Fall 12-2003

Class II Molecules of the Major Histocompatibility Complex

Abbey Elizabeth Jones
University of Tennessee - Knoxville

UNIVERSITY HONORS PROGRAM

SENIOR PROJECT - APPROVAL

Name: Abbey Jones

College: Arts and Sciences Department: Biochemistry, Cellular and Molecular Biology

Faculty Mentor: Dr. Jim Hall

PROJECT TITLE: Class II Molecules of the Major Histocompatibility Complex

I have reviewed this completed senior honors thesis with this student and certify that it is a project commensurate with honors level undergraduate research in this field.

Signed: _____, Faculty Mentor

Date: 12-08-03

Comments (Optional):

Class II Molecules of the Major Histocompatibility Complex

Abbey Jones

The University of Tennessee Honors Program

Senior Honors Thesis

November 13, 2003

Class II Molecules of the Major Histocompatibility Complex

ABSTRACT:

Within the study of genetic polymorphism, species diversity, and natural selection, a common factor continually takes the lead in the global demand for the attention of geneticists and other scientists, as well as medical doctors. This common factor is the major histocompatibility complex, or the MHC. In this thesis I address the relevance of the MHC to the common layman as well—for the highly studied, multi-talented MHC has also been shown to influence attraction between members of the opposite sex. In order to describe how the MHC affects everyday life, I will first describe exactly what it is, how it works, and the processes that occur when the MHC does not function properly.

The major histocompatibility complex is a vital component of the immune system. It is a genetic complex located on the short arm of chromosome 6. Two protein strands comprise the MHC, corresponding to two different classes of MHC genes, class I and class II. Class II molecules are the focus of my paper and presentation. Class II genes are composed of the human leukocyte antigens (HLA-) –DP, -DQ, and –DR. Each class II gene has two strands, and each strand has two domains. Similarly, Class I has two strands, and multiple domains. The class I and class II genes encode for proteins which aid in distinguishing "self" particles from "non-self" particles within the body. Class I molecules are present on all "self" cells, widely distributed throughout the body, and class II molecules are present on antigen-presenting cells of the immune system. These cells, when complexed with MHC class II serve to label "non-self" particles for destruction by the immune system.

When the MHC is mutated, absent, or otherwise altered, multiple and complex problems may result, from the so-called "bare lymphocyte syndrome," where MHC II molecules may be completely absent (resulting in a general immune deficiency), to generation of autoimmune diseases such as lupus and various forms of arthritis. Population-based control studies have been performed which indicate the involvement of the MHC as a cause in many such polygenic autoimmune diseases. On a lighter note, studies with a focus on natural selection have also been performed which propose the MHC as a factor in determining attraction to members of the opposite sex in humans.

In summation, the MHC is an integral factor in determining immune health, predisposition to autoimmune disease and susceptibility to various treatments; it also affects aspects of everyday life such as whom you flirt with next. Current research is uncovering more every day about this portion of the genome which appears to have great power and influence in such diverse instances.

Scientists have historically searched for a biological basis for the differences observed among members of the human race. What is it truly that sets each person apart? How could it be that every human responds to the environment, with its numerous pathogens, in a distinctly different way? Often groups of people with similar backgrounds or genetic histories may respond to a certain pathogen drastically differently—or not at all—than another population of people whom the same pathogen decimates.

The answer to this complex is a complex within itself—the major histocompatibility complex, or MHC. The MHC is an integral component of the immune defense system. Antigen presentation to T cells, as well as T cell development and activation, is highly regulated by genes of the MHC. Two major types of molecules compose the MHC: class I and class II. Class I molecules are present on all nucleated cells throughout the body. These are the molecules used to communicate "self" peptides to the immune system. Class II molecules, on the other hand, the focus of this paper, are usually limited to expression upon antigen-presenting cells such as macrophages, monocytes, dendritic cells, and B cells, as well as thymic epithelium. Cells which are not antigen-presenting cells may be subjected to the cytokine interferon gamma (IFN-γ) and thus induced to express class II molecules of the MHC (Dorak 2003, Masternak et al. 2000).

The MHC molecules were identified because of their significance in organ transplantation rejection and correlation to many autoimmune diseases. A transplanted organ may be rejected if the MHC proteins of the two individuals differs by a single amino acid sequence. In 1951, geneticist George D. Snell of the Jackson Laboratory in Bar Harbor, Maine, instigated investigation into the major histocompatibility complex with his publication of two papers (Klein 2001).

At that time, only the histocompatibility complex of a laboratory mouse had been identified. It was referred to as the H2 system, for the name major histocompatibility complex did not enter the field until the 1970s, when more knowledge of the subject was unearthed, and it emerged that many vertebrates possessed genetic complexes that were a great deal similar to the H2 system of the laboratory mouse. Research on the MHC has progressed exponentially from its beginning in 1951, when only two scientists were actively involved—Snell and another by the name of Peter A. Gorer in London. Few others were even researching MHC-relevant issues (Klein 2001).

