and cause foodborne illness in humans. Sprouts can become contaminated with viruses in a number of ways, such as:

- **A Production Worker** - who handles seed or sprouts and is shedding (excreting the virus in their stool). If the person practices poor hygiene, he or she may transfer the virus to sprouts or other foods.
- **Contaminated Water** - used to irrigate or wash sprouts.
- **Cross-Contamination** - of sprouts by contaminated food.
- **Insects** - can transport viruses as they move about.
- **Seeds** - may transmit virus infection either due to external contamination of the seed with virus particles, or due to infection of the living tissues of the embryo. The latter only occurs in plant pathogens. Chlorine is ineffective as a sanitizer against viruses.

How It Causes Disease

A free virus particle may be thought of as a packaging device by which viral genetic material can be introduced into appropriate host cells, which the virus can recognize by means of proteins on its outermost surface. A bacterial virus generally infects the cell by attaching fibers of its protein tail to a specific receptor site on the bacterial cell wall and then injecting the nucleic acid into the host, leaving the empty capsid outside.

Within the cell the virus nucleic acid uses the host machinery to make copies of the viral nucleic acid as well as enzymes needed by the virus and coats and enveloping proteins, the coat proteins of the virus. Release of virus particles from the host may occur by lysis of the host cell, as in bacteria, or by budding from the host cell's surface that provides the envelope of membrane-enveloped forms.

More simply put, viruses cause disease in humans by tricking healthy cells into duplicating the virus's nucleic acid instead of its own, which lets the virus multiply. Once the virus is duplicated, the healthy cell usually dies.

A retrovirus (HIV) is thought to cause AIDS, several viruses cause particular forms of cancer in humans, and many have been shown to cause tumors in animals. Other viruses that infect humans cause measles, mumps, smallpox, yellow fever, rabies, influenza, and the common cold.

Some Examples of Human Viruses:

- Influenza (causes the flu)
- HIV (causes AIDS)
- Polio (causes poliomyelitis)
- Rhinovirus (causes colds)
- Rubella (causes German measles)

Some Examples of Foodborne Human Viruses:

- Norwalk Virus and other Norwalk-like viruses
- Hepatitis A
- Rotavirus (mainly affects young children)

Some Examples of Plant Viruses:

- Mosaic virus
- Mottle virus
- Ringspot virus

What's the difference between viruses and bacteria?

The differences between viruses and bacteria are numerous. Viruses are the smallest and simplest life form known. They are 10 to 100 times smaller than bacteria. The biggest difference between viruses and bacteria is that viruses are parasites, so they must have a living host - like a plant or animal - to multiply, while most bacteria can grow on non-living surfaces.

Also, unlike bacteria, which attack the body like soldiers mounting a pitched battle, viruses are guerilla fighters. They don't attack so much as infiltrate. They literally invade human cells and turn the cell's genetic material from its normal function to producing the virus itself. In addition, bacteria carry all the machinery needed for their growth and multiplication, while viruses carry mainly information - for example, DNA or RNA, packaged in a protein and/or membranous coat. Viruses harness the host cell's machinery to reproduce. In a sense, viruses are not truly "living," but are essentially information (DNA or RNA) that float around until they encounter a suitable living host.

Which sprouts are especially "friendly" to viruses?

- Sprouts that are grown in uncompensated manure or unsanitary soil.
- Sprouts irrigated with contaminated water.
- Sprouts harvested by production workers with poor hygiene practices.
- The complex, multi-layered surfaces of sprouts are more difficult to clean than, for example, the surface of an apple or potato. Finally, because many sprouts are eaten raw, there is no heating step that would inactivate the viruses. (From International Specialty Supply website, http://www.sproutnet.com/Reports/viruses.htm).

Moral/Ethical/Dilemma Scenario:

Case Study: Rabbit Calicivirus Disease: Magic Bullet or Pandora's Box? A Case Study on Biological Controls by Gary M. Fortier, Delaware Valley College

The characters in this dilemma case, representing the scientific community and government, must make a decision about whether or not to release a virulent pathogen into the environment in order to control the rapidly expanding population of European rabbits in New Zealand. As they

work through the case, students grapple with the complex issues associated with introduced species and biological controls.

Smallpox: To Be or Not to Be?

Smallpox disease was eliminated in 1979 after a worldwide effort to inoculate every man, woman, and child on Earth. Two collections of frozen smallpox virus have been preserved, one in Atlanta and the other in Moscow. Have students form discussion groups to talk about what they would do with the two collections. Students should list the pros and cons of keeping the smallpox virus in research laboratories, focusing on the ethics of eradicating life-forms that threaten the human population. Students can continue their study by researching how the CDC (Centers for Disease Control and Prevention) in Atlanta works to protect us from other viral invaders. They might also create fictional stories or plays exploring possible consequences that could result if smallpox should ever be reintroduced into society.

Divergent and Creative Thinking Activity:

- Explain the way in which a virus is able to reproduce and cause disease in a host.
- Explain how World War I contributed to the flu pandemic of 1914. If there were no war, what probably would have happened to the flu strain? Give supporting statements to back your explanation.
- Compare and contrast the work of Edward Jenner to that of Jonas Salk. How can the triumphs of these two virologists set an example for modern scientists researching new threats?
- How might viruses help cure genetic diseases?

- Describe two instances from the documentary in which disease was used as a weapon. How effective were the weapons? Is this practice still in use today? What are some of the potential consequences of using viruses in this manner?
- How might the destruction of rain forests help spread new viral diseases?

Laboratory Component:

Microscopy Skills Observing cell samples on slides and in culture plates

- Plaque formation
- Syncytia
- Fluorescent and confocal microscopy
- Electron microscopy
- Counting cells with a hemocytometer and calculating concentrations in cell suspension.

Unit 2: Molecular Biology

Worthy of Note:

The genetic material is able to specify a large variety of proteins. The nature of the genetic material was unknown for a long time. The cell is composed of lipids , carbohydrates , and nucleic acids , which do not have a lot of variety, and proteins which do have a lot of variety. It was thought for a long time that only proteins had enough diversity to give the orders to a cell to make other proteins. Then it was suggested that the information to build protein could be carried in a coded form. Such a coding mechanism could impart a lot of information with very simple building blocks. Consider for example that a computer codes all information in sequences of O's and 1's. With such simple coding, it is possible to display this text on the computer screen as well as diagrams and photographs.

Both proteins and DNA satisfy the fundamental requirement of the genetic material, and both are found in the chromosomes. Which one are genes made of?

Where does the genetic information reside? What gives a cell orders to make proteins? Several teams of researchers worked hard to help us understand what carries the information.

- Griffith, 1928 : the transforming principle is genetic material
- Avery, McLeod, and McCarty, 1940 : the transforming principle is DNA
- Hershey and Chase, 1952 : **only** DNA enters the cell

Moral/Ethical/Dilemma Scenario:

Case Study: Tazswana's Story: How Alternative mRNA Splicing Leads to Genetic Disease and Cure by S. Catherine Silver Key, Department of Biology, North Carolina Central University, Durham, NC. While students easily grasp the concepts of gene mutation, their effect on protein function, and their association with disease states, the concept of RNA processing is often

foreign and less easily understood. In this directed case study, students read about a little girl with β-thalassemia, a life-threatening disease. Through a series of increasingly complex activities, they learn how alternative pre-mRNA processing (splicing) has caused her disease and how gene therapy that targets the process may provide a cure. The case was developed for a junior-level genetics course, but could be modified for use in a cell, molecular genetics, or molecular biology course.

Divergent and Creative Thinking Activity:

Upon course acceptance, to be taught as a professional development course, this portion of the curriculum will be completed.

Laboratory Component:

How to Extract DNA and the Purpose – Biotechnology Laboratory Exercises

- From plant tissues
- From animal tissues/cells
- PCR and sequencing
- A comparison of DNA extraction methods used in research labs as opposed to classroom labs.
- DNA precipitation
- Washing and Resuspension
- Basic protocols
- Amplifying DNA by PCR
- Checking for quality using electrophoresis
- Analyzing DNA samples in a classroom lab

Possible laboratory exercises:

Laboratory One: Safety, laboratory equipment and introduction to pipetting

Description: Designed as a hands-on training session that introduces all students to the equipment in a modern Molecular Biology. Future labs build upon this initial training, such that teachers (will refer to as a student from this point on in the laboratory components) from diverse educational backgrounds start with a level playing field. Each student practices using three single channel, and two multi-channel pipettors (one manual and one electronic). Each student also receives instruction using basic laboratory equipment such as micro-centrifuges, centrifuges, a balance and programming a PCR machine.

Learning Objective: Students are given a first iteration of hands-on use of common molecular biology laboratory equipment. The procedures are designed to give the instructor the maximum amount of time to identify those students who need immediate, one on one instruction.

Laboratory Two: Collection of samples, extraction of genomic DNA and polymerase chain reaction setup

Description: Students are taken outside and encouraged to select plants from a region of campus that has several different plants. In addition, each student is required to draw the area from which they took their sample and a digital picture is taken of the plant. Students then extract DNA from his/her plants using a single tube extraction protocol. Dilutions of these DNA extractions are used in PCR with different RAPD primers (RAPD primers are used for PCR setup due to their likelihood of success on unknown genomic DNA).

Learning Objective: This is the first step in getting the student personally involved in learning molecular biology. The student picks their plant, draws the area that they have chosen

and extracts DNA from their plant. This is also the first chance to actively use the skills introduced in lab 1 and to improve documentation skills.

Laboratory Three: Electrophoresis of collected PCR samples, Design assigned gene primers and practice for lab practical.

Description: Students load their RAPD PCR samples from lab 2, then design primers for assigned gene(s). Students are currently assigned multiple genes to increase their probability of success.

Learning Objective: This is usually the first time that each student uses online scientific research databases and tools. This is also the first time that students may have to make a decision on what organism to use if there is not a representative gene from soybean. This is the first reinforcement of the concept that genes can be highly conserved between organisms. This protocol personally involves the student in the research to be performed in the lab.

Laboratory Four: Lab Practical Exam

Description: Placement of the lab practical at this point in the course assures the instructor that the student is ready for the next phase of the project, which requires competency in laboratory techniques and forces the student to learn proper techniques earlier, rather than later. Timing the practical after the primer design lab also allows additional time for the primers to be ordered and delivered

Laboratory Five: Primer dilution and initial PCR test of Soybean and Pea

Description: Primers ordered from lab three are processed by re-suspension in TE, then dilution to a working concentration of 10 mM. An initial test of these primers is conducted on soybean and Pea DNA. The students are given a PCR protocol and are required to program the PCR machines de-novo. Learning Objective: Resuspension of primers, pipetting accuracy

Laboratory Six: Electrophoresis of initial PCR test: PCR setup based on electrophoresis results

Description: Students load PCR product from lab five. After electrophoresis, the amplification profile is analyzed and based on these data, each student could begin an independent study project if they choose. The course can end at this time, or can continue by screening all functional primer pairs on a larger population of organisms. Learning Objective: Electrophoresis, post-translational modifications (product is larger than predicted from EST sequence), gel loading and pipetting

Laboratory Seven: Electrophoresis of populations.

Description: Electrophoresis of PCR product from lab six. Collect and analyze data as instructor desires (phylogeny trees, statistical analysis, etc)

Other Laboratory Skills to be discussed if time allows

How and Why an SDS-Polyacrylamide Gel is Used to Separate Proteins?

- Background on SDS-PAGE
- Basic Procedure
- Chemical ingredients and their roles such as SDS and DTT or beta-mercaptoethanol
- Stacking and resolving gels
- Chemicals for processing and visualization using BPB and coomassie blue

What is a Western Blot, Why is it Used?

- A way to detect proteins using antibodies
- Steps in tissue preparation, gel electrophoresis, transfer, blocking, detection (one step or two steps) and analysis.
- Discuss how chemiluminescence works

Preparation and dilutions of solutions

- How do you use a dilution factor
- How to use concentrations
- How to use an amount
- Serial dilutions
- Pipetting skills
- Reagent preparation

Unit 3: Immunology

Worthy of Note:

To study immunology today is to be on the cutting edge of research that is important in our lives. Immunology is revealing astonishing things about the system in our body that defends us from foreign germs that could do us harm, much like a country's military defends the country from invaders.

