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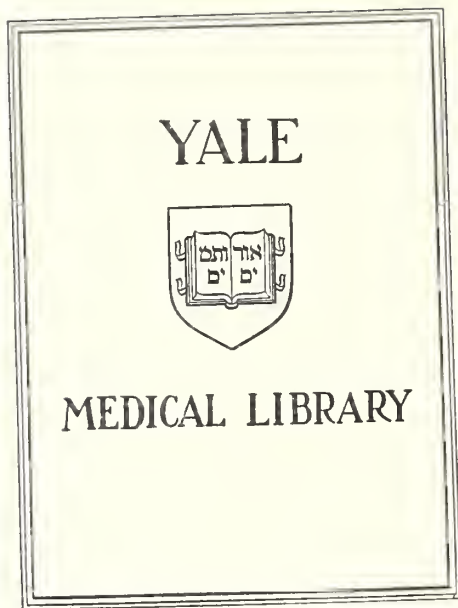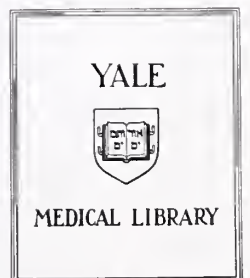
THE ROLE OF IMMUNOGLOBULIN E (IgE) IN RESPONSE TO NEOPLASIA

A thesis submitted to the Faculty, Department of Internal Medicine,
Yale University, in partial fulfillment of the requirements for the
degree, Doctor of Medicine

James T. Rosenbaum
A.B. Harvard University 1971

April 1, 1975

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Acknowledgements

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One individual deserves special mention. Her thinking contributed substantially to the content of this work. Her technical skills were welcomed. Her patience and her impatience were tested many times. To Sandy, who understands, this work is dedicated.

List of Abbreviations Used

PCA -passive cutaneous anaphylaxis

ACA -active cutaneous anaphylaxis

P-K -Prausnitz-Kustner

PBS -Phosphate buffered saline

i.v.-intravenous

i.p.-intraperitoneal

s.c.-subcutaneous

i.d.-intradermal

Ig -immunoglobulin

OA -ovalbumin

RIA -radioimmunoassay

SRS-A -slow reacting substance of anaphylaxis

CBH -cutaneous basophil hypersensitivity

AMP -adenosine monophosphate

ECF -eosinophilic chemotactic factor

SREC -sheep red blood cells

H&E -Hematoxylin and Eosin

PHA -Passive hemagluttination

BGG -Bovine gamma globulin

PREFACE

As a first year medical student I naively wondered, what is the relationship between allergy and cancer? After all, I reasoned with a limitless lack of sophistication, the immune system must have something to do with both diseases. When I posed this question two years later to John Dwyer, my adviser, I received the answer that stimulated much of what I now report. His reply was, "The question has merit. Go to the library. Find out what you can. Report back to me." Dutifully I began to search the journals trying to resolve such broad issues as do atopics develop cancer as often as normals?; could those with allergy have a genetic selective advantage in immunosurveillance, the rejection of malignant clones before their clinical presentation?; what is the role of IgE, that immunoglobulin class primarily responsible for allergy, in responding to neoplasia? Armed with questions like these, a pattern was established. I would read and report; John Dwyer would listen, question, suggest. Slowly we elaborated projects to resolve specific issues.

The laboratory in which these experiments were undertaken possesses many skills in immunological research. In particular, the lab is expert in assessing T and B cell function and investigating cutaneous basophil hypersensitivity responses. The laboratory's lack of prior experience with research on IgE provided me with great latitude in experimentation, but this also meant that a substantial amount of time had to be devoted to basics before the proposed tumor work could be successfully engineered.

The thesis then contains three natural divisions. Part I stems directly from that first naive question; it is a review of the literature regarding the relation between IgE and neoplasia. Part II derives from the need to understand some basic properties of IgE. It describes studies that have no direct relation to tumor immunology but includes such fundamental topics as the assay of IgE and the ontogenetics of IgE production. Finally, Part III concerns specifically the relation between IgE and tumors.

In many ways, this work is unfinished. This is not the study's defect, but rather its virtue. The topics explored were constantly demanding clarification of results, suggesting further study, and requiring refinement of techniques. This thesis project resembles the process of education by representing the construction of a starting gate from which to conduct a marathon, not a dash. While the finish line has not yet been reached, many hurdles have been cleared and most important, a direction in which the runners can head has been established.

The naive medical student has changed little since his initial question, but he has discovered that even questions asked out of ignorance may merit exploration.

PART I: A Review of the Literature

Few topics in medicine are currently generating as much interest as the relationship between the immune system and malignancy. Recent reviews[192,208,245] have covered such relevant issues as blocking anti-bodies, cell-mediated immunity, tumor associated antigens, immunosurve-illance, immunodeficiency, and immunotherapy. With such a plethora of interests demonstrating a definite relationship between the immune system and cancer, not surprisingly all reviews omit any mention of the ambig-uous role that immunoglobulin E or IgE plays in response to neoplasia. Yet much suggests the importance of just this subject.

Background

Basic facts about IgE, which is sometimes called reaginic, skin-sensitizing, or homocytotropic antibody, have been reviewed thoroughly and the reader is referred elsewhere[24,92] for detailed information. In brief, as recently as ten years ago, the antibody responsible for immed-iate wheal and flare skin reactions was thought to be a subclass of IgA. Then, in 1966, careful work by K. Ishizaka and his colleagues showed reaginic antibody to be a unique class of immunoglobulin.[93,94] This was confirmed shortly thereafter by the discovery of an IgE myeloma,[104] and the demonstration that antisera to the myeloma protein could absorb re-aginic activity from serum.[254] Several parasitic infections[171,196,210] and atopic diseases[210] including asthma,[103,121] allergic rhinitis,[134] and atopic eczema[106] are often characterized by elevated levels of IgE. Reagin is thought to be primarily responsible for immediate type hyper-senstivity responses such as anaphylaxis. Some evidence suggests that IgE might have a role in such diverse entities as Graves' disease[239] and the nephrotic syndrome.[70,133] IgE is present in the serum in ex-

tremely low concentrations,[105] nanograms per milliliter, and is also
tissue bound to basophils and mast cells.[15,99] An IgE-antigen reac-
tion can trigger the release of potent mediators including hista-
mine,[255] slow reacting substance of anaphylaxis(SRS-A)[255], and
eosinophilic chemotactic factor.[110]

Intuitive Arguments

The idea that individuals with atopy may have a significantly
lower incidence of malignancy has occurred to many investigators. One
intuitive argument could be stated as a syllogism: Cancer begins as a
failure of the immune system, an immunodeficiency; in allergy, the im-
mune system is over-active or hypersensitive; therefore, perhaps the
immunosurveillance system of an atopic is more likely to reject a clone
of malignant cells before a cancer is clinically apparent.

In rejecting this logic, a response might stress that the patho-
genesis of atopy should not be considered merely as an excess of IgE or
an exaggeration of the normal immune response. First, no study has
shown that all those with asthma, allergic rhinitis, or atopic eczema,
have increased total IgE; roughly thirty to sixty per cent of atopics
can be characterized as such,[34,105,106,121,134,210] although an eleva-
tion of antigen-specific IgE antibodies does correlate more closely
with the incidence of allergic symptoms.[134] Second, atopics clearly
have other immune aberrations. Recent studies have suggested that T-
cell function, both as tested in vivo by delayed hypersensitivity skin
testing and in vitro as tested by mitogen stimulation, is depressed in
atopic disease,[75,138,155,262] although studies in our lab and else-
where have not confirmed these findings.[38,52,55,65] Serafini has shown

that allergic patients have markedly increased autoantibodies of the thyro-gastric group.[203] Evidence suggests that reaginic antibodies are qualitatively and not just quantitatively abnormal in atopic persons.[251] Some have contended that the primary immune disturbance in allergic individuals is actually a transient IgA deficiency, not an IgE elevation.[217,226] And numerous excellent studies in animals are demonstrating the role of the T-cell in regulating IgE.[79,81,174-176, 222-225]

A second intuitive argument considers genetic laws. The incidence of atopy may be as high as twenty-four percent of the population.[207] Allergy has long been recognized as familial[45] and now evidence has mounted that both in[20,21,130,150] man and mouse[151,229,230] IgE levels are genetically determined. One would hope to find then some selective advantage in being allergic, but none has been proven. While IgE levels are clearly elevated in many parasitic infestations, only debatable arguments claim any benefit from this elevation.[34,171, 125,126] Some have suggested that IgE protects against respiratory infections if IgA is absent[7,87] and that possibly the IgE system serves as a warning about pollutants, but equally good data indicate that IgE in the presence of IgA deficiency predisposes to pulmonary disease.[184] Individuals apparently perfectly normal have been shown to be IgE deficient.[132,184] Although without substantiation, the argument is tantalizing. If a quarter of the population has a genetic defect, namely the tendency to develop allergic symptoms, then there must be an unrecognized selective advantage somehow involved with this "defect".

Hoping to show that this selective advantage involves the response to tumors, at least nine separate investigations have been published on

the incidence of allergy or atopy in patients with malignan-
cies.[56,63,66,139,141,156,159,204,228] The first such study[139] pub-
lished in 1953 actually concluded that patients with cancer, mainly
carcinoma of the cervix in this report, were far more likely to have
allergies (27%) as compared to controls (11%). The study has several
major defects, however, including the authors' effort to prove an
hypothesis that they had formulated before the investigation, thus
biasing their inquiries, and the lack of age or sex matched controls.

Of the eight subsequent studies along this line, five concluded
that patients with malignancy had a significantly decreased incidence
of atopy, while three studies disclosed no difference between normals
and patients with cancer. These studies all differ markedly in such
essential parameters as the definition of allergy or atopy, the care
in matching controls, the range in types of malignancy studied, and
the size of the population. Some of the findings of all nine studies
are summarized in Table I. The publication of so many reports has
served to confuse rather than clarify, as evidence accumulates that
atopy is both relevant and irrelevant to the incidence of carcinoma.

Total Serum IgE and Malignancy

With the ability to measure serum IgE, however, it seemed that
the controversy could be settled more definitively by simple assaying
IgE in the serum of normals and those with cancer. Indeed, at least
four groups of investigators have tried this approach, but here, too,
opinion is not unanimous.

Augustin and Chandradasa[13] were first to publish their results on
serum IgE in patients with neoplasia in 1971. Using a radial immuno-
diffusion technique to measure IgE, they found that the mean serum value

TABLE I STUDIES ON ATOPY AND CANCER INCIDENCE

AUTHOR & DATE	RANGE OF ATOPY OR ALLERGY	NUMBER OF SUBJECTS	DESCRIPTION OF SUBJECTS	% CANCER PTS ATOPY	% CONTROL ATOPY	COMMENTS
LOGAN & SAKER 1953	ASTHMA, HAY FEVER, ECZEMA	CANCER -195	MOSTLY CERVI- CAL CARCINOMA	27	11	ONLY STUDIED TO CONCLUDE THAT ALLERGICS HAVE INCREASED INCI- DENCE OF MALIGNANCY
DWORIN ET AL 1955	HAY FEVER, AS- THMA, URTICAR- IA ECZEMA, ANGI- ONEUROTIC EDEMA	TOTAL -200	HODGKIN'S DISEASE ONLY	13	Not Done	NO CONTROLS BUT CONCLUDED THAT INCIDENCE OF MAJOR ALLERGY WOULD PROBABLY HAVE BEEN SIMILAR
FISHER- MAN 1960	BRONCHIAL ASTHMA SEASONAL RHINITIS ECZEMA	CANCER 1185 CONTROL 294	PATIENTS PRESEN- TING TO CLINICS; WIDE RANGE OF TU- MORS INCLUDED	3.2	12.9	MANY ATOPICS HAD REMISSION OF THEIR SYMPTOMS BEFORE CANCERS DEVELOPED; UTERUS, PROSTATE, & BREAST HAD ESPECI- ALLY LOW INCIDENCE OF ATOPY
MACKAY 1967	ASTHMA, HAY FEV- ER, NETTLE RASH, ECZEMA	CANCER 150 CONTROL 150	LEUKEMICS EXCLUDED	9.3	20.7	FEMALES WITH TUMORS WERE ESPECIALLY UNLIKELY TO BE ALLERGIC
URE 1969	NASAL ALLERGY	TOTAL 140	ALL PATIENTS ON A GYNECOLOGY WARD	0	20	ALLERGY AND MALIGNANCY WERE MUTU- ALLY EXCLUSIVE IN THIS SMALL STUDY OF PATIENTS ON A GYNECOLOGY WARD
SHAPIRO ET AL 1971	HAY FEVER, SKIN PROBLEMS, INJEC- TION REACTIONS	CANCER 605 CONTROL 3310	PATIENTS FROM 3 BOSTON HOSPITALS	7.1	6.6	ATOPY DETERMINED BY NURSE'S ADMIS- SION DATA AND THE FIRST FOUR DIS- CHARGE DIAGNOSES
GABRIEL ET AL 1972	ASTHMA, HAY FEV- ER, ECZEMA, URTICAR- IA, FOOD REACTION	CANCER 150 CONTROL 150	ALL LUNG CANCERS, CONTROLS WERE OUT- PATIENTS	2	14	CONTROLS AGE MATCHED WITH SIMILAR SMOKING INCIDENCE AS CANCER GROUP
MCKEE ET AL 1967	SEASONAL RHINITIS OR ASTHMA	TOTAL 403 CANCER 104 CONTROL 299	PATIENT WERE PRE- OPERATIVE WITH A VARIETY OF TUMORS	13.4	15	ALL SUBJECTS WERE INTERVIEWED PRIOR TO SURGERY SO THAT NEITHER PATIENT NOR INTERVIEWER KNEW IF THE DIAGNO- SIS WAS CANCER
MEERS 1973	ASTHMA OR HAY FEVER	CANCER 546 CONTROL 533	PATIENTS UNDER GOING RADIOTHER- APY	4.9	15	SUGGEST THAT IgE MAY SUPPRESS A MALIG- NANCY VIA " VASCULOCELLULAR DAMAGE"

of this immunoglobulin was depressed in 65 patients with cancer as compared to 50 controls. But IgE levels were also decreased in 48 hospitalized surgical patients. No statistically significant difference was found between the cancer group and the surgery controls or between the surgery patients and the normals.[263] Still, this report is often cited as one that shows decreased IgE in patients with cancer.

In 1972, Jacobs et al.[102] measured IgE levels in 50 patients with allergy, 100 normals, 200 with untreated advanced carcinoma, and 200 with carcinoma undergoing chemotherapy with cyclophosphamide. Both a radial immunodiffusion and solid phase radio-immunoassay (RIA) were used. Most of the tumors were bronchogenic carcinomas. While the majority of cancer paitents had low levels of IgE, many of the untreated subjects had falsely elevated serum levels as shown by the high levels by RIA and undetectable values by immunodiffusion. This false elevation may be due to an auto-antibody which is also present in normal sera and is of unknown significance.[160] Other authors have also found autoantibodies against IgE in normal serum.[1,242]

Arbesman et al.[9] utilized their own radio-radial immunodiffusion technique to evaluate IgE levels in patients with various diseases including malignancy. Of 282 patients with cancers, 32 had what the authors considered to be an elevated level, greater than 300 units per milliliter. Like the two previous studies, they conclude that IgE seems to be decreased with malignancy, but no statistically significant difference was proven between the cancer group and the blood bank donor controls.

The study of Waldmann et al.[231] was most recently published. The main advantage of their work is the use of a double antibody radio-

immunoassay[72] which is particularly sensitive when low values of IgE
or non-specific interference with the assay are possibilities.[185]
Their data is also submitted to statistical analysis and includes only
immunoglobulin levels of patients on first admission to the hospital,
thereby eliminating the complication of therapy possibly altering IgE
levels. Studying 331 cases of neoplasia, the authors conclude that
IgE levels are generally normal with cancer. However, patients with
B-cell malignancies such as multiple myeloma and chronic lymphatic
leukemia do have depressed levels of serum IgE just as they have de-
pression of other immunoglobulin classes.[231] This is often explained
as a crowding out of the normal B-cell or antibody-producing precursors
by neoplastic cells. Finally, and most intriguingly, patients with
Hodgkin's disease have clearly elevated levels of IgE which the authors
suggest may be due to a depression of T-cell function. Arbesman et al.[9]
had likewise shown elevated levels correlating with the stage of the
disease.
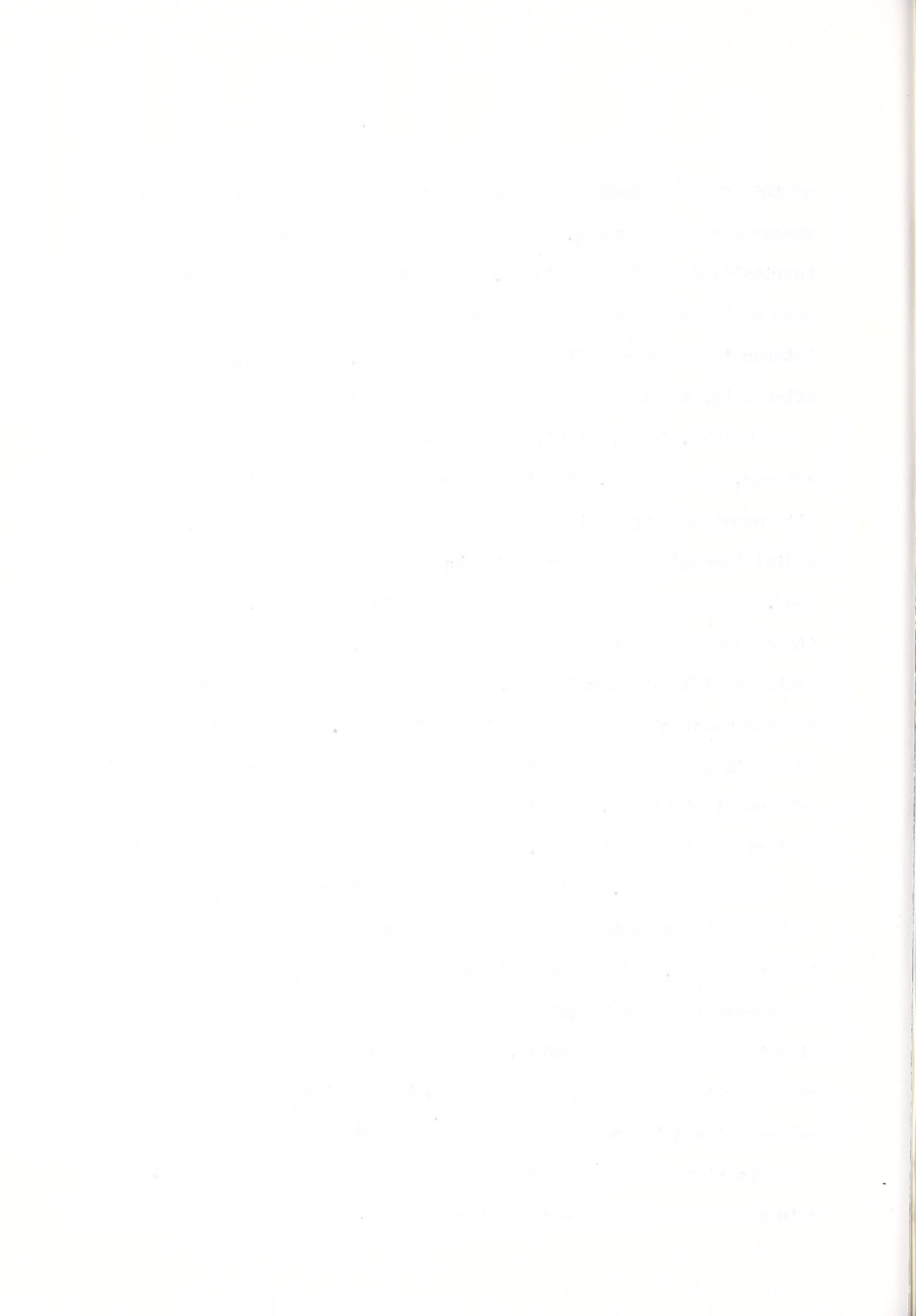
Numerous investigators are actively examining the role of the T-cell
in regulating IgE responses. Most manipulations that destroy T-cell
function including thymectomy,[175] irradiation,[173,175] and treatment with
anti-lymphocyte serum[240] seem to favor IgE production. One group has
isolated a soluble factor from thymocytes that can suppress an IgE res-
ponse.[223] Since Hodgkin's disease has decreased T-cell function with the
incidence of anergy correlating with the stage of the disease, a possible
cause of the elevated IgE, especially in stage III and IV disease, becomes
apparent.[198]

Although the association may just be coincidental, Hodgkin's dis-
ease is associated with two phenomena also related to IgE. The neph-

rotic syndrome not infrequently accompanies Hodgkin's disease, and as
IgE may be a causative factor in some forms of nephrosis, at least one
physician has speculated that the nephrotic syndrome in Hodgkin's
might be related to an IgE response.[41] Second, fifteen to twenty per-
cent of Hodgkin's disease patients have eosinophilia,[56] some to a very
marked degree,[250,258] and the speculative possibility exists that this
is related to reagin.