Rose O. Payne, a hematology department technician at the Stanford University School of Medicine, had just begun a foray into the question of whether thrombopenias and leukopenias had an immunological basis. She soon discovered that what she had thought were self antibodies had actually been induced by foreign white blood cells from blood transfusion donors. Later Payne, in collaboration with Walter Bodmer, using an improved antibody source, identified the antigens referred to as LA; this antigen set eventually became the LA portion of the human MHC human histocompatibility leukocyte antigen identity (HLA) (Klein 2001).

During these advancements, Gorer and Snell continued their research in the pursuit of the MHC. They were specifically concerned with the role of circulatory antibodies in their research. Hypothesizing that there might be a connection between allogenic transplant rejection factors and antigens of the blood, Gorer's reasoning was that response to transferred foreign blood was similar to the rejection response of grafted tissue (Klein 2001). Snell, on the other hand, left the mechanism study to Gorer and instead focused on the loci makeup involved in such tissue rejection. The location and characteristics of the histocompatibility loci became Snell's focus; he soon realized that he needed a way to differentiate among the loci. Through the production of coisogenic mouse strain pairs, which had many problems with the experiment itself, Snell was eventually able to determine important differences in the characteristics of the multiple H loci. Snell also discovered a "marker gene" for a specifically influential H locus, which greatly aided in his research (Klein 2001).

Thus Snell was another historical proponent of the MHC identity. Differing between "major" and "minor" H loci, and discovering that the H2 locus was most significant and hence the "major;" led to use of "major" as the first word of MHC. Snell's later research illustrated the complexity of the genetics of the H2 locus, in turn initiating addition of the word "complex" in MHC (Klein 2001).

After discovery of the polymorphism, complexity, and high influence on transplant rejection which the MHC possesses, its popularity soared. Scientists everywhere got involved, from clinicians who discovered links from diseases to specific HLA alleles, to frustrated transplant surgeons, to immunologists who came to realize how

important the MHC was in the role of adaptive immunity, to geneticists intrigued by the notion of genetic analysis experiments in human systems (Klein 2001).

Every MHC class II molecule is composed of two chains—an α and a β, which are combined as a heterodimer. The α chain is slightly larger, about 3kDa more, than the β chain due to glycosylation characteristics. There are also two α domains (α1 and α2) and two β domains (β1 and β2) that comprise each of the α and β chains of these molecules. The binding site formed by the configuration of these domains is the basis for the extensive polymorphism of these molecules. The β domain forms eight antiparallel fragments, which combine to become a foundation for the α domain formation of two antiparallel loops; with a disulfide bond for attachment of the domains, one solitary peptide binding site is the end product. However, this binding site has the ability to attach to many different peptides—so-called "high affinity and low specificity" perhaps due in part to the hydrogen bonds responsible for maintaining attachment of the peptide to the side chains lining the class II molecule. Binding of the class II classical molecules to the peptides is moderated greatly by the invariant nonclassical molecules of HLA-DM and HLA-DO (Ting and Trowsdale 2002).

The MHC Class II complex may be found upon dendritic cells, which are among the most important APCs due to their high effectiveness. In circulation, a dendritic cell greatly resembles an irregularly nucleated lymphocyte containing many mitochondria. Dendritic cells are often found attached to fibroblastic reticular cell conduits (FRCCs), which are fiber-filled tubes in which the manufacturing of cytokines and related products

occurs. FRCCs are effective messengers and pathways for migrating cells. The site formed when dendritic cells attach to FRCCS encourages antigen presentation to migrating lymphocytes. These lymphocytes are continuously traveling from the lymph to the blood and back again. In the blood, lymphocytes are found to be spherical in shape, with many surface microvilli. Lymphocytes may be found at rest; once they have been instigated to emigrate throughout the body, a polarization process begins to aid the lymphocyte on its way. Chemokines signal a lymphocyte to polarize its organelles as well as its cytoskeleton. Actin becomes concentrated in the anterior lamellipodium, and tubulin bundles becomes concentrated in the posterior uropod. Intermediate filaments of vimentin then quickly collapse, plectin is expressed, and becomes involved with lamin B, actin, and vimentin in the lymphocyte, resulting in full polarization and thus activation of the lymphocyte (Madigan et al. 2000; Clausen et al. 1998).

Other cells upon which antigens may be presented include macrophages, monocytes, and B cells. Monocytes differentiate to become macrophages, which aid in the specific immune response as well as the destruction of most pathogens they encounter. Macrophages are found primarily in the spleen and lymphoid tissue, whereas the precursor monocyte is found circulating throughout the blood and lymph. Once phagocytosis by an APC such as a macrophage has taken place, that cell becomes primed to phagocytize much more efficiently (Madigan et al. 2000).

B cells are not only important in antigen presentation, but they are also critical antibody factories and instigators of the memory function of the immune system. Immunoglobulin molecules found on the surface of B cells separates them from other

immune cells. These immunoglobulins are the key to antigen recognition; once exposed to an antigen, B cells may proliferate to become long-lasting memory cells, which readily reproduce at the first sign of reexposure to the same antigen. B cells are normally found in large numbers in lymph node cortexes; this location is crucial for interaction with antigens (Madigan et al. 2000).