The microbial world is continuously evolving and producing new infectious threats. In this unit, you will learn that the immune system has many strategies designed to cope with these threats as they evolve. Our immune defense is intricate, cunning, and in many ways beautiful (The Dana Foundation, 2008). All living organisms need to protect themselves against infections. In multi-cellular organisms this defense system is normally called the immune system. The immune system consists of a number of organs, cells and soluble factors. These components cooperate to detect and to remove infectious organisms like viruses, bacteria and parasites. The immune cells of mammals usually have very different functions. Neutrophilic granulocytes are, for example, very important for our defense against bacteria, and cytotoxic T cells are essential for our defense against many viruses. The immune system can be separated into two branches, innate and adaptive immunity. Adaptive immunity differs from innate immunity by the ability to acquire memory and to develop specificity against different molecular structures. Innate immunity does instead react against common structures that we during millions of years have learned to recognize as typical for certain groups of microorganisms, as for example cell wall components of bacteria or double stranded RNA from viruses. In mammals the adaptive immunity consist primarily of two cell types, T and B lymphocytes. To recognize different pathogens these cells use highly variable molecules, i.e. antibodies and T-cell receptors.

The immune system is normally sufficient to protect us from different infections, but there is a constant battle between host and infectious organism, an arms race, where the immune systems always have to be prepared to encounter new and more aggressive pathogens. The immune system is a very powerful system that normally works in silence. However this system sometimes overreacts, which may cause us large medical problems, such as allergies and autoimmune diseases.

The Big Picture

Germs breaches the body's natural barriers. This alerts the immune system and triggers the first line of defense (innate immune system). If the first line needs reinforcement, it recruits and triggers the second line of defense (adaptive immune system) to ultimately eliminate the pathogens and generate ~~–m~~emory".

Considering a daily barrage of microbes from all directions, it's a wonder that we aren't sick all the time. Fortunately, the human immune system has evolved to handle this onslaught, operating like a highly efficient killing machine to fend off germs wherever and whenever they appear. Although the immune system employs a variety of weapons and strategies based on the specific threat, in general your immune system mounts a three-step defensive process.

Step 1: Sounding the Alarm

The first defenders on the scene of a germ attack are components of your innate immune system. The white blood cells that make up the innate immune system circulate throughout the body constantly, much like police on patrol, always on the lookout for biological suspects. These patrolling white blood cells belong to the phagocyte family, but they have many subtypes (just as a police force consists of detectives, sergeants, captains, and patrol officers). Phagocytes consist of macrophages, dendritic cells, and granulocytes. In various ways, the different types of

phagocytes identify, engulf, and ingest germs and other invaders. Phagocytes lead the way in many critical innate reactions.

Knowing that where one harmful virus or germ lurks more may be hiding, phagocytes sound chemical alarms to bring the more specialized immune cells to the scene. First, dendritic cells display an antigen—a chemical that identifies the invader—so that the appropriate immune system specialist cells are able to recognize the culprit. The specialists, which consist of B and T cells, are known collectively as lymphocytes, and they make up the body's adaptive immune system. As its name implies, the adaptive immune system adapts and adjusts to specific threats as the need arises, whereas the innate immune system is pre-existing and less specific. Although B cells can recognize and respond to antigens without much assistance, T cells require a second ~~"d~~anger" signal in the form of a biological flag, known as an MHC molecule, which an antigen-presenting cell (such as a macrophage or dendritic cell) uses to clearly designate that an invader is foreign. The phagocytes also release chemical messengers known as cytokines.

A foreign substance triggers the innate and subsequently the adaptive immune systems. In a successful response the pathogens are killed and antibodies are produced to more efficiently respond to future attacks.

Step 2: The Battle Escalates

Lymphocytes mount a two-pronged attack, one directed at infected cells and the other at hostile microbes circulating in the blood. The cell-targeting attack is directed by T cells. Killer T cells directly kill infected cells that have been marked for destruction by the phagocytes, while helper T cells coordinate the attack and send for reinforcements as needed. Meanwhile, B cells produce antibodies that bind to free-floating microbes circulating in the blood so that they cannot infect other cells. Phagocytes then engulf and destroy the antibody-studded invaders. Antibodies

also activate complement proteins, which destroy microbes by punching holes in them. As the battle rages at the microscopic level, you may start to be aware that something is amiss. If you've been infected with a cold virus, for example, your throat will become sore, your eyes watery, and your sinuses congested. These are the physical signs of inflammation, the buildup of fluid and cells that occurs as the immune system fights a hostile invader.

Step 3: Remember and Recover

Once all invaders and infected cells have been destroyed, the immune system soldiers that once multiplied so quickly decline in number. Inflammation subsides, and symptoms gradually disappear. But certain memory B and T cells remain, to remember how to attack the invader if it returns. Even with all these physiological weapons at our disposal, microbes occasionally manage to outsmart our immune sentries or elude detection. One reason is that microbes evolve rapidly and humans do not, giving the germs an advantage over our immune defenses. Moreover, if our defense system is impaired in any way—either by an inherited condition or because we've been exposed to certain environmental toxins or medical treatments that suppress immunity—we are more susceptible to infection (The Dana Foundation, 2008).

Failing to Protect: Immune Dysfunction Spells Trouble

For all its highly evolved mechanisms for identifying and fighting off the daily onslaught of germs, the immune system sometimes fails to provide the protection we need. Because the system is so complex, relying on a battalion of specialized units (in the form of T cells, B cells, macrophages, and an arsenal of antibodies and other biochemicals) to perform specific defense tasks and to coordinate with one another, dysfunction in any one of these units can render the entire system inadequate.

One might compare the immune system to the U.S. Department of Defense. When waging a war, the Defense Department relies on all of its forces—Army, Navy, Air Force, Marines, as well as specialized intelligence units, paramilitary forces, antiterrorist teams, etc., together in a coordinated fashion to defeat the enemy. It also relies heavily on its weapons—bombs, missiles, guns, tanks, battleships, warplanes, and so forth. As with the immune system, the effectiveness of the war machine, as a whole, depends largely on the ability of each component to efficiently fulfill its individual role. If a reconnaissance plane spies an enemy battalion approaching, but it can't get the message to troops on the ground, or if the troops don't have the ammunition or armor they need to fend off the attack, the enemy is free to wreak havoc.

In the same way, if any one component of the immune system is not up to par, germs can quickly get the upper hand. This is the case in immune deficiency diseases (IDDs), which result when one or more parts of the immune system are missing or defective. An IDD can be inherited or acquired through an infection (such as HIV) or illness, or it can result as a side effect of certain immunosuppressive medical treatments, or treatments that suppress natural immune responses. Acquired immunodeficiency syndrome (AIDS) is the best-known immune deficiency disease but there are many other less well-known and less prevalent examples. Some cancer treatments, including chemotherapy drugs, radiation, and high doses of a group of medicines called steroids, can weaken the immune system and render a person more vulnerable to infection.

When the Problem is in Your Genes

Primary immune deficiency (PID) diseases are the result of an inherited genetic defect that interferes with the immune system's normal development in one way or another. More than eighty different PID diseases have been identified, each one producing a constellation of symptoms depending on which piece or pieces of the defensive system are faulty. Symptoms of

individual diseases can range from mild or nonexistent to devastatingly severe. About 25,000 to 50,000 Americans have been diagnosed with a severe form of primary immune deficiency disease, but there are probably many thousands more who have milder forms.

One of the most common of these diseases, affecting about 1 in 600 Americans, is selective immunoglobulin A (IgA) deficiency. IgA is a particular type of antibody that defends against infections at mucous membranes that line the mouth, airways, and digestive tract. IgA deficiency results when B cells do not mature properly and fail to produce IgA antibodies at the levels required. Many people with the deficiency remain healthy; others may suffer recurrent infections of the ear, sinuses, or lungs.

Living in a Bubble

Far less common is severe combined immunodeficiency disease (SCID), sometimes called ―bubble boy" disease, which affects about one child in a million and is usually fatal. Infants born with this inherited condition have dramatic abnormalities in both innate and adaptive immunity, leaving them utterly defenseless against infections of any type and necessitating that they remain in a germ-free environment (the plastic ―bubble" in which such children often must live acts as a barrier to infectious microbes).

At least a quarter of SCID cases are linked to a defect in a gene that specifies the genetic code for a particular enzyme, adenosine deaminase (ADA). The absence of ADA interferes with metabolic processes within the cell, setting off a cascade of molecular events that are particularly lethal to T and B cells. Scientists have tested controversial gene therapy approaches to treating SCID, with the goal of replacing the missing gene and restoring production of ADA. While this approach has proven successful in some cases, treated children died as a result of complications

from the therapy, prompting an end to clinical trials until scientists conduct further laboratory research

Inducing Disease: "Friendly Fire" from the Immune System

Like tragic cases of "friendly fire" on the battlefield of war, sometimes the immune system mistakenly attacks "self" tissue. This self-attack can cause symptoms that range from the annoyance of a runny nose, as in allergies, to the devastating progressive degeneration of joints and organs associated with rheumatoid arthritis.

A disease in which the immune system attacks the body is called an autoimmune disorder. Autoimmune disorders comprise at least 80 different conditions that together affect 5 to 9 percent of Americans, or between 14 million and 22 million people. The misdirected attack on self tissue may target one or several body parts, depending on the disease. For example, in type 1 diabetes the immune system zeroes in on the pancreas, damaging cells that secrete the hormone insulin and rendering the body inefficient at processing glucose (a type of sugar). In multiple sclerosis, the objects of attack are cells in the central nervous system (the brain and spinal cord), particularly those in myelin, the fatty substance that sheathes nerve fibers and enhances the transmission of nerve signals. In psoriasis, skin cells are targeted. Systemic lupus erythematosus, on the other hand, is an example of a non-organ-specific autoimmune disease: organs and tissues throughout the body may be affected, and different people experience different sets of symptoms.

No Tolerance for Self

Each autoimmune disorder is associated with a unique combination of health problems. All of them seem to result from the malfunctioning of immune tolerance mechanisms. "Tolerance"

refers to the process by which the developing immune system normally eliminates any immune cells that are auto reactive, that is, immune cells that mount a response to the body's own tissue. Tolerance is a multilayered system, with a series of checks and balances built in to prevent self-attack from either innate or adaptive immune cells. In some cases, the self-reactive cells are removed or deleted. In other cases, they are silenced by immune cells called regulatory or suppressor cells.

Unfortunately, the process is somewhat leaky, and each of us ends up having a subpopulation of auto reactive immune cells floating around our body. If at some point in our life we are exposed to a microbe that happens to carry antigens resembling those on a particular organ—a situation immunologists call molecular mimicry—then the self-reactive cells that slipped through the checkpoints of tolerance mistakenly take aim at the body, striking out against the very tissue that they are supposed to be defending. Some scientists reason that such a scenario may trigger multiple sclerosis, for example.

A combination of many factors, inherited and environmental, increases one's susceptibility to autoimmune disease. On the genetic side, scientists are trying to identify the specific genes involved in various disorders, but that search has proved difficult because these disorders do not appear to result from single genetic defects. Rather, it is likely that a combination of genes, each of which may increase one's vulnerability, interact with environmental and lifestyle factors to produce disease. Environmental risks for autoimmune disease may include exposure to certain toxins or chemicals that are in the air, the ground, or the food we eat, or to certain viral infections. For reasons that are not clear, autoimmune disorders strike women more than men.

Some scientists believe the female hormone estrogen may contribute to this increased

incidence among women, but estrogen's role in immune function is complex and still being sorted out.

In 2002 the National Institutes of Health committed $51 million to a five-year research initiative aimed at unraveling the puzzle of autoimmune disorders (Dalakas *et al.*, 2001). The plan, which established nine Autoimmune Centers of Excellence at large academic research institutions, funds both basic research on the underlying biology of these disorders and clinical trials to test potential treatments. The goal is to speed the translation of scientific discoveries about the basic biology of autoimmunity into new therapies that will benefit patients.

Sneezing and Wheezing

Though they are not technically autoimmune disorders, allergies and asthma also result from an inappropriate immune response. An allergic reaction occurs when normally harmless substances, such as pollen or chemicals in pet dander, trigger an immune response. In people prone to allergies, the response produces an overabundance of the antibody called IgE, or immunoglobulin E, which in turn triggers plasma cells in the blood and mast cells in the skin, tongue, lungs, nose, and intestinal tract to release histamine, a biochemical that produces common allergy symptoms

Interleukins are proteins that help regulate the immune system, particularly the interaction among white blood cells, or leukocytes. B cells recognize the allergen and produce antibodies. A reaction follows, producing the symptoms commonly referred to as allergies. (Adapted from NIAID, NIH).