Hodgkin's Disease in an Animal Model

The SJL mouse strain develops a spontaneous neoplasm often consi-
dered to be an animal model of Hodgkin's disease.[3] Paradoxically, in
published reports so far, this mouse has been shown to be a very poor
producer of reagin[131] and it has even been noted that its failure to
make IgE might relate to its tendency to develop tumors.[129] Despite
its seeming inability to produce IgE, the SJL mouse has been reported
to be among the most susceptible mice to anaphylaxis,[227] although mice
have reaginic antibody other than IgE and non-reaginic factors may also
mediate anaphylaxis.

Haran-Ghera et al.[82] have made a thorough study of the SJL mouse
in order to consider the possibility of predicting by immune parameters
which individuals would develop the spontaneous reticulum cell neoplasm
resembling Hodgkin's. Examining such criteria as presence of antinuclear
antibodies, antibody response to sheep red blood cells, and rejection of
skin grafts, they were unable to distinguish those animals that were to
develop tumors. Warren[235] has shown that SJL mice make a poor immune
response to schistosoma mansoni eggs when compared to DBA/2 controls.
His criterion for response was the size of granulomata in the lung six-

teen days after the eggs were given intravenously. As many believe that
IgE is a crucial factor in responses to parasites, a poor reaginic res-
ponse might account for the smaller granuluma in SJLs.

With the evidence that humans with Hodgkin's disease have increased
IgE but that animals that supposedly develop a Hodgkin's-like tumor have
low IgE levels, further study is needed. What, for example, are the IgE
levels in the mouse after the onset of tumor? Could variability in mak-
ing IgE have predictive value as to which animal will develop the neo-
plasia? These studies would be facilitated by the ability to measure
total serum IgE in the mouse. This can be done in the rat[22],[42] thanks
to the discovery of IgE myeloma proteins in this species,[23] but comparable
tumors have not been described in the mouse. At present mouse IgE levels
can be titered only in relation to a specifice antigen by the method of
passive cutaneous anaphylaxis. (See methods in Part II.)

Tumor-Specific IgE Responses

Since nine studies have failed to show conclusively if atopics have
a decreased incidence of carcinoma, and four studies on serum IgE seem
now to point to normal levels of IgE in malignancies except in Hodgkin's
disease (for which a few authors would even question its status as a
neoplasia)[4], can IgE still be playing a significant role in response to
neoplasia? Some experimental work published in 1958 strongly suggests
that IgE may play a critical role in responding to some tumors.

Seventeen years ago, Grace and Kondo[74] reported on eight patients,
give with breast cancer, two with Hodgkin's and one with a lymphoma, all
of whom had "heat, edema, and tenderness" locally about their tumor, With
extracts of each tumor, they produced a wheal and flare reaction within
fifteen to twenty minutes after an intradermal injection into the patient's

own skin, an immediate hypersensitivity response as is mediated by IgE.
As controls, patients had no reactions to saline or normal tissue. As
added proof, the reaction could be passively transferred by first in-
jecting a normal volunteer with appropriate serum intradermally and
one day later challenging that site with solubilized tumor antigen.
This classic Prausnitz-Kustner (P-K) reaction following a twenty-four
hour sensitization must be mediated primarily by IgE,[91,186,211] but as
IgE was not known to be a distinct antibody class until 1966, the true
significance of this report has been overlooked. The paper is usually
cited only to show that antibodies, but not specifically reaginic anti-
bodies, were made against the tumor. A P-K reaction was also demonstrated
by Grace and Dao[73] in a patient with breast cancer and dermatomyositis, and
later by Curtis et al.,[49] again in a paitent with dermatomyositis, though
this patient had a primary carcinoma in the signoid colon and a metastasis
in the lung from which the extract was made. Grace was unable to pro-
duce the reaction unless the patient had some sort of cutaneous mani-
festation of his malignancy.

The existence of a tumor-specific IgE responses is not at all in
conflict with the concept that total serum IgE is normal in malignancy.
Normal levels of IgE vary widely. For example, the lower limit of normal
was one hundred and thirty times less than the upper limit of normal in
one study.[72] Obviously a very significant amount of tumor-specific IgE
could be produced and total serum levels of IgE might still remain within
a normal range.

Only in 1972 was an animal study published proving that a mouse
could produce IgE specific for a tumor. Bartholomaeus and Keast[16] achi-
eved this with the B16 melanoma and C57Bl/6 mice and also showed that
IgE could be produced against alloantigens in a transplant situation.

No data in man or animal has as yet been published to suggest if this IgE response is of benefit to the host.

Some evidence in animals, however, might be related. Parasitic infestations have been shown to act similarly to an adjuvant in stimulating an animal to rpoduce IgE against various antigens.[42] Keller et al.[111] reported that mice infected with Nippostrongylus brasielensis, a nematode, were resistant to growth of a syngeneic mammary adenocarcinoma. In rats, the Walker sarcoma could be inhibted or enhanced depending on the timing of the parasitic infection and tumor challenge. These authors suspected reagin but were unable to demonstrate it. Another study shows that mice with trichinella spiralis have a longer survival and a decreased incidence of spontaneous mammary tumors.[238] Infections with trypanosoma cruzi may similarly affect spontaneous breast cancer in mice. As an adjuvant, Bordetella pertussis augments IgE responses greatly[44] and also has value in tumor immunotherapy in animal[14,137] models and possibly in man.[77] Some speculate that IgE is the factor responsible for prolonged survival.[14,137] For all these observations, IgE, however, has not been proven to be the factor responsible for the tumor resistance and certainly multiple factors have been affected by the experimental manipulations.

The Implications of an IgE Response:

Some Hypotheses

At least five distinct but possibly related, hypothetical mechanisms might account for the role of IgE in response to malignancy.

The Gatekeeper Theory

An obvious explanation for the role of IgE is that the release of

histamine and other vasoactive substances triggered by IgE could be
causing a vascular change that would hasten tumor expulsion or des-
truction. As such, IgE or the mediators released from a degranulating
basophil might not have a direct effect on the tumor but would act
as a catalyst or "gatekeeper",[213] altering vessel permeability and
thus, allowing other mediators, immunoglobulins, or chemotactic sub-
tances to interact with the neoplasm. Although at first not believed
to activate complement, IgE has now been found capable of activating
the alternate pathway.[30,100] By virtue of its altering permeability,
IgE is said to be responsible for a model of immune complex disease
in rabbits.[25] Perhaps the destruction of tissue in immune complex
disease is not too dissimilar from the destruction of a malignant
clone.

Histamine

A second mechanism for the role of IgE involves histamine. Bourne[37]
and Lichtenstein[136] have elegantly reviewed the evidence that hista-
mine, by stimulating cyclic AMP, can modulate immune responses, in-
cluding basophil degranulation, cell-mediated immunity, antibody produc-
tion, and neutrophil phagocytosis. In in vitro experiments, histamine
retards all of these processes. If IgE can be directed specifically
against a tumor, it would be expected that the IgE response would lib-
erate histamine. Indeed, in a rat model it has been shown that after
immunization with tumor and B. pertussis adjuvant, tumor challenge
seven days later will release histamine from peritoneal exudate cells,
presumably via reagin.[220]

If in vivo studies confirm the in vitro work on histamine, the
first hypothesis might be that histamine release should potentiate

tumor growth by inhibiting lymphocyte mediated cytolysis. However, histamine seems to have so many possible roles that the inhibition of tumoricidal lymphocytes might be balanced by other positive factors. Histamine receptors have recently been shown to be of at least two types, H-1 and H-2.[31] Conventional antihistamines compete for H-1 receptors such as those responsible for smooth muscle contraction. The receptors for histamine on the basophil and the lymphocyte are of the H-2 type, that is blockable by the experimental pharmacologic agents, burinamide or metiamide. With the characterization of histamine receptors, work should now proceed rapidly elucidating the role of histamine vis à vis the immune response.

Cutaneous Basophil Hypersensitivity

Understanding a third possible interaction between IgE and tumor tissue may come with a greater knowledge of cutaneous basophil hypersensitivity (CBH).[53] This skin reaction to antigen is characterized by a delayed onset in time similar to classic delayed hypersensitivity, but the histology is marked by a high percentage of mast cells or basophils. CBH can be transferred with serum,[11] but since heating the serum to 56°C does not destroy the capacity to transfer,[12] IgE alone would not seem to be the serum factor. Still, with the surface of the basophil marked primarily by IgE, it is hard to believe that the basophil could be involved without the principle immunoglobulin on its surface membrane somehow participating, possibly even in a negative sense.

Further, in animal models CBH has clearly been demonstrated to have a role in tumor rejection.[54] In man the potential for CBH reactions can be monitored by a method called the skin window similar to delayed hyper-

sensitivity skin testing. Antigen is placed over a skin scratch and the area covered by a coverslip. Periodically the cellular exudate collecting on the slip is examined and the percentage of basophils counted. Using this method, Black and Leis[32,33] have found that patients with breast cancer react to their autologous tumor with a basophil-laden exudate. They have found that the percentage of basophils correlates with the sinus histiocytosis in the regional lymph nodes at the time of surgery which in turn correlates with favorable prognosis. The crucial role of the basophil awaits fuller clarification, but surely IgE will be found to have a hand in its work.

The Eosinophil

Fourth, IgE has been closely linked with eosinophilia. Eosinophils are prevalent in allergies and parasitic infections, and a high peripheral eosinophil count is not uncommon with neoplasia.[50,89] The eosinophil produces an enzyme, arylsulfatase, that neutralizes SRS-A[236] and eosinophils may inhibit histamine relase from basophils.[88] Kay and Austen[110] have demonstrated that IgE can cause the release of an eosinophilic chemotactic factor (ECF) and ectopic production of ECF has been shown in a case of carcinoma.[237] In a phenomenon termed radiation-related eosinophilia, patients with pelvic or abdominal carcinoma who receive supervoltage irradiation may develop an eosinophilia which actually correlates with their prognosis.[71] The evidence is only circumstantial but it is tempting to speculate that radiation stimulates IgE perhaps by altering the antigenicity of the tumor or by reducing suppressor T-cells. The increased IgE might cause the eosinophilia and either the IgE itself or the eosinophil might favor the host response over the tumor. Such reasoning is

plausible but highly speculative, and eosinophils, like IgE, may be
working toward the patient's detriment rather than his health. Also,
although only studied in four patients, total serum IgE has not been
found elevated in radiation-related eosinophilia.[243]

T-cell Function

Finally, unraveling the relationship between IgE and the T-cell
should be crucial in understanding the role of IgE in response to malig-
nancy. Generally the T-cell is thought to be primarily responsible for
cell-mediated immune responses that can aid the host by destroying tumor
cells, while a humoral or B-cell response to a tumor can have a variable
prognostic effect and may account for enhancement of tumor growth via
blocking antibodies.

Besides Hodgkin's disease, other illnesses with decreased T-cell
function may have elevated IgE. In infectious mononucleosis, which is
frequently characterized by anergy,[143] IgE may be transiently elevated.[168]
In sarcoidosis, which is known to be associated with frequent anergy,
German investigators found IgE higher than in normal and tuberculous
controls.[256] And in atopy, as previously alluded to, while most think of
the primary immune dysfunction as increased IgE, several have found de-
creased T-cell function.

If decreased T-cell function is the primary defect causing elevated
IgE, several paradoxes arise. First, besides being responsible for
suppressing IgE, the T-cell bears a factor that enhances IgE production,[225]
so one must posit that this function is intact while the suppressive func-
tion is abrogated. Second, many diseases can be shown to have diminished
T-cell function, including commonly most malignancies.[209,246] Why should

not any tumor with depressed cell-mediated immunity have increased IgE?

Third, how can measles vaccine simultaneously suppress both atopic sym-

toms and T-cell function?[265]

Possibly IgE accounts for negative feedback to the T-cell. Acting

via histamine or other mediators, it may be IgE capable of suppressing

the T-cell as well as the T-cell capable of suppressing IgE.

Conclusions

At present, the relationship between IgE and other functions of

the immune system is confusing. No unifying hypothesis has been able

to relate IgE, the T-cell, CBH, histamine, and the eosinophil. Consider

these puzzles.

Many patients with ataxia-telangiectasia have absent IgE[7,184] and

this disease has a high rate of lymphoma at very young ages.[233] On the

other hand, some of the highest IgE levels have been recorded for patient's

with Wiskott-Aldrich syndrome[29,232] and they, too, develop neoplasia early

in life.[233] If IgE abets a host response toward the tumor, why is T-cell

function supposedly depressed in atopic eczema and why does histamine turn

off tumor cell lysis? Are atopics more likely than others to produce an

IgE response toward a tumor? If a tumor-specific IgE response is demon-

strable, what effects does it have on the prognosis? What is unique about

Hodgkin's disease when other malignancies do not have an elevated IgE?

An intriguing area for studying the role of IgE in response to neo-

plasia might come from understanding the pathophysiology of dermatologic

manifestations of malignancy. Although its role is uncertain, IgE has been

implicated in a variety of dermatidides, not just atopic eczema.[47] In

diseases like dermatomyositis, erythema multiforme, and pemphigoid,

diseases which may herald an occult malignancy, IgE may prove to be active in the pathogenesis of skin signs. In all the reports of either Grace or Curtis in which P-K reactions were demonstrated for tumor patients, the individuals had some cutaneous manifestation of the tumor. Arbesman has found that IgE is commonly elevated in patients with pemphigoid and in the fluid of its bullae.[8] All of this information suggests that the area of IgE, carcinoma, and skin signs demands further exploration.

While many riddles remain to be solved and questions need to be answered, the role of IgE in response to neoplasia is emerging as a provocative topic for study.

Experimental Design and Rationale

Desiring to clarify the relationship between IgE and neoplasia, we designed experiments to help resolve some of the specific issues raised in this literature review. Some of the studies explored directly the relation between IgE and a tumor; these are described in Part III. Other studies stemmed from a need to understand certain essentials of the IgE response and to perfect a serum assay. These studies were logical preliminaries to those described in Part III and will be considered in Part II. The value of undertaking these preliminary projects can perhaps best be demonstrated by first explaining the projects with a direct relation to tumors.

In Part III, one finds experiments to: 1) demonstrate an IgE response to tumor in animals, 2) evaluate the ability of an animal bearing tumor to make reagin 3) measure IgE levels in children with neoplasms and 4) show that humans can make a tumor-specific IgE response.

Demonstrating IgE responses to tumor in animals was a repetition
of the work of Bartholomaeus and Keast, but it was essential in order
to evaluate what effect this IgE response had on the course of the
malignancy. Evaluating the ability of an animal with tumor to make
reagin was designed to answer whether low serum IgE in patients with
cancer or high serum IgE in Hodgkin's disease was a cause or effect of
the tumor. Measuring IgE levels in children with cancer was a test of
the genetic hypothesis that atopics have a selective advantage in resis-
tance to neoplasms. Finally, showing that man can make tumor-specific IgE
was a way of duplicating the work of Grace and others without asking
healthy volunteers to accept injections of tumor antigen as is done in
the P-K reaction.

With these projects in mind, we undertook the preliminary work des-
cribed in Part II. Obviously in order to do the work on childhood neo-
plasia, we needed to be able to measure serum IgE. So our work with a
RIA for IgE is the first study described. Second, as we were going to
be measuring IgE, we wished to be certain that the time of day that the
sample was drawn was not influencing the serum level. So ruling out a
diurnal rhythm for IgE was a necessary undertaking. Finally we conducted
experiments to delineate the ontogenetics of IgE production in the neonatal
mouse, that is, the relation of IgE to IgM, G, and A. We realized that
such a project would not directly reveal the interplay between IgE and
tumor, but understanding the normal physiology of the IgE response will be
essential in understanding the factors that determine an IgE response in
a patient with cancer. For example, the ontogenetic model that we devised
should allow us to study the relationship between IgE and IgA deficiency.

The breast can produce secretory immunoglobulin and evidence suggests that an IgA response may be prognostically significant in women treated with radiation following mastectomy.[161] On the other hand, Grace's work showing P-K reactions in five patients with breast cancer and Black's studies that show that a basophilic response is prognostically significant in breast carcinoma, both suggest that IgE may be important in breast tumors. Our work on ontogeny could help elucidate the interplay of these two secretory immunoglobulins, E and A. Just as the explanation for elevated IgE levels in patients with Hodgkin's disease has derived from studies that furthered the knowledge of the normal physiology of the IgE response but had no direct bearing on tumor research at the time they were performed, so we hoped that our work on the ontogeny of IgE would further our knowledge of the normal physiology of the IgE response and perhaps eventually prove relevant to tumor immunology.

PART II: Basic Studies

(A) Radioimmunoassay

One of the first problems to be solved in order to study IgE was
the perfection of a method to assay its concentration in serum. A num-
ber of techniques have been described including a solid phase radio-
immunoassay (RIA) using sepharose particles (RIST),[105] a RIA using
antibody bound to bromacetyl cellulose,[21] a double antibody RIA,[72] radio-
radial diffusion,[9] immunodiffusion,[102] and mixed immunosorbent RIA.[1]
Of the two methods available commercially at the time of this writing,
immunodiffusion can only detect elevated levels of IgE. The other,
Pharmacia's radioimmunosorbent test (RIST) (Phadebas Test, Pharmacia,
Piscataway, N.J.) is a radioimmunoassay essentially identical to that
used by Johansson et al. shortly after their having described the first
known case of an IgE myeloma.

With practice, the Pharmacia method proved adequate for measuring
serum IgE in the high normal to elevated range. However, for values
in the range of 10 to 100 units/ml, still within normal limits, counts
from standards with known, differing concentrations of IgE began to
overlap and values were not reproducible. This is illustrated by the
typical standard curve shown in Figure 1. It demonstrates graphically
the difficulty in using this assay. Even a group that included Johansson
himself has cautioned against the method for measuring low levels of IgE[216]
and a comparison study of three methods concluded that the double antibody
RIA is the superior method for both reproducibility and assessment of low
levels.[185]

FIGURE I

Radioimmunoassay of IgE
STANDARD CURVE

1 international unit ≅ 2.4 ng

FIGURE I
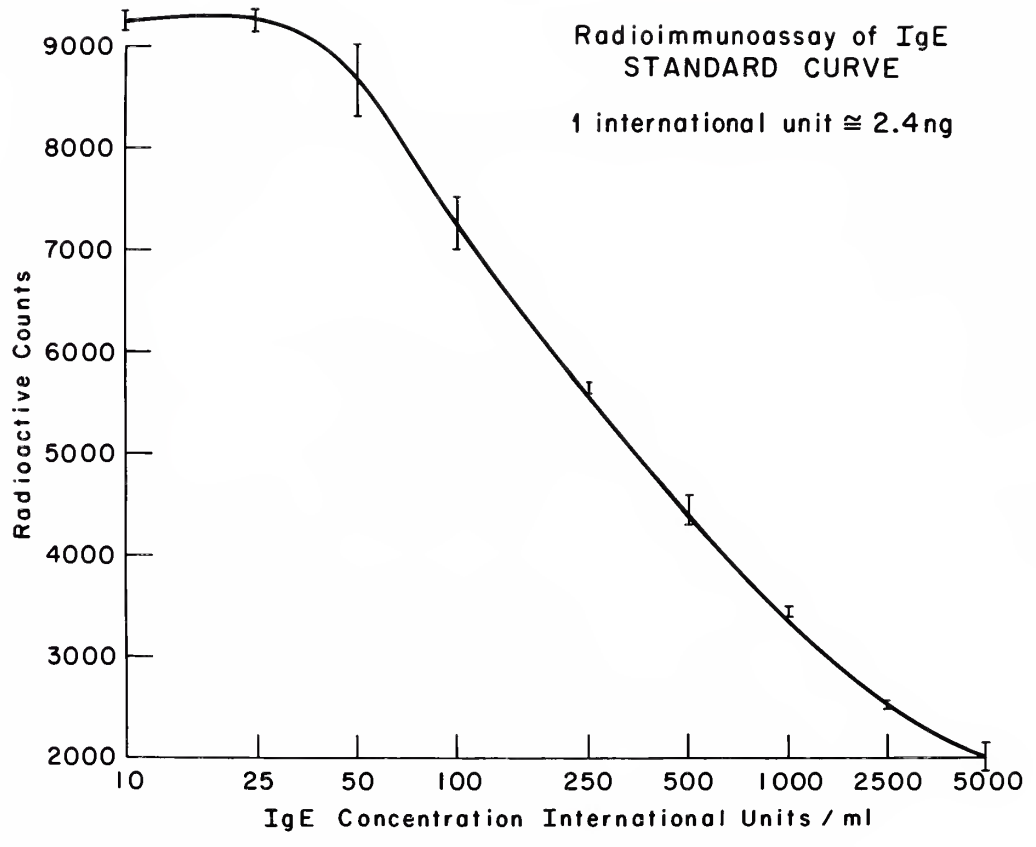
(B) Circadian Rhythms

Wishing to measure serum IgE, we need to explore the possibility that serum levels might have a diurnal variation. While other immunoglobulins are not known to have daily fluctuations, IgE is unique in being present in such low concentrations, having the shortest half life,[234] and being tissue bound.[101] The symptoms of allergy commonly vary with the time of day. And many factors possibly related to allergic symptomatology do have daily patterns including pollen counts, plasma cortisols, and circulating eosinophil counts.[200] Conceivably, then, serum IgE itself might have a diurnal rhythm, perhaps in atopics if not in normals, and possibly correlating with eosinophil counts.