Antigen presenting cells process and deliver their antigen package to T lymphocytes. Each T cell possesses a T cell receptor (TCR) that is highly specific to a particular antigen. The two major types of T cells are classified by their cell surface proteins (either CD4 or CD8). T helper cells, composed of Th1 and Th2 groups, possess the CD4 protein. This set of T helper cells is the primary group of T cells that interacts with class II molecules. CD8 T helper cells, conversely, are associated with the MHC class I gene complex (Madigan et al. 2000).

Three general isotypes of class II molecules exist in humans, referred to as the human histocompatibility leukocyte antigens (HLAs): HLA-DP, HLA-DQ, and HLA-DR. These human leukocyte antigens are found on the short arm of chromosome 6 (6p21.31) (Dorak 2002). Class II molecules of the MHC are responsible for commencing the humoral immunity via the coding of heterodimeric glycoproteins. These glycoproteins are then responsible for the presentation of foreign antigens to the aforementioned CD4 T cells. Class II molecules also fill a directoral role in developing as well as activating these T cells, and they are crucial to gene transcription regulation. This control of the immune response system is a result of a critical regulation of class II molecular expression. The genetic factors associated with activating the MHC are class

II transactivator (CIITA) and regulatory factor X (RFX). Both are crucial transactivators of genes, and both factors may be subject to a "genetic complementation cloning" method for effective isolation (Boss et al. 2002).

The class II transactivator is a non-DNA binding control factor of MHC expression, while regulatory factor X is part of the nuclear complex upon which DNA binding relies. RFX has three subunits referred to as RFXANK (or –B), RFX5, and RFXAP. These transcription factors bind the cis-acting sequence, the conserved X1 box, found in promoters of the MHC class II isotypes (HLA-DQ, -DR, and –DP). CIITA also is believed to associate with certain constituents of the class II promoters, and through its acidic activation domain activate transcription. The presence of the class II transactivator as a "molecular switch" is thus illustrated (Boss et al. 2002).

The promoters of the class II isotypes include the W, X1, X2, and Y boxes. Regulation is coordinated through these cis-acting elements, which are located within a few hundred base pairs upstream from each of the different isotypes of class II molecules. These promoter factors, as well as the structural genes for MHC class II loci are uniquely conserved (Ting and Trowsdale 2002). Other class II transcription factors are CREB, which binds the X2 box motif, and NF4, which binds to the Y box motif (Boss et al. 2002).

The position of the class II transactivator in initiating gene expression is a result of CIITA's involvement with transcription machinery factors and the enzyme acetyltransferase. Not only does CIITA express acetyltransferase activity, but it also causes acetylation of nucleosomes via compelling histone acetyltransferases. CIITA also

possesses down-regulation properties, most likely because of its titration of CBP, the CREB-binding protein, from locations on certain genes; specifically, the promoting and enhancing regions of such genes (Ting and Trowsdale 2002).

This titration of transcription factors and regulation of MHC class II expression illustrates two methods by which CIITA may exert its power. CIITA has been extensively studied as a gene regulator that more than likely influences a great deal of gene activity (Boss et al. 2002).

Much of the research for MHC class II regulation has been based upon studies done with humans presenting a genetic deficiency, either in CIITA or RFX complex units and binding. MHC class II deficiency is often referred to as bare lymphocyte syndrome, because of the usual absence of class II expression on lymphocytes in the bodies of these patients. This immunodeficiency is an extremely rare form of an autosomal recessive genetic disease. Two general forms of the MHC class II deficiency have been distinguished. One form is a result of CIITA mutation, and the other is a result of defects in the RFX complex formation or defects in any aspect of subunit binding. These mutations are labeled as different types depending upon their complementation group (Alcaide-Loridan et al. 2001; Mach et al. 1996).

Complementation group refers to the unaltered phenotype of fusions of paired cells; genes for these complementation groups encode integral proteins needed for transcription of MHC II genes. Complementation group A genetic defect has been found to encode for CIITA; and only recently, geneticists from the Howard Hughes Medical

Institute in San Francisco have determined that complementation groups B, C, and D are associated with genetic mutation in RFX subunits RFXANK, RFX5, and RFXAP, respectively. A little-known controversial complementation group E has been speculated which encodes a chromatin organizational protein (Jabrane-Ferrat et al. 2000).

The RFX complex, which binds to the MHC class II promoters at the X and S (Y) boxes, is comprised of the three subunits RFXANK (B), RFXAP, and RFX5. These proteins differ in size, with RFXANK the smallest at 33 kDa, RFXAP in the middle size category at 41 kDa, and RFX5 the largest at 75 kDa. A DNA-binding domain is present upon RFX5, (although it cannot bind DNA even in a long fragment by itself) and RFXAP has been shown to bind slightly to RFX5 in vivo (Jabrane-Ferrat et al. 2000; Brown et al. 1997).