Asthma results when mast cells in the lungs and airways are provoked into producing histamine as a result of contact with an allergen, or because of some other precipitating factor such as exercise. The airways can become constricted, causing the difficult breathing, wheezing,

and coughing that are typical of an asthma attack. Without treatment, asthma can be deadly. Although the overall number of deaths from asthma is low, at least 17 million Americans have asthma, and its prevalence appears to be increasing. Researchers are trying to understand why. (The Dana Foundation, 2008)

Losing Battles: Why the Immune System Can't Beat Every Enemy

If the immune system is such an efficient germ-killing machine, why do so many people get sick with the flu at certain times of the year? Or why, if our immune system can fight off cancer cells and other life-threatening illnesses, it can't protect us from the common cold. Why do we hear so much in the news these days about "new" viral diseases (such as West Nile, SARS, and bird flu) and resurging "old diseases" (such as tuberculosis, malaria, and whooping cough)? Despite the remarkable efficiency of the normally functioning immune system, people who manage to escape unscathed from every infectious microbe they encounter are rare. The highly adaptable nature of germs means that new threats are always emerging as microbes reinvent themselves, learn to outsmart our medicines, and jump from other species to humans.

Adapting to Change

Perhaps the biggest reason our immune systems can't seem to keep up with every "bug" out there waiting to infect us is a simple one: microbes are more adaptable to changing environments than we are. Bacteria, parasites, and viruses replicate rapidly in response to environmental pressures and, while doing so, alter their structures in subtle ways that make them undetectable to the immune system.

Flu viruses are among the most changeable of microbes; the genes that compose them are in a continual state of flux and reassortment. This is why last year's flu vaccine is unlikely to protect fully against this year's dominant strain (or strains) of flu, and why the flu vaccine is

reformulated every year. It is also why, without immunization, we are more likely to catch the flu: since the virus has morphed into a new composition, our immune system doesn't recognize it, and we end up suffering the classic symptoms: fever, chills, muscle aches, and fatigue. These symptoms are the result of our immune system trying to rid the body of the virus. Annually, many Americans come down with the flu and an average of 20,000 to 40,000 people die from it. The elderly, young children, and people whose immune systems are compromised are most at risk for serious complications from the flu.

The common cold, like the flu, is the result of a viral infection, usually called a rhinovirus. Scientists have identified more than 110 different strains of rhinovirus and at least another hundred viruses that cause colds in humans. Nearly half of all adult colds are of unknown origin, which makes the sought-after ~~–e~~ure for the common cold" a distant goal. School-age children get colds most often, probably because they are in such close proximity to one another. Children have an average of six to ten colds a year, compared with adults' two to four a year, on average. People over 60 have even fewer colds—less than one a year, on average—which may simply reflect the fact that many older people have less day-to-day contact with other people who could infect them with a cold virus.

Pandemics and Emerging Microbes

Every few decades a particularly virulent strain of the flu emerges and produces a pandemic, an epidemic that occurs across a wide geographic area. The last flu pandemic occurred in 1968, and many scientists believe we are long overdue for the next. Global health experts are keeping a close watch on bird flu (also known as avian flu), which many fear might produce a pandemic. First identified in chickens, this flu strain began causing alarm because it was found in humans

for the first time in 1997, and by 2005, many dozens of human cases had been documented in several countries.

What Are "Emerging Microbes"?

One of the biggest subjects in contemporary microbiological research (the study of microbes) is that of "emerging microbes." In the last two decades alone, more than thirty newly recognized infectious diseases have emerged, including AIDS, toxic shock syndrome, Lyme disease, hantavirus, hepatitis C, and SARS. In most cases, these diseases are not really new, but rather their incidence is rising more rapidly than ever, making them an ever greater threat to humans. Also of concern are old diseases that are now re-emerging, even though they were mostly wiped out thanks to improved public health measures and widespread immunization. Tuberculosis is one example. Driving the spread of these diseases is increasing globalization, with the growing world population traveling greater distances than ever before, carrying disease-causing microbes with them. Climate changes and the widespread logging of Earth's forests are also factors in the spread of infectious diseases to new geographic areas and populations.

Moral/Ethical/Dilemma Scenario:

Both an injectable inactivated (killed) vaccine and an oral attenuated (live) vaccine are available for immunization against polio-virus, which can cause paralysis by destroying nerve cells in the brain and spinal cord. The oral vaccine is no longer recommended in western countries, where polio has been eradicated, because the live virus in this vaccine may mutate to a more virulent form, and be reintroduced into the population. However, the oral vaccine continues to be used in countries where polio persists because it is easy to administer (no needles) and highly effective. Moreover, the attenuated virus can spread to (and immunize) unvaccinated individuals. Do you feel this risk of mutation to virulence (about 1 in 12 million) is acceptable

when compared with the benefits of oral vaccination? How do you think public health decisions of this type should be made?

Divergent and Creative Thinking Questions:

Case Study: To Spray or Not to Spray: A Debate Over Malaria and DDT by Frank J. Dinan and Joseph F. Bieron, Canisius College. In this case study, students grapple with the complex issues surrounding the use of DDT to control malaria. In their examination of the issue, students study the cause of malaria, the life cycle of the protozoan, how it affects the human host and why there are no vaccines for malaria. Students consider risk/benefit analysis and the precautionary principle, two techniques used when making policy decisions involving the impact of science and technology on society.

Science, Technology and Society: The ability of the pathogen *Plasmodium* to evade the human immune system is one reason developing a malaria vaccine is so difficult. Another reason is that less money is spent on malaria research than on research into disease that affects far fewer people, such as cystic fibrosis. What are the possible reasons for this imbalance in research effort? What could a group of high school students do to help in the fight against Malaria in the world?

Case Study: The Case of a Tropical Disease and Its Treatment: Science, Society, and Economics by Cathy Santanello and Jennifer Rehg, Southern Illinois University at Edwardsville. Designed for a Costa Rican study abroad course, but appropriate for traditional science courses as well, this case highlights the epidemiological and socioeconomic factors associated with Chagas disease. Adrian is a banana plantation worker who develops a mysterious illness. By reading his story, students learn about infectious diseases, pathogens, and vectors endemic to this area of Central America. Students are asked to diagnose Adrian's illness and consider his dilemma with

respect to treatment options. Students also examine alternate approaches to treating this illness that plagues thousands of Central and South American citizens.

Science, Technology and Society: Kidneys were the first organs to be successfully transplanted. A donor can live a normal life with a single kidney making it possible for individuals to donate a kidney to an ailing relative or even an unrelated individual with a similar tissue type. What are some ethical problems that arrive as a result of being able to successfully transplant so many types of tissues in this age of medicine? What are some of the implications for the recipient of a donated organ or tissue?

Laboratory Component:

Discuss the use for monoclonal antibodies: for research, medicine, diagnosis, therapy, analysis and measurement of biological molecules. Include discussion and demonstrations of hemagllutination, ELISA, immunoblotting, immunofluorescence, and describe flow cytometry, with output data samples; practice interpreting results.

Connect with the Internet

- In the past few years, two diseases have received a lot of media attention: West Nile virus and SARS. They received so much attention because they were new, at least to the United States. Using the Internet, research one of these diseases and find out how it infects people and how it spreads. Based on your research, create a poster that provides information on how to protect yourself from the disease you select. Your poster should include information on how people are infected by the virus and steps for preventing the spread of the disease.
- Multiple sclerosis (MS) is an autoimmune disorder that affects the central nervous system. You may recall that autoimmune disorders are diseases where the immune system attacks the body. Use the Internet to research information in order to write a report about MS. In your

report you should include how the disease affects the human body and treatments that combat symptoms. You may want to use the following Web sites as resources for your research: the National Multiple Sclerosis Society (http://www.nationalmssociety.org), the National Library of Medicine (http://www.nlm.nih. gov), and the Multiple Sclerosis Foundation (http://www.msfacts.org).

- In order for children to remain healthy, it is important that they receive certain immunizations. Using the Internet as a resource, create a poster that explains to parents why and when their children should receive certain immunizations. Helpful sources for your poster include: the U.S. Centers for Disease Control and Prevention (http://www.cdc.gov), the American Academy of Pediatrics (http://www.aap.org), and KidsHealth® (http://www.kidshealth.org) Web sites.
- About two-thirds of the people in the world who are infected with HIV/AIDS live in sub-Saharan Africa. Imagine that you are in charge of a fundraiser to support the fight against AIDS in Africa. Using the Internet as a resource, design a brochure that will encourage people to contribute to your cause. Your brochure should inform people of the serious conditions in Africa. Information for your brochure can be found on Web sites for groups such as the World Health Organization (http://www.who.org), The World Bank (http://www.worldbank.org), and One: The Campaign to Make Poverty History (http://www.one.org).
- Avian (bird) flu is considered to be a potentially dangerous strain of influenza. Use the U.S. Centers for Disease Control and Prevention (http://www.cdc.gov) and the World Health Organization (http://www.who.org) Web sites to find information about avian flu. Create a

presentation that explains the origins of bird flu and why there is potential for this virus to be the next worldwide pandemic.

- Until the 1950s, there was no way to prevent polio, a viral disease that mostly affected children and could cause paralysis. Use the Internet to find out how this disease was virtually eliminated in the United States and what is being done today to eradicate the disease throughout the world.

Unit 4: Pathogenesis and Society

Worthy of Note:

Upon course acceptance, to be taught as a professional development course, this portion of the curriculum will be completed.

Moral/Ethical/Dilemma Scenario:

- Explain how the excessive or inappropriate use of antibiotics poses a health hazard for a human population. Give current examples of this problem.
- Humans have engaged in genetic manipulation for millennia, producing plant and animal varieties through selective breeding and hybridization processes that modify the genomes of organisms on a significant level. Why do you think modern genetic engineering, which often entails introducing or modifying only one or a few genes, has met with so much public opposition? Should some forms of genetic engineering be of greater concern than others? Explain (Campbell and Reece, 2005).
- Both an injectable inactivated vaccine and an oral attenuated vaccine are available for immunization against polio-virus, which can cause paralysis by destroying nerve cells in the brain and spinal cord. The oral vaccine is no longer recommended in western countries, where polio has been eradicated, because the live virus in this vaccine may mutate to a more virulent form, and be reintroduced into the population. However, the oral vaccine continues to be used in countries where polio persists because it is easy to administer (no needle) and is highly effective. The attenuated virus can spread to and immunize unvaccinated individuals. Do you feel this risk of mutation to virulence (about 1 in 12 million) is acceptable when compared with the benefits of oral vaccination? Explain.

Divergent and Creative Thinking Activity:

Case Study: Is Guaiacum sanctum Effective Against Arthritis? An Ethnobotany Case by Eric Ribbens, Barbra Burdett, and Angela Green, Western Illinois University. Dr. Beth Tonoany, a tropical population ecologist, is studying an unusual tree, *Guaiacum sanctum*, in the tropical forests of Central America. Interestingly, several local Ticos have told her that they use the tree for medicinal purposes. Students read the case and then answer questions designed to explore the process of screening and testing the medicinal value of plants identified as having potential health benefits.

Case Study: MDR Tuberculosis: A Case Study for Non-Science Majors Focused on Social Justice by Katayoun Chamany, Eugene Lang College of the New School University. In this case on multi-drug resistant (MDR) tuberculosis. students consider ways in which to preserve health as a human right without subjecting already marginalized communities susceptible to the disease to further discrimination. Students learn about the science behind TB diagnostics and current treatment protocols as well as the political and social history of TB outbreaks and the development of MDR TB. The case makes use of video clips, news stories, public health press releases and reports, and other secondary and primary literature.

Case Study: Biological Terrorism: The Anthrax Scare of 2001 by Kathleen A. Cornely, Providence College. In the weeks following the September 11, 2001, terrorist attacks on the World Trade Center and the Pentagon, anthrax-laced envelopes were mailed to individuals in government and the news media by an as-yet-unidentified bioterrorist. Thousands were treated for exposure, and five people were killed. At the same time, scientists solved the last remaining pieces of the anthrax puzzle, and the mechanism of infection of the anthrax toxin is now well understood. This case presents students with a wealth of biochemical, microbiological, and

immunological material to analyze while exploring important societal issues related to national preparedness against bioterrorist attacks, funding for bio-defense research, and the use and misuse of antibiotic therapy.