Multiple determinations, however, were performed on a single unknown sample and counts for an individual determination differed by as many as 1200. With this range of values on a single unknown, it seemed doubtful that the method would be reproducible enough to detect the relatively subtle changes that might occur throughout the course of a day.

Testing the hypothesis of circadian rhythms of IgE must await either 1) demonstration of greater reproducibility of values of unknown sera or 2) perfection of an alternate method to assay IgE.

(C) Ontogeny of the IgE Responses:
The Effect of Anti-Heavy Chain Antisera

Investigating the ontogeny of the IgE response in mice was a major concern of this thesis project. While the results could not directly resolve the relation between IgE and neoplasia, furthering the understanding of IgE production cannot help but bring us closer to elucidating the

role of IgE in host reactions to malignancies.

Kincade and Cooper first described treating chickens with anti-heavy chain immunoglobulin.[113,114] When given in ovo injections of anti-mu, that is antisera directed against the heavy chain of chicken IgM, bursectomized chickens not only failed to produce IgM, but also could not synthesize IgG. Working with neonatal, germ-free, Balb/C mice, treatment with anti-mu chain immunoglobulin also prevented production of the immunoglobulin classes,[122,123] M,G, & A. Treatment with anti-gamma chain prevented IgG and IgA responses, but not the response of IgM. Finally, intraperitoneal injections of anti-alpha chain precluded the appearance of IgA, but did not affect IgM or IgG. The explanation offered by the authors is that ontogenetically the mouse's B-cells progress from a sequence of producing IgM to IgG to IgA. The anti-heavy chain antisera interrupts this sequence theoretically either by having a lytic effect on the B lymphocytes or by interfering with their membrane receptors.

This work has been repeated by other investigators in mice that were not kept in a germ-free environment[145,148] and in random bred animals.[166] The effect of anti-heavy chain has also been studied on lymphocytes grown in vitro.[178,179] Studies have confirmed the initial reports with the modification that IgA producing cells may come directly from IgM precursors.[144,151] No reported study has concerned itself with relationships of IgE to the sequential development of immunoglobulins M, G & A, but the question has considerable clinical importance.

Several investigators have measured IgE concentrations in patients with hypogammaglobulinemia, including common variable 'acquired' and Bruton's sex-linked hypogammaglobulinemia.[215,232,184,87,269] Each study has shown that greater than ninety percent of hypogammaglobulinemic sub-

jects have depressed levels of IgE. However, low levels of IgE, as has been mentioned, are difficult to measure. Normal serum levels of IgE are less well established and vary more widely than the normal levels of other immunoglobulins. Subjects who are apparently completely normal and who have absent IgE have been reported.[132,184] Possibly some of these depressed levels of IgE in these hypogammaglobulinemic subjects are within normal range.

Several pieces of evidence suggest that IgE might have an ontogenetic development independent from the M to G to A progression. First, Ishizaka et al. have studied the production of IgE in vitro in primed lymphocytes treated with anti-mu sera.[95] These lymphocytes were able to produce IgE despite the presence of anti-heavy chain sera and the reduction of other immunoglobulins. However, because this work was done with primed lymphocytes, i.e. lymphocytes that had been exposed to antigen prior to the treatment with anti-mu and re-exposure to antigen, the study merely indicates that the cells producing IgE were unaffected by anti-mu but says nothing about the possible ontogenetic precursors of these IgE-bearing cells.

Second, although acquired hypogammaglobulinemia is not a single entity, but a group of disorders with a variety of causes,[2,69] some hypogammaglobulinemic subjects retain IgE despite loss of other immunoglobulin classes. In an experimental model in rabbits, it is possible to stimulate IgE without detectable antibodies of other classes appearing.[268] In doing immunofluorescence studies on lymphoid tissue, Tada and Ishizaka reported on one hypogammaglobulinemic subject who appeared to retain a normal number of IgE-bearing lymphocytes despite markedly depressed

plasma cells or B-cells of other immunoglobulin classes.[221] Two other similar patients were devoid of IgE as well as IgG, M, or A bearing cells. A recent case report describes a hypogammaglobulinemic patient who retained normal levels of IgE and suffered from a malabsorption syndrome.[128]

Third, many patients with hypogammaglobulinemia develop anaphylactoid symptoms when being treated with passively administered gamma globulin.[59] Both these observations suggest that the synthesis of IgE may be preserved while other immunoglobulins are absent. Schumacher and Ellis,[201] however, have studied several hypogammaglobulinemics and demonstrated that their basophils do not release histamine in the presence of foreign gamma globulin indicating that the anaphylactoid responses may not be due to IgE-mediated reactions.

Fourth, Gajl-Peczalska et al.[67] reported a case of Bruton's sex-linked agammaglobulinemia in which the subject had demonstrated allergic symptoms and had apparently a normal number of circulating IgE-bearing lymphocytes while B-cells of other immunoglobulin classes were virtually absent. Because of the possible confusion between basophils and IgE-bearing lymphocytes when studied by immunofluorescence, the conclusions of this study have been questioned.[195] A second case report[78] describes another child with Bruton's hypogammaglobulinemia who had positive immediate hypersensitivity skin tests and who reacted with a wheal and flare to injections of anti-IgE, thus demonstrating the presence of IgE on his mast cells despite absent serum levels of other immunoglobulins.

Finally, occasionally patients with severe combined immunodeficiency, a condition with depressed T-cell as well as B-cell function, will have

an elevated serum IgE,[267] while other immunoglobulin levels are
typically depressed.[85]

With this conflicting evidence relating to the ontogeny of IgE,
we studied the production of IgE in mice treated with anti-mu sera
from birth.

Methods:

Antisera- Goat anti-mouse mu sera (lot 40667 and/er lot 40900) was
purchased commercially from Meloy laboratories, Springfield, Virginia.
An absence of anti-light chain activity, kappa or lambda, or anti-heavy
chain activity other than anti-mu was demonstrated with Ouchterlony gel
diffusion plates. The purchased antisera was preserved with sodium
azide and was dialyzed against sterile saline to remove this preservative
prior to injection into animals. Control animals were injected with bevine
gamma globulin (BGG), Cohn Fraction II, Sigma Chemical Co. BGG was dissol-
ved in sterile saline at a concentration of 10 mg/ml, passed through a
0.20 micron millipore filter, and stored in sterile glass syringes until
ready for use.

Animals- Mice were provided from the specific pathogen free colony of
the Biobehavioral Science Research Center of the University of Connec-
ticut, Storrs, Connecticut, at a nominal cost. In the preliminary work,
DBA/1 mice were selected for treatment with anti-mu as these mice are
especially reactive to evalbumin (OA), an antigen for which IgE anti-
bodies can be readily demonstrated by passive cutaneous anaphylaxis (PCA)
(see below).

A number of difficulties forced us to abandon the use of DBA/1
mice. First as mothers, these mice are particularly temperamental and
it was necessary to use surrogate C57Bl/10 or C57Bl/6 lactating females.

Second, the animals produce small litters. Third, the animals are relatively small in size. And fourth, these mice were especially sysceptible to stress and infection. Although we began with roughly forty DBA/1 infants, only seven (four experimentals and three controls) were able to survive to the end of the experiment thirty-four days after birth.

Of these experimental animals that did survive, all were runted when compared to the controls. The weights of the two groups were statistically different by Students' T test, $p < 0.0001$. Thus, it was impossible to tell if the immunoglobulin titers produced by the experimentals reflected the effect of immunosuppression by anti-mu or the effect of the runting itself.

These problems were minimized in subsequent experiments with the use of the first generation products of the mating of DBA/1 males and C57Bl/10 females. These animals had a number of advantages. The litter size was larger. The animals were healthier. The need for surregate mothers was obviated. And the reactivity of these animals to OA was at least as great as the reactivity of the DBA/1s.[230]

Male and female six week old Swiss mice from the Sterling Hall of Medicine colony, Yale University Medical School, were used as recipients of sera to titer IgG1 antibodies by PCA. Preliminary work had shown these mice to be comparable to CFW mice (Carworth Farms, New City, New York). CFW are a standard breed used by other investigators for PCA work.[131] Two hundred gram male Sprague-Dawley rats were purchased from Charles River Laboratories, and used as recipients for IgE titration by PCA as described by Mota and Wong[164] and Konig et al.[119]

Injection schedule- The schedule for treatment with anti-mu varied

slightly from litter to litter, but in most cases, neonatal DBA/1

x C57Bl/10 mice received their first injection, either antisera or

BGG, within twenty-four hours of birth. This was considered to be

day one. Animals were given 0.05 cc on days one through four intra-

peritoneally (i.p.) and 0.10 cc on days 9, 16, and 26. The total dose

was therefore 0.50 cc. On day 21 all animals were given an i.p.

injection of ovalbumin (OA) (twice recrystallized, Worthington Bio-

chemical, Freehold, N.J.) 0.1 ug dissolved in saline plus alum, 1.12mg,

in a total volume of 0.1cc. The alum was prepared as described by Levine

and Vaz[131] and had a dry weight of 28 mg/ml. On day 34, thirteen days

after exposure to antigen, all animals were placed under light ether

anesthesia and bled repeatedly through the retro-orbital plexus with

glass pipettes. Approximately 0.20 cc of serum was obtained per animal.

Titration of immunoglobulin levels-

A) PCA: This technique has been well described by Ovary[177] and others.[44]

[131] In brief, mouse sera contains two immunoglobulin classes of reaginic

antibody, IgE and IgG1.[34,189] Reaginic antibodies can be thought of as

those immunoglobulins that can passively sensitize the surface of mast

cells or basophils and which cause the release of serotonin, histamine,

and/or other vasoactive substances from these cells when exposed to

appropriate antigentic stimulus. Unlike IgG1, IgE activity can be destroyed

by heating at 56°C or by treatment with 2-mercaptoethanol.[34] In mice,

activity of passively injected IgE will persist after 48 hours by which

time essentially all IgG1 activity has disappeared.

To titer IgG1, sera was diluted with saline in a two fold manner

beginning at a concentration of one to five. The backs of Swiss mice were shaved and after light ether anesthesia, 0.03 cc of diluted serum was injected intradermally into the shaved surfaces. Up to four injections of varying dilutions were given per back. After two hours, the recipient mouse was again given ether anesthesia and 0.15cc of saline with 0.7% CA and 0.4% Evans blue (Matheson, Coleman, and Bell, E. Rutherford, N. J.) was injected intravenously via the retro-orbital plexus. One half hour after that, the animals were sacrificed and reactions read by removing the skin from the back of the animal and observing the blueing at the injection site. Theoretically immunoglobulin from the passively administered serum will sensitize the mast cells in the recipient animal. When appropriate antigen is given, the mast cell will degranulate, releasing histamine and/or serotonin. This alters vascular permeability and allows the extrusion of Evans blue, causing the local blueing which could be easily read as a positive reaction. The titer of antibodies was considered to be the highest dilution of serum causing blueing of 5 mm or more in diameter in at least two of three mice. If a dilution of 1:5 failed to produce a reaction in a majority of mice, the titer was considered to be zero.

Titration of IgE antibodies in mouse sera can be done in either of two ways by using PCA. Recipient animals can either be mice or rats. For the mice, administering antigen i.v. 48 to 72 hours after the skin has been sensitized will detect essentially only reagin of the IgE class. An alternative method is to use rats, as mouse IgE, but apparently not IgG1, will bind readily to rat mast cells.[119]

Male Sprague-Dawley rats were anesthetized with sodium pento-
barbital (Diabutal, Diamond Labs, Des Moines, Iowa) approximately
0.80 cc/kg i.p. The back of the rat was shaved and up to 24 in-
jections of 0.05 cc of varying dilutions of sera were given per
animal. Two hours later the animal was anesthetized with ether and
injected i.v. through the dorsal vein of the penis with 1 cc of saline
containing 0.4% Evans blue and 0.7% OA. Half an hour later animals
were sacrificed under ether and their dorsal skin removed. The titer
was considered to be the highest dilution causing blueing of at least
5 mm in two of three animals.

B) Ouchterlony plates: Presence of IgG1 and IgG2 was determined by
appearance or absence of a precipitation line when serum diluted 1:5
was allowed to raact on a gel diffusion plate with the appropriate
class specific antiserum provided by Dr. Philip Askenase. This method
is described by Arnason.[10]

C) Passive hemagglutination: IgM and IgG antibody levels to OA were
measured by their ability to agglutinate sheep red blood cells (SRBC)
coated with antigen. Blood was defibrinated and the cells washed. Red
cells were removed and mixed for four minutes at room temperature with
3 mg 0.1% $CrCl_3.6H_2O$ (Malinckrodt Chemical, St. Louis) and 3 ml of OA at
a concentration of 10 mg/ml. The treated cells were washed five times
and resuspended in normal saline at a concentration of 5%. This suspen-
sion was diluted 1:5 in phosphate buffered saline.

Serum to be evaluated for antibody was tested in serial two fold
dilutions beginning at a dilution of 1:10. Rabbit hyperimmune antisera
to OA was provided by Dr. Philip Askenase and used for control. Untreated

serum was considered to reflect both IgM and IgG antibodies. To measure just IgG, serum was reacted with mercaptoethanol (Pierce Chemical Company, Rockford, Ill.) and tested at dilutions beginning with 1:20. To 0.1 ml of serum to be tested, 0.3 ml of 0.133 M mercaptoethanol was added and incubation carried out at room temperature for two hours.

Serial dilutions of serum were added to autotiter hemagglutinating plates containing the treated SRBCs and the agglutination end point read. Antibody titers are expressed as the dilution for which agglutination ended. Fifteen dilutions were tested for each sample. If no agglutination occurred at the lowest dilution tested, the titer was considered to be zero.

Splenic histology- Spleens were removed immediately after sacrifice and frozen in liquid nitrogen. Spleens were stored at $-90^{\circ}C$ until ready for sectioning and staining. Microtome sections of four to six microns were prepared. Tissue was stained with hematoxylin and eosin and evaluated with light microscopy.

Animal survival precautions- Obviously the survival of the animals was crucial in the performance of this experiment. Absorption of injections without rupture of bowel is a delicate technique in a newborn. These injections were skillfully given by Sandra Rosenbaum. To minimize the risk of cannabalization, we handled all animals with gloves, and during the first few days of life, rubbed the newborns' backs with Vicks Vapo Rub (Vicks Chemical Co, New York City, NY) immediately after injection in an effort to discourage the mother from injuring the litter and to minimize odor from handling.

Results:

The results of treating DBA/1 X C578El/10 mice with anti-mu
antisera and then immunizing with OA are given in Table II. Mean
values and standard deviations for the BGG treated and the anti-mu
treated groups are given in Table III.

Immunosuppression clearly was achieved. By Students' T test,
the difference between the two groups for IgG1 values as determined
by PCA was significant with p < 0.025. Similarly, comparing the IgM
and IgG values as determined by PHA showed p < 0.005. P < 0.01 for the
comparison of IgG levels as measured by PHA. In contrast, the IgE
values did not differ significantly between the two groups ($p > 0.3$).

Splenic histology is pending.

TABLE II. RESULTS OF ANTI-MU TREATMENT ON

IMMUNOGLOBULIN PRODUCTION

Litter	Animal	Treatment	Weight (gms.)	Sex	IgE by PCA	IgG+IgM by PHA	IgG by PHA	IgG1 by PCA	IgG1 by Ouchterlony[1]	IgG2 by Ouchterlony[1]
1	A	ANTI-MU	5.8		0	0	0	0	+	0
	B	ANTI-MU	9.3		40	0	0	0	+	0
	C+D[4]	BGG	4.2 5.2		20	0	0	0	N.T.[5]	N.T.[5]
	E	BGG	10.8		1280[2]	5000[3]	640	160	++	++
2	A	ANTI-MU	15.0		160	80	0	0	±	±
	B	ANTI-MU	19.0		40	0	0	0	+	±
	C	BGG	18.2		320	1280	80	5	+	±
	C	BGG	16.9		160	640	40	0	+	±
3	A	ANTI-MU	14.9		320	320	80	0	±	±
	B	BGG	13.6		640	5000[3]	1280	80	+	±
	C	ANTI-MU	14.2		1280[2]	160	0	10	0	0
	D	ANTI-MU	16.3		320	10	0	0	0	0
	E	BGG	15.7		80	10,000[3]	1280	40	+	0
	F	BGG	14.9		80	5000[3]	320	40	+	0
4	A	ANTI-MU	17.0		640	1280	160	40	+	0
	B	BGG	15.8		1280[2]	5000[3]	160	160	+	+
	C	ANTI-MU	15.9		640	160	0	0	+	±

1 All sera tested at 1:5 Dilution
2 Not tested at dilution greater than 1280
3. Values over 5000 are rounded to the nearest thousand
4 The small size of these two animals necessitated combining their sera for
5 NT = Not tested   titration

TABLE III   MEAN VALUES AND STANDARD DEVIATIONS FOR

IMMUNOGLODULIN LEVELS OF ANTI-MU AND BGG TREATED MICE

Treatment	IgE by PCA	IgG by PCA	IgG + IgM BY PHA	IgG by PHA
Anti-Mu	382 ± 391	5.6 ± 12.6	223 ± 387	27 ± 53
BGG	482 ± 495	60.6 ± 62.7	3990 ± 3055	475 ± 502

Discussion:

The data indicate that while treatment with anti-mu produced a statistically significant reduction in anti-OA antibodies of the IgM and IgG class, anti-OA of the IgE class was not similarly affected. The lack of dramatic differences between the two groups in total serum IgG1 and IgG2 as measured by Ouchterlony technique can be explained by maternal antibodies, especially as animals were either not weaned or weaned just prior to the termination of the experiment.[145]

While a number of clinical and experimental observations on individuals with hypogammaglobulinemia as referred to earlier might have suggested this result, the outcome nonetheless seems counter to the fact that most hypogammaglobulinemic persons have a significant reduction in IgE.

Another piece of experimental evidence would certainly seem to suggest that anti-mu treatment would decrease serum IgE. Taniguchi and Tada have recently shown that a solubilized fraction of T-cells can serve a helper function in immunoglobulin production.[225] This helper molecule can be absorbed by anti-mu. They believe that the helper activity may be an IgT, an immunoglobulin on the surface of the T-cell. Unlike IgG, passively administered IgM in small amounts acts to enhance the production of immunoglobulin[83] including IgE.[218] One speculation would be that this effect is mediated by priming helper T-cells.

One would expect then that treatment with anti-mu would exert a

significant effect on IgE production if only by virtue of its potential action on T-cells.

Abundant evidence, however, indicates that the effect of anti-mu is independent of T-cells. In in vitro spleen cell cultures, T-cells can be removed, but the effect of anti-mu will not be diminished.[178] Anti-mu antibodies have a suppressive effect even if the antigen is thymus-independent such as ferritin.[123] Anti-mu antibodies can prevent the transplantation of such B-cell tumors as Friend leukemia virus leukemia[149] or myelomas.[146] And anti-mu does not effect such T-cell functions as skin graft rejection.[147] and possibly helper function itself.[265]

Still specific T cell functions may be attributable to subpopulations of T-cells[205,257] and a subset responsible for assisting or suppressing the ontogenetic development of the B-cell line might be affected by anti-mu while other T-cell functions are undisturbed. If work substantiates that some T-cells bear an IgT with mu chain determinants,[225,261] one would predict that anti-mu treatment should affect T-cell function, including helper function in the production of IgE. In our work, either helper function was unaltered or a second effect of anti-mu compensated for diminution in helper function and permitted normal serum IgE levels.

While treatment with anti-mu suppresses IgM, IgG, and IgA, a return to normal levels of IgA despite prolonged treatment with antisera has been reported.[145,148] It is unlikely that such a rebound is also occuring with IgE because the return of IgA values was observed only

after six weeks of treatment whereas at three weeks, the crucial time in this experiment when animals were exposed to antigen, IgA levels were always still markedly depressed. Since, however, the timing and extent of the reappearance of IgA is dependent on the dose of anti-mu,[146] we are conducting studies to determine if increasing the dosage of antiserum might result in a significant depression of IgE.

This experimental model utilizing anti-heavy chain immunoglobulin has potential for elucidating other aspects about the physiology of IgE. For example, the question of affecting IgE levels by selectively suppressing IgA is especially relevant as some have suggested that the true immunological defect in atopy is not an excess of IgE but a transient deficiency of IgA, which may be the only truly significant blocking antibody.[217,226] As IgE and IgA may both be important factors in the immune response in breast carcinoma (see p. 19), this work could help elucidate the relationship of these two immunoglobulins in response to malignancy.

