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ANNUAL REPORT

Conference on Immunology

Title: Normal and Abnormal Aspects of Immunologic Regulation

by

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MEETING REPORT

MIDWINTER CONFERENCE OF IMMUNOLOGISTS

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The Seventeenth Midwinter Conference of Immunologists was held January 21-24, 1978, at the Asilomar Conference Grounds, Pacific Grove, California, with Eli Benjamini and Norman Talal as co-chairs. The subject of the conference, "Normal and Abnormal Aspects of Immunologic Regulations," was discussed during five half-day sessions by invited speakers.

One evening of the conference was devoted to a speaker provided by the Graduate Students and Postdoctoral Immunologists Association. Their speaker was Dr. Mark Lappe', who spoke on the "Regulation of Scientific Research."

The Fourth Annual Dan H. Campbell Memorial Lecture was given by Baruj Benacerraf (Harvard Medical School). His lecture was preceded by a few introductory remarks by George Feigen (Stanford University), a close associate and friend of Dr. Campbell for many years. H. O. McDevitt (Stanford University) introduced Dr. Benacerraf, whose topic for the opening talk was "The Regulation of Immune Responses by Specific Suppressor T Cells and Suppressor Factor."

Dr. Benacerraf presented data demonstrating the induction of suppressor T cells to the antigen [poly (Glu^{60} Ala^{30} Tyr^{10})] (GAT), and that the induction of suppressor cells is under the control of immune response (Ir) genes. A soluble factor extracted from suppressor T cells was described, and the properties of the suppressor factor were discussed. The suppressor factor: (1) binds antigens, (2) has a molecular weight between 40,000 and 50,000, (3) is coded for by the Ir gene, (4) is active both in vivo and in vitro, and (5) can function across the H-2 barrier.

Dr. Benacerraf presented data that indicate the soluble suppressor factor also may induce normal nonimmune spleen cells to produce suppressor T cells. Because the suppressor factor combined with antigen, the role of antigen-bound T suppressor factor was explored. Affinity chromatography was used for removal of antigen from the antigen-suppressor factor complex to attain antigen-free soluble suppressor factor. Antigen-free suppressor factor cultured with normal spleen cells did not induce the formation of suppressor T cells.

Experiments designed to determine if the in vivo administration of antisera against the I-J region could deplete the recipient of suppressor T cells and permit a normal immune response were presented, and the potential application of such antisera to such various disease states was discussed.

The first session of the conference, convened by Harvey Cantor (Harvard Medical School), dealt with general aspects of immunologic regulation. Dr. William Paul (National Institutes of Health), the first speaker, talked about immunologic recognition in cellular interaction.

The activation of antigen-specific thymus-dependent (T) lymphocytes for in vitro proliferation depends upon the interaction of these cells with antigen-presenting cells. The latter are Ia^+, Ig^-, $Thyl^-$, adherent radioresistant cells that are probably a subset of cells within the macrophage-monocyte lineage. The activation of primed T lymphocytes by such antigen-presenting cells is most efficient if the donor of the T lymphocytes and the donor of the antigen-presenting cells possess a common allelic form of the I-A subregion of the major histocompatibility complex (MHC). Furthermore, in in vitro responses that are controlled by MHC-linked specific immune response (Ir) genes, the donor of the antigen-presenting cells must possess a responder allele of the

Ir gene controlling the response if it is to stimulate the response of primed T lymphocytes. These results suggest that the primed T lymphocytes detect two characteristics of the antigen-presenting cell; the antigen that it bears and the Ia molecules expressed on it. Furthermore, Ir genes express their functions at least within antigen-presenting cells.

Recent studies have examined the "rules" for cellular interactions and Ir gene product expression in the more complex dual Ir gene system controlling the immune response to poly-(Glu, Lys, Phe) (GLϕ). Paul and his associates have concluded that both Ir genes must be expressed during lymphocyte activation, that both control cell surface phenomena, that both are expressed in the same cell, and that the antigen-presenting cell is a site of expression of these genes. These conclusions were based on studies on the inhibition of activation by anti-Ia sera, immune responsiveness of appropriate radiation chimeras, and presentation functions of antigen-presenting cells.

The joint recognition by T lymphocytes of GLϕ and the gene products of two separate I regions places very serious constraints on immunologic recognition mechanisms of T lymphocytes and on antigen-processing mechanisms of antigen-presenting cells. On the whole, these data are most consistent with either "linked dual recognition" or "complex antigenic determinant recognition." They strongly suggest that the antigen-processing event is a locus of Ir gene product expression.