The mechanistic formation of this RFX complex has been excessively researched to no major avail. It has been shown that the RFX complex may be nucleated without the presence of DNA via the weakly linked interactions in vitro between RFXAP and RFXANK. Using E. Coli to express the RFXANK binding protein, while performing in vitro translation of the wild-type proteins of RFXAP and RFX5, it was shown by researchers at the Howard Hughes Medical Institute that this binding protein could repair MHC II expression on RFXANK-deficient cells. When the RFXAP and RFX5 were mixed with this binding protein, there was no binding of RFX5 to the binding protein; however, there was a significant amount of binding between RFXAP and the binding protein of RFXANK. To illustrate that this same binding situation occurs in vivo, plasmid-transfected cells were subjected to expression of these proteins; the plasmids

used were predetermined RFXANK protein regulators, HA-labeled RFXAP protein, or a mixture of the two. Western blot using antibody to HA was performed to detect RFXAP; cells transfected with a plasmid of either RFXANK protein regulator or HA-labeled RFXAP protein expressed no RFXAP. However, if a transfected cell possessed a plasmid containing both, then the anti-HA antibody was expressed to a high degree along with RFXAP (Jabrane-Ferrat et al. 2000).

Using a dual hybrid mammalian system, experiments have further illustrated that there is no observed fusion between RFX5 and RFXANK and a very small amount is observed between RFXAP and RFX5; however, it has also been suggested that perhaps RFX5 binds to a combined form of RFXANK and RFXAP. Another opinion suggests that perhaps RFXAP is a connector for the three by joining both RFXANK and RFX5 (Jabrane-Ferrat et al. 2000).

When the RFXAP and RFXANK proteins become mutated, the assembly of the RFX complex cannot be completed, even when DNA is available. When proteins from RFXAP are mutated, they are C-terminally deficient and as a result cannot bind to RFXAP. In a similar fashion, RFXANK mutant proteins are not able to bind to RFXAP. Thus if a mutation occurs in one of these B and D complementation groups, binding to the other group is hindered entirely; the very beginning of the RFX complex assembly is aborted. A summary of the RFX complex assembly and resultant DNA binding is illustrated as follows:

> "RFXANK and RFXAP assemble first and represent a scaffold that attracts RFX5. Upon binding, the conformational change of RFX5 exposes its

DNA-binding domain, which anchors the RFX complex to X and S boxes. The final shape of the RFX complex also allows RFXAP to make extensive contacts with DNA. Another part of the RFX5 protein touches CIITA, which is attracted to MHC II promoters by a combinatorial surface formed on [conserved upstream sequences]." (Jabrane-Ferrat et al. 2000)

Experiments in this aspect of the RFX complex assembly illustrated that the presence of DNA does not aid or support formation of the RFX complex, especially when there exists a mutation in the RFX subunits. The subunits cannot independently bind to DNA or form a proper RFX complex, nor can any double subunit formation. The correctly and wholly assembled nuclear complex from the wild-type forms of the RFX subunits must be formed in order for proper DNA binding. Any individual protein binding of RFXANK, RFXAP, or RFX5 are not associated with DNA binding (Jabrane-Ferrat et al. 2000).

Other studies have attempted to illustrate that it is the ankyrin repeats of the RFXANK subunit that may be most significant in MHC class II deficiency. Angela M. DeSandro, Uman M. Nagarajun, and Jeremy M. Boss of the Emory University School of Medicine's Department of Microbiology and Immunology performed RFX gene mutagenesis experiments which analyzed and isolated RFX subunit domains, particularly those responsible for accurate assembly of the nuclear RFX complex. Yet these researchers also discovered that these factors alone are not enough for proper expression of MHC class II molecules. Promoters of MHC class II must associate with CIITA to

ensure proper transcription activation. When mutations in CIITA occur, the class II promoters have filled X1, X2, and Y boxes; however, if the mutation occurs in the RFX binding, the X1, X2, and Y boxes are empty (Boss et al. 2000).

These researchers (Boss et al.) hypothesized that the RFX complex mutation may have many different aspects, depending upon mutations within each subunit of the complex. Experiments were undertaken to identify integral components of each of the RFX subunits required for the various mechanisms of MHC class II expression. The gene encoding the RFXANK subunit was extensively analyzed because of its suspected and potentially influential impact upon the remaining two subunits. RFXANK has a C-terminal region, which consists of three ankyrin repeats; at this point in MHC research, the precise function of these was not known. The researchers at Emory designed mutation experiments in which deletions were made in regions of RFXANK. These mutants were then analyzed in the context of MHC class II expression reinstatement in vivo. Cell lines to be transfected were obtained from bare lymphocyte syndrome patients whose cells demonstrated splice site mutation homozygosity, with wild type expression for genes encoding RFX5, RFXAP, and CIITA. Results of this experiment illustrated that proper RFXANK utility is much more dependent upon the region C-terminal or N-terminal to the ankyrin repeats than it is dependent upon its amino-terminal portion (Boss et al. 2000).