Treating mice with anti-gamma 1 and/or anti-gamma 2 may help clarify the relation between IgG and IgE. A tradiational view holds that IgG is the principle blocking antibody and that IgG levels may be inversely related to IgE. Support for this hypothesis came when Tada and Okumura showed that rats given IgG passively produced markedly depressed IgE titers.[222] However, this report could not be reproduced by the Ishizaka and Okudaira[96] in mice, nor could the Ishizakas show an inverse relationship between IgG and IgE levels during the course of long term hyposensitization immunotherapy.[91] By treating slightly older
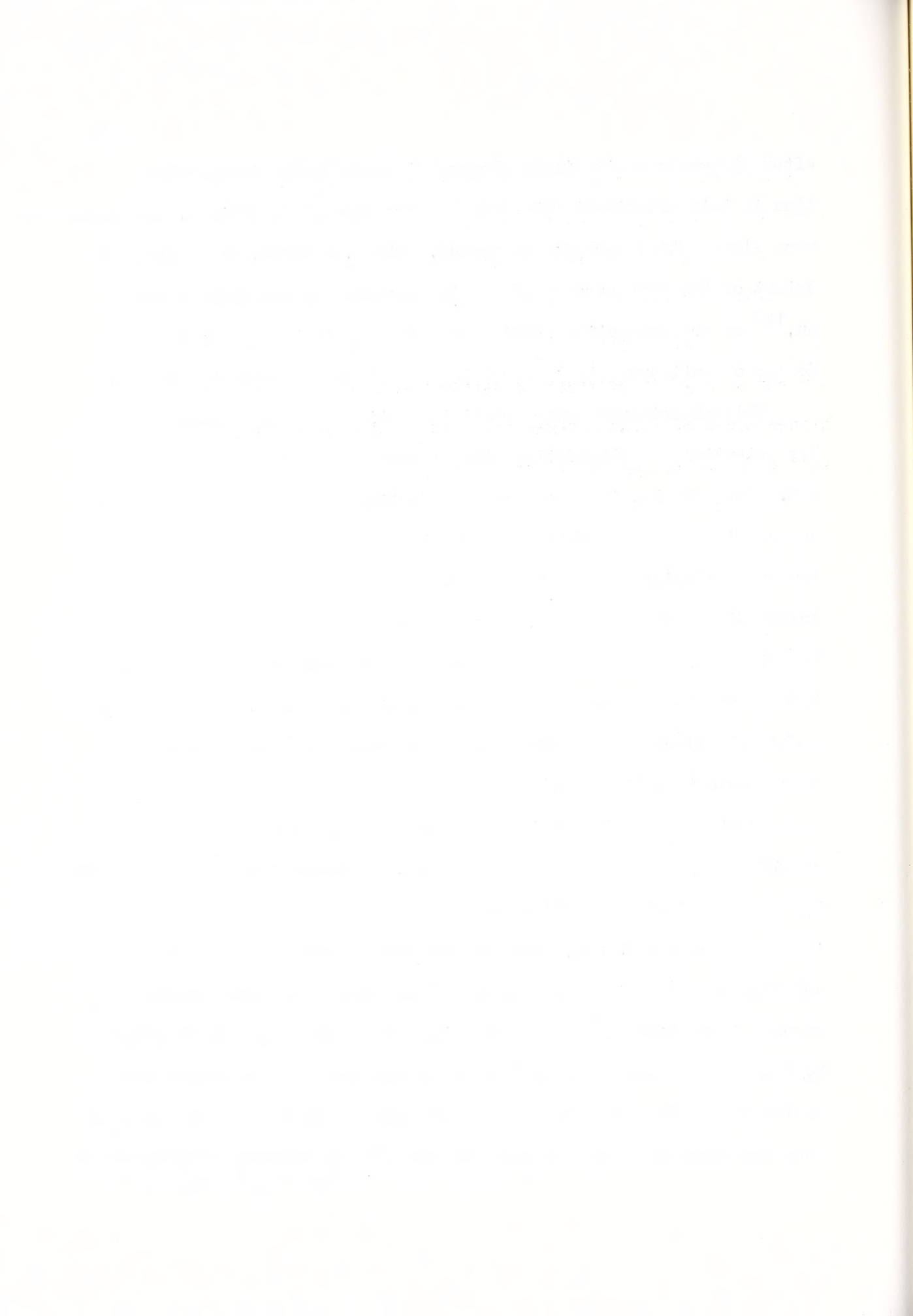
mice with anti-gamma we might be able to inhibit IgG selectively and analyze the relationship between 'blocking' IgG and IgE.

Thus, results so far indicate that synthesis of IgE is either independent of the ontogenetic progression of other B lymphocytes from IgM to IgG to IgA or IgE may actually precede IgM in ontogeny. Current work is in progress to verify these results, especially with higher doses of immunosuppression, and to elucidate the effect of anti-alpha antiserum on IgE production.

PART III: Tumor-Related Studies

(A) IgE Responses to Tumor in Animals

As discussed in the literature review, Bartholomaeus and
Keast in 1972 reported that mice could make IgE responses to
tumors.[16] We attempted to repeat their work as a preliminary
to answering some of the following questions: Does an IgE res-
ponse alter the course of the malignancy? Could passive transfer
of IgE affect tumor challenge or the course of an already trans-
planted tumor? Is the immunotherapeutic effect of certain adju-
vants like B. pertussis related to is effect on IgE response?
While Bartholomaeus and Keast had shown that IgE could be pro-
duced, they had not addressed themselves to any of the above ques-
tions.

To assess the role of IgE we attempted to produce a variable
antibody response by using a range of immunizing doses of tumor
and two different adjuvants.

Methods:

Animals- Male and female AKD2Fl/J, DBA/2J, and C5781/6J mice were
purchased from Jackson laboratories, Bar Harbor, Maine. Swiss mice
were obtained from the Sterling Hall of Medicine colony, Yale Medical
School. CFW mice were purchased from Carworth Farms, New   City,
New York. Lewis rats were purchased from Microbiological Associates.
Recipient animals for PCA titrations were either Swiss or CFW mice

or the rats. The Lewis rats had been shown to be comparable to Sprague-Dawley rats in PCA reactions to OA as described in the previous section.

Tumors- A murine lymphoma, L5178Y, maintained in vivo was provided for us by Dr. Robert Capizzi. This tumor was initially reported in DBA/2 mice and in our hands took readily in AKD2F1/J mice at i.p. doses of 10^3 to 10^6 cells. Death preceded by ascites occurred regularly in 10 to 18 days depending upon the number of malignant cells initially injected. This tumor was selected because some immunological aspects of its growth had previously been studied[6,60,61,244] and it was clear that injecting animals with irradiated cells could produce immunity.[6]

The murine melanoma, B16, was given to us by Dr. Ted Reid, who maintained an in vitro cell line. This tumor took readily in C57Bl/6 mice and caused death in 17 to 35 days. It is the same tumor as initially reported upon by Bartholomaeus and Keast.

Immunization of animals and serum collection- For the work with L5178Y, a variety of mice strains including AKD2F1/J, DBA.2J, CFW, Swiss, and C57Bl/6J were given injections of tumor in doses ranging from 1.4×10^1 to 2.8×10^6 cells. The cells were usually viable, but in some instances they were irradiated prior to injection. Either an i.p. or a subcutaneous (s.c.) route of injection was used. Cells were either given without adjuvant, with 1 mg of alum, or with killed B. pertussis organisms (Eli Lilly and Co., Indianapolis, Ind.) with the number of bacteria varying from 9.6×10^6 to 4.4×10^9 depending on the trial. Alum was prepared as previously described.[131] Mice were bled from seven to nineteen days after an

an initial injection of cells or following a booster injection of
tumor.

For work with the B16 melanoma, 6 C57B1/6J mice given a s.c. in-
jection of 3×10^5 cells plus 1 mg. of alum. Serum was obtained from
these mice one and two weeks after immunization.

For all animals, bleedings and i.v. injections were usually
performed with the animal under light ether anesthesia.

Irradiation- When cells were irradiated, a Semans x-ray machine was
used with a 2 Al filter. When set at 250 KV and 15 mA, this machine
delivers 500 rads/min. Three to five thousand rads were given cells
depending on the trial.

Passive Cutaneous Anaphylaxis (PCA)- PCA reactions were performed
as previously described with recipient animals including Swiss and
CFW mice and Lewis rats. Antigen prepared from tumor cells was given
in the solubilized forms as described below. Antigen was usually
given i.v., but in some trials the antigen was given intradermally (i.d.)
directly into the site that had previously received serum passively.
When antigen was given in this manner, a control injection of antigen
into unsensitized skin was also tested.

Active Cutaneous Anaphylaxis (ACA)- In a few instances, antibody res-
ponse was assayed by injecting an animal with antigen i.d. some time
after that animal had been actively immunized with tumor cells. The
animal receiving the i.d. injection would first be shaved, receive the
injection of intradermal antigen, then immediately receive an injection
of Evans blue with saline i.v. Thirty minutes later tha animal would
be sacrificed and reactions judged from the underside of the skin.

Control reactions included injection of saline instead of tumor

antigen and injection of tumor antigen into non-immunized animals.

Positive reactions may be attributable to either IgE or IgGl.

Solubilization of tumor antigen- A positive reaction in PCA requires

that antigen react on with IgE on the surface of a basophil or mast

cell. For steric reasons, we believed that this reaction would be

difficult to elicit without first solubilizing the tumor antigen. Al-

though in a few instances whole tumor cells were used to attempt to

elicit the PCA reaction, we generally solubilized the antigen by one

or more of the following procedures: rapid freezing and thawing in a

dry ice-acetone bath; ultrasonication with a sonifier cell disruptor,

Model W185D, Heat systems-ultrasonics, Inc.; ultracentrifugation at

30,000 RPM or at 30,000 g for one hour; irradiation with three to five

thousand rads; and extraction with 3 M KCl as described by Meltzer et

al.[162] In order to elicit PCA reactions, antigen was administered i.v. via

the retro-orbital plexus in the mouse or via the dorsal vein of the

penis in the rat. Either Evans blue was added directly to the tumor

antigen or a second injection of saline plus Evans blue was given imme-

diately before or after the injection of tumor antigen. An initial con-

centration of greater than 10^6 cells/ml. and usually greater than 10^7

cells/ml was always used to begin the solubilization process.

Foot pad testing- An index of delayed hypersensitivity response to

L5178Y was assessed by injecting appropriate antigen into the foot pad of

immunized mice. Antigen injected into one foot was 0.04 ml of irradiated

cells at a concentration of 1.25×10^7 cells/ml. The other foot pad re-

ceived 0.03 ml of tumor antigen extracted with 3 M KCl. Foot pad thick-

ness was measured prior to injection and twenty-four hours after

antigen. Mice tested included 3 AKD that had received 10^3 L5178Y

plus 10^8 B. pertussis, 3 Swiss that had received 10^3 L5178Y twice

with two weeks separating the immunizations, and 3 Swiss mice that

had received no exposure to tumor. Mice that had been immunized were

tested twenty days after the initial injection of tumor.

Results:

Using the tumor, L5178Y, despite the variety of animals immunized,

the range of bleeding times, the types of adjuvants, the varied routes

and doses of immunogen, and the alternative methods for solubilizing the

antigen, we were unable to produce consistent PCA reactions for either

IgE or IgG1.

Results are summarized in Tables IV and V.

A description of a typical experiment should clarify the procedure

followed.

Five groups of four AKD2F1/J mice were given i.p. injections of

irradiated (3000 rads) L5178Y cells in doses ranging from 3×10^1 to

3×10^5 cells. Cells were given with 1 mg of alum in an i.p. injection

of 0.2 cc. Although 3,000 rads has been reported to destroy the activity

of L5178Y,[6] by day 18, the majority of animals having received the

maximum tumor dose were dead. The survivors of this group were bled on

this day and all other groups were bled the next day. Mice in all other

groups showed no signs of illness and were disease free for at least an

additional six weeks. Serum was pooled and frozen at -20°C until ready

for use. L5178Y cells in a concentration of 9.6×10^6 cells/ml. were

TABLE IV PCA REACTIONS TO L5178Y FOLLOWING

PRIMARY EXPOSURE TO TUMOR ANTIGEN

Mouse Strain and Number of Animals	Tumor immunizing Dose (cell#) ?adjuvant ?irradiation	⇐Day after immunization	serum collected	PCA test antigen(s)	Comments
4AKD	3×10^1 l.p. +alum irradiated	19	1	Freeze/thaw x 3; Ultrasonicate; Utracentrifuge 30,000 RPM	No morbidity from tumor
4AKD	3×10^2 l.p. +alum	19			No morbidity from tumor
4AKD	3×10^3 l.p. +alum	19			No morbidity from tumor
4AKD	3×10^4 l.p. +alum	19			One of three animals ⊕ for IgE at 1:3 Dilution No morbidity
4AKD	3×10^5 l.p. +alum	19			No morbidity from tumor
5AKD	2.5×10^2 l.p. + Pertussis irradiated	18			Animals dead or moribund by Day 18
3AKD	10^3 l.p.	7	1	Whole cells, 10^8/ml	Animals dead Day 15
3AKD	10^3 l.p. + 10^8 pertussis	7	2 3	Irradiated cells Sonicated cells	No morbidity from tumor
3 SWISS	10^3 l.p.	7			No morbidity from tumor
3 SWISS	10^3 l.p. + 10^8 pertussis	7			No morbidity from tumor
3AKD	2.8×10^6 l.p.	9	1	Freeze/thaw x 3; Ultrasonicate; Ultracentrifuge 30,000 g	Also negative at 14 days by ACA
4AFW	1.4×10^3 l.p.	9			Also negative at 14 days by ACA
4CFW	1.4×10^2 l.p.	9	2	Whole cells	
4CFW	1.4×10^1 l.p.	9			Also negative at 14 days by ACA
5C57B16	10^6 + alum S.C.	10	1	Freeze/thaw x 5	Minimum dilution tested 1:2; No morbidity from tumor
4DBA/2	10^6 + alum S.C.	8to10			Minimum dilution tested 1:2; Death 12 days
6CFW	10^6 + alum l.p.	10			Minimum dilu. tested 1:2; No morbidity from tumor
3AKD	10^3 + 10^8 pertussis l.p.	14	1 2	Irradiated cells KCl extract	No morbidity from tumor
3AKD	10^3 l.p		3	Intradermal KCl Extract	Animals dead Day 15

TABLE V PCA AND ACA REACTIONS TO L5178Y FOLLOWING SECOND EXPOSURE TO TUMOR ANTIGEN

Mouse Strain and Number of Animals	Initial immunization Cell #	Day of Boost	Booster Dose	Day after Boost Serum Collected	PCA Test Antigen	ACA Test Antigen
4AKD	3×10^1 +alum irradiated i.p.	28	Repeat Initial Dose	19	1 Freeze/thaw x 3; Ultrasonicate; Ultrocentrifuge @ 30,000g for 1 Hour	
4AKD	3×10^2 +alum irradiated i.p.	28	Repeat Initial Dose	19	2 Whole cells	
4AKD	3×10^3 +alum irradiated i.p.	28	Repeat Initial Dose	19		
4AKD	3×10^4 +alum irradiated i.p.	28	Repeat Initial Dose	19		
4AKD	2.5×10^2 irradiated $+1.9 \times 10^7$ pertussis i.p.	28	2.25×10^2 4.4×10^9 pertussis i.p.	19		
3AKD	$10^3 + 10^8$ pertussis i.p.	24	2.8×10^6 i.p.	9		
3 SWISS	10^3 i.p.	12	Repeat Initial Dose			1 Irradiated Cells
3 SWISS	$10^3 + 10^8$ pertussis	12 12	Repeat Initial Dose			2 KCl Extract Both 12 Days Following Boost

solubilized by freezing and thawing three times, ultrasonicating
with the sonifier intensity set at 4.5 and 7 bursts of 3 to 5 seconds
given to the suspension of cells surrounded by an ice water bath, and
ultracentrifuging at 30,000 RPM for one hour. The supernatant was
used as the antigen to elicit PCA reactions.

Each of the Swiss and CFW mice recipients for PCA titration re-
ceived 0.1 ml. of antigen i.v. This administration was given either
two or 48 hours after the animal had been passively skin sensitized
with a dilution of serum of at least 1:5 from the actively immunized ani-
mals. With one exception no positive reactions were elicited. The
one exception occurred after a 48 hour sensitization period at a
dilution of 1:5 with serum from the group that had received 3×10^4
cells, but as this reaction occurred in only one of three animals, it
was not scored as positive when judged by criteria generally used for
scoring PCA reactions, namely a positive response in the majority of
animals tested.

ACA responses in Swiss mice that had received 10^3 leukemic cells
26 and again 12 days previously were tried with irradiated cells and
with the hypertonic KCl extract. Reactions in the immunized mice did
not differ from controls.

Experimentation with the B16 melanoma also did not produce consis-
tently psoitive results. As described in the methods section, 6 C57
mice were immunized with a s.c. dose of melanoma and alum and then bled
7 and 14 days after immunization. The sera was pooled from each bleeding
and diluted 1:5 with saline. Tumor antigen was solubilized by harvesting

5×10^6 cells growing in vitro and freezing and thawing them
rapidly five times in a dry ice-acetone bath. Four Swiss mice
were skin sensitized and two hours later each received an injection
of Evans blue i.v. followed by 0.15 cc of the solubilized antigen.
In each case the mouse receiving antigen died within ten minutes of
the intravenous injection of an apparent cardiorespiratory arrest.
Death was clearly not due to the ether anesthesia that had been used
to facilitate the giving of i.v. injections as usually the animal was
visibly recovering from the ether before respiratory difficulties
developed. Death was also not due to the volume of fluid adminis-
tered as mice in our hands had been shown to tolerate far greater fluid
loads quite well. Pulmonary emboli from particles in the antigenic
suspension was considered a possibility, but centrifuging the antigenic
fluid to remove possible particles did not seem to affect the toxicity
of the challenge antigen. This antigenic suspension was also lethal to
animals that had received no passive serum.

Thirty-four days after receiving the s.c. melanoma injection, three
of the survivors received an i.d. injection of tumor cells that had been
ultrasonicated. One of the three animals produced a reaction markedly
larger than the response produced by a similar injection into unimmunized
controls.

Discussion:

While the lack of consistent positive results prevented us from ass-
essing the effect of an IgE response on tumor growth in an animal sys-
tem, useful information did emerge from this work.

In their report on mice producing an IgE response to the B16
melanoma, Bartholomaeus and Keast solubilized the tumor antigen by a
process of freezing and thawing, ultrasonication, and ultracentrifugation.[16]
While we wished to duplicate their work as closely as possible, we were
loathe to ultrasonicate and ultracentrifuge the tumor cells. Ultra-
sonication, if done at too high a frequency, can serve to destroy anti-
genicity rather than solubilize it.[108] Unfortunately, in their report
Batholomaeus and Keast did not specify the frequency with which they
sonicated. We also reasoned that ultracentrifugation would only cause
the loss of some antigen. For these reasons, in most of our work we
solubilized the B16 cells only by rapid freezing and thawing, and the
intravenous administration of this suspension proved lethal to passively
sensitized animals.

A personal communication from Bartholomaeus clarified some of our
difficulties.[17] First, the frequency of their ultrasonication was re-
ported as ranging from 16 to 24 KCS. As the ultrasonifier that we
employed performed at 20 KCS, ultrasonication would probably not have
destroyed antigenicity. The positive active cutaneous anaphylaxis res-
ponse obtained in one of three animals using ultrasonicated antigen is
encouraging and confirms the idea that sonication would not destroy all
antigenicity.

Second, the author reported that while they had been success-
ful in demonstrating IgE responses with a variety of tumor systems,
they had been unsuccessful with all leukemias and lymphomas. Hence,
our failure with L5178Y despite careful adherence to the methods of
Bartholomaeus and Keast in some of the trials with this tumor be-
comes understandable.

In trying to interpret our negative results, we could not be
sure if they arose from a failure of the immunized mice to produce an
antibody response or from a failure of our antigen to be solubilized.
The communication from Bartholomaeus re-emphasized the necessity of
using some solubilization procedure to elicit the PCA. We had some
evidence that mice were making an immune response to the tumor, namely
the mouse's ability to survive tumor challenge. For example, 3 AKD2F1/J
mice were given 10^3 L5178Y i.p. and three were given an equal number
of tumor cells plus 10^8 heat killed Bordetella pertussis. 16 to 18 days
after this injection, the animals from the first group were dead. Eight
weeks after the injection the animals that had received tumor plus B.
pertussis showed no evidence of disease. As shown in Table IV, despite
the acquired immunity, we were unable to demonstrate the presence of
either IgG1 or IgE antibodies directed against the tumor in these mice.
Foot pad testing likewise failed to demonstrate the acquired immunity.