Laboratory Component:

Scientific Inquiry: In April 1989, an accident at a nuclear power plant in Chernobyl, Ukraine, scattered radioactive fallout for hundreds of miles. In assessing the biological effects of the radiation, researcher found mosses to be especially valuable as organisms for monitoring the damage. Radiation damages organisms by causing mutations. Explain why the genetic effect of radiation can be observed sooner in bryophytes than in plants from other groups. Imagine that you are conducting tests shortly after a nuclear accident. Using potted moss plants as your experimental organism, design an experiment to test the hypothesis that the frequency of mutations decreases with the organism's distance from the source of radiation.

Evolution Connection: The history of life has been punctuated by mass extinction events. Fossil records indicate that many forms of animal life were wiped out yet plants were less severely affected by mass extinctions. What adaptations may have enable plants to withstand these disasters better than animals?

Summary

High school teachers play a vital role in the preparation of students for college science courses. Through inquiry, content integration, epistemological pedagogy techniques, relevant case studies, augmenting organizational tools and resources, and fostering interdependence among professional scientists and educators, students most assuredly will be more successful in science courses and approach science careers as a possibility. In this course, teachers are encouraged and offered organizational tools, case studies, and inquiry activities in more depth, without sacrificing content to meet state standards and core objectives. By fostering independent thinking and encouraging students to actively work towards understanding the material, teachers prepare students better able to approach the material rationally. Not only will these skills help our students be successful in college, but will enhance their success in later life as well as provide the pathway for lifelong learning.

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Table 1: Present Life Science Teacher Education Programs in Utah and Nevada

Brigham Young University	Utah Valley University	Utah State University	University of Utah	University of Nevada
BIO 220A : Biological Diversity: Animals.	BIOL 1610/1615 College Biology 1 and lab	BIOL 1610 Biology I	BIOL 1200 General Biology	BIOL 190 Intro to Cell and Molecular Biology
BIO 220B : Biological Diversity: Plants.	BIOL 1620/1625 College Biology 2 and lab	BIOL 1620 Biology II	BIOL 2010 Evolution and Diversity of Life	BIOL 192 Intro to Organismal Biology
BIO 350 : Ecology	BIOL 3500 Genetics	BIOL 2220 General Ecology	BIOL 2020 Principles of Cell Biology	BIOL 192 Biological Investigation
BIO 420 : Evolutionary Biology	BIOL 3700 General Ecology	BIOL 2420 Human Physiology	BIOL 2030 Genetics	BIOL 223 Human Anatomy and Physiology
BIO 421 : Evolutionary Biology Laboratory	BIOL 4200 Teaching Methods in Science	BIOL 3060 Principles of Genetics	BIOL 3215 Cell Biology Lab	BIOL 251 General Microbiology
MMBIO 240 : Molecular Biology	BIOL 4500 Principles of Evolution	BIOL 3065 Genetics Laboratory	BIO 3900 Teaching of Biology	BIOL 200 Principles of Genetics
PDBIO 120 : Science of Biology	BIOL 4500 Principles of Evolution	BIOL 3220 Field Ecology	BIOL 5900 Teaching Experience	BIOL 314 Ecology and Population Biology
PWS 340 : Genetics	BIOL 494R Seminar	BIOL 3330 General Microbiology	BIOL 3230 Developmental Biology, OR BIOL 3310 Comparative Vertebrate Morphology, OR BIOL 3320 Comparative Physiology, OR BIOL 3330 Behav Neurobiology, OR BIOL 3350 Plant Physiology, OR BIOL 3370 Microbial Biology, OR BIOL 5364 Plant Structure	EDSC 464 Environmental Science Tchg Mthds
BIO 235 : Field Botany	MICR 2060 Microbiology for Health Professionals OR MICR 3450 General Microbiology	BIOL 5250 Evolutionary Biology	15 elective credit (Rogers and Abell) hours in Biology, two courses with labs and one at a 5000 level.	9 more credits (3 classes in biology electives)
BIO 370 : Bioethics	ZOOL 2320 Human Anatomy	BIOL 4400 Plant Physiology OR BIOL 5300 Microbial Physiology		
BIO 380 : Comparative Animal Physiology and	ZOOL 2420 Human Physiology	BIOL 5100 Neurobiology OR BIOL 5600 Comparative		

Anatomy		Animal Physiology OR BIOL 5620 Medical Physiology		
BIO 441 : Entomology	ZOOL 3100 Vertebrate Zoology	BIOL 5610 Animal Physiology Lab		
BIO 470 : History and Philosophy of Biology	ZOOL 3200 Invertebrate Zoology	SCI 4300 Science in Society		
Recommended: BIO 352 Introduction to Marine Biology BIO 430 Plant Classification BIO 443 Ichthyology, BIO 445 Herpetology, BIO 446 Ornithology BIO 447 Mammology	BOT 2050 Field Botany OR BOT 2100 Flora of Utah OR BOT 4300 Wildland Shrubs	Other upper division courses, advisor recommended		
	BOT 3340 Plant Biology OR another approved upper-division botany course			

Highlighted areas indicate courses in molecular or microbiology required.

Table 2: Science Concepts in this Course

COURSE MATERIAL INCLUDED IN THIS COURSE	CONTENT CONCEPTS AND INTEGRATION	INQUIRY AND EXPERIMENTATION
VIROLOGY	CORE INFORMATION -Components, genomes and types of viruses, historical view, connect to molecular biology, replication, transcription, translation, and protein struct. polymerases, RNA splicing, frame shifting, promoters, codons, cistronic, use of antibodies in study of virology,	Cell culturing, observing cytopathic effects, setting up a lab activity using cells and viruses, lab notebook keeping and organization
BACTERIOLOGY	CORE INFORMATION- Components, specific glycoproteins, cell walls, bacteriophages, relate to immune responses, how bacteria are used by molecular biologist.	Bacteria cell culture, use of bacteria for cDNA library, setting up a lab activity using bacteria, lab notebook keeping and organization
PATHOGENESIS	Diseases, relates with virology, bacteriology, parasitology, relating to immune response	Writing case studies, integrating immunology, virology, bacteriology
MOLECULAR BIOLOGY	REVIEW CORE INFORMATION- Components of DNA, RNA, RNA processing, transformations	Immunofluorescence, ELISA, PCR, microscopy, electrophoresis, setting up a lab activity using cells molecular tools, writing case studies,
IMMUNOLOGY	CORE INFORMATION – plants,, invertebrates, vertebrate HUMAN, mechanisms in response to pathogens, knock-out mice	Background on Flow Cytometry, how to read output data, lab notebook keeping, writing case studies

Appendix

Divergent and Creative Thinking Models:

The following are examples of free response prompts that can be used for the trainer and the teacher in the classroom. These prompts will encourage divergent and creative thinking and, when used with the case studies included in this course, will lead to interactive discussions and opportunities for the learner to make connections to the content materials.

The Brainstorm Model

- Brainstorm all of the _____
- Brainstorm as many _____as you can think of.
- How many ways can you come up with to _____?

Viewpoint Model (Human or Animate)

- How would _____look to a(n) _____?
- What would a _____mean from the viewpoint of a(n)_____?
- How would _____view this?

Involvement Model (Personification/Inanimate object brought to life)

- How would you feel if you were _____?
- If you were a _____, what would you see, taste, smell, feel, etc.?

The Forced Association Model

- How is _____like _____?
- Get ideas from _____to improve _____.
- I only know about _____. Explain _____to me.

Reorganization/Synectics Model

- What would happen if _____?
- Suppose _____happened. What would be the consequences?
- What would happen if there were no _____?

Possible Assessment Products:

This list of informal assessment instruments may help the trainer and teacher create alternative assessments in their classrooms.

A Dance/A Letter
A Lesson
Advertisement
Animated Movie
Annotated Bibliography
Art Gallery
Block Picture Story
Bulletin Board
Bumper Sticker
Chart
Choral Reading
Clay Sculpture
Code
Collage
Collection
Comic Strip
Computer Program
Costumes
Crossword Puzzle
Database
Debate
Demonstration
Detailed Illustration
Diorama
Diary
Display
Edibles
Editorial Essay
Etching
Experiment
Fact Tile
Fairy Tale
Family Tree
Fiction Story
Film
Filmstrip
Flip Book
Game
Graph
Hidden Picture
Illustrated Story
Interview
Jingle
Joke Book
Journal
Labeled Diagram
Large Scale Drawing
Learning Center
Letter to the Editor
Map with Legend
Mazes
Mural
Museum Exhibit
Musical Instruments
Needlework
Newspaper Story
Non-Fiction
Oral Defense
Oral Report
Painting Pamphlet
Pantomime
Paper Mache
Petition
Photo Essay
Pictures
Picture Story for Children
Plaster of Paris Model
Play
Poetry
Political Cartoon
Pop-Up Book
Postage Stamp, Commemoratives
Press Conference
Project Cube
Prototype
Puppet
Puppet Show
Puzzle
Rap
Radio Program
Rebus Story
Recipe
Riddle
Role Play
Science Fiction Story
Sculpture
Skit
Slide Show
Slogan
Soliloquy
Song
Sound
Story Telling-Tall Tales
Survey
Tapes–Audio–Video
Television Program
Timeline
Transparencies
Travel Brochure
Venn Diagram
Web Home Page
Working Hypothesis
Write a new law
Video Film

Literature / Language Arts - "Read A Book" List

The following list sets out to describe a selection of books that include immunology among their themes and serve as additional sources for descriptions of diseases, what it is like to live with such diseases, and the battle against them that rages in the body and in the laboratory. This list is by no means exhaustive, but these readable books are a good place to begin your exploration of immunology.

VIROLOGY

West Nile Story

By Dickson Despommier. Apple Trees Productions LLC, 2001, 134 pgs.

In 1999, infection with the West Nile virus killed several people in New York City. Despommier describes these cases and discusses theories on the spreading of disease, including details about specific mosquito species and the migratory patterns of crows. The book helps explain an outbreak of a somewhat mysterious disease and also delves into the manner in which the virus moves through the population.

When Plague Strikes: The Black Death, Smallpox, AIDS

By James Cross Giblin. HarperCollins, 1995, 212 pgs.

Giblin compares the development and impact of the three diseases, starting with the bubonic plague of the fourteenth century. He also compares perceptions and treatment of victims of the diseases, citing similarities between the treatment of people living with AIDS and that of people who had plague and smallpox in previous centuries.

Pox Americana: The Great Smallpox Epidemic of 1775–82

By Elizabeth Anne Fenn. Hill & Wang, 2001, 370 pgs.

Fenn takes a close look at smallpox outbreaks during the Revolutionary War and describes General George Washington's efforts to contain their spread. She also examines the results when these efforts were insufficient, including an analysis of the impact of the disease on the indigenous American population. The book is a case study in early epidemic response techniques.

The Demon in the Freezer

By Richard Preston. Random House, 2002, 240 pgs.

Smallpox, at one time a leading cause of death around the world, was eradicated in the twentieth century. Preston weighs the pros and cons of destroying the remaining stocks of smallpox virus. Some are worried that scientific testing involving the virus will set off a biological-weapons arms race. Others are concerned that if the stocks are destroyed, efforts to find a cure for the disease will be hampered.

The Hot Zone: A Terrifying True Story

By Richard Preston. Anchor, 1995, 442 pgs.

In the winter of 1989 the Ebola virus—airborne, very contagious, and lethal to 90 percent of its victims—made an appearance in a primate laboratory in suburban Washington, D.C. Soldiers and scientists working at an army research facility worked feverishly and in secret to stop the out break. *The Hot Zone* gives a gripping account of the historical occurrence of rare and lethal viruses among humans.

The Great Influenza: The Epic Story of the Deadliest Plague in History

By John M. Barry. Viking, 2004, 546 pgs.

Barry provides a look at a global health crisis. He describes how the frequently mutating influenza virus, coupled with the world's preoccupation with World War I, resulted in the

influenza pandemic of 1918. The response from the scientific community laid the groundwork for the modern flu vaccine.