The Emory team of researchers then went on to perform further analysis of mutant RFXANK proteins; both DNA binding characteristics and association with other subunits was assessed. Using a coupled T7 transcription-reticulocyte translation kit, or

IVT, the subunits were each synthesized and submitted to coimmunoprecipitations using anti-RFX5 antibody. Three deficient mutants of RFXANK were also submitted to this same test. Mutant RFX-BΔ1 had 39 amino acids deleted from its C-terminus, and mutant RFX-BΔ2 had 122 amino acids deleted from its N-terminus, while mutant RFX-BΔ3 had a deletion of sequences slightly N-terminal to the ankyrin repeats. The normal RFXANK proved to fully associate with the remaining two RFX subunits, which were labeled for the purposes of the experiment. The three mutants, two with their N-terminal regions removed (which contains the ankyrin repeat sequence linkages) and the other with a C-terminal deletion that allowed the ankyrin repeats to remain, did not associate very well with the normal RFXAP and RFX5. As compared to the fully normal RFXANK, which immunoprecipitated 55% of its received substance, the mutants only associated at 43%, 12%, and 0.8% of input, with the least percentage of association corresponding to the RFXANK mutant that actually contained the three ankyrin repeats but possessed a deletion in another region. The higher association represented by the first two mutants mentioned, which were deficient in the first three ankyrin repeats but maintained a region with a weaker fourth ankyrin repeat, insinuated that perhaps this region of the subunit plays a critical role as an association domain (Boss et al. 2000).

Further experimentation using RFX5 attempted to analyze more specific transactivation functions of the gene. Multiple deletions within the 5′ and 3′ regions were enacted, and the resulting mutants were tested for efficiency in transactivation of an MHC II promoter. Assays involving transfections similar to those the Emory geneticists used for the RFXANK analysis were performed on these RFX5 mutants. Evidence

appeared to show that mutagenesis greatly affects the properties of RFX5. With the wild-type RFX5, expression of HLA-DR may be significantly reinstated; however, every mutant but one showed extremely reduced or no expression restoration. Less than five percent of normal (wild-type) RFX5 activity was illustrated in the first five mutants, and only twenty five percent of normal expression was displayed by the sixth mutant. The most likely reason for the difference was found in the specific type of mutation constructed. The mutants performing less than five percent of normal were missing their DNA binding domain and possibly others, contributing to transactivation or interaction with other subunits (Boss et al. 2000).

These RFX5 mutants were also analyzed for their efficiency of interaction with RFXAP and RFXANK wild types. RFXAP was translated in vitro and incubated with recombinant RFXANK, and then each mutant of RFX5 was subjected to this incubated mixture. After purification, the proteins that had formed complexes were analyzed. The results proved to be different than for the transactivation experiment discussed just previously; the majority of the RFX5 mutants illustrated effective interaction with subunits RFXANK and RFXAP. The amounts of interactions and the relation to each specific mutant led the researchers to believe that RFX5 contains two essential regions for RFXANK and RFXAP association. One of these regions is believed to be in the N terminus, within a 92-amino acid sequence, while the other region exists between the 201 and 410 amino acids. The mutant with a deletion in the region of the N terminus displayed an extremely reduced amount of interaction as compared to the other mutants (Boss et al. 2000).

Geneticists at the University of Geneva Medical School in Switzerland proved that the majority of the RFXAP gene is not necessary for proper expression of its isotype HLA-DR; however, a larger portion of the gene is necessary for proper HLA-DQ and –DP expression. This experiment illustrated that the domain of RFXAP observed is specific to each particular isotype, as each isotype required different portions and lengths of the RFXAP gene. It was also suggested by these geneticists that this difference in isotype need was based upon the distinctions among the reliance that CIITA had upon this RFXAP domain (Barras et al. 2001). Genetic complementation assay experiments with MHC II deficient patients that possessed mutations in the RFXAP gene illustrated classification of RFXAP mutation into complementation group D. Using the RFXAP wild-type gene, transfection was successfully performed within these patients' cells. The result was complete reinstatement of class II expression upon the cells used (Fischer et al. 1997).

Further experimentation involving the RFXAP subunit resulted in key clues about RFX subunit mechanism. Analysis of RFXAP involved mutating the acidic-, amino acid- and glutamine-rich regions that differ greatly from the other RFX proteins. Here the X1 box seemed to play a role, for it was hypothesized that a base pair association existed between RFXAP and the X1 box. Three RFXAP mutants were constructed for this experiment—one altered at the C terminal and two altered at the N terminal. The N terminal deficient mutants were observed to maintain association levels comparable to the wild-type gene; whereas the C terminal deficient mutant maintained no association.

The glutamine region, located on the C terminal part of the protein, was therefore suggested to be critical for wild-type displayed activity levels (Boss et al. 2000).

Experiments involving the association between the RFX complex and CIITA were constructed to determine which, if any, subunit of the nuclear RFX complex played a critical role. Because transactivation of this association and indeed the entire system is dependent upon CIITA's activating position, the connection between CIITA and the RFX complex was considered extremely pertinent. There is no direct association between DNA and the class II transactivator; however, the N terminal region of CIITA consists of a particularly strong domain for transcription activation. Many research groups have hypothesized that MHC class II factors associate with CIITA, and thus the gene expression pathway is triggered, but small amount of evidence has actually proved this theory. The researchers at Emory wanted to test their theory on specific regions of RFX5—particularly, the C terminus and proline-rich regions; the experiments performed indeed suggested that these regions of RFX5 were accountable for instigating system activation. (Boss et al. 2000). And thus another question was raised—does CIITA association with RFX5 depend upon these two specific regions—the C terminus and proline-rich region—of RFX5?