While B pertussis has a variety of effects on the immune system,
many investigators have utilized its potent adjuvant effect on IgE
responses. Furthermore, several authors have noted that like bacille

calmette guerin[19,76,127] and corynebacterium parvum,[48] B. per-
tussis may be used to prolong survival in animals bearing tumors.[13,
14,137,247] It has also been tried in immunotherapy of human neo-
plasia.[77] Like other immunotherapeutic agents,[180] timing of its
administration in certain ways may also enhance tumor growth.[249]
Some authors have speculated that the effect of pertussis on IgE and
the effect on survival after tumor challenge might be related. With
this in mind, to attempt to demonstrate IgE in pertussis immunized
animals seemed logical. Negative results, especially as all the work
with pertussis was done only in the lymphoma system, do not rule out
the possibility that IgE might be an important factor in immunotherapy
with B. pertussis.

An outgrowth of these experiments was the observation that when the
B16 melanoma was admixed with alum prior to its inoculation, the sur-
vival time of the mice seemed to be prolonged 50%. The alum was pro-
bably not toxic to the tumor cells prior to the injection, as the alum
was buffered and in an isosmotic medium, although this possibility was
not ruled out by examining the viability of tumor cells combined with alum
in vitro. Alum itself might prove to have some use in tumor immunotherapy.
We are unaware of other reports suggesting prolonged survival using
alum as an adjuvant.

In summary, in an effort to investigate how an IgE response against
a tumor alters the course of the malignancy, an attempt to show that
mice could make an IgE response against a tumor was undertaken. Exten-
sive work concerned the lymphoma, L5178Y, and a lesser portion of study

was devoted to the melanoma, E16. IgE responses were not consis-
tently demonstrated despite the prolonged survivals. indicating an
apparent immune response, and the use of adjuvants, B. pertussis and alum,
which are known to enhance IgE production. Clarification of methods
in a report describing an IgE response to a tumor was obtained. This
information along with the knowledge gained in the unsuccessful trials
should enable this work to be successfully completed in the future.

(B) IgE Responses to Ovalbumin

in Tumor-Bearing Animals

Several reports have stated that individuals with neoplastic
disease have depressed circulating levels of serum IgE.[9,13,102] If
correct, these reports leave unanswered the question of cause of ef-
fect. Has a low level of IgE predisposed to the malignancy or did the
tumor result in depressed IgE? Only a very large prospective study
would really answer these questions, but we attempted to gain some
clues by studying IgE responses in mice bearing tumors and examining
the effect of tumor inoculation on pre-existing IgE levels in mice. We
are especially interested in reports of increased IgE in patients with
Hodgkin's Disease.[9,198,231] As this is usually attributed to decreased
T-cell function in what is thought by many to be a T-cell malignancy,[3]
we studied IgE responses in mice bearing a theta positive lymphoma,
L5178Y. The theta antigen is found on cells of thymic origin. We also
assessed the effect of a melanoma on IgE responsiveness.

Methods:

Animals- Mice given living tumor cells were C57Bl/6J mice for

the melanoma studies or AKD2F1/J mice for the lymphoma studies.
Both were purchased from Jackson laboratories, Bar Harbor, Maine.
PCA reactions were titered in Swiss mice from the Sterling Hall of
Medicine colony (2 hour IgG1 reactions) or in Sprague-Dawley rats
(IgE reactions) from either Camm Research (Wayne, N.J.) or Charles
River Labs (Wilmington, Mass.).

Tumors- Either the L5178Y lymphoma or the B16 melanoma were used as
previously described. While the lymphoma was usually transferred from
an in vivo cell line, one group of experiments made use of a transfer
from an in vitro line also maintained by Dr. Robert Cappizzi.

Irradiation- Cells were irradiated with 3000 rads as previously des-
cribed. Irradiated cells were used only in Experiment I.

Antigen- Ovalbumin (2X recrystallized, Worthington Biochemical, Free-
hold, N.J.) was given i.p. in saline at a dose of 1 ug with approxi-
mately 1 mg of alum, synthesized as previously described. Total injec-
ted volume was 0.1 ml.

PCA Reactions- These were run as previously described with passively
sensitized mice or rats given i.v. injections of saline plus 0.7 % OA and
0.4% Evans blue two hours after skin sensitization. Mice were skin sen-
sitized with 0.03 ml of diluted serum while 0.10 ml was used in the rats.

Results:

Experiments were designed to test if the transplanatation of tumor
would affect the IgE response to ovalbumin. Experiments I through III
involve the lymphoma L5178Y. In experiment I, tumor was given to mice
that had a pre-existing, high titer of anti-OA antibodies. In experiment

II, the tumor was given just prior to the booster antigen. And in experiment III, tumor was transplanted just before or after the initial exposure to antigen. Experiments IV and V are similar except that they involve the melanoma B16. In the fourth, animals with a well established tumor were exposed to OA for the first time in the fifth, animals were given the tumor after a high, boosted antibody titer had been established. The data are summarized in the five accompanying tables (VI through X), with the data from Table VI also represented graphically in Figures II and III. Essentially animals bearing tumors were able to make IgE and IgGl responses comparable to controls and once an animal had been immunized, transplantation of a tumor did not greatly reduce the antibody response, although a tendency toward depression of the antibody response was seen in some instances.

A few specific comments about each of the experiments are in order. In the first, (Table VI), high PCA titers were established by immunizing mice with OA and boosting them 28 days later. Several days after the booster dose, tumor was transferred either in a dose of 10^5 or 10^2 cells with one group receiving 10^5 cells that had been irradiated and one group receiving only saline. The graphs (Figures II and III) pictorialize that all four groups were within a two fold range of IgE and IgGl concentrations at the time of tumor transplantation. The mice that received 10^5 cells had all died within 10 days. Irradiation only slightly delayed the death so that no animals in this group were living at day 15 after tumor transfer. All mice that received NaCl or 10^2 cells survived.

Effect of L5178Y on ongoing, boosted Antibody Response

Table VI Experiment I

# of Animals	Strain	Tumor Dose Cell #	Day Antigen Given	IgE Titer-Day*					IgG$_1$ Titer-Day*				
				7	12	17	22	27	7	12	17	22	27
6	AKD	.1cc NaCl	-35,-7	640	320	160	160	160	1280	1280	1280	2560	1280
6	AKD	10^5 1.p.	-35,-7	640	160	80			640	1280	320		
6	AKD	10^2 1.p.	-35,-7	640	320	160	80	80	640	1280	640	1280	1280
6	AKD	10^5 1.p. irradiated	-35,-7	320	80	40	40**		1280	1280	640	640**	

*Number of day is relative to last exposure to antigen
 Serum from Day 7 was collected prior to transfer of tumor
**Serum was collected on Day 21 as animals were moribund at
 the time and all dead by Day 22
 Blank spaces in table indicate all animals were dead by
 that bleeding date

FIGURE II

THE EFFECT OF L5178Y ON BOOSTED
IgG$_1$ RESPONSE

Saline control
10^2 tumor cells - No deaths
10^5 tumor cells - All dead
    by day 10
10^5 irradiated tumor cells -
    All dead by day 14

Tumor (saline) given

Day Following Booster Dose of Antigen

IgG$_1$ Titer
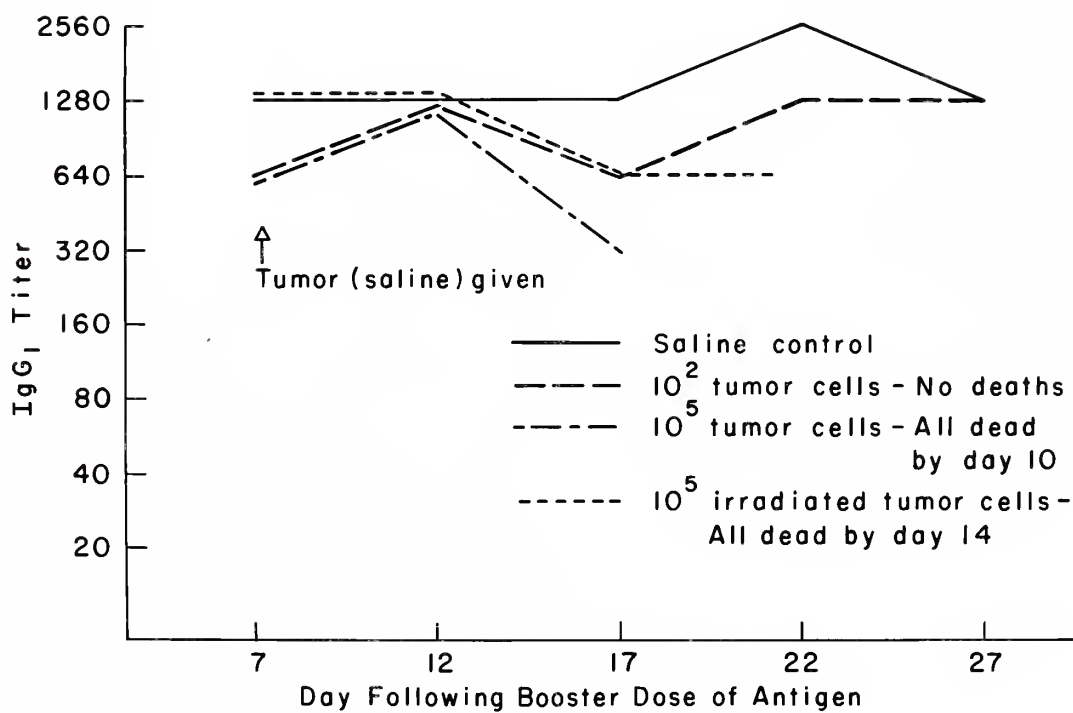
FIGURE II

FIGURE III

THE EFFECT OF L5178Y ON ONGOING,
BOOSTED IgE RESPONSE

Saline control
10^2 tumor cells – No deaths
10^5 tumor cells – All dead by day 10
10^5 irradiated tumor cells – All dead by day 14

Tumor (saline) given

IgE Titer

Day Following Booster Dose of Antigen

FIGURE III
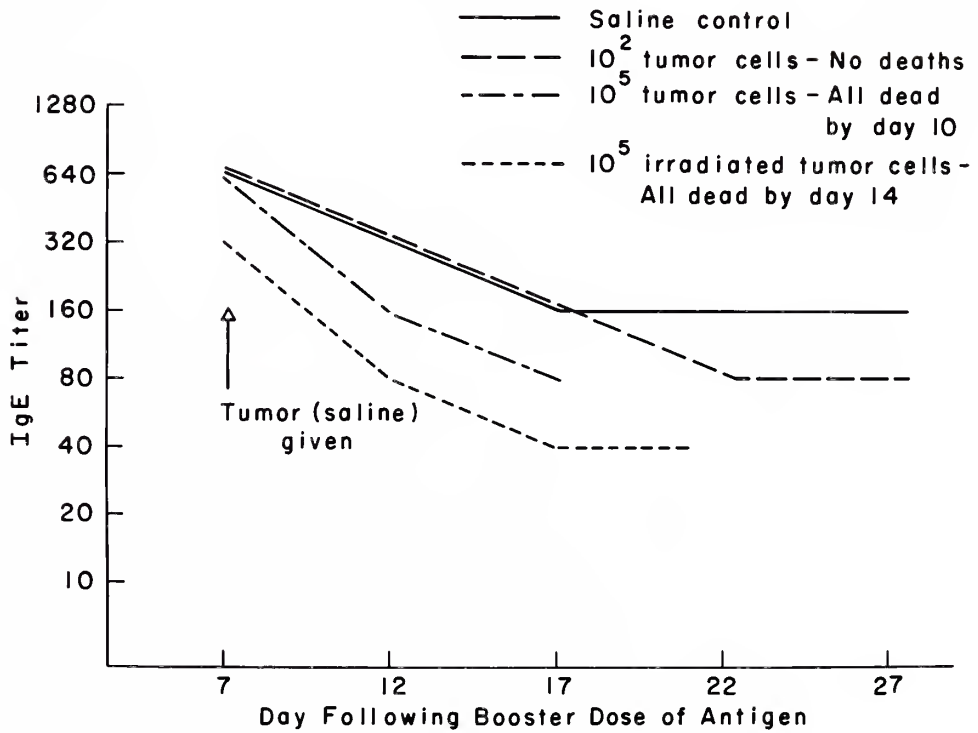
As can be seen, the IgE levels did tend to drop faster and to lower levels in those mice dying from tumor, but as two fold differences were obtained in identically treated groups prior to tumor transplantation, the four fold differences seen after tumor implantation are suggestive but may not be significant. IgG1 levels tended to decline even less as compared to controls.

In experiment II (Table VII), antigen was given and then tumor was transferred just prior to the booster dose of antigen. The mice receiving tumor were dead or grossly ascitic fourteen days after tumor transfer. While the IgE levels do not differ significantly between the two groups, a marked decline in IgG1 levels is noted in the tumor-bearing mice just prior to death.

In experiment III (Table VIII), responses to the primary exposure to antigen were assessed, that is, not booster doses of OA were given. Tumor that had been maintained in vitro was injected either two days after antigen, the same day, or three days before. One group received the tumor s.c., a route of injection that permits slightly longer survival, and then was not given antigen until 12 days later when the tumor had been well established. One group was given tumor cells that had been frozen several months. As can be seen from the chart, all groups except the one receiving frozen cells produced IgE and IgG1 responses varying at most by a factor of two when compared with the controls that had received no tumor. The large majority of these animals died within 18 days of exposure to tumor with one animal surviving completely and one surviving to 22 days. If an animal did not appear ascitic when others in

Transfer of L5178Y Prior to Secondary Exposure to Antigen

Table VII Experiment II

# of Animals	Strain	Tumor Dose # of cells	Day Antigen Given Relative to tumor transfer	IgE titer		IgG_1 titer	
				*Day 5	*Day 11	*Day 5	*Day 11
3	AKD	1.2×10^5 l.p.	-24, + 3	320	160	160	160
3	AKD	None	-24, + 3	320	160	320	1280

*Number of day is relative to last exposure to antigen

Effect of L5178Y on primary exposure to Antigen

Table VIII Experiment III

# of Animals	Strain	Tumor Dose # of cells	Day Ag Given Relative to Tumor transfer	IgE titer		IgG_1 titer	
				*Day 12	*Day 17	*Day 12	*Day 17
5	AKD	2×10^4 1.p.	0	20		5**	
4	AKD	2×10^4 1.p. Frozen	0	5		0	
5	AKD	2×10^4 1.p.	-2	80	10	10	0
5	AKD	2×10^4 1.p.	+3	80		10	
5	AKD	2×10^4 1.p.	+12	40		10	
4	AKD	None	0	40	10	5**	5

*Number of day is relative to last exposure to antigen
**Only two animals tested; dilution positive in 1 of 2

the group were clearly moribund, it was not bled. This was true
of the one animal that survived the course of the experiment. Animals
that received s.c. tumor died at about thirty days after inoculation
and animals that had received frozen tumor cells also tended to die
by this date. The death of those animals receiving frozen cells and
their apparent lack of an IgE response will be commented upon in the
discussion.

Experiments IV and V involved the melanoma B16. In the fourth
(Table IX), we were attempting to confirm some preliminary data that
suggested that mice bearing this tumor might be able to make a greater
IgE response than controls. In this preliminary trial, a group of
three mice had been given B16 plus a alum and three weeks later were
immunized with OA plus alum. When they were bled one week later,
their serum had an IgE titer of 1:20 while controls that had just re-
ceived a comparable OA injection had no detectable titer. Neither group
had detectable IgG1. Experiment IV could not confirm this finding as
all groups had no measurable titers seven days after exposure to anti-
gen. Thirteen days after antigen the groups still remained comparable,
except that animals that had received s.c. saline did make a signifi-
cantly higher IgG1 titer. No effort was made to repeat this and no
explanation for it other than individual variation is readily apparent.
While IgE concentrations for Day 13 were not tested at dilutions greater
than 1:160, the qualitative response at high dilutions can give an
approximation of the antibody titer and it was evident that these groups
would have at most a two fold difference in IgE concentrations.

Effect of B16 on primary response to Antigen

Table IX Experiment IV

# of Animals	Strain	Tumor Dose # of Cells	Day Antigen Given Relative to tumor transfer	IgE titer		IgG$_1$ titer	
				*Day 7	*Day 13	*Day 7	*Day 13
4	C57	9×10^4 S.C.	+20	0	All animals dead	0	All animals dead
4	C57	9×10^4 S.C. + Alum S.C.	+20	0	≥ 160	0	10
4	C57	Alum S.C.	+20	0	≥ 160	0	10
4	C57	Saline S.C.	+20	0	≥ 160	0	80

*Number of day is relative to last exposure to antigen

In the final experiment (Table X), mice that had been immunized with OA were given a booster dose 28 days later and a s.c. dose of tumor six days after that. On the day that the tumor was given, the controls had a two fold lower concentration of IgE and a four fold higher concentration of IgGl. These differences tended to disappear after one group was exposed to tumor. It should be noted that some of the titrations in this trial did not always establish the precise IgGl concentration. The qualitative response at high dilutions suggested that IgGl concentrations did not differ widely. As the primary purpose of the series of experiments was to examine IgE responses with IgGl responses used as control data, precise titration of the IgGl response was unnecessary if the IgE concentrations were within a two fold range.

Discussions:

The data lend little support to the idea that low levels of IgE arise from depressed IgE responsiveness during malignancy, but pre-existing antigen-specific IgE titers do tend to fall after a tumor is transplanted. However, in interpreting the data one should consider that: first, the method, may not be sensitive enough to detect decreased IgE responsiveness. For example, in experiment I four fold differences were noted between the IgE levels of tumor-bearing mice and the controls. These are suggestive differences, but as two fold differences occurred among groups of animals treated identically just prior to the tumor transfer, this four fold difference might be within experimental limits. IgGl levels supposedly reflect levels of total antibody response.[230] Our data also suggest that tumor-bearing mice made a decreased IgGl response, but again the limited sensitivity of the method makes such a

Effect of B16 on secondary response to antigen

Table X Experiment V

# of Animals	Strain	Tumor Dose # of cells	Day Antigen Given Relative to tumor transfer	I_gE titer			IgG_1 titer		
				6	*Day 13	20	6	*Day 13	20
4	C57	10^7 S.C.	-34,-6	160	40	10	40	$\geq$ 320	< 160
4	C57	None	-34,-6	80	40	20	160	$\geq$ 320	160

*Number of day is relative to last exposure to antigen
Titers from Day 6 were obtained from serum just prior
to tumor transplant
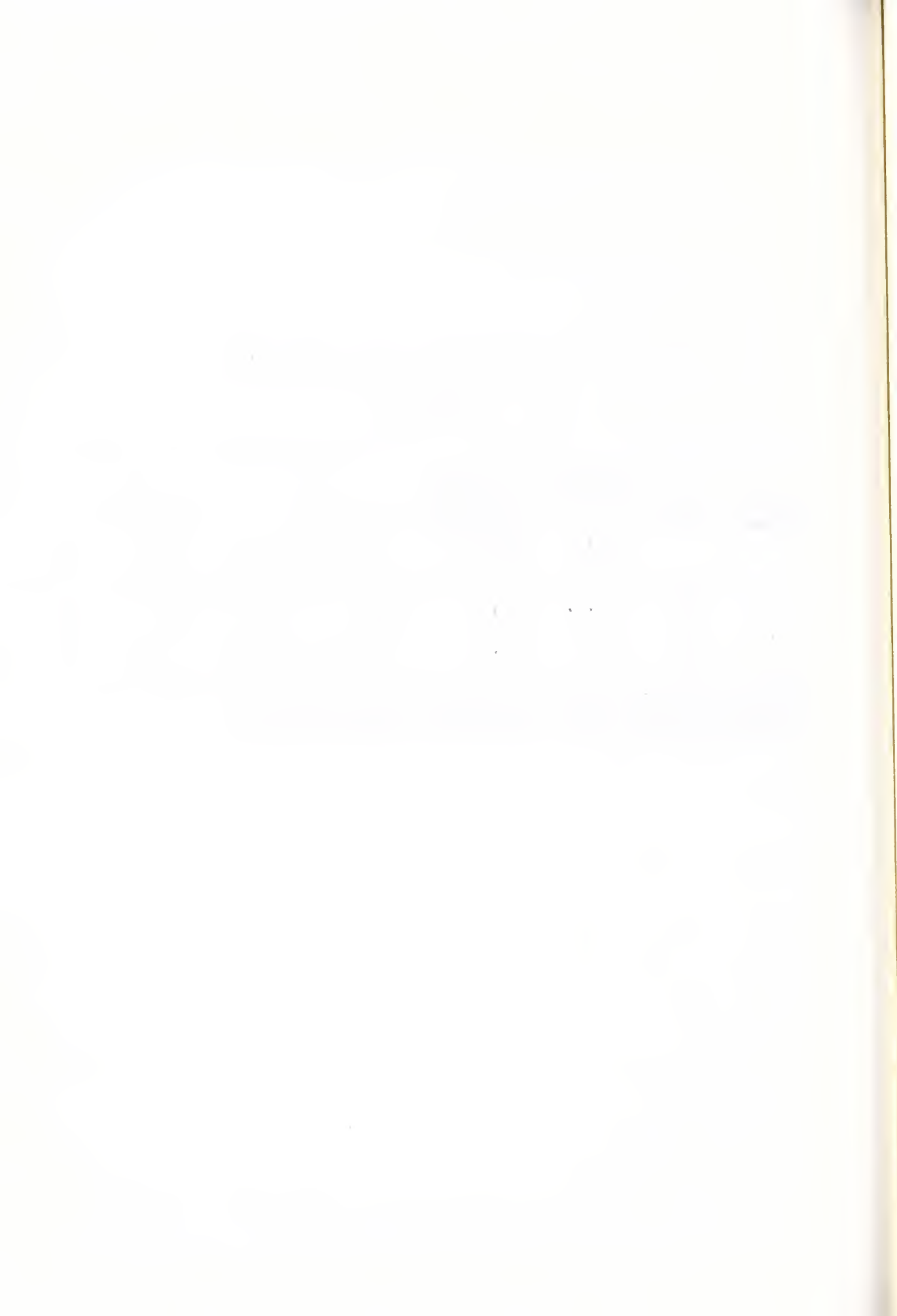
conclusion difficult. Evidence in man supports the idea of a de-
creased antibody response in individuals with malignancy,[140] but
reports of normal antibody responses have also been published.[209]
Second, with PCA, ECA, one measures IgE titers for a specific antigen
while reports in the literature have been concerned with total serum
IgE. Several studies have shown generally normal or elevated levels
of other immunoglobulins in most paitients with cancer,[259,260] but
this may not be indicative of responsiveness to a specific antigen.
Finally, to draw conclusions about human responses on the basis of
animal work can be difficult. The most recently published study sug-
gests that IgE levels are normal in patients with cancer.[231] Our data
are not inconsistent with this concept.