Dr. Richard Gershon (Yale University School of Medicine) next discussed various aspects of immunologic regulation in cellular interactions.

Dr. Gershon and his associates have shown (a) that purified helper T cells induce cells of another T cell set, expressing the Lyl,2,3$^+$ Qal$^+$ surface phenotype, to exert potent suppressive activity, (b) that this T-T

interaction plays an important role in regulating in vivo immune responses, and (c) that this interaction represents an important barrier to procedures intended to augment the immune status of individuals by adoptive (or active) immunotherapy. Their results also indicate that the Lyl,2,3^{+} T cell set mediating feedback suppressions in vivo is sensitive to both low doses of cyclophosphamide and removal of the thymus in adult life. The importance of this T-T interaction to normal physiological regulation of the immune response is emphasized by the finding that the major T cell deficit of NZB mice (an inbred strain of New Zealand Black mice in which an autoimmune disorder spontaneously developes) is the absence and/or the malfunction of Lyl,2,3^{+} T cell set responsible for feedback inhibition.

The third paper, by Tadamitsu Kishimoto (Osaka University Medical School), discussed regulation of immunoglobulin class by T lymphocytes.

Previously, Dr. Kishimoto and his associates had shown the requirement of distinctive helper T cells for the activation of precursors of cells that secrete IgG and IgE. Several recent studies have also suggested that helper T cells are uniquely specific in their interaction with precursors of antibody-forming cells that bear a given class or subclass of Ig or even a given allotype of an IgG class. These results suggest that suppressor T cells for the IgE antibody response may also be distinct from those for the IgG antibody response and that the Ig-class antibody response may be regulated by Ig-class specific helper and suppressor T cells.

Dr. Kishimoto summarized some recent experiments about IgE-class specific suppressor T cells and their soluble products. Pretreatment of BALB/c mice with DNP-Mycobacterium suppressed induction of the anti-DNP IgE antibody response with DNP-OA, whereas the IgG antibody response was enhanced by the

pretreatment. The selective suppression of the IgE antibody response was shown to be due to the induction of DNP-reactive IgE-class specific suppressor T cells with DNP-Mycobacterium.

The suppressor function of IgE-class specific suppressor T cells was mediated with soluble products released from these cells upon stimulation with DNP-antigen. Antigen-specific stimulation was required for the induction of suppressor factor(s), but the suppressor activity of the factor(s) was not antigen-specific and was not adsorbed with DNP-antigen. The suppressor factor (or factors) has a determinant encoded with the MHC gene and did not exert its effect across the MHC barrier. The suppressor activity was adsorbed with spleen cells that had been primed with DNP-OA for induction of an IgE antibody response, but the activity was not adsorbed with nylon-purified T cells, a fact that shows the target cells were B or nylon-adherent T cells precommitted to the IgE antibody response. One clone of T cell hybrids between BW 5147 and T cells from DNP-Mycobacterium-primed BALB/c mice continuously secretes IgE-class specific suppressor factor. The hybrid T cells expressed Thy1,2, H-2K^{d}, and H-2D^{d} on their surface and had 62 chromosomes. The T cell hybrid that shows the function of IgE-class specific suppressor T cells will be a useful tool in the study of the mechanism of Ig-class specific regulation of an antibody response by T cells.

The first session concluded with a paper by Thomas A. Waldmann (National Institutes of Health) on disorders of regulatory T cells in human disease.

A series of suppressor cell systems regulates virtually all immunologic processes. Recently, it has been recognized that many immunodeficiency, autoimmune, and malignant diseases are associated with disorders of these negative regulatory or suppressor cell systems. In addition, T cell leukemias