Flu: The Story of the Great Influenza Pandemic of 1918 and the Search for the Virus That Caused It

By Gina Kolata. Farrar, Straus and Giroux, 1999, 330 pgs.

Kolata explains how the plague spread, examines its possible origins, and describes the search for a strain of the virus once science had advanced, making it possible to better investigate the virus. She also considers whether health officials today are prepared for a similar threat.

The Swine Flu Affair: Decision-Making on a Slippery Disease

By Richard E. Neustadt and Harvey V. Fineberg. University Press of the Pacific, 2005, 189 pgs.

In 1976, the U.S. government launched a nationwide vaccination campaign to respond to what it thought was a resurgence of the flu strain that caused the 1918 epidemic. The side effects patients suffered as a result of taking the vaccine caused some to sue the federal government for millions of dollars and now serve as a cautionary tale as the government addresses the prospect of new microbial threats.

Living with Hepatitis C: A Survivor's Guide

Third revised edition

By Gregory T. Everson and Hedy Weinberg. Hatherleigh Press, 1997, 274 pgs.

Nearly 4 million Americans are estimated to have hepatitis C, a viral infection that targets the liver. Everson and Weinberg provide much information about the disease, including means of transmission, how to avoid it, signs of abnormal liver function, and the latest in research. Their overview of the disease also suggests how to deal with it.

Hepatitis C: The Silent Killer

By Carol Turkington. Contemporary Books, 1998, 188 pgs.

Turkington describes for the general reader the potentially fatal illness hepatitis C, one of several related viral diseases that attack the liver. Doctors are still learning how best to treat the disease, and the author describes the current methods being used.

MOLECULAR BIOLOGY

Timebomb: The Global Epidemic of Multi-Drug-Resistant Tuberculosis

By Lee B. Reichman with Janice Hopkins Tanne. McGraw Hill Companies, 2002, 240 pgs.

Tuberculosis, a terror of the past and once thought to be near eradication, has recently experienced a rebirth of sorts. Reichman suggests that the seeds for a pandemic of multi-drug-resistant tuberculosis are being sown in, among other places, Russia, where the disease is thriving. This epidemic is being worsened by the presence of HIV, which enhances TB's impact. *Timebomb* provides an introduction to the science and history of the disease, as well as a review of current attempts to control its spread.

Conquering Rheumatoid Arthritis: The Latest Breakthroughs and Treatments

By Thomas F. Lee. Prometheus Books, 2001, 255 pgs.

Lee, who has worked on the Human Genome Project, provides the reader with an up-to-date account of research efforts to combat the autoimmune disease, including several molecular and gene-based therapies. He also explores the ethical implications for future human genome research. The book is a detailed look at a relatively prevalent autoimmune disorder.

The Malaria Capers: More Tales of Parasites and People, Research and Reality

By Robert S. Desowitz. W. W. Norton, 1991, 288 pgs.

Desowitz cites the shortcomings of research as he explains worsening health and health systems, especially in poorer countries. He says a vaccine against malaria has not been found because of misrepresentation, poor use of funds, and blatant incompetence.

The Miraculous Fever-Tree: Malaria and the Quest for a Cure That Changed the World

By Fiammetta Rocco. HarperCollins, 2003, 348 pgs.

Rocco follows the intricate history of the tree from which quinine, the most common antidote to malaria, was produced. A native Kenyan who was infected with malaria as a child, she combines her experience with the history of quinine and its development as a medicine.

E. coli 0157: The True Story of a Mother's Battle with a Killer Microbe

By Mary Heersink. New Horizon Press, 1996, 303 pgs.

Heersink describes her son's experience dealing with the severe illness he developed after eating an undercooked hamburger contaminated with drug-resistant E coli 0157. The child beats the sickness, but not before several of his organs are threatened by the bacterium. Heersink, who is not confident about the effectiveness of testing procedures for meat, has testified before Congress about the matte

Parasite Rex: Inside the Bizarre World of Nature's Most Dangerous Creatures

By Carl Zimmer. Free Press, 2000, 298 pgs.

Zimmer reviews the history of the study of parasites, starting with discoveries made in the nineteenth century about their strange life cycles. He discusses how parasites travel from one host to another, and the battle the immune system wages against them, concluding with a look at the role of parasites in human medicine and agriculture.

The Coming Plague: Newly Emerging Diseases in a World Out of Balance

By Laurie Garrett. Farrar, Straus, and Giroux, 1994, 750 pgs.

Garrett documents the history of global epidemics while introducing her reader to new, increasingly threatening diseases. In this telling investigation, she explores the effects of social development on ecosystems, suggesting that these disruptions may be the cause for the spread and creation of infectious diseases. Garrett concludes with a sobering proposal, calling for the creation of a global early warning system that would bolster detection in an effort to prevent the spread of infectious diseases.

IMMUNOLOGY

Fatal Sequence: The Killer Within

This novel by Kevin Tracey, a neurosurgeon and immunologist, describes the case of a 1-year-old burn victim, Janice.

How the Immune System Works

By Lauren Sompayrac. Blackwell Publishers, 2003, 100 pgs. Second edition

In this overview of the body's immune functions, Sompayrac avoids the intricacies of the immune system and the exceptional phenomena that can sometimes occur and instead focuses on the big picture, providing the lay reader with a basic foundation in immunology.

The Eradication of Smallpox: Edward Jenner and the First and Only Eradication of a Human Infectious Disease

By Hervé Bazin. Academic Press, 2000, 246 pgs.

Bazin describes the history of smallpox, a disease that ravaged the world in the 1700s and 1800s, and examines the life of Edward Jenner, who first proposed "vaccination" with cowpox. He follows the fight against smallpox through its 1979 eradication and covers the debate about whether to maintain the stocks of smallpox virus in the United States and Russia.

Plague Time: How Stealth Infections Cause Cancers, Heart Disease, and Other Deadly Ailments

By Paul W. Ewald. The Free Press, a division of Simon and Schuster, 2000, 288 pgs.

Ewald asserts that a wide variety of ailments, including breast cancer, diabetes, and schizophrenia, are caused, at least in part, by microbes. Further, he says that there is strong resistance to this idea within the medical community and that it is preventing progress in efforts to combat these diseases. The book highlights the potential importance of a better understanding of immunology as a field.

My Adventure with Lupus: Living with a Chronic Illness

By Robert L. Yocum. Griffin Publishing Group, 1995, 172 pgs.

Yocum recounts his own experience with lupus. Since there is no cure for the disease, which inflames connective tissue in the body, living with lupus is a continuous battle. Yocum describes how lupus develops and suggests how best to cope with it.

SARS: A Case Study in Emerging Infections

Edited by Angela R. McLean, Robert M. May, John Pattison, and Robin A. Weiss. Oxford University Press, 2005, 133 pgs.

The editors use the SARS outbreak of 2002 as a model for examining worldwide response capabilities in the face of infectious-disease epidemics. The authors look at issues of trans-global travel and isolation of infected people in an increasingly integrated world. Highlighting many global public health issues, the book offers a model for addressing future problems.

Multiple Sclerosis: The History of a Disease

By T. Jock Murray. Demos Medical Publishing, 2005, 580 pgs.

Murray's book outlining the history of multiple sclerosis is written for laypeople. It describes early cases from the days before MS was a defined disease and chronicles the development of the various treatments in use today. The book provides an overview of the disease and its past.

Commotion in the Blood: Life, Death, and the Immune System

By Stephen S. Hall. Henry Holt, 1997, 544 pgs.

Hall traces the history of immune therapies for cancer, describing complex immunological processes in simpler terms for the lay reader. He is critical of making too much of research success and does not see a definite cure on the horizon.

At War Within: The Double-Edged Sword of Immunity

By William R. Clark. Oxford University Press, 1995, 276 pgs.

Highlighting several threats to public health, including tuberculosis, AIDS, and allergies, Clark examines each in detail and describes the immune response. Among the varied reactions are those that end up harming the body: so-called autoimmune responses. Clark also provides a basic history of immunology.

Tao of Immunology: A Revolutionary New Understanding of Our Body's Defenses

By Marc Lappé. Plenum Press, 1997, 317 pgs.

Lappé, a toxicologist, challenges the pop-culture approach of taking supplements to bolster the immune system, arguing that too strong an immune system can be harmful. He cites the numerous diseases in which the immune system attacks the body it is meant to protect. Essentially the book argues that artificially manipulating the immune system does more damage than good.

Inside AIDS: HIV Attacks the Immune System

By Conrad J. Storad. Lerner Publications Co., 1998, 96 pgs.

The book (current through 1997) describes how HIV is transmitted, how it infiltrates the human immune system, and its subsequent effect on the body. Storad focuses on the scientific and microbial level rather than the public health level, though he does discuss methods for preventing the spread of the disease. Storad also offers a history of AIDS, beginning in the early 1980s.

Internet Resources

These are websites that may be of interest for further research and information about topics that may come up in the course. I have compiled some of these from Biology by Campbell and on-line search engines.

General Resources

A comprehensive site covering signal transduction pathways, offered from *Science*, includes articles, reviews and experimental protocols. http://stke.sciencemag.org/

A National Library of Medicine dataset of more than 9 million publications is the world's most comprehensive bibliographic database for biological and biomedical literatures.

American Academy of Family Physicians: www.aafp.org

American Association of Immunologists http://www.aai.org

American Association of Immunologists: www.aai.org

American Association of Veterinary Immunologists: cvm.missouri.edu/aavi

American Cancer Society, updated regularly http://www.cancer.org/docroot/home/index.asp

American Lung Association: www.lungusa.org

American Society for Histocompatibility & Immunogenetics (A National Research Council): www.ashi-hla.org

Arthritis Foundation: www.arthritis.org

Associated with the Kuby Immunology Text www.whfreeman.com/kuby

Association of Medical Laboratory Immunologists: www.amli.org

Association of Medical School Microbiology and Immunology Chairs: www.amsmic.org

British Aerobiology Federation: www.pollenuk.co.uk/baf/BAF.html

British Society for Immunology: immunology.org Department of Health (UK): www.dh.gov.uk/Home/fs/en

Canadian Institutes of Health Research—Institute of Infection and Immunity: www.cihr-irsc.gc.ca/e/13533.html

Canadian Society for Immunology: www.csi.ucalgary.ca/

Cells Alive sub-site has time-lapse video of cytotoxic T lymphocytes (CTLs) recognizing, attacking, and killing a much larger influenza-infected target. http://www.cellsalive.com/ctl.htm

Centers for Disease Control and Prevention: www.cdc.gov

Clinical Immunology Society: www.clinimmsoc.org

Clinical Ligand Assay Society: www.clas.org

Comprehensive information about many types of cancer and good source of information about cancer research and advances in cancer therapy http://www.oncolink.upenn.edu/

Department of Health and Human Services: www.hhs.gov

eMedicine.com: www.emedicine.com

Environmental Protection Agency: www.epa.gov

Every Child by Two offers useful information on childhood vaccination, including recommended immunization schedules http://www.ecbt.org/

Federation of American Societies for Experimental Biology: www.faseb.org

Free software for visualizing molecular structures that can be run on Windows-based, Macintosh, or Unix PCs, to view 3-D structures of many types of molecules, including proteins and nucleic acids. http://www.umass.edu/microbio/rasmol/

Health Canada: www.hc-sc.gc.ca

Health Protection Agency: www.hpa.org.uk

Home page for the National Institute for Arthritis and Musculoskeletal and skin Diseases http://www.nih.gov/niams/

Home page for the National Institute for Diabetes and Digestive and Kidney Diseases http://www.niddk.nih.gov/

Home page for the New England Biolabs, a molecular biology company, gives useful information concerning restriction enzymes http://www.neb.com/

Home page for the World Health Organization, international organization that monitors infectious diseases worldwide http://www.who.int/

Home page of Global Alliance for Vaccines and Immunization, a source of information about vaccines in developing countries and worldwide efforts at disease eradication http://www.VaccineAlliance.org/

http://www.ultranet.com~jkimball/BiologyPages/B/B_andTcells.html

Images of major inflammatory cells involved in acute and chronic inflammation, as well as example of specific inflammatory diseases.http://cpmcnet.columbia.edu/dept/curric-pathology/pathology/pathology/pathoatlas/GP_I_menu.html