The kinetics of the normal IgE response should be compared to
the IgG1 response. IgE levels declined steadily after the booster dose
of antigen, while the level of IgG1 tended to maintain itself or even
rise slightly. The data suggest a more marked decline in IgE than IgG1.
This might be explained in part by the much shorter half life of IgE,[234]
at least in human studies, so that decreased immunoglobulin production
following antigen exposure would become evident sooner for IgE than IgG1.

As mentioned in the literature review, Hodgkin's disease is unique
among malignancies in that IgE levels are clearly elevated. Debate
exists as to whether Hodgkin's disease is a T-cell or B-cell malignancy.
The traditional view was that Hodgkin's disease must be a T-cell malig-
nancy because T-cell function both as judged in vivo[39] and in vitro is
depressed.[3,68] Recent evidence, however, suggests that T-cells in

Hodgkin's disease may be quite normal, only depleted in peripheral blood.[152] Some claim that the relative number of B-cells is also often diminished[68] and others suggest that the characteristic Reed-Sternberg cell has surface immunoglobulin and thus is of B-cell origin.[124] Perhaps the concept of T or B-cell malignancy is simply not relevant to this disease.

Regardless of which cell is primarily involved, unquestionably a depressed level of T-cell response often characterizes the disease. A wealth of excellent experimental data is now accumulating to elucidate the role of T-cells in controlling IgE responses[203,224,225] and the depressed T-cell function is one explanation of the elevated IgE.[198] [231] Unfortunately, as alluded to previously, studying IgE responses in the mouse model of Hodgkin's disease, the SJL, is most difficult because at present they are thought to make poor IgE responses. The use of the lymphoma, L5178Y, seemed to offer a possible way to abrogate the T-cell control of IgE responses in mice. This lymphoma is theta positive;[169] that is, it bears an antigen marker attributed to lymphocytes of thymic origin. Speculation prior to the experiment was that a T-cell malignancy might be characterized by heightened IgE production. Most manipulations that destroy T-cell function including radiation,[175] anti-thymocyte serum,[176] and thymectomy,[175] generally predispose to increased IgE production. Increased IgE production clearly was not found in our experiments. Several explanations for this seem reasonable.

First, although T-cells may be affected by L5178Y, their function,

-54-

or at least their suppressor cell function, may be preserved. Second, as T-cells are known to exert both a helper[225] and a suppressor[223] effect over IgE production, perhaps affecting both equally allowed relatively normal IgE production. Third, the malignancy may have affected only a subset of all T-cells, leaving intact the subset vital for IgE response. Fourth, a tumor maintained in vivo or in vitro over a period of years may change its antigenic properties. Although we used the 'same' tumor that other investigators have found to be theta positive, only our own testing could verify this for our cell line. Finally, a transplanted malignancy might proliferate but leave the animal's indigenous lymphocytes functioning relatively normally.

Acute lymphocytic leukemia may be derived from T-cells.[112] In the few patients in whom it has been studied, IgE levels seem normal in this disease.[231] The tumor most clearly bearing T-cell determinants in man is the Sezary cell.[57] We are unaware of IgE levels having been measured in this disorder.

Finally, the decreased IgE levels in the mice given frozen tumor suggests a very speculative explanation. Viral infections, with measles being one example under study,[62,165] are known to affect immune responsiveness and remission of allergic symptoms has been described following measles vaccine.[265] Perhaps the diminished IgE response and subsequent death of these animals was due to an activation of a latent virus from the frozen, lysed cells.

(C) Serum levels of IgE in childhood neoplasia

If IgE confers a genetic selective advantage, this survival benefit must be present prior to child-bearing age. We wished, therefore, to measure IgE levels in children with neoplasias. As children normally have low levels of IgE which rise gradually into adolescence, we needed a radioimmunoassay that was particularly sensitive to low serum values. As explained in Part II, such an assay was not commercially available at the time of this study and so serum from children with a variety of tumors was collected and stored at -20°C for future investigation.

Some limitations on the measurement of total serum IgE in cases of neoplasia deserve comment. First, neoplasms represent a variety of entities, each with a theoretical variety of causes. Even limiting study to childhood neoplasia, we had collected samples which included acute lymphocytic leukemia, acute undifferentiated leukemia, lymphosarcoma, neuroblastoma, osteogenic sarcoma, and thyroid carcinoma. If total serum IgE is significant for any specific entity, this might be overlooked by considering all neoplasms together. Second, total serum IgE may be irrelevant as it might not reflect tumor-specific IgE. Normal levels of IgE vary widely so that a great deal of tumor-specific antibody might be present but not be reflected in total serum levels. Finally, finding abnormal levels of IgE would still leave the cause a mystery. Did low (or high) IgE predispose to malignancy or arise as a result?

(D) Tumor-Specific IgE Responses in Man

Almost a decade before the discovery of IgE as a unique class of immunoglobulins, a report was published indicating that IgE could be involved in the anti-tumor immune response of some individuals. The work of Grace,[73,74] Curtis[49] and co-workers showing that patients with cutaneous manifestations of malignancy had immediate wheal and flare reactions to their tumors established that IgE was involved, even though IgE was not known to be a separate antibody class at the time.

Our studies designed to demonstrate that some individual do make an IgE response against tumors had two purposes. First, we merely wished to verify the previous reports. Second, if we could readily develop a method to determine if a given individual was making an IgE response, then the implications of this response could be systematically evaluated. For example, what is the effect of IgE on the course and prognosis of the tumor? What characterizes the tumors and the individuals who tend to produce the IgE response? Does the presence of an IgE response suggest therapy?

The same method available to Grace and Curtis, namely the P-K reaction, was not available to us. Current ethical standards would not permit the transfer of solubilized human tumors to volunteers. Therefore, two alternative methods to establish the presence of an IgE response were evaluated, immunofluorescence and histamine release. The results with the former are described; the latter method is currently being investigated.

Most of the studies involve breast carcinoma because 1) five
of Grace's patients had this disease,[74] 2) Black's data on skin
windows shows that basophils are prognostically significant in breast
cancer,[32,33] and 3) the disease is common enough so that tissue was
readily available. We were unable to obtain tissue on any patient
who had a cutaneous sign of malignancy.

Methods:

Tissue— All tissue was obtained either from surgical pathology or
directly from the operating room usually within sixty minutes of its
removal and often in less time. Tissue from 21 patients was obtained.
Malignant tissue came from nine females with breast carcinoma and one
with a breast sarcoma. Control tissue included sections from two re-
duction mammoplasties, biopsies from five cases of fibrocystic dis-
ease, breast tissue from a make with gynecomastia, and a spleen free
of tumor from a patient with Hodgkin's disease. Tonsils and nasal
polyps were obtained to provide positive controls. Immediately after
its receipt, all tissues were frozen in liquid nitrogen and then
stored at -90°C. Within a few weeks of its surgical removal, tissues
were allowed to come to a temperature of -20°C and sectioned at a
thickness of four to six microns with a cryostat. These sections were
placed on microscope slides and stored at -90° C in sealed containers until
ready for use, usually within a week or two. On one occasion, tissue
from a patient with fibrocysiic disease was obtained directly from sur-
gical pathology and immunofluorescence was performed without the inter-
vening storage of tissue.

Antisera- Commercial antisera to human immunoglobulin G and E
prepared in goats were purchased from Meloy Laboratories, Spring-
field, Va. These antisera were fluoresceinated with a fluorescein
to protein ratio of 3.3 ug/mg for the anti-IgE and 6.2 ug/mg for
the anti-IgG. Fluoresceinated antisera to human IgA and to rat
immunoglobulin produced in goats were given to us by Dr. Michael
Kashgarian. These had been purchased from Hyland Laboratories. All
antisera were diluted with saline at a ratio of 1:5 except for the
anti-IgG which was diluted 1:10.

Sera- Serum from the respective patients was obtained from the blood
bank. Serum was diluted 1:10 with saline before use.

Immunofluorescence- Both direct and indirect immunofluorescence studies
were usually performed. For indirect immunofluorescence one step in the
procedure must involve incubation of the tissue with serum prior to the
incubation with antiserum. For direct immunofluorescence this step is
omitted. The direct technique detects antibody on the surface of the
cell, while the indirect procedure looks for antibody in the serum that
has become attached to cellular antigen

Slides were taken from the freezer at -90° C and allowed to come
to room temperature before removing them from their sealed containers.
Slides were then washed with agitation in phosphate buffered saline (PBS)
for ten minutes. Slides were dried and placed over a moistened piece
of filter paper. The tissue was then covered with the appropriate serum
or antiserum. After a thirty minute incubation at 37° C slides were
washed twice for ten minute periods in PBS while being shaken on a

platform. For direct immunofluorescence, slides were next dried,
the tissue covered with a drop of phosphate buffered glycerine, and
a coverslip put in place. For indirect studies, slides were subjected
to a second incubation at $37^{\circ}C$, this time with appropriate antisera,
and then two more ten minute washes were performed prior to being
covered. Slides were viewed within hours of completion of the staining
with a Zeiss microscope equipped with ultraviolet light.

Histology- Hematoxylin and eosin (H&E) sections and phase contrast
microscopy were performed with some of the sections in order to localize
the areas of fluorescence.

Results:

Initial studies involved only the Meloy antisera to IgG and IgE. These
studies indicated that the sections from four of six patients with
malignancy stained with both of the fluoresceinated antisera after
direct and indirect immunofluorescence. Tissue from the five patients
with fibrocystic disease and the two patients with reduction mammoplas-
ties stained with anti-IgG consistently, but never significantly with
anti-IgE. Both benign and malignant tissue stained occasionally with
anti-IgE in areas of vascular smooth muscle. Such staining was not
considered positive.

With the help of Dr. Michael Kashgarian, H & E stains of the slides
were prepared. Examining the slides under light and phase contrast
microscopy verified that the immunofluorescence in the positive specimens
was predominantly over the area of malignant cells. However, when the
control of fluoresceinated anti-rat immunoglobulin was added to the

procedure, this antiserum too stained the areas with malignant

tissue in a pattern identical to that obtained with anti-IgE.

Some of the tissues collected including the sarcomatous breast

and the spleen from the paitent with Hodgkin's disease have not

been sectioned but remain in storage in -90°C. Their ultimate use

will depend upon the progress of further immunofluorescence studies.

Discussion:

Immunofluorescence as a technique for demonstrating a humoral

immune reaction to a tumor was first described by Klein et at.[118] in

patients with Burkitt's lymphoma. While immunofluorescence has been

used to locate IgE in such diverse studies as those on nasal polyps,[18]

the thyroid in Graves' disease,[239] the kidney in the nephrotic syndrome,[70]

[133] and peripheral blood lymphocytes in a variety of disease states,[5,67,]

[120,155,181] we are unaware of any previous study that has used immuno-

fluorescence to investigate the role of IgE in response to a solid tumor.

Our initial results were extremely encouraging, but performance

of adequate controls indicated that this enthusiasm was premature. The

artifactual staining of tumor tissue may be due to a number of factors

including necrosis of the cells despite our diligence in freezing the

tissue as rapidly as possible. The malignant cells may be more sensi-

tive to the experimental conditions and thus they attracted the anti-IgE

while the benign tissue samples may have had less necrosis. Breast tissue

does have significant local immunoglobulin production[193,194] and this

might explain the staining for anti-IgG in all tissues, benign and

malignant. Another possibility is that these malignant cells are especi-

ally cytophilic for proteins.

Immunofluorescence studies not involving IgE have been performed with breast carcinoma.[188] However, these studies involved tumor cells that have been grown in culture. With such a system, one can more easily control the staining procedure and artifact by using a set number of cells for each trial. As human tumors including breast carcinoma[252] tend to have common antigenic determinants, a single tumor growing in culture could be used to test sera of numerous patients with the technique of indirect immunofluorescence. Such testing would assume that the common antigen(s) is the one recognized by IgE. While breast carcinoma is not considered one of the easier tumors to grow in culture, cells obtained from pleural effusions secondary to the tumor do grow fairly readily in vitro.

We are currently approaching the problem of demonstrating that some individuals produce tumor-specific IgE with the use of a histamine release assay. Basophils in high concentration can be found among the leukocytes from blood sedimented with dextran or Ficoll-Hypaque.[264] If a paitent has produced IgE specific for his tumor, this IgE should have passively sensitized his own basophils. Adding solubilized tumor antigen to these leukocytes should cause the release of histamine as the cell degranulates.

If human tumors do cause the release of histamine, the implications may relate to both pathogenesis and therapeutics. Histamine or other vasoactive substances released by IgE may be implicated in the cutaneous signs of some tumors. And substances that block or neutralize the pro-

ducts of an IgE-antigen reaction would be logical pharmacologic agents to evaluate.

Paradoxes in evaluating the putative IgE response to tumors are plentiful. The data on CBH suggests that the basophil can play a role in tumor rejection, while the present understanding of histamine's effect on the immune response suggests that it should hamper tumor rejection. Work with adjuvants like B. pertussis implies to some that a tumor-specific IgE response is beneficial, but reports on cutaneous manifestations of malignancy suggest that IgE may be the culprit. IgE is elevated in Hodgkin's disease presumably secondary to depressed T-cell function, but lowered T-cell function is found often in malignancy and IgE levels are either normal or low. Is atopy related to the tendency to make a tumor-specific IgE response? Do skin signs appear in the absence of an IgE response? Does an IgE response occur in the absence of skin signs? Why should IgE be related to dermatologic disease? A histamine release assay is a reliable, reproducible technique that should greatly facilitate the answering of these questions.

SUMMARY

The possible role of IgE in response to neoplasia is reviewed with speculations offered about the possible implications of this response. Experimental efforts were divided bwtween basic research on IgE and studies directly involving tumors. Of the basic studies, A) the use of a solid phase radioimmunoassay (RIA) was perfected; B) evaluation of diurnal rhythms awaits commercial availability or a more sensitive, reproducible assay; and C) preliminary work on the ontogenetics of IgE suggests that its synthesis may be independent of the prior maturation of IgM bearing cells. Of the tumor-related studies, A) the demonstration in animals of IgE specific for tumor antigen has progressed so that its accomplishment should be attainable; B) the studies on IgE responses to ovalbumin in tumor-bearing mice suggest that the tumor-bearing animals make antibody responses nearly comparable to controls even with the transplantion of a theta positive lymphoma; C) the work on IgE levels in childhood malignancies must await the aforementioned RIA; and D) studies now in progress are attempting to demonstrate IgE responses to tumors in human subjects with the use of immunofluorescence and a histamine release assay.

BIBLIOGRAPHY

1. Aalberse, RC, Loghen, EV, Van Munster, PJJ, et al, Human antibodies to IgE: A simple method to avoid ambiguous results of the radioimmunosorbent test (RIST), J Lab Clin Med, 83: 831, 1974.

2. Abdou, NI, Casella, SR, Abdou, NL, et al, Comparative study of bone marrow and blood B cells in infantile and acquired agammaglobulinemia, J. Clin Invest, 52:2218, 1973.

3. Aisenberg, AC, Malignant Lymphoma, New Engl J Med, 288:883, 1973.

4. Aisenberg, AC, Hodgkin's Disease, in Immunological Diseases, Samter, M-editor, Little, Brown & Co., Boston, 1971.

5. Aisenberg, AC, Bloch, Immunoglobulins on the surface of neoplastic lymphocytes, N Engl J Med, 287:272, 1972.

6. Alexander, P, Connell, DI, Mikulska, ZB, Treatment of a murine leukemia with spleen cells or sera from allogeneic mice immunized against the tumor, Cancer Res, 26:1508, 1966.

7. Amman, AJ, Cain, WA, Ishizaka, K, et al, IgE deficiency and Ataxia-Telangiectasia, N Engl J Med, 281:469, 1969.

8. Arbesman, CE, General discussion, in The Biological Role of the Immunoglobulin E System, Ishizaka, K, Dayton, DH-editors, U.S. Goverment Printing, Washington, 1972, pp. 265-272.

9. Arbesman, CE, Wypych, JI, Reisman, RE, Serum IgE in Human Diseases, in Mechanisms in Allergy, Reagin-Mediated Hypersensitivity, Goodriend, L. et al-editors, Marcel Dekker, New York, 1973.

10. Arnason, BG, deVaux St. Cyr, C. Relyveld, EH, Role of the thymus in immune reactions in rats IV. Immunoglobulins and antibody formation, Int Arch Allerg, 25:206, 1964.

11. Askenase, PW, Cutaneous basophil hypersensitivity in contact-sensitized guinea pigs I. Transfer with immune serum, J. Exp Med, 138:1144, 1973.

12. Askenase, PW, Haynes, DH, unpublished data, personal communication.

13. Augustin, R. Chandradasa, KD, IgE levels and allergic skin reactions in cancer and non-cancer patients, Int Arch Allerg, 41:141, 1971.

14. Augustin, R. West, CR, Sparshott, SM, et al, The effect of alterations in tumor cell surface topography on surface charge, cell viability and immunogenicity, in Immunity and Tolerance in Oncogenesis, Severi, L-editor, Perugia, 1970, p. 937.

15. Bach, MK, Brashler, JR, On the nature of the presumed receptor for IgE on mast cells II. Demonstration of the specific binding of IgE to cell free particulate preparations from peritoneal mast cells, J Immunol, 111:324, 1973.

16. Bartholomaeus, WM, Keast, D, Reaginic antibody to tumor and alloantigens in mice, Nature-New Bio, 239:206, 1972.

17. Bartholomaeus, WM, personal communication.

18. Bass, RM, Potter, EV, Braney, PL, Immunofluorescent localization of immunoglobulins in nasal polyps, Arch Otolaryng, 90:446, 1974.

19. Bast, RC, Zbar, B, Borsos, T, et al, BCG and Cancer, New Engl J Med, 290:1413 & 1458, 1974.

20. Bazaral, M, Orgel, HA, Hamburger, RN, Genetics of IgE and allergy: Serum IgE levels in twins, J Allerg, 54:288, 1974.

21. Bazaral, M. Orgel, HA, Hambruger, RN, IgE levels in normal infants and mothers and an inheritance hypothesis, J Immunol, 107:794, 1971.

22. Bazin, H, Beckers, A, Querinjean, P, Three classes and four (sub) classes of rat immunoglobulins: IgM, IgA, IgE, and IgG1, IgG2a, IgG2c, Eur J Immun, 4:44, 1974.

23. Bazin, H, Querinjean, P, Beckers, A, et al, Transplantable immunoglobulin-secreting tumors, Immunol, 26:713, 1974.

24. Bennich, H, Johansson, SGO, Structure and function of human IgE, Advances in Immun, 13:1, 1971.

25. Benveniste, J, Henson, PM, Cochrane, CG, A possible role for IgE in immune complex disease, in The Biological Role of the Immunoglobulin E System, Ishizaka, K, Dayton, DH-editors, US Government Printing, Washington, 1972.

26. Berg, JW, Morpholocial evidence for immune response to breast cancer, Cancer, 28:1453, 1971.

27. Berg, T. Johansson, SGO, Immunoglobulin levels during childhood with special regard to IgE, Acta Pediat Scand, 58:513, 1969.

28. Berg, T, Johansson, SGO, IgE concentrations in children with atopic disease, Int Arch Allerg, 36:219, 1969

29. Berglund, G. Finnstrom, O, Johansson, SGO, et al, Wiskott-Aldrich syndrome: A study of 6 cases with determination of the immuno-globulins A,D,G,M, and ND, Acta Pediat Scand, 57:89, 1968.

30. Berrens, L, Rijswijk-Berbeck, JV, Inactivation of complement (C3) in human serum by atopic allergens, Int Arch Allerg, 45:30, 1973.