have been identified that represent monoclonal expansion of either the helper or suppressor T cell systems. To analyze these defects of immunoregulatory cells, Waldman and his associates utilized a technique for the study of differentiation of peripheral blood B lymphocytes in vitro with the polyclonal stimulant pokeweed mitogen to drive B cells into terminal maturation into immunoglobulin-synthesizing cells. A modified version of this technique was utilized in the assessment of helper T cell and suppressor T cell activities. When assessed with these techniques, the majority of patients with common variable hypogammaglobulinemia or with selective IgA deficiency were demonstrated to have an intrinsic defect of the B cell - plasma cell system. However, in a subset of patients a disorder of the suppressor cell system appeared to play a more significant role in the pathogenesis of the immunologic defects. An abnormal number of activated suppressor T cells was demonstrated in this group of patients with common variable hypogammaglobulinemia. The coculture of cells from the patients of this group with normal cells suppressed immunoglobulin synthesis by the normal cells. In addition, the purified B cells from these patients were able to synthesize immunoglobulin molecules normally when separated from their circulating suppressor T cells. Some patients with a selective IgA deficiency had an associated circulating IgA-class specific suppressor T cell. Non-T-cell suppressor cells that appear to play a role in a polyclonal deficiency associated with multiple myeloma have been demonstrated in the circulation of patients with this disorder. At the other end of the spectrum of immunologic response, a reduction in the capacity to generate suppressor T cells and suppressor T cell products has been implicated in the pathogenesis of autoimmune diseases in mice.

Waldman and co-workers have studied T cell leukemias of man to define homogeneous T cell populations with retained immunologic activity. When analyzed by the in vitro biosynthesis technique, peripheral blood leukemic T cells of patients with Sezary syndrome could not synthesize immunoglobulin molecules, nor could they be activated to become suppressor cells; however, the leukemic cells of some (but not all) patients with this disorder were able to act as the helper cells required for the maturation of B cells to immunoglobulin-synthesizing cells in pokeweed-mitogen-stimulated cultures. Thus, the leukemic cells of some patients with the Sezary syndrome originate from a subset of T cells programmed exclusively for helper interactions with B cells in their production of immunoglobulin molecules. In general, acute lymphocytic lymphoblastic leukemic T cells do not synthesize immunoglobulin molecules nor demonstrate helper or suppressor T cell function. Waldman et al. observed an interesting exception to this generalization in a patient with leukemia and profound hypogammaglobulinemia. The leukemic cells from this patient acted as a potent suppressor of immunoglobulin synthesis when cocultured with normal cells. These leukemic cells required a cooperating subset of normal T cells in order to express a full suppressor effect. These observations are consistent with the view that two T cells, a prosuppressor and a suppressor activator, must interact for the generation of suppressor effector cells. Leukemic cells of this patient were presumably of either the prosuppressor or the suppressor activator class but were not suppressor effector cells. It is hoped that the study of malignant T cells and their products may eventually prove to be as rewarding in resolving questions regarding the T cell regulation of humoral immune responses as the study of myelomas and their paraprotein products has proved to be in resolving questions concerning humoral immunity.

The second session, on the immunoregulatory effects of the histocompatibility system, was chaired by Hugh McDevitt (Stanford University School of Medicine). It opened with Paul Terasaki (University of California at Los Angeles), who discussed the association of HLA antigens on B lymphocytes with disease susceptibility.

The highest association of an HLA antigen with disease is found with HLA-B27 and ankylosing spondylitis. People with B27 have a relative risk (RR) of 88, i.e., they are 88 times more susceptible to having ankylosing spondylitis than non-B27 people. Other diseases, e.g., Reiter's disease, psoriatic arthritis, and anterior uveitis, are associated with B27. The largest group of diseases associated with a single HLA antigen is that with HLA-B8. This group includes celiac, dermatitis, myasthenia gravis (MG), Sjogren's disease, Addison's disease, Graves' disease, juvenile onset diabetes, and chronic active hepatitis. The RRs for these diseases range from 2 to 9. There are only a few other diseases for which associations with HLA are now well established: hemachromatosis with A3 and B14 (RR = 9); psoriasis with B13, B17, and B37 (RR = 5), and more strongly with CW6; and multiple sclerosis with A3 and B7 (RR = 2). There is a long list of about 50 diseases that are not associated with HLA or are only weakly associated.

Recently at the Seventh International Histocompatibility Workshop, it was established that HLA-D antigens previously defined by mixed lymphocyte culture (MLC) or lymphocyte defined (LD) typing could now be defined by serologic means. These antigens are DRW1 through DRW8. Some of the DRW types are more closely linked to disease than the previously known aA or B loci antigens, and HLA-B8 diseases are more closely associated with DRW3 than with B8. The RRs are two to five times higher in most of the diseases studied thus far. Multiple

sclerosis is more closely associated with DRW2 than with HLA-A3 or B7. Rheumatoid arthritis seems to be closely associated with DRW4, particularly in females; the HLA-ABC antigens have weaker associations.