Immune Deficiency Foundation: www.primaryimmune.org

ImmuneDisease.com: www.immunedisease.com

Immunization Action Coalition: www.immunize.org

Information about the global AIDS epidemic http://hivinsite.ucsf.edu/

Jeffrey Modell Foundation: www.jmfworld.com

Journal of Immunology: www.jimmunol.org

KidsHealth: www.kidshealth.org

MayoClinic.com: www.mayoclinic.com

Medinfo (UK): www.medinfo.co.uk

Molecular Biology web site, updated regularly

http://www.public.iastate.edu/~pedro/research_tools.html

Molecular Immunology Foundation: www.mifoundation.org

National Biological Information Infrastructure: www.nbii.gov

National Center for Biotechnology Information: www.ncbi.nlm.nih.gov

National Coalition for Adult Immunization: www.nfid.org/ncai

National Foundation for Infectious Diseases: www.nfid.org

National Heart, Lung and Blood Institute: www.nhlbi.nih.gov

National Human Genome Research Institute: www.genome.gov

National Institute of Arthritis and Musculoskeletal and Skin Diseases: www.niams.nih.gov

National Institute of Neurological Disorders and Stroke: www.ninds.nih.gov

National Library of Medicine: www.nlm.nih.gov

National Network for Immunization Information: www.immunizationinfo.org

National Primary Immunodeficiency Resource Center: npi.jmfworld.org

Primary Immunodeficiency Association: www.pia.org.uk

Society for Mucosal Immunology: www.socmucimm.org

The Animal Diversity Web (Taylor *et al.*) is based at the University of Michigan, comprehensive database of animal classification as well as a source of information on animal natural history and distribution. http://animaldiversity.ummz.umich.edu/site/index.html

The Antibodies: Structure and Sequence Web site summarizes useful information on antibody structure and sequence. http://www.bioinf.org.uk/abs/

The Global Fund: www.theglobalfund.org/en

The National Center for Infectious Diseases home page. Great site for monitoring emerging diseases and many other links http://www.cdc.gov/ncidod/

The Online Mendelian Inheritance in Man Web site contains a sub-site that features 10 different inherited diseases associated with defects in the TCR complex or associated proteins. http://www.ncbi.nlm.nih.gov/Omim/

The SCID home page http://www.scid.net/

The site for the Lupus Foundation of America http://www.lupus.org/index.html

The University of Pittsburgh Center for Biosecurity Web site provides information about select agents and emerging diseases that may pose a security threat http://www.upmc-biosecurity.org/

These sub-sites of John Kimball's' Biology Pages Web site provide a clear introduction to T-cell biology and a good basic discussion of apoptosis

http://www.ultranet.com/~jkimball/BiologyPages/A/Apoptosis.html

United Scleroderma Foundation: www.scleroderma.com

World Health Organization: www.who.int/en

AIDS

See HIV.

Allergies and Asthma

Allergy & Asthma Network Mothers of Asthmatics: www.aanma.org/headquarters

Allergy Society of South Africa: www.allergysa.org

American Academy of Allergy, Asthma & Immunology: www.aaaai.org

American Lung Association: www.asthmacontrol.com

Asthma and Allergy Foundation of America: www.aafa.org

Asthma UK: www.asthma.org.uk

European Academy of Allergology and Clinical Immunology: www.eaaci.net/site/homepage.php

European Federation of Allergy and Airway Diseases: www.efanet.org

Global Initiative for Asthma: www.ginasthma.com

Hispanic American Allergy and Immunology Association: www.haama.org

Joint Council of Allergy, Asthma and Immunology: www.jcaai.org

Lung Association (Howe): www.lung.ca/asthma

My Asthma: www.myasthma.com

National Asthma Council (Australia): www.nationalasthma.org.au

National Institute of Allergy and Infectious Diseases: www.niaid.nih.gov

Texas Allergy, Asthma and Immunology Society: www.taai.org

World Allergy Organization: www.worldallergy.org

Amyotrophic lateral sclerosis (American Association for the Advancement of Science)

ALS Association: www.alsa.org/

ALS Society of Canada: www.als.ca/

Ataxia-Telangiectasia

A-T Children's Project: www.atcp.org

Ataxia-Telangiectasia Society (UK): www.atsociety.org.uk

Autoimmune diseases

American Autoimmune Related Diseases Association: www.aarda.org

Bird Influenza

Agriculture Department—Animal Production and Health Division (Italy):

www.fao.org/ag/againfo/subjects/en/health/ diseases-cards/special_avian.html

Prevention of Avian Influenza (Hong Kong): www.info.gov.hk/info/flu/eng

Cancer

American Association for Cancer Research: www.aacr.org

American Cancer Society: www.cancer.org

Cancer Research and Prevention Foundation: www.preventcancer.org

Cancer Research UK: www.cancerresearchuk.org

Dana-Farber Cancer Institute: www.dfci.harvard.edu

Memorial Sloan-Kettering Cancer Center: www.mskcc.org/mskcc/html/44.cfm

National Cancer Institute: www.nci.nih.gov

Chronic granulomatous disease

Immune Deficiency Foundation: www.primaryimmune.org

Crohn's disease

Crohn's & Colitis Foundation of America: www.ccfa.org

Diabetes

American Diabetes Association: www.diabetes.org

Canadian Diabetes Association: www.diabetes.ca

Children with Diabetes: www.childrenwithdiabetes.com

Joslin Diabetes Center: www.joslin.org

Juvenile Diabetes Research Foundation International: www.jdf.org

Graft-versus-host disease

National Marrow Donor Program: www.marrow.org

Hepatitis A

Hepatitis Foundation International: www.hepfi.org

Hepatitis Neighborhood: www.hepatitisneighborhood.com

Hepatitis.org: www.hepatitis.org

Hepatitis Magazine: www.hepatitismag.com

HepNet (Canada): www.hepnet.com

Hepatitis C

See Hepatitis A.

Hepatitis C Information Center: www.hepatitis-central.com

Herpes

American Herpes Foundation: www.herpes-foundation.org

Herpes.com: www.herpes.com

HIV

Aegis-AIDS Education Global Information System: www.aegis.com

AIDSmap: www.aidsmap.com

HIV InSite: hivinsite.ucsf.edu

Joint United Nations Programme on HIV/AIDS: www.unaids.org/en/default.asp

The Body: www.thebody.com

Lyme disease

Lyme Disease Association: www.lymediseaseassociation.org

Lyme Disease Foundation: www.lyme.org

Lyme Info: www.lymeinfo.net

LymeNet: www.lymenet.org

Malaria

Africa Fighting Malaria: www.fightingmalaria.org

Malaria Foundation International: www.malaria.org

Malaria Journal: www.malariajournal.com

Malaria Vaccine Initiative: www.malariavaccine.org

Measles

Measles Initiative: www.measlesinitiative.org

Multiple sclerosis

All About Multiple Sclerosis: www.mult-sclerosis.org

Consortium of Multiple Sclerosis Centers: www.mscare.org

MS Australia: www.msaustralia.org.au

Multiple Sclerosis Association of America: www.msaa.com

Multiple Sclerosis Foundation: www.msfacts.org

Multiple Sclerosis International Federation: www.msif.org

Multiple Sclerosis Resource Centre (UK): www.msrc.co.uk

Multiple Sclerosis Society of Canada: www.mssociety.ca

National Multiple Sclerosis Society: www.nmss.org

Rabies

Rabies.com: www.rabies.com

Respiratory syncytial virus (RSV)

RSV Info Center: www.rsvinfo.com

Rheumatoid arthritis

American College of Rheumatology: www.rheumatology.org

Arthritis Foundation: www.arthritis.org

Rotavirus

Rotavirus Vaccine Program: www.rotavirusvaccine.org

SARS

SARSReference: www.sarsreference.com

Severe combined immunodeficiency disease (SCID) SCID.NET: www.scid.net

Sjögren's syndrome

Sjögren's Syndrome Foundation, Inc.: www.sjogrens.com

Systemic lupus erythematosus

Alliance for Lupus Research: www.lupusresearch.org

Lupus Foundation of America: www.lupus.org/

Toxic shock syndrome

Toxic Shock Syndrome Information Service: www.toxicshock.com

Tuberculosis

Tuberculosis.net: www.tuberculosis.net

Information on diseases not listed can usually be found on the general information or government resources Web sites.

Key Immunology Terms:

By From the Dana Sourcebook of Immunology, free access on-line (The Dana Foundation, 2005).

active immunity: usually long-lasting immunity that is acquired through the production of *antibodies* and memory T cells within the organism in response to the presence of *antigens*.

adaptive immune system: also called the acquired immune system, this component of the immune system comprises *white blood cells*, particularly *lymphocytes.* When it is presented with a new microbe or vaccine, it may take days or weeks to respond or adapt, but the resultant improved immune readiness, or ~~"~~memory," is sustained for long periods (years).

adenosine deaminase (ADA): an enzyme found in mammalian tissues that is capable of catalyzing the process in which adenosine is split into inosine and ammonia. A deficiency can cause problems with metabolic reactions in cells, which leads to the destruction of *B* and *T cells*. ADA deficiency can lead to one form of severe combined immunodeficiency disease.

allergy: a misguided reaction by the immune system to harmless foreign substances.

antibody: a protein on the surface of *B cells* that is also secreted in large amounts into the blood or lymph in response to an *antigen*, a component within an invader such as a bacterium, virus, parasite, or transplanted organ. Antibodies neutralize the antigen, and thereby the invader, by binding to it, often directing it toward a macrophage for destruction. Also called an immunoglobulin.

antigen: a foreign substance (usually a protein or carbohydrate) capable of triggering an immune response in an organism.

antiretroviral drugs: drugs that act against *retroviruses* (such as HIV).

autoimmune disorders: conditions in which the body's own immune system acts against it.

autoreactive: describes immune cells that mount a response against the body's own cells or tissues.

B cell: a type of *lymphocyte* that produces *antibodies*, which bind to free-floating microbes circulating in the blood so that they cannot infect other cells.

biochemicals: chemicals produced within living organisms. Many coordinate to fight off invasion in an immune response.

biological barriers: the body's first layer of protection against harmful microbes; skin is a prime example.

blood-brain barrier: a tight layer of cells and tissue that separates the brain from the rest of the body; a physical roadblock that normally keeps immune cells outside the brain.

blood-forming stem cells: immature cells in the bone marrow that multiply extensively and produce *red* and *white blood cells* and platelets.

CD4+ helper T cells: *T cells* with CD4 receptors that respond to *antigens* on the surface of specific molecules by secreting a certain type of cytokine that stimulates *B cells* and killer *T cells.* Helper T cells are infected and killed by *HIV;* people who develop *AIDS* have no more than one-fifth the normal number of helper T cells.

central nervous system: the brain and spinal cord, to which sensory impulses are transmitted and from which motor impulses emanate. The central nervous system supervises and coordinates the activity of the entire nervous system and interacts with the immune system.

clones: copies that viruses make of themselves.

cytokine: a class of substance secreted by cells of the immune system to regulate immune cells.

dendritic cell: an *antigen*-presenting immune cell that initiates the immune response by activating *lymphocytes* and stimulating the secretion of *cytokines.* Dendritic cells also prevent autoimmune reactions by instructing the T lymphocytes to be silent or tolerant to the body itself.

DNA (deoxyribonucleic acid): nucleic acid that carries the cell's genetic information and is capable of self-*replication* and the synthesis of RNA.

DNA vaccine: vaccines that often use ~~n~~aked" *DNA* (DNA not associated with a cell or a virus) with instructions for making protective *antigens*. When injected, the DNA is taken in by other cells, which then produce protective antigens.