31. Black, JW, Duncan, WAM, Durant, CJ, et al, Definition and anta-gonism of histamine H2-receptors, Nature, 236:385, 1972.

32. Black, MM, Leis, HP, Cellular responses to autologous breast can-cer tissue, Cancer, 28:263, 1971.

33. Black, MM, Leis, HP, Cellular responses to autologous breast can-cer tissue, Cancer, 32:384, 1973.

34. Bloch, KJ, Reaginic and other homocytotropic antibodies: Diverse immunoglobulins with common function, in Mechanisms inAllergy: Reagin-Mediated Hypersensitivity, Goodfriend, L, et al-editors, Marcel Dekker, New York, 1973.

35. Bloch, KJ, Cygan, RW, Waltin, J, The IgE system and parasitism: Role of mast cells, IgE, and other antibodies in host response to primary infection with nippostrongylus brasiliensis, in The Bio-logical Role of the Immunoglobulin E System, Ishizaka, K, Dayton, DH-editors, US Government Printing, Washington, 1972.

36. Bloch, KJ, Ohman, JL, Waltin, J. et al, Potentiated reagin res-ponse: Initiation with minute doses of antigen and alum followed by infection with nippostrongylus brasiliensis, J Immunol, 110:197, 1973.

37. Bourne, HR, Lichtenstein, LM, Melmon, KL, et al, Modulation of in-flammation and immunity by cyclic AMP, Science, 184, 19, 1974.

38. Branch, LB, Nelson, HS, Liptak, R, Serum IgA, and delayed hyper-sensitivity skin tests in allergic patient's, Abstracts-American Academy of Allergy Meeting, Feb., 1975, p. 56.

39. Brown, RS, Haynes, HA, Foley, T, et al, Hodgkin's Disease: immuno-logic, clinical, and histoligic features of 50 untreated patients, Ann Int Med, 67:291, 1967.

40. Buckley, RH, Wray, BB, Belmaker, EZ, Extreme hyperimmunoglobulin-emiaE and undue susceptibility to infection, J. Allerg, 47:108, 1971, abstract.

41. Carpenter, CB, Weiss, MA, Case Records of the Massachusetts General Hospital, Nephrotic syndrome followed by Hodgkin's disease, New Engl J Med, 289:1241, 1973.

42. Carson, D, Metzger, H, Bloch, KJ, Serum IgE levels during the potentiated reagin response to egg albumin in rats infected with nippostrongylus brasiliensis, Abstracts-American Academy of Allergy Meeting, Feb., 1975, p. 81.

43. Carswell, EA, Wanebo, HJ, Old, LJ, et al, Immunogenic properties of reticulum cell sarcomas of SJL/J mice, J Nat'l Cancer Inst, 44:1281, 1970.

44. Clausen, CR, Munoz, J, Bergman, RK, Reaginic-type of antobody in mice stimulated by extracts of B. pertussis, J Immunol, 103:768, 1969.

45. Cohen, C, Genetic aspects of Allergy, Med Clin NA, 58:25, 1974.

46. Cooper, MD, Lawton, AR, Kincaide, PW, A two-stage model for development of antibody-producing cell, Clin Exp Imm, 11:143, 1972.

47. Cormane, RH, B and T cells in dermatitis, Mayo Clin Proc, 49:531, 1974.

48. Currie, GA, Bagshawe, Active immunotherapy with corynebacterium parvum and chemotherapy in murine fibrosarcoma, Brit Med J, 1:541, 1970.

49. Curtis, AC, Heckaman, JH, Wheeter, AH, Study of the autimmune reaction in dermatomyositis, JAMA, 178:571, 1961.

50. Donohygh, DL, Eosinophils and eosinophilia, Calif. Med, 104:421, 1966.

51. Dorf, ME, Newburger, PE, Hamaoka, T, et al, Characterization of an immune response gene in mice controlling IgE and IgG antibody responses to ragweed pollen extract and its 2,4 dinitrophenylated derivative, Eur J Imm, 4:346, 1974.

52. Dupree, E, Friedman, JM, Land, RN, et al, Cell-mediated immunity in atopic dermatitis, Abstracts-American Academy of Allergy Meeting, Feb., 1975, p. 41.

53. Dvorak, HF, Dvorak, AM, Cutaneous Basophil Hypersensitivity, in Progress in Immunology Vol. 3, Brent, L, Holborrow, J-editors, North-Holland Publishing, 1974.

54. Dvorak, HF, Dvorak, AM, Churchill, WH, Immunologic rejection of **diethylnitrososomine induced hepatomas in strain** 2 quinea pigs, J Exp Med, 137:751, 1973.

55. Dwyer, JMD, personal communication.

56. Dworin, M, Diamond, HD, Craver, LF, Hodgkin's disease and allergy, Canoccer, 8:128, 1955.

57. Edelson, RL, Kirkpatrick CH, Shevach, EM, et al, Preferential cutaneous infiltration by neoplastic thymus-derived lymphocytes, Ann Int Med, 80:685, 1974.

58. Edynak, EM, Lardis, MP, Vrana, M, Antigenic changes in human breast neoplasia, Cancer, 28:1457, 1971.

59. Ellis, EF, Henney, CS, Adverse reactions following administration of human gamma globulin, J. Allerg, 43:45, 1969.

60. Evans, R, Alexander, P, Cooperation of immune lymphoid cells with macrophages in tumor immunity, Nature, 228:620, 1970.

61. Evans, R, Alexander, P, Rendering macrophages specifically cytotoxic by a factor release from immune lymphois cells, Transplant, 12:227, 1971.

62. Fireman, P, Friday, G. Kumate, J, Effect of measles vaccine on immunologic responsiveness, Pediatrics, 43:264, 1969.

63. Fisherman, EW, Does allergic diathesis influence malignancy?, J. Allerg, 31:74, 1960.

64. Fishkin, BG, Orloff, N, Scaduto, LE, et al, IgE multiple myeloma: a report of the third case, Blood, 39:361, 1972.

65. Franz, ML, Galant, SP, Correlation of Serum IgE and cutaneous delayed hypersensitivity in atopic children, Abstracts-American Academy of Allergy Meeting, Feb, 1975, p. 55.

66. Gabriel, R, Dudley, EM, Alexander, WD, Lung cancer and allergy, Brit J Clin Pract, 26:202, 1972.

67. Gajl-Peczalska, KJ, Fallow, M, Hansen, JA, et al, IgE-bearing lymphocytes and atopy in a patient with X-linked infantile agammaglobulinemia, Lancet, i:1254, 1973.

68. Gajl-Peczalska, KJ, Hansen, JA, Bloomfield, et al, B lymphocytes in untreated patients with malignant lymphoma and Hodgkin's Diesease, J Clin Invest, 52:3064, 1973.

69. Geha, RS, Schneeberger, E, Menler, E, et al, Heterogeneity of 'Acquired' or common variable agammaglobulinemia, New Engl J Med, 291: 1, 1974.

70. Gerber, MA, Paronette, F, IgE in glomeruli of patients with nephrotic syndrome, Lancet, i:1097, 1971.

71. Ghossein, NA, Stacey, P, The prognostic significance of radiation-related eosinophilia, Radiology, 107:631, 1973.

72. Gleich, GJ, Averbeck, AK, Swedlund, HA, Measurement of IgE in normal and allergic serum by radioimmunoassay, J Lab Clin Med, 77:690, 1971.

73. Grace, JT, Dao, TL, Dermatomyositis in cancer: A possible etiological mechanism, Cancer, 12:648, 1959.

74. Grace, JT, Kondo, T, Investigations of host resistance in cancer patients, Ann Surg, 148:631, 1958.

75. Gupta, S, Grieco, MH, T and B lymphocyte rosettes in bronchial asthma, Abstracts-American Academy of Allergy Meeting, Feb., 1975, p. 38.

76. Gutterman, JU, McBride, C. Freireich, EJ, et al, Active immunotherapy with BCG for recurrent malignant melanoma, Lancet, i:1208, 1973.

77. Guyer, RH, Growther, D, Active immunotherapy in treatment of active leukemia, Br. Med J, 4:406, 1969.

78. Haddad, ZH, Osgood, K, Korotzer, et al, Immune deficiency and IgE, Lancet, i:627, 1970.

79. Hamaoka, T, Katz, DH, Benacerraf, B, Hapten-specific IgE antibody responses in mice II. Cooperative interactions between adoptively transferred T and B lymphocytes in the development of IgE response J Exp Med, 138:538, 1973.

80. Hamaoka, T, Katz, DH, Bloch, KJ, et al, Hapten-specific IgE antibody responses in mice I. Secondary IgE responses in irradiated recipients of syngeneic primed spleen cells, J Exp Med, 138:306, 1973.

81. Hamaoka, T, Newburger, PE, Katz, DH, et al, Hapten-specific IgE antibody responses in mice III. Establishment of parameters for generation of Helper T cell function regulating the primary and secondary responses of IgE and IgG B lumphocytes, J Immunol, 113:958, 1974.

82. Haran-Ghera, N, Ben-Lakov, M, Peled, A, et al, Immune status of SJL/J mice in relation to age and spontaneous tumor development, J Nat'l Cancer Inst, 50:1227, 1973.

83. Henry, C, Jerne, NK, Competition of 19S and 7S antigen receptors in the regulation of the primary immune response, J Exp Med, 128:133, 1968.

84. Herberman, RB, Hollinshead, AC, Alford, TC, et al, Delayed cutaneous hypersensitivity reactions to extracts of human tumors, Nat'l Cancer Inst Mono, 37:189, 1973.

85. Hitzwig, WH, Congential thymic and lymphocytic deficiency disorders, in Immunologic disorders in infants and children, Stiehm, ER, Fulfiniti, VA-editors, Saunders, Philadelphia, 1973.

86. Holt, JE, Yeu-Tsu, NL, Peripheral lymphocyte counts and results of therapeutic castration for advanced mammary carcinoma, Ann Surg, 175:403, 1972.

87. Hong, R, Amman, AJ, Cain, et al, The biological significance of IgE in chronic respiratory infections, in The Secretory Immunologic System, Dayton, DH, et al-editors, US Government Printing, Washington, 1969.

88. Hubscher, T, Eisen, AH, A possible immunopharmacological role of human eosinophils in allergic reactions, in Mechanisms in allergy: Reagin-mediated hypersensitivity, Goodfriend, L, et al-editors, Marcel Dekker, New York, 1973.

89. Isaacson, AH, Rapoport, P, Eosinophilia in malignant tumors, its significance, Ann Int Med, 25:893, 1946.

90. Isersky, C, Kulczycki, A, Metzger, H, Isolation of IgE from reaginic rat serum, J Immunol, 112:1909, 1974.

91. Ishizaka, K, Ishizaka, T, Role of IgE and IgG antibodies in reaginic hypersensitivity in the respiratory tract, in Asthma, Physiology, Immunology, Pharmacology, and Treatment, Austen, KF, Lichtenstein, LM, - editors, Academic Press, New York, 1973.

92. Ishizaka, K, Ishizaka, T, IgE and reaginic hypersensitivity, Annals NYas, 190:443, 1971.

93. Ishizaka, K, Ishizaka, T, Identification of Gamma E Antibodies as a carrier of reaginic activity, J Immunol, 99:1187, 1967.

94. Ishizaka, K, Ishizaka, T, Hornbrook, MM, Physiochemical properties of human reaginic antibody, J Immunol, 99:75, 1966.

95. Ishizaka, K, Kishimoto, T, Hornbrook, MM, Physiochemical properties
     of human reaginic antibody, J Immunol, 99:75, 1966.

96. Ishizaka, K, Okudaira, H, Reaginic antibody formation in the mouse
     J Immunol, 109:84, 1972.

97. Ishizaka, K, Okudaira, H, Reaginic antibody formation in the mouse:
     II. Enhancement and suppression of anti-hapten antibody forma-
     tion by priming with carrier, J Imm, 110:1067, 1973.

98. Ishizaka, K, Tomioka, T, Ishizaka, T, Mechanisms of passive sensi-
     tization, I. Presence of IgE and IgG molecules on human leucocytes,
     J Immunol, 105:1459, 1970.

99. Ishizaka, T, DeBernardo, R. Tomioka, H, et al, Identification of
     basophil granulocytes as a site of allergic histamine release, J
     Immunol, 108:1000, 1972.

100. Ishizaka, T, Sian, CM, Ishizaka, K, Complement fixation by aggre-
     gated IgE, J Immunol, 108:848, 1972.

101. Ishizaka, T. Soto, CS, Ishizaka, K, Mechanisms of passive sensi-
     tization III. Number of IgE molecules and their receptor sites
     on human basophil, J Immunol, 111:500, 1973.

102. Jacobs, D, Landon, J, Houri, M, et al, Circulating levels of IgE
     in patients with cancer, Lancet, ii:1059, 1972.

103. Johansson, SGO, Raised levels of a new immunoglobulin class (IgND)
     in asthma, Lancet, ii:951, 1967.

104. Johansson, SGO, Bennich, H, Immunological studies of an atypical
     (myeloma) immunoglobulin, Immunol, 13:381, 1967.

105. Johansson, SGO, Bennich, H, Wide, L, A new class of Immunoglobulin
     in human serum, Immunol, 14:265, 1968.

106. Johansson, SGO, Juhlin, L, Immunoglobulin E in 'healed' atopic
     dermatitis and after treatment with corticosteroids and azathio-
     prine, Br J Derm, 82:10, 1970.

107. Jones, VE, Edwards, AJ, Preparation of an antiserum specific for
     rat reagin (rat Gamma E?), Immunol, 21:383, 1971.

108. Kahan, BD, Reisfeld, RA, Transplantation Antigens, Science, 164:
     514, 1969.

109. Katz, DH, Hamaoka, T, Newburger, PE, et al, Hapten-specific IgE
      antibody responses in mice IV. Evidence for distinctive sensi-
      tivities of IgE and IgG B lymphocytes to the regulatory influence
      of T cells, J Immunol, 113:974, 1974.

110. Kay, AB, Austen, KF, The IgE mediated release of an eosinophil
      leukocyte chemotactic factor from human lung, J Immunol, 107:899,
      1971.

111. Keller, R, Ogilvie, BM, Simpson, E, Tumor growth in nematode in-
      fected animals, Lancet, i:678, 1971.

112. Kersey, JH, Nesbit, ME, Luckason, JR, et al, Acute lymphoblastic
      leukemic and lymphoma cells with thymus-derived (T) markers. Mayo
      Clin Proc, 49:584, 1974.

113. Kincade, PW, Cooper, MD, Development and distribution of immuno-
      globulin containing cells in the chicken; an immunofluorescence
      analysis using purified antibodies to mu, gamma, and light chains,
      J Immunol, 106:371, 1971.

114. Kincade, PW, Lawton, AR, Bockman, DE, Suppression of immunoglobulin
      systhesis as a result of antibody-mediated suppression of immuno-
      globulin M synthesis in chickens, Prov Nat'l Acad Science, 67:
      1918, 1970.

115. Kind, LS, Macedo-Sobrinho, B, Reaginic antibody formation in mice
      injected with rabbit anti-mouse thymocyte serum, Int Arch Allerg,
      45:780, 1973.

116. Kind, LS, Macedo-Sobrhino, B, Heterologous adoptive cutaneous ana-
      phylaxis: A method for detecting reaginic antibody formation by
      cells of the mouse, J Immunol, 111:638, 1973.

117. Kishimoto, T, Ishizaka, Regulation of antibody response in vitro
      VII. Enhancing soluble factors for IgG and IgE antibody response,
      J Immunol, 111:1194, 1972.

118. Klein, G, Clifford, P, Klein, E, et al, Search for tumor specific
      immune reactions in Burkitt lymphoma patients by the membrane
      immunofluorescence reaction, Proc Nat'l Acad Science, 55:1628, 1966.

119. Konig, W, Okudaira, H, Ishizaka, K, Specific binding of mouse IgE
      with rat. mast cells, J Immunol, 112:1652, 1974.

120. Kubo, RT, Grey, HM, Pirofsky, B, IgD: A major immunoglobulin on the
      surface of lymphocytes from patients with chronic lymphatic
      leukemia, J Immunol, 112:1952, 1974.

121. Kumar, L, Newcomb, RW, Ishizaka, K, et al, IgE levels in sera of children with asthma, Pediatrics, 47:848, 1971.

122. Lawton, AR, Asofsky, RM, Davie, JM, et al, Suppression of Immunoglobulin synthesis in mice: Age dependence of anti-mu suppression and effects of anti (gamma 1 & gamma 2), Anti-gamma 2, and anti-alpha, Red Proc, 32:1012, 1973.

123. Lawton, AR, Asofsky, R, Hylton, MB, et al, Suppression of immunoglobulin class synthesis in mice I. Effects of treatment with antibody to mu chain, J Exp Med, 135;277, 1972.

124. Leech, J, Immunoglobulin positive Reed-Sternberg cells in Hodgkin's Disease, Lancet, ii:265, 1965.

125. Leid, RW, Williams, JF, The immunological response of the rat to infection with taenia taeniaformis, Immunol, 27:209, 1974.

126. Leid, RW, Williams, JF, The immunological response of the rate to infection with taenia taeniaformis, Immunol, 27:195, 1974.

127. Lemonde, P, Clode-hyde, M, Influence of Bacille Calmette-Guerin infection on polyoma in hamsters and mice, Cancer Res, 26:585, 1966.

128. Levin, DM, Lipsky, PE, Kirkpatrick, CH, Selective hypogammaglobulinemia with persistence of IgE, malabsorption, and a nutritionally dependent, reversible defect in cell-mediated immunity, Am J Med, 58:129, 1975.

129. Levine, BB, Atopy and mouse models, Int Arch Allerg, 41:88, 1971.

130. Levine, BB, Stember, RH, Fotino, M, Ragweed Hay Fever: Genetic control and linkage to HL-A Haplotypes, Science, 178:1201, 1972.

131. Levine, BB, Vaz, NM, Effects of comfinations of inbred strains, antigen, and antigen dose on immune responsiveness and reagin production in the mouse, Int Arch Allerg, 39:156, 1970.

132. Levy, DA, Chen, J, Healthy IgE deficient person, New Engl J Med, 238:541, 1970.

133. Lewis, EJ, Kallen, RJ, Glomerular localisation of IgE in lipoid nephrosis, Lancet, i:1395, 1973.

134. Lichtenstein, LM, Ishizaka, K, Norman, PS, et al, IgE antibody measurement in ragweed hay fever, J Clin Invest, 52:472, 1973.

135. Lichtenstein, LM, Osler, AG, studies on the mechanism of hypersensitivity phenomena IX. Histamine release from human leukocytes by ragweed pollen antigen, J Exp Med, 120:507, 1964.

136. Lichtenstein, IM, Plaut, M, Hennery, C, et al, The role of H2-receptors on the cells involved in hypersensitivity reactions, in <u>International Symposium on Histamine H2-Receptor Antagonists</u>, Smith, Kline, and French, London, 1973.

137. Likhite, VV, The delayed and lasting rejection of mammary adeno-carcinoma cell tumors in DBA/2 mice with the use of killed bordetella pertussis, Cancer Res, 34:1027, 1974.

138. Lobitz, WC, Honeyman, JF, Winkler, nW, Suppressed cell-mediated immunity in two adults with atopic dermatitis, Br J Derm, 86:317 1972.

139. Logan, J, Saker, D, The incidence of allergic disorders in cancer, New Zealand Med J, 52:210, 1953.

140. Lytton, B, Hughes, LE, Fulthorpe, AJ, Circulating antibody response in malignant disease, Lancet, i:69, 1964.

141. Mackay, WD, The incidence of allergic disorders and cancer, BrJ Cancer, 20:434, 1966.

142. Markari, JF, Detection of antigens in sera of patients with neo-plastic disease by Schultz-Dale Test, Br Med J, 2:359, 1958.

143. Mangi, RJ, Niederman, JC, Kelleher, JE, et al. Depression of cell-mediated immunity during acute infectious mononucleosis, New Engl J Med, 291:1149, 1974.

144. Manning, DD, Receovery from anti-immunoglobulin induced immuno-suppression: Implications for a model of immunoglobulin secret-ing cell development, J Immunol, 113:455, 1974.

145. Manning, DD, Induction of temporary IgA deficiency in mice inject-ed with heterologous anti-immunoglobulin heavy chain antisera, J Immunol, 109:1152, 1972.

146. Manning, DD, Jutila, JW, Prevention of myeloma growth in Balb/C mice by injection of anti-immunoglobulin antisera, J Immunol, 113:78, 1974.

147. Manning, DD, Jutila, JW, Effect of anti-immunoglobulin antisera on homograft rejection in mice, Nature (New Bio), 237:58, 1972.

148. Manning, DD, Jutila, JW, Immunosuppression of mice injected with heterologous anti-immunoglobulin heavy chain antiera, J Exp Med, 135, 1316, 1972.

149. Manning, DD, Koultab, NM, Jutila, JW, Effect of anti-mu specific immunosuppression on Friend virus leukemia, J Immunol, 112:1698, 1974.

150. Marsh, DG, Bias, WB, Hsu, SH, et al, Association of the HL-A7 cross reacting group with a specific reagin antibody response in allergic man, Science, 179:691, 1973.