Further analysis of direct CIITA interaction with synthesized RFX subunits revealed individual and separate association between CIITA and RFX5. When CIITA was likewise incubated with RFXANK and RFXAP, no association whatsoever was observed. When a wild type RFX complex was used in the incubation analysis with CIITA, predictably, a strong association was observed (Boss et al. 2000).

Another study done by a slightly different group of researchers in Atlanta confirmed that the majority of patients exhibiting MHC class II deficiency or bare lymphocyte syndrome possessed mutations in the RFXANK gene. The experiments performed involved purification and sequencing of the proteins comprising the RFX complex. The isolated RFXANK sequences were then tested for ability to reinstate MHC class II expression upon cells derived from bare lymphocyte syndrome patients experiencing a complementation group D deficiency. Expression upon the cells was restored; the mutation that was covered by the tested RFXANK was found to include a deletion at a splice site that produced an irregular protein instead of the ankyrin repeats often deemed crucial to subunit association (Boss et al. 1999).

The experiment proceeded further to illustrate relation of RFXANK function necessity for the promoters of MHC II genes; tests were undergone which involved the role of activating a protein reporter containing W, X, and Y box sequences, as well as a protein reporter without these sequences. When RFXANK was transfected with the control reporter (no W, X, and Y box sequences), protein expression was very small; however, when RFXANK was transfected with the reporter containing the box sequences, a high level of protein was expressed. These results indicated the ability of RFXANK to perform via the promoter of MHC II; indicating therefore that RFXANK may be a gene regulator elsewhere besides the class II MHC genes (Boss et al. 1999).

Other studies confirming the theory that RFXANK mutation causes the majority of bare lymphocyte syndrome patients have also assessed slightly different facets of the RFXANK role in nuclear complex formation. Geyer et al. of the Howard Hughes

Medical Institute isolated and identified four ankyrin repeats from the RFXANK subunit. Using as a reference other proteins with known ankyrin repeats, the three-dimensional structure of RFXANK was determined. With a model of the exposed surfaces, the actual binding sites to CIITA and RFXAP were observed and recorded. Instead of continuous amino acid chains as has been previously theorized, it was determined that the RFXANK ankyrin repeat surfaces contain irregular, discontinuous residues. The β loops of the first three repeats, as well as a groove helix, were the association points for fusion with RFXAP. Multiple residues of the external helices of the opposite side of RFXANK were the attachment points for CIITA (Geyer et al. 2001).

In relation to bare lymphocyte syndrome, when the deficiency is due to RFXANK, the alteration consists of a single point mutation that causes a shift in amino acid production. This critical alteration occurs upon the third ankyrin repeat (necessary for RFXAP binding), inside the helix structure. Thus when this mutation occurs, it prevents crucial association between RFXAP and RFXANK, and therefore prevents formation of the nuclear RFX complex. In this same report, the researchers experimented with RFX5/RFXANK interactions, concluding in a similar statement to other reports on RFX5/RFXANK binding: that is, that the proper RFX complex formation needs RFX5; however, in order to bind, RFX5 must be presented to the combined RFXANK and RFXAP surfaces; only then will a proper full complex develop (Geyer et al. 2001).

Though bare lymphocyte syndrome is a major concern and area of focus in MHC class II research, other immunodeficiencies are linked to MHC class II molecules and mechanistic pathways. Perhaps the most notorious is HIV infection. Similar to BLS,

human immunodeficiency virus, the causative agent of acquired immunodeficiency syndrome (AIDS), results in a combined immunodeficiency involving CD4 T cells. By infecting and altering T cells, HIV impairs the processing and presentation of antigens to the immune system. In this way HIV has also been discovered to hinder MHC class II determinant transcription (Kanazawa et al. 2000).

A strong transcriptional transactivator encoded by HIV, referred to as Tat, has been studied for its role in binding MHC II gene transcription. To determine the promoter target of Tat, Kanazawa et al. performed cotransfection experiments involving plasmids encoding HLA-DRA promoter-linked chloroamphenicol acetyltransferase, Tat, and CIITA. Since it was known that this HLA-DRA promoter was associated with transcription activation through CIITA, it was subjected to coexpression with Tat. The result was an extremely reduced level of chloroamphenicol acetyltransferase activity as compared to the control containing HLA-DRA promoter linked without Tat. A mutated form of Tat subjected to the same coexpression test failed to significantly reduce activity; also, without CIITA present, Tat also failed to significantly reduce activity (Kanazawa et al. 2000).

Multiple Sclerosis (MS) is another immune deficiency which has been linked to chromosome 6 (via genome scans), and then more specifically, MHC class II alleles. Family-based association studies performed on data from the MS Society of Tasmania have clearly illustrated the inheritance factor involved with MS; certain patterns of HLA alleles were discovered among family lines of MS patients. These HLA patterns were not observed, however, in the control populations (Genethics 2003).