E. coli: a bacterium that is used in public health as an indicator of fecal pollution (as of water or food) and in medicine and genetics as a research organism. *E. coli* occurs in various strains that may live as harmless inhabitants of the human lower intestine or may produce a toxin causing intestinal illness.

enzymes: complex proteins produced by living cells and that catalyze specific reactions from *biochemicals.*

epidemic: an outbreak of disease that simultaneously affects an atypically large number of individuals within a population, community, or region.

estrogen: a *steroid* hormone produced chiefly by the ovaries, responsible for promoting development and maintenance of female secondary sex characteristics. Estrogen may play a role in certain immune system diseases.

genetic engineering: deliberate alteration of genetic material by intervention in genetic processes.

granulocyte: a type of *phagocyte* with cytoplasm that contains grain like particles.

highly active *antiretroviral therapy* (HAART): a treatment to combat AIDS using several antiretroviral drugs at the same time.

histamine: a compound found in mammalian tissues that causes stretching of capillaries, contraction of smooth muscle, and stimulation of gastric acid secretions; released during allergic reactions.

immune deficiency diseases (IDDs): diseases that result when one or more parts of the immune system are missing or defective.

immunoglobulin E (IgE): a class of *antibodies* that function in allergic reactions.

immunosuppressive: describes a treatment that suppresses natural immune responses—for example, chemotherapy for cancer.

inactivated vaccines: *vaccines* made by growing and purifying large numbers of the target organism in the laboratory and then killing them with heat, radiation, or chemicals. The immune system reacts to the dead microorganisms, producing immunity.

inflammation: a buildup of fluid and cells that occurs as the immune system fights a hostile invader.

innate immune system: component of the immune system that consists of a set of genetically encoded responses to pathogens and does not change or adapt during the lifetime of the organism. Innate immunity involves quickly mobilized defenses triggered by receptors that recognize a broad spectrum of microbes; in contrast to adaptive immunity, it does not acquire memory for an improved response during a second exposure to infection.

killer T cell: a type of *lymphocyte* that directly attacks and kills infected cells or other targets, including tumor cells and even one's own tissues. Killer cells are generated by the coordinated action of *dendritic cells* and *CD4+ helper T cells*.

knockout: term used in genetic engineering when a specific gene is deliberately removed in order to create an organism unable to carry out the functions the gene codes for; knockouts are used by immunologists to determine the functions of specific genes that encode immune proteins.

latency: the state or period in which a virus has invaded a host but is not actively multiplying, and during which symptoms of the infection are not seen. "Microbial latency" means the microbe is not multiplying, as occurs in some cells in HIV infection, while "clinical latency" means that the patient does not have symptoms of disease even though the virus is multiplying and damaging the immune system. In HIV, clinical latency precedes the AIDS stage.

lymph nodes: small, rounded structures in the lymphatic system that contain disease-fighting *white blood cells*, especially *lymphocytes*, and filter out harmful microbes and toxins. Lymph nodes may become enlarged when they are actively fighting infection.

lymphocyte: a type *of white blood cell* involved in the human body's immune system, of which there are two broad categories, *T cells* and *B cells*. Lymphocytes are an integral part of the body's defenses because they are highly specific for antigens associated with microbes, tumor cells, transplants, allergies, and tissues attacked in autoimmune diseases. The immune system comprises clones of lymphocytes, each with a single specificity, and exposure to antigens leads to clonal expansion, the acquisition of helper and killer functions, and formation of immune memory.

lysozyme: an *enzyme* in saliva and tears that destroys bacteria.

macrophages: large *phagocyte* cells that remove harmful microbes from the body.

major histocompatibility complex (MHC) molecules: a group of molecules that help the immune system distinguish between harmful and safe foreign substances in the body. Recent research suggests some classes of MHC molecules also play an essential role in brain function.

mast cells: large cells, found in connective tissues, that mediate allergic reactions. Mast cells play an important protective role in wound healing and defend against *pathogens*.

memory B and T cells: *B* and *T cells* that remain in the body after the completion of an immune response to ward off future attacks by the same microbe. Memory is imparted by the increased size in the *antigen*-specific B or T cell clone, as well as improved function of individual cells within the clone.

microglia: specialized immune cells, related to macrophages, that protect the *central nervous system.*

molecular mimicry: an occurrence in many *autoimmune disorders* in which a microbe carries *antigens* that resemble those on a particular organ, causing the immune system to attack the body.

monoclonal antibodies: *antibodies* derived from a single cell and used against a specific *antigen* such as a cancer cell. Rituxan and Herceptin are monoclonal antibodies used in the treatment of lymphoma and breast cancer, respectively.

mutation: a process in which a microbe or organism undergoes a permanent change in hereditary material. When viruses or bacteria mutate they are no longer recognized by the immune system and become resistant to previously administered *vaccines* and drugs.

myelin: a white, fatty material that sheathes nerves and enhances the transmission of signals between the brain and the body. In *multiple sclerosis*, an *autoimmune disorder,* immune cells attack myelin, affecting the transmission of nerve signals.

pandemic: an outbreak of disease occurring over a wide geographical area and affecting an exceptionally high proportion of the population.

passive immunity: immunity acquired by the transfer of *antibodies* (as by injection of serum from an individual with active immunity).

pathogen: a specific causative agent of disease, such as a bacterium or a virus.

penicillin: a mixture of nontoxic antibiotics produced by mold and used regularly to treat harmful bacteria.

phagocyte: a cell such as a *white blood cell* that engulfs and consumes foreign material, such as microorganisms.

plasma cell: an *antibody*-producing l*ymphocyte* derived from a *B cell* upon reaction with a specific *antigen*.

protease: an *enzyme* that catalyzes the splitting of proteins into smaller molecules. To treat AIDS, scientists have designed drugs that interfere with protease made by the HIV virus, which is essential to its replication.

reassortment: the constant state of flux and rearrangement seen in the genes of viruses.

red blood cells: any of the hemoglobin-containing cells that carry oxygen to the tissues and are responsible for the red color of vertebrate blood..

regulatory T cells (Treg cells): special *T cells* that regulate or suppress immune responses, preventing autoimmunity for example.

replication: process by which an organism produces a copy of itself—for example, the way microbes reproduce.

respiratory syncytial virus (RSV): a virus that forms masses, or syncytia, in tissue culture and that is responsible for severe respiratory diseases.

retrovirus: a type of *RNA* virus (such as HIV) that reproduces by transcribing itself into *DNA* (using *reverse transcriptase*). The resultant DNA inserts itself into a cell's DNA and is reproduced by the cell.

reverse transcriptase (RT): an *enzyme* that catalyzes the formation of *DNA* using *RNA* as a template.

RNA (ribonucleic acid): a group of molecules similar in structure to a single strand of *DNA*. The function of RNA is to carry the information from the DNA in the cell's nucleus into the body of the cell to assemble proteins.

rotavirus: a *retrovirus* with a double-layer protein shell and a wheel-like appearance. Rotaviruses cause diarrhea, especially in infants.

stem cell transplants: a kind of *passive immune therapy* that transfers cells instead of antibodies. Stem cells have the capacity to give rise to all elements of the immune system, such as many types of *lymphocytes* and *phagocytes*.

steroids: a large family of chemical substances, comprising many hormones, body constituents, and drugs; they are often *immunosuppressive*.

subunit vaccines: *vaccines* that contain only a part of the target microorganism.

synapses: specialized junctions at which cells of the nervous system signal to one another and to nonneuronal cells, such as those of muscles and glands.

T cell: a type of lymphocyte that possesses highly specific cell-surface antigen receptors; types include *CD4+ helper T cells, regulatory T cells*, *and killer T cells*.

tolerance: the capacity of the body to become less responsive to a substance or a physiological insult. Tolerance to components of the self prevents or suppresses autoimmunity.

toxoid vaccine: an inactivated and weakened version of the disease-causing toxin a microbe produces; it is still capable of inducing the formation of *antibodies* when injected.

transgenic technology: technology used to deliberately alter the genome of an organism by the transfer of a gene or genes from another species or breed.

two-photon microscopy: an imaging technique using high-powered laser microscopes to examine immune response in the nervous system.

vaccine: killed microorganisms, weakened living organisms, fully virulent living organisms, or subunit proteins of a microbe, administered to produce or artificially increase immunity to a particular disease.

vector vaccines: *vaccines* made by inserting protective *antigen* genes into harmless bacteria or viruses (vectors). As the vectors multiply in the body, they expose the immune system to protective antigens, stimulating *active immunity* against the harmful organism.

white blood cells: any of the blood cells that are colorless, lack hemoglobin, and contain a nucleus. They include the *lymphocytes, dendritic cells*, monocytes, neutrophils, eosinophils, and basophiles; also called leukocytes

Selected Diseases Related to the Immune System:

By From the Dana Sourcebook of Immunology, free on-line access

acquired immunodeficiency syndrome (AIDS): a disease of the immune system that is caused by infection with *human immunodeficiency virus (HIV),* commonly transmitted in blood and body secretions such as semen. People with AIDS are highly vulnerable to life-threatening infections.

amyotrophic lateral sclerosis (ALS): a rare, fatal, progressive degenerative disease that affects pyramidal motor neurons (responsible for all voluntary movements), characterized especially by increasing and spreading muscular weakness. ALS, also called Lou Gehrig's disease, usually appears in middle age.

asthma: a condition often of allergic origin that is marked by labored breathing accompanied by wheezing and a sense of constriction in the chest, and often by attacks of coughing or gasping.

athlete's foot: a foot rash caused by a fungus.

autism: a mental disorder that appears at a young age and is characterized by self-absorption, inability to interact socially, repetitive behavior, and language difficulty.

bird flu: (also avian influenza); any of several highly variable diseases of domestic and wild birds, caused by viruses and usually characterized by respiratory symptoms.

cancer: a malignant tumor of potentially unlimited growth that expands locally by invasion and can spread throughout the body in a process called metastasis.

chicken pox: a contagious disease, usually in children, caused by a virus and marked by low-grade fever and the formation of blister like spots; also called varicella.

chronic lung disease: one of a group of recurrent diseases of the lower respiratory tract.

coronary artery disease: a condition that reduces the blood flow through the coronary arteries to the heart muscle; also called coronary disease, coronary heart disease.

cowpox: a mild disease in cows that is caused by a poxvirus and that protects against a related virus, s*mallpox*, when injected as a vaccine into humans.

Crohn's disease: a disease of the small intestine that often spreads to the colon, part of the large intestine. Crohn's disease is characterized by diarrhea, cramping, and loss of appetite and weight, with local abscesses and scarring.

diabetes: abnormal condition characterized by a lack of insulin or a resistance to insulin. The excretion of excessive amounts of urine is an early symptom.

gonorrhea: a contagious *inflammation* of the genital mucous membrane caused by the gonococcus bacterium.

hantavirus: any of a group of closely related viruses that cause a respiratory disease or fever accompanied by leakage of plasma and red blood cells through the lining of blood vessels and by the death of kidney tissue.

hepatitis A: a usually benign *inflammation* of the liver, caused by an *RNA*-containing virus that does not persist in the blood serum. Hepatitis A is transmitted especially in food and water contaminated with fecal matter.

hepatitis C: a disease that inflames the liver, caused by a single-stranded *RNA*-containing virus and usually transmitted by exposure to blood or blood products. Hepatitis C leads eventually to scarring in the liver and liver cancer.

herpes: inflammatory diseases of the skin caused by one of several herpes viruses and characterized by clusters of blisters.

human immunodeficiency virus (HIV): a *retrovirus* that infects and destroys *CD4+ helper T ce*lls in the immune system, causing the marked reduction in their number and thus lowering resistance to life-threatening infections that characterize *AIDS*.

Lyme disease: an acute inflammatory disease that is usually characterized initially by skin lesions, fatigue, fever, and chills, and if left untreated may later manifest itself in cardiac and neurological disorders, joint pain, and arthritis. Lyme disease is caused by a bacterium transmitted by the bite of a tick.

malaria: a disease caused by the presence of the organism *Plasmodium* in human or other vertebrate red blood cells, usually transmitted to humans by the bite of an infected female mosquito that previously sucked blood from a person with malaria. The disease is characterized by episodic severe chills, high fever, and exhaustion; it can be fatal.

measles: a contagious viral disease that begins with conjunctivitis (pinkeye), coughing, and spots in the mouth. Measles is marked by the appearance on the third or fourth day of an eruption of distinct red circular bumps that gradually diminish after another four days, and much less frequently by serious *inflammation* of the brain (encephalitis).

mononucleosis: an infectious disease associated with the Epstein-Barr virus; characterized by fever, swelling of lymph nodes, and an abnormal increase of single-nucleus white blood cells (accounting for the prefix ~~–m~~ono-").

multiple sclerosis: a disease marked by patches of hardened tissue in the brain and on the spinal cord that causes the destruction of the nerves' protective myelin sheath. Partial or incomplete paralysis and jerking muscle tremor can result.

mumps: a contagious disease caused by a paramyxovirus and marked by fever and swelling, especially of the parotid glands.