151. Martin, LN, Leslie, GA, IgM-forming cells as the immediate precursor of IgA producing cells during ontogeny of the immunoglobulin - producing system of the chicken, J Immunol, 113:120, 1974.

152. Matchett, KM, Huang, AT, Kramer, WB, Impaired lymphocyte transformation in Hodgkin's Disease, evidence for depletion of circulating T lymphocytes, J Clin Invest, 52:1908, 1973.

153. McGredie, JA, Inch, WR, Sutherland, RM, Peripheral blood Lymphocytes and breast cancer, Arch Surg. 107, 162, 1973.

154. McGeady, SJ, Personal communication, March 12, 1974.

155. McGeady, SJ, Buckley, RH, Studies of cell-mediated immune function in atopic eczema, J. Allerg, 53:72, 1974. (abstract)

156. McKee, WD, Arnold, CA, Perlman, MD, A double blind study of the comparative incidence of malignancy and allergy, J Allerg, 39: 294, 1967.

157. McKelvey, EM, Fahey, JL, Immunoglobulin changes in disease, J Clin Invest, 44:1778, 1965.

158. Meacock, SCR, Marsden, CH, The effects of adjuvants and stress on the production of IgE antibody in rats, Int Arch Allerg, 45:136, 1973.

159. Meers, PD, Allergy and cancer, Lancet, i:884, 1973.

160. Merrett, TG, Personal communication, Feb. 22, 1974.

161. Meyer, KM, Mackler, GL, Beck, WC, Increased IgA in women free of recurrence after mastectomy and radiation, Arch Surg, 107:159, 1973.

162. Meltzer, MS, Leonard, EJ, Rapp, HJ, et al, Tumor-specific antigen solubilized by hypertonic potassium chloride, J Nat'l Cancer Inst, 47:703, 1971.

163. Miller, DL, Hirvonen, T, Gitlin, D, Synthesis of IgE by the human conceptus, J Allerg, 52:188, 1973.

164. Mota, I, Wong, D, Homologous and heterologous passive cutaneous anaphylactic activity of mouse antisera during the course of immunization, Life Sciences, 8:813, 1969.

165. Munyer, TP, Measles virus induced anergy: A clinical and ex-
     perimental study, Yale University Medical School Thesis, 1974.

166. Murgita, RA, Mattioli, CA, Tomasi, TB, Production of a runting
     syndrome and selective Gamma A deficiency in mice by the admin-
     istration of anti-heavy chain anti sera, J Exp Med, 138:209,
     1973.

167. Newcomb, RW, Ishizaka, K, Skin reaction to anti-gamma E antibody
     in atopic and non-atopic and immunologically deficient children
     and adults, J Allerg, 43:292, 1969.

168. Nordbring, F. Johansson, SGO, Apsmark, A, Raised serum levels of
     IgE in infectious mononucleosis, Scand J Infect Dis, 4:119, 1972.

169. Nowakowski, M, Feldman, JD, Kano, S, et al, The production of
     vesicular stomatitis virus by antigen or mitogen-stimulated
     lymphocytes and continuous lymphoclastoid lines, J Exp Med,
     137:1042, 1973.

170. Ogawa, M, Kochwa, S. Smith, C, et al, Clinical aspects of IgE my-
     eloma, New Engl J Med, 28:1217, 1969.

171. Ogilvie, BM, Jones, VE, Immunity in the parasitic relationship
     between helminths and hosts, Progr Allerg, 17:93, 1973.

172. Okudaira, H, Ishizaka, K, Reaginic antibody formation in the mouse
     III. Collaboration between hapten-specific memory cells and
     carrier specific helper cells for secondary anti-hapten antibody
     formation, J Immunol, 111:1420, 1973.

173. Okudaira, H, Ishizaka, K, Reaginic antibody formation in the
     mouse, IV. Adoptive anto-hapten IgE antibody response in irradiated
     recipients of hapten primed cells and carrier specific cells, J
     Immunol, 113:563, 1974.

174. Okumura, K, Tada, T, Regulation of homocytropic antibody formation
     in the rat IX. Further characterization of the antigen specific
     inhibitory T-cell factor in hapten specific homocytotropic response,
     J Immunol, 112:783, 1974.

175. Okumura, K, Tada, T, Regulation of homocytotropic antibody forma-
     tion in the rat, J Immunol, 107:1682, 1971.

176. Okumura, K, Tada, T, Ochiai, T, Effect of antithymocyte serum on
     reaginic antibody formation in the rat, Immunol, 26:257, 1974.

177. Ovary, Z, Passive cutaneous anaphylaxis, in Immunological Methods,
     Ackroyd, JF-editor, Blackwell Scientific Publications, Oxford, 1964.

178. Pierce, CW, Solliday, SM, Asofsky, R, Immune responses in vitro IV. Suppression of primary Gamma M, G. and A plaque-forming cell responses in mouse spleen cell cultures by class-specific antibody to mouse immunoglobulins, J Exp Med, 135:675, 1972.

179. Pierce, CW, Solliday, SM, Asofsky, R, Immune responses in vitro V. Suppression of Gamma M, G. and A plaque-forming cell responses in cultures of primed mouse spleen cells by class-specific antibody to mouse immunoglobulins, J Exp Med, 135:698, 1974.

180. Piessens, Wf, Lachapelle, FL, Legros, N, et al, Facilitation of rat mammary tumor growth by BCG, Nature, 228:1210, 1970.

181. Piessens, WF, Schur, PH, Moloney, WC et al, Lymphocyte surface immunoglobulins: Distribution and frequency in lymphoproliferative diseases, New Engl J Med, 288;176, 1973.

182. Plaut, M, Lichtenstein, LM, Gillespie, E, et al, Studies on the mechanism of lymphocyte-mediated cytolysis IV. Specificity of the histamine receptor on effector T cells, J Immunol, 111:389, 1973.

183. Polmar, SH, Comments on serum IgE in DiGeorge syndrome, in The Biological Role of the Immunoglobulin E System, Ishizaka, K, Dayton, DH-editors, US Government Printing office, Washington, 1972, p. 146.

184. Polmar, SH, Waldmann, TA, Balestra, ST, et al, IgE in immunologic deficiency disease I. Relation of IgE and IgA to respiratory tract disease in isolated IgE deficiency, IgA deficiency, and Ataxia-Telangiectasia, J Clin Invest, 51:326, 1972.

185. Polmar, SH, Waldmann, TA, Terry, WD, A comparison of 3 radio-immunoassay techniques for the measurement of serum IgE, J Immunol, 110:1253, 1973.

186. Parish, WE, Raginic and non-reaginic antibody reactions on ana-phylactic participating cells, in Mechanisms in Allergy: Reagin Mediated Hypersensitivity, Goodfriend, L, et al, editors, Marcel Dekker, New York, 1973.

187. Prehn, RT, Immunosurveillance, regeneration, and oncogenesis, Prog Exp Tumor Res, 14:1, 1970.

188. Priori, ES, Seman, G, Dmochowski, L, L. et al, Immunofluorescence studies on sera of patients with breast carcinoma, Cancer, 28:1462, 1971.

189. Prouvost-Danon, A, Characterization of mouse IgE and mast cell reactions, in Mechanisms in Allergy, Reagin-Mediated Hyper-sensitivity, Goodfriend, L, et al - editors, Marcel Dekker, New York, 1973.

190. Rachelefsky, GS, Ferguson, AC, Stiehm, ER, et al, IgE and allergy in antibody immunodeficiencies, J Allerg, 51:111, 1973. (abstract)

191. Reed, CE, The pathogenesis of asthma, Med Clin NA, 58:55, 1974.

192. Ritts, RE, Neel, HB. An overview of cancer immunology, Mayo Clin Proc, 49:118, 1974.

193. Roberts, MM, Bass, EM, Studies of the immune response in breast cancer, Br J Cancer, 28:77, 1973. (abstract)

194. Roberts, MM, Bass, EM, Wallace, IWJ, et al, Local immunoglobulin production in breast cancer, Br J Cancer, 27:269, 1973.

195. Rosenbaum, JT, Basophil or IgE-bearing lymphocyte:, New Engl J Med, 291:678, 1974.

196. Rosenberg, EB, Whalen, GE, Bennich, et al, Increased circulating IgE in a new parasitc disease-Human Intestinal Capillariasis, New Engl J Med, 283:1148, 1970.

197. Roy, LP, Westberg, NG, Michael, AP, Nephrotic syndrome: No evidence for a role for IgE, Clin Exp Immun, 13:553, 1973.

198. Rubenstein, E. Reisman, RE, Sokal, JE, et al, Relationship of serum total IgE and cell-mediated immunity in patients with Hodgkin's Disease, Abstracts-American Academy of Allergy Meeting, Feb., 1975, p. 57.

199. Ruckdeschel, JC, Codish, SD, Stranahan, A, et al, Postoperative empyema improves survival in lung cancer, New Engl J Med, 287, 1013, 1972.

200. Rud, F, The eosinophil count in health and mental disease, A biometrical study, Acta Psychiatric Et Neuro, Supp, 40, 1947.

201. Schumacher, MJ, Ellis, EF, Leucocyte histamine release studies and skin tests with aggregated gamma globulin in hypogammaglobulinemia, Aust N Z J Med, 4:356, 1974.

202. Schwartz, DP, Buckley, RH, Serum IgE concentrations and skin re-activity to anti-IgE antibody in IgA deficient patients, New Engl J Med, 284:513, 1974.

203. Serafini, U, Organ specific autoantibodies in reagin-containing sera from allergic patients, Lancet, ii:821, 1965.

204. Shapiro, S, Heinonen, OP, Siskind, U, Cancer and Allergy, Cancer 28:396, 1971.

205. Silver, J, Benacerraf, B, Dissociation of T cell helper function and delayed hypersensitivity, J Immunol, 113:1872, 1974.

206. Smith, HJ. Ozkaragoz, K, Gokcen, M, A simplified radioimmuno-assay technique for measuring human IgE, J Allerg, 50:193, 1972.

207. Smith, JM, Incidence of atopic disease, Med Clin N A, 58:3, 1974.

208. Smith, RT, Possibilities and problems of immunologic intervention in cancer, New Engl J Med, 287:439, 1972.

209. Southam, CM, The immunologic status of patients with nonlymphomatous cancer, Cancer Res, 28:1433, 1968.

210. Spitz, E, Gelfand, EW, Sheffer, AL, et al, Serum IgE in clinical immunology and allergy, J Allerg, 49:337, 1972.

211. Stanworth, DR, Humphrey, JH, Bennich, H, et al, Inhibition of Prausnitz-Kustner reaction by proteolytic cleavage fragments of human myeloma protein of class immunoglobulin E, Lancet, ii:17, 1968.

212. Stechschulte, DT, Orange, RP, Austen, KF, Immunochemical and biologic properties of rat IgE: I. Immunochemical identification of rat IgE, J Immunol, 105, 1082, 1970.

213. Steinberg, P, Ishizaka, K, Norman, PS, Is IgE a 'gatekeeper' for other antibodies?, J Allerg, 51:109, 1973. (abstract)

214. Stewart, THM, Orizaga, M, The presence of delayed hypersensitivity reactions in patients toward cellular extracts of their malignant tumors III. The frequency, duration, and cross reactivity of this phenomenon in patients with breast cancer, and its correlation with survival, Cancer, 28:1472, 1971.

215. Stites, DP, Ishizaka, K, Fudenberg, H, Serum IgE concentrations in hypogammaglobulinemia and selective IgA deficiency, Clin Exp Imm, 10:391, 1972.

216. Stokes, CR, Hosking, CS, Turner, MW, et al, Urinary IgE: a reappraisal, Eur J Immunol, 3:241, 1973.

217. Stokes, CR, Taylor, B, Turner, MW, Association of house-dust and grass pollen allergies with specific IgA antibody deficiency, Lancet, ii:485, 1974.

218. Strannegard, O, Belin, L, Enhancement of reagin formation in rabbits by passively administered 19S antibody, Immunol, 20:427, 1971.

219. Strom, TB, Sytkowski, AJ, Carpenter, CB, et al, Cholinergic augmentation of lymphocyte mediated cytotoxicity. A study of the cholinergic receptor of cytotoxic T lymphocytes, Proc Nat'l Acad Science, 71:1330, 1974.

220. Sweeney, WT, Seibel, HR, Histamine release from peritoneal mast cells of tumor susceptible rats following periods of tumor growth and sensitization with tumor antigen and B pertussis, Int Arch Allerg, 48:289, 1973.

221. Tada, T, Ishizaka, K, distribution of Gamma E forming cells in lymphoid tissues of the human and monkey, J Immunol, 104:377, 1970.

222. Tada, T, Okumura, K, Regulation of homocytotropic antibody formation in the rat, J Immunol, 106:1002, 1971.

223. Tada, T, Okumura, K, Taniguchi, M, Regulation of homocytotropic antibody formation in the rat VIII. An antigen specific T cell factor that regulates anti-hapten homocytotropic antibody response, J Immunol, 111:952, 1973.

224. Tada, T, Okumura, K, Taniguchi, M, Cellular basis of IgE antibody response in the rat, in The Biological Role of the Immunoglobulin E System, US Government Printing office, Washington, 1972.

225. Taniguchi, M, Tada, T, Regulation of homocytotropic antibody formation in the rat X. IgT-like molecule for the induction of homocytotropic antibody response, J Immunol, 113:1757, 1974.

226. Taylor, B, Norman, AP, Orgel, HA, et al, Transient IgA deficiency and pathogenesis of infantile atopy, Lancet, ii:111, 1973.

227. Treadwell, PE, The inheritance of susceptibility to anaphylaxis in inbred mice and their hybrid progenies, J Retic Endo Soc, 6:343, 1969.

228. Ure, DMJ, Negative association between allergy and cancer, Scot Med J, 14:51, 1969.

229. Vaz, NM, DeSouza, CM, Maic, LCS, Genetic control of immune responsiveness in mice responsiveness to ovalbumin in (C57Bl X DBA/2) F1 Mice, Int Arch Allergy, 46:275, 1974.

230. Vaz, NM, Phillips-Quagliata, JM, Levine, BB, et al, H-2 linked genetic control of immune responsiveness to ovalbumin and ovomucoid, J Exp Med, 134:13335, 1971.

231. Waldmann, TA, Bull, JM, Bruce, RM, et al, Serum immunoglobulin E levels in patients with neoplastic disease, J Immunol, 113:379 1974.

232. Waldmann, TA, Polmar, SH, Balestra, ST, et al, IgE in immunologic deficiency diseases II. Serum IgE concentration of patients with acquired hypogammaglobulinemia, thymoma and hypogammaglobulinemia, myotonic dystrophy, intestinal lymphangiectasia, and Wiskott-Aldrich Syndrome, J Immunol, 109:304, 1972.

233. Waldmann, TA, Strober, W, Blaese, RM, Immunodeficiency disease and malignancy, Ann Int Med, 77:605, 1972.

234. Waldmann, TA, Strober, W. Polmar, SH, et al, IgE levels and metabolism in immune deficiency diseases, in The Biological Role of the Immunoglobulin E System, Ishizaka, K, Dayton, DH-editors, US Government Printing Office, Washington, 1972.

235. Warren, KS, Inhibition of granuloma formation around schistosoma mansoni eggs, Am J Path, 56:293, 1969.

236. Wasserman, SI, Goetzl, EJ, Austen, KF, Inactivation of human SRS-A by intact human eosinophils and by eosinophil arylsulfatase, Abstracts American Academy of Allergy Meeting, Feb, 1975, p. 4.

237. Wasserman, SI, Goetzl, EJ, Ellman, L, et al, Tumor-Associated eosinophilotactic factor, New Engl J Med, 290:420, 1974.

238. Weatherly, NF, Increased survival of swiss mice given sublethal infections of trichinella spiralis, J Parasitol, 56:748, 1970.

239. Werner, SC, Wegelius, O, Fierer, JA, et al, Immunoglobulins (E,M,G) and complement in the connective tissues of the thyroid of Graves Disease, New Engl J Med, 287:421, 1972.

240. White, GJ, Holm, MS, Induction of reagin synthesis in the rat with associated and dissociated hemocyanin: Effect of antilymphocyte serum, J Immunol, 110:327, 1973.

241. Wide, L, Bennich, H, Johansson, SGO, Diagnosis of allergy by an in vitro test for allergen antibodies, Lancet, ii:1105, 1967.

242. Williams, RC, Griffiths, RW, Emmons, JD, et al, Naturally occurring antiglobulins with specificity Gamma E, J Clin Invest, 51:955, 1972.

243. Wohl, H, Ghossein, NA, Complement levels before and after radiotherapy in cancer patients, Oncology, 25:344, 1971.

244. Wolf, A, The activity of cell-free tumor fractions, in inducing immunity across a weak histocompatibility barrier, Transplantation, 7:49, 1969.

245. Woods, AH, The immunology of cancer, Avenues to therapy, Ariz Med, 30:463, 1973.

246. Wybran, J, Fudenberg H, Thymus derived rosette forming cells in human disease states: cancer, lymphomas, viral and bacterial infections, and other diseases, J Clin Invest, 52:1026, 1973.

247. Zbar, B, Tanaka, T, Immunotherapy of cancer: Regression of tumors after intralesional injection of living mycobacterium bovis, Science, 172:271, 1971.

248. Zweiman, B, Miller, MF, Characteristics of measles virus-induced lymphocyte depression, J Allerg, 51:126, 1973. (abstract)

249. Floersheim, GL, Facilitation of tumor growth by bacillus pertussis, Nature, 216:1235, 1967.

250. Major, RH, Leger, LH, Marked eosinophilia in Hodgkin's disease, JAMA, 112:2601, 1939.

251. Michael, JG, Bernstein, IL, Greenert, S, et al, Reaginic antibodies in non-atopic individuals, Fed Proc, 32:999, 1973.

252. Perlin, E, McCoy, JL, Dean, JH, et al, Tumor-associated antigen in femal and male breast cancer, New Engl J Med, 292:45, 1975.

253. Peterson, DAR, Page, AR, Good, RA, Wheal and erythema allergy in patients with aggmmaglobulinemia, J Allerg, 33:406, 1962.

254. Bennich, H, Ishizaka, K, Ishizaka, K, Ishizaka, T, et al, A comparative antigenic study of gamme E-globulin and myeloma-IgND, J Immunol, 102:826, 1969.

255. Ishizaka, T, Ishizaka, K, Orange, RP, et al, The capacity of human IgE to mediate release of histamine and SRS-A from monkey lung, J Immunol, 104:335, 1970.

256. Bergmann, K-C, Zaumseil, I, Lachman, B, Quantitative IgE-Bestimmung bei Sarkoidose, Zeitschrift fur Erkrankungen der Atmungsorgane, 137:351, 1973.

257. Segal, S, Cohen, IR, Feldman, M, Thymus-derived lymphocytes: Humoral and cellular reactions distinguished by hydrocorisone, Science, 175:1126, 1972.

258. Sears, WG, The blood in hodgkin's disease with special reference to eosinophilia, Guys Hosp Report, 82:40, 1932.

259. Rowinska-Zakrewska, E, Lazar, P, Burtin, P, Dosage des immuno-globulines dans le serum des cancereux, Ann Inst Pasteur, 119:621, 1970.

260. Hughes, Nr, Serum concentration of Gamma G, A, and M immunoglobukins in patients with carcinoma, melanoma, and sarcoma, JNCI, 46:1015, 1971.

261. Greaves, MF, Hogg, NM, Immunoglobulin determinants on the surface of antigen binding T- and B-lymphocytes in mice, in Progress in Immunology, Amos, B-editor, Academic Press, New York, 1971.

262. Gottlieb, RR, Hanifin, JM, unpublished data as cited in Cormane, RH, B and T cells in dermatitis, Mayo Clin Proc, 49:531, 1974.

263. Augustin, R. personal communication.

264. Boyum, A, Isolation of mononuclear cells and granulocytes from human blood, Scan J Clin Lab Invest, 21: Suppl 97:77, 1968.

265. Friday, GA, Kumate, J, Fireman, P, Suppression of delayed hyper-sensitivity and atopic manifestations by measles vaccine, J. Allerg, 41:111, 1968 (abstract).

266. Aden, DP, Manning, DD, Reed, ND, unpublished data, cited in Manning, DD, Jutila, JW, Prevention of Myeloma growth in Balb/C mice by injection of anti-immunoglobulin antisera, J Immunol, 113:78, 1974.

267. Polmar, SH, General discussion, in The Biological Role of the immunoglobulin E system, Ishizaka, K, Dayton, DH-editors, US Goverment Printing Office, Washington, 1972, p. 146.

268. Pinckard, RN, Halonen, M, Meng, AL, Preferential expression of anti-bovine serum albumin IgE homocytotropic antibody synthesis and anaphylactic sensitivity in the neonatal rabbit, J Allerg, 49: 301, 1972.

269. McLaughlan, P, Stanworth, DR, Webster, ADB, Asherson, G. L., Serum IgE in Immune Deficiency Disorders. Clin Exp Immunol 16:375 1974.