A review of case studies was performed in Mexico by Flores-Dominguez et al. to analyze the involvement of class II alleles in Takayasu arteritis (TA), an inflammatory disease of the arteries. Studies performed on populations varying from Asian, Arab, North American, and Mexican mestizo were compiled and the results indicated patterns within populations, but not as significantly across populations. Human leukocyte antigens -A31, -B52, -B39, -B5, and –DR2 were found to be associated with TA in Asian populations; HLA-A2, -A9, -B35, and –DR7 were found to be associated with Arab populations, HLA-DR4 was found to be associated with North American populations, and HLA-B5, -B52, and –DR6 were found to be associated with Mexican Mestizo populations. It was also speculated in this review that the alleles associated may instigate disease in these genetically susceptible individuals via presentation of an antigen not yet discovered (Flores-Dominguez et al. 2002).

The University of California-Los Angeles School of Medicine's Division of Rheumatology has also linked autoimmune disease to class II alleles. Case-control studies of human systemic lupus erythematosus (SLE), involving so-called "multiplex families," have led to the association between SLE and its multiple-factor causes, including MHC class II alleles, as well as complement mutations and Fc gamma receptor gene polymorphism. Current research performed at this institution involves mapping of these regions (which are presumably linked) in order for further identification (UCLA 2002).

The MHC has also been significantly linked to leukemia; in mice, spontaneous leukemia development has been illustrated via injecting newborn, low-incidence strains

with high-incidence strain leukemic filtrates. Conversely, the same experiment performed on adult strains of the same mice resulted in no spontaneous leukemia development. The difference was attributed to the homozygosity of the newborns' H2 types and the inoculate, which resulted in leukemia. Strains of mice with heterozygosity for their H2 type produced no leukemia when inoculated (Dorak 2003).

Similar experiments with enzootic bovine leucosis, the highest incidence neoplasia observed in cattle, appear to illustrate the retrovirus associated--the bovine leukemia virus. Lymphocytosis may result in persistent infections, followed by lymphosarcoma. In determining susceptibility factors, bovine genetics was assessed, and the major histocompatibility complex was identified as the critical flaw source in these infections; specifically, linkage disequilibrium existence with the bovine MHC loci (Dorak 2003).

Reports have also illustrated that MHC variability may influence mating choices in mice. The MHC of mice, usually referred to as the H2 locus, is able to be differentially distinguished by mice and select other highly trained species. Female mating preference experiments were performed in which both H2 identical and H2 differing males were presented. Twenty-nine out of thirty-nine females selected males different from them at the H2 locus as their primary mating choice. These results translate into a ratio of 1 (H2 different) to 0.344 (H2 identical) for mating choice. Natural population observation by other researchers has supported this evidence by discovering a homozygote deficiency of 27%; with lack of other influential factors, this

data indicates that female mating selection for males is greatly influenced by genetic differences at the H2 locus (Hedrick 1992).

Interestingly enough, a similar experiment has been performed on humans with analogous results. Claus Wedekind, a zoologist from the University of Bern, wanted to determine if differences at MHC loci were involved in attraction between the sexes. He provided forty-four men with tee shirts, instructing them to wear the shirt for two consecutive nights. The men were also instructed to abstain from the use of soap, deodorants, or any other "perfumed cosmetics" during the time the shirts were worn. After collecting the shirts, Wedekind then presented them to forty-nine female volunteers, with instructions to smell and then rank the "odourific attractiveness" of the shirts. As was discovered in the mice studies, the women in this study rated as most attractive the smells of the shirts worn by males with a largely differing MHC from their own. The biological basis theorized was that partnering with a human whose MHC is greatly different results in offspring with a greater amount of MHC variability—thus increasing the child's immune defense system and chance for survival (Wholistic Research Co. 2002).

The only contrast to these conclusive results was the portion of women in the volunteer group who were taking birth control pills. This group of women actually rated higher the smells of males having similar MHCs. Due to the pregnancy-imitating effects of birth control pills upon the female body, it was speculated that attraction to a male of similar MHC is indicative of the natural desire for aid and benefit of family (similar

MHC) support when pregnant, also a factor that may enhance survival of the offspring (Wholistic Research Co. 2002).

Thus the importance and influence of the major histocompatibility complex, as well as its major molecular pathways, its influence on immune function, and resulting significance in disease, have been illustrated in an attempt to further educate interested readers. Though the MHC is a relatively new topic in research, many steps have been accomplished in recent years to further understand this integral immune component. More research is needed to fully understand many of the interactions that occur, but new information is published very often, and technological advances have an enormous impact on such developments. And the next time you find yourself particularly attracted to someone of the opposite sex, you will understand the biological basis for the attraction, and perhaps trust your sense of smell more often.

Literature Cited

Alcaide-Loridan, Catherine; Wojciech Wizniewski, Marie-Claude Fondaneche, Francoise Le Deist, Maria Kanariou, Francoise Selz, Nicole Brousse, Viktor Steimle, Giovanna Barbieri, Dominique Charron, Alain Fischer, and Barbara Lisowska-Grospierre. "Mutation in the Class II trans-Activator Leading to a Mild Immunodeficiency." *The American Association of Immunologists.* 23 May 2001.