Parkinson's disease: a chronic, progressive nervous system disease that usually appears later in life and is linked to decreased dopamine production in the substantia nigra brain region. Parkinson's disease is marked by tremor and weakness of resting muscles and by a shuffling gait.

primary immune deficiency (PID) diseases: diseases caused by an inherited genetic defect that interferes with the immune system's normal development.

rabies: highly fatal infectious disease transmitted by the bite of infected animals, including dogs, cats, foxes, raccoons, and bats, and caused by a virus in the central nervous system and the salivary glands. The symptoms are characteristic of a profound disturbance of the nervous system: excitement, aggressiveness, and madness, followed by paralysis and death.

rheumatoid arthritis: a usually chronic *autoimmune disorder* characterized especially by pain, stiffness, *inflammation,* swelling, and sometimes destruction of joints.

rubella (German measles): a contagious viral disease that is milder than typical *measles* but is damaging to the fetus when it occurs early in pregnancy.

scleroderma: a disease, usually slow to progress, characterized by fibrous connective tissue in the skin and, frequently, internal organs. Symptoms include sensitivity to cold and tightening and thickening skin.

selective immunoglobulin A deficiency (selective IgA deficiency): a condition that results when B lymphocytes, a type of *white blood cell*, do not mature properly and fail to produce immunoglobulin A *antibodies* at the levels required. People with the condition can be healthy or suffer recurrent lung, ear, and sinus infections.

severe acute respiratory syndrome (SARS): a severe form of pneumonia, caused by a virus, that appeared in outbreaks in 2003.

severe combined immunodeficiency disease (SCID): a rare, congenital disorder of the immune system characterized by the inability of *B cells* and *T cells* to produce a normal complement of antibodies; usually results in early death.

smallpox: a contagious disease characterized by fever, pus-filled bumps on the skin, separation of dead tissue, and scar formation; caused by a poxvirus that is believed to exist now only in lab cultures.

systemic lupus erythematosus: an inflammatory autoimmune disease of unknown cause that leads to the production of autoantibodies, or antibodies that recognize and attack the body's own components. Occurring chiefly in women, it is characterized especially by fever, skin rash, and arthritis; often by anemia, in which red blood cells are destroyed; by small hemorrhages in the skin and mucous membranes; by *inflammation* of the pericardium (the sac around the heart); and in serious cases by involvement of the kidneys and central nervous system.

toxic shock syndrome: a sometimes fatal disease characterized by fever, nausea, diarrhea, skin redness, and shock. The syndrome is associated especially with the presence of the bacterium *Staphylococcus aureus,* which produces a toxic protein, and occurs especially in menstruating females using tampons.

tuberculosis: a highly variable disease caused by the tubercle bacterium or, rarely in the United States, a related bacterium. Usually transmitted by inhalation of airborne bacteria, it affects the lungs but may spread to other areas, especially the brain, from local lesions or by way of the lymph or blood vessels. Tuberculosis is characterized by fever, coughing, difficulty in breathing, and *inflammation*.

turista: also called traveler's diarrhea; intestinal sickness typically caused by ingesting microorganisms such as *E. Coli.*

West Nile: disease caused by a virus spread chiefly by mosquitoes that causes an illness marked by fever, headache, muscle ache, skin rash, and sometimes encephalitis or meningitis.

whooping cough: also called pertussis; an infectious disease especially of children that is caused by a bacterium and is marked by a convulsive, spasmodic cough, sometimes followed by a shrill intake of breath. Whooping cough is one of the targets of the diphtheria-pertussis-tetanus (DPT) vaccine.

Alignment with Utah State Performance Benchmarks with Standards:

The following are benchmarks, standards and objectives set out by the Utah State Board of Education for secondary biology teachers. This Core was designed using the American Association for the Advancement of Science's *Project_2061: Benchmarks For Science Literacy* and the National Academy of Science's *National Science Education Standards* as guides to determine appropriate content and skills. The Utah Science Home Page at http://www.usoe.k12.ut.us/curr/science is an ongoing report of resources available and aligned to the Biology Core Curriculum.

* This professional development curriculum encompasses many of the items delineated from the State Science Core but at a molecular and microbial level. When possible, the trainer should refer to the State Core Standards and Objectives as he/she moves through the course with the teachers. Emphasis in molecular biology and microbiology will not add more to the curriculum. Instead, whenever the core refers to —organism" the trainer should point out that viruses, bacteria and host cells of pathogens can just as easily be used to deal with state mandated objectives.

Benchmark 1: Ecosystems are shaped by interactions among living organisms and their physical environment. Ecosystems change constantly, either staying in a state of dynamic balance or shifting to a new state of balance. Matter cycles in ecosystems, and energy flows from outside sources through the system. Humans are part of ecosystems and can deliberately or inadvertently alter an ecosystem.

STANDARD I: Students will understand that living organisms interact with one another and their environment.

Objective 1: Summarize how energy flows through an ecosystem.

a. Arrange components of a food chain according to energy flow.

b. Compare the quantity of energy in the steps of an energy pyramid.

c. Describe strategies used by organisms to balance the energy expended to obtain food to the energy gained from the food.

d. Compare the relative energy output expended by an organism in obtaining food to the energy gained from the food.

e. Research food production in various parts of the world.

Objective 2: Explain relationships between matter cycles and organisms.

a. Use diagrams to trace the movement of matter through a in a variety of biological communities and ecosystems.

b. Explain how water is a limiting factor in various ecosystems.

c. Distinguish between inference and evidence in a newspaper, magazine, journal, or Internet article that addresses an issue related to human impact on cycles of matter in an ecosystem and determine the bias in the article.

d. Evaluate the impact of personal choices in relation to the cycling of matter within an ecosystem.

Objective 3: Describe how interactions among organisms and their environment help shape ecosystems.

a. Categorize relationships among living things according to predator-prey, competition, and symbiosis.

b. Formulate and test a hypothesis specific to the effect of changing one variable upon another in a small ecosystem.

c. Use data to interpret interactions among biotic and abiotic factors (e.g., pH, temperature, precipitation, populations, diversity) within an ecosystem.

d. Investigate an ecosystem using methods of science to gather quantitative and qualitative data that describe the ecosystem in detail.

Benchmark 2: Cells are the basic unit of life. All living things are composed of one or more cells that come from preexisting cells. Cells perform a variety of functions necessary to maintain homeostasis and life. The structure and function of a cell determines the cell's role in an organism. Living cells are composed of chemical elements and molecules that form large, complex molecules. These molecules form the basis for the structure and function of cells.

STANDARD II: Students will understand that all organisms are composed of one or more cells that are made of molecules, come from preexisting cells, and perform life functions.

Objective 1: Describe the fundamental chemistry of living cells.

a. List the major chemical elements in cells (i.e., carbon, hydrogen, nitrogen, oxygen, phosphorous, sulfur, trace elements).
b. Identify the function of the four major macromolecules.
c. Explain how the properties of water contribute to maintenance of cells and living organisms.
d. Explain the role of enzymes in cell chemistry.

Objective 2: Describe the flow of energy and matter in cellular function.

a. Distinguish between autotrophic and heterotrophic cells.
b. Illustrate the cycling of matter and the flow of energy through photosynthesis.
c. Measure the production of one or more of the products of either photosynthesis or respiration.

Objective 3: Investigate the structure and function of cells and cell parts.

a. Explain how cells divide from existing cells.
b. Describe cell theory and relate the nature of science to the development of cell theory.
c. Describe how the transport of materials in and out of cells enables cells to maintain homeostasis.
d. Describe the relationship between the organelles in a cell and the functions of that cell.

e. Experiment with microorganisms and/or plants to investigate growth and reproduction.

Benchmark 3: Structure relates to function. Organs and organ systems function together to provide homeostasis in organisms. The functioning of organs depends upon multiple organ systems.

STANDARD III: Students will understand the relationship between structure and function of organs and organ systems.

Objective 1: Describe the structure and function of organs.

a. Diagram and label the structure of the primary components of representative organs in plants and animals
b. Describe the function of various organs
c. Relate the structure of organs to the function of organs.
d. Compare the structure and function of organs in one organism to the structure and function of organs in another organism.
e. Research and report on technological developments related to organs.

Objective 2: Describe the relationship between structure and function of organ systems in plants and animals.

a. Relate the function of an organ to the function of an organ system.
b. Describe the structure and function of various organ systems (i.e., digestion, respiration, circulation, protection and support, nervous) and how these systems contribute to homeostasis of the organism.
c. Examine the relationships of organ systems within an organism and describe the relationship of structure to function in the relationship.
d. Relate the tissues that make up organs to the structure and function of the organ.

e. Compare the structure and function of organ systems in one organism to the structure and function in another organism.

Benchmark 4: Information passed from parent to offspring is coded in DNA (deoxyribonucleic acid) molecules. The fundamental DNA structure is the same for all living things; the sequence of DNA differs between each organism and each species. Changes in the DNA sequence may alter genetic expression. The genetic information in DNA provides the instructions for assembling protein molecules in cells. The code used is virtually the same for all organisms. There are predictable patterns of inheritance. Sexual reproduction increases the genetic variation of a species. Asexual reproduction provides offspring that have the same genetic code as the parent.

STANDARD IV: Students will understand that genetic information coded in DNA is passed from parents to offspring by sexual and asexual reproduction. The basic structure of DNA is the same in all living things. Changes in DNA may alter genetic expression.

Objective 1: Compare sexual and asexual reproduction.

a. Explain the significance of meiosis and fertilization in genetic variation.

b. Compare the advantages/disadvantages of sexual and asexual reproduction to survival of species.

c. Formulate, defend, and support a perspective of a bioethical issue related to intentional or unintentional chromosomal mutations.

Objective 2: Predict and interpret patterns of inheritance in sexually reproducing organisms.

a. Explain Mendel's laws of segregation and independent assortment and their role in genetic inheritance.

b. Demonstrate possible results of recombination in sexually reproducing organisms using one or two pairs of contrasting traits in the following crosses: dominance/recessive, incomplete dominance, codominance, and sex-linked traits.

c. Relate Mendelian principles to modern-day practice of plant and animal breeding.

d. Analyze bioethical issues and consider the role of science in determining public policy.

Objective 3: Explain how the structure and replication of DNA are essential to heredity and protein synthesis.

a. Use a model to describe the structure of DNA.

b. Explain the importance of DNA replication in cell reproduction.

c. Summarize how genetic information encoded in DNA provides instructions for assembling protein molecules.

d. Describe how mutations may affect genetic expression and cite examples of mutagens.

e. Relate the historical events that lead to our present understanding of DNA to the cumulative nature of science knowledge and technology.

f. Research, report, and debate genetic technologies that may improve the quality of life (e.g., genetic engineering, cloning, gene splicing).

Benchmark 5: Evolution is central to modern science's understanding of the living world. The basic idea of biological evolution is that Earth's present day species developed from earlier species. Evolutionary processes allow some species to survive with little or no change, some to die out altogether, and other species to change, giving rise to a greater diversity of species. Science distinguishes itself from other ways of knowing and from other bodies of knowledge through the use of empirical standards, logical arguments, and skepticism, as science strives for explanations of the world.

STANDARD V: Students will understand that biological diversity is a result of evolutionary processes.

Objective 1: Relate principles of evolution to biological diversity.

a. Describe the effects of environmental factors on natural selection.

b. Relate genetic variability to a species' potential for adaptation to a changing environment.

c. Relate reproductive isolation to speciation.

d. Compare selective breeding to natural selection and relate the differences to agricultural practices.

Objective 2: Cite evidence for changes in populations over time and use concepts of evolution to explain these changes.

a. Cite evidence that supports biological evolution over time (e.g., geologic and fossil records, chemical mechanisms, and DNA structural similarities, homologous and vestigial structures).

b. Identify the role of mutation and recombination in evolution.

c. Relate the nature of science to the historical development of the theory of evolution.

d. Distinguish between observations and inferences in making interpretations related to evolution.

e. Review a scientific article and identify the research methods used to gather evidence that documents the evolution of a species.

Objective 3: Classify organisms into a hierarchy of groups based on similarities that reflect their evolutionary relationships.

a. Classify organisms using a classification tool such as a key or field guide.

b. Generalize criteria used for classification of organisms (e.g., dichotomy, structure, broad to specific).

c. Explain how evolutionary relationships are related to classification systems.

Justify the ongoing changes to classification schemes used in biology.