Barras, E., M. Peretti, J. Villard, M. Zufferey, and W. Reith. "Expression of the Three Human Major Histocompatibility Complex Class II Isotypes Exhibits a Differential Dependence on the Transcription Factor RFXAP." *Molecular and Cellular Biology.* Sep 2001; 21(17).

Boss, Jeremy M.: Angela M. DeSandro, and Uma M. Nagarajan. "Associations and Interactions between Bare Lymphocyte Syndrome Factors." *Molecular and Cellular Biology.* Sept 2000.

Boss, Jeremy M.; Uma M. Nagarajan, Pascale Louis-Plence, Angela DeSandro, Roger Nilsen, and Alyssa Bushey. "RFX-B Is the Gene Responsible for the Most Common Cause of the Bare Lymphocyte Syndrome, an MHC Class II Immunodeficiency." *Immunity.* Vol 10; Feb 1999.

Boss, Jeremy M., Uma M. Nagarajan, and Alyssa Bushey. "Modulation of Gene Expression by the MHC Class II Transactivator." *The Journal of Immunology.* 28 Aug 2002.

Boss, J.M., J.A. Brown, E.M. Rodgers, and C.S. Moreno. "Regulatory Factor X, a Bare Lymphocyte Syndrome Transcription Factor, is a Multimeric Phosphoprotein Complex." *Journal of Immunology.* 15 Jun 1997; 158 (2).

Clausen, B.E.; JM Waldburger, F. Schwenk, E. Barras, B. Mach, K. Rajewsky, I. Forester, and W. Reith. "Residual MHC Class II Expression on Mature Dendritic Cells and Activated B Cells in RFX5-Deficient Mice." *Immunity.* Feb 1998.

Dorak, M. Tevfik. *Origin of the MHC.* World Wide Web. 2003. http://www.openlink.org/dorak

Dorak, M. Tevfik. *Major Histocompatibilty Complex.* World Wide Web. 2002. http://www.dorakmt.tripod/com/mhc.html

Dorak, M. Tevfik. *MHC and Leukemia.* The World Wide Web. 2003. http://home.att.net/~dorak/hla/mhcleuk.html

Masternak, K., A. Muhlethaler-Fischer, A., J. Villard, B. Lisowska-Grospierre, P. van den Elsen, W. Reith, and B. Mach. " Mutation of RFXAP, a Regulator of MHX Class II Genes, in Primary MHC Class II Deficiency." *New England Journal of Medicine.* 11 Sep 1997; 337 (11)

Flores-Dominguez, C., G. Hernandez-Pacheco, J. Zuniga, and R. Gamboa. "Alleles of the Major Histocompatibility Complex Related with the Susceptibility to the Development of Takayasu Arteritis." *Gac Med Mex.* 2002; 138 (2).

Geyer, Matthias; Nekrep, Nada, Nabila Jabrane-Ferrat, and B. Matija Peterlin. "Analysis of Ankyrin Repeats Reveals How a Single Point Mutation in RFXANK Results in Bare Lymphocyte Syndrome." *Molecular and Cellular Biology.* Aug 2001.

Genethics. "More Information on Multiple Sclerosis." The World Wide Web. 2003. http://www.genecrc.org/site/rl/rl2c5.htm

Hedrick, Philip W. "Female Choice and Variation in the Major Histocompatibility Complex." *The Genetics Society of America.* 10 Jun 1992.

Klein, Jan. "Perspectives: George Snell's First Foray Into the Unexplored Territory of the Major Histocompatibility Complex." *The Genetics Society of America.* 2001.

Jabrane-Ferrat, Nabila; Nada Nekrep, and B. Matija Peterlin. "Mutations in the Bare Lymphocyte Syndrome Define Critical Steps in the Assembly of the Regulatory Factor X Complex." *Molecular and Cellular Biology.* Jun 2000.

Kanazawa, Satoshi; Takashi Okamoto, and B. Matija Peterlin. "Tat Competes with CIITA for the Binding to P-TEFb and Blocks the Expression of MHC Class II Genes in HIV Infection." *Immunity.* Vol 12; Jan 2000.

Madigan, Michael T., John M. Martinko, and Jack Parker. *Brock Biology of Microorganisms.* Ninth Edition. Prentice Hall, Upper Saddle River, NJ. 2000.

Mach, B., V. Steimle, E. Martinez-Soria, and W. Reith. "Regulation of MHC Class II Genes: Lessons From a Disease." *Annual Review of Immunology.* 1996; 14.

Mottet, J. W. Reith, and J. Villard. "Molecular Genetics of the Bare Lymphocyte Syndrome." *Review of Immunogenetics.* 2000; 2(2).

Ting, Jenny Pan-Yun, and John Trowsdale. "Genetic Control of MHC Class II Expression." *Cell.* Vol. 109; Apr 2002.

UCLA School of Medicine, Department of Medicine, Division of Rheumatology. "An update on genetic studies of systemic lupus erythematosus." *Current Rheumatology.* Vol 4; 4 Aug 2002.

Wholistic Research Co. "Perfume: recent studies on the underlying reasons for preference." The World Wide Web. 2002. http://www.wholisticresearch.com/info/artshow.php3?artid=314. (First reported in The Journal of Chinese Medicine News, 1990s)