The earlier finding by Terasaki et al. that an antigen on B lymphocytes detected by cold incubation at $5^{o}C$ in the antibody step is of low frequency in leukemic cells has been confirmed; leukemic cells do not seem to react to these cold antibodies by B lymphocytes from normal persons.

Studies are now being carried out in which HLA types are being correlated to the antibody response to vaccines. Terasaki and co-workers have shown that BW16 persons, unlike non-BW16 persons, do not respond to influenza vaccine. Vaccination with rubella results in high titers in HLA-B14 and B22 volunteers. The associations will require testing in other series for confirmation. They also have studied HLA association with immune response to HLA antigens themselves. They examined human kidney transplant patients who have had allograft survival for more than 12 months (2,074 patients) and compared them to those in whom transplants have failed between 1 and 3 months after transplantation (1,444 patients). There were no differences in the frequency of the HLA antigens in these groups. In addition, the survival rates of patients with each of the HLA antigens were determined and found not to differ significantly. Therefore, the responder status is not directly associated with a patient's HLA type. In a study on specific nonresponsiveness, the incompatibilities for each of the HLA specificities were examined in over 5,000 patients.

There were some differences in various combinations, but in general it was difficult to establish that one combination of HLA incompatibilities would result in failures or successes. Patients who produced large amounts of cytotoxic antibodies differed from those who did not have any cytotoxic

antibodies in their serum in the frequency of a few HLA specificities. HLA-A3 was high and HLA-A9 was low in the high responders to HLA antigens.

The paradox of high survival rates of kidney transplants after blood transfusions has been confirmed in 18 different studies. Recently, Terasaki et al. were able to associate the presence of cold B lymphocyte cytotoxins with high survival rates. Thus, it is possible that enhancing antibodies induced by the transfusions may possibly be antibodies against suppressor cells or anti-idiotype antibodies detected by cold B lymphocyte cytotoxicity.

The second speaker, Robert Winchester (Rockefeller University), spoke on human Ia determinants. He focused his discussion on the differentiation alloantigens relevant to disease in human beings.

There is considerable evidence that proteins in the major class of human lymphocyte membrane glycoproteins that are selectively expressed on B but not T cells are homologues of the murine Ia system. Thus, they are termed Ia-like or, for convenience, Ia determinants. The two types of reagents used for their recognition are relatively well defined in terms of the nature of the molecular system with which they react. Heteroantisera raised to a mixture of all B cell specific alloantigens and alloantisera both recognize a 65,000-dalton noncovalently linked complex of 28,000- and 37,000-dalton polypeptide chains. Chain dissociation occurs at 100^{o}C but results in a loss of antigenicity.

Definition of specificities of the Ia alloantisera can readily be accomplished with the use of a panel of B lymphoid lines derived from donors who are homozygous for determinants of the D locus. Certain alloantisera give reactivities that enable them to be used as a parallel method for the recognition of determinants closely related to the D locus, the DR specificities. Of particular interest are antisera that recognize determinants exclusively

expressed on either DW4 or DW7 homozygous cells or those reagents that react with an antigen shared by both DW4 and DW7 individuals. Absorption experiences provide evidence of the single specificity of the sera reactive with both DW4 and DW7. In tests of patients with rheumatoid arthritis, 80% reacted with the DRW4-7 specificity reagents; only 20% of normals did. The MLC testing of these patients demonstrated significant increases in both DW4 and DW7 in rheumatoid arthrisis patients; 78% of patients but only 31% of controls had DW4 and/or DW7. Winchester discussed examples of patients reacting with the DRW4-7 reagents who nevertheless were negative for the MLC types DW4 or 7. There was an association of rheumatoid arthritis with a related family of D loci alleles, rather than with a single determinant DW4 as originally described by Janway.

The Ia system was viewed in the context of a differentiation alloantigen on hematopoietic cells not usually considered to be part of the immune system. The model system of granulocyte differentiation was used. Ia determinants were detected on committed precursors (CFU-C) as well as on myeloblasts and a few promyelocytes. Evidence that these were the same molecular system expressed on B cells was provided by absorption experiments, parallel reactivity of alloantisera, and chemical evidence of the same 28,000- to 37,000-dalton profile obtained with B lymphocytes. It was thought that the Ia determinants function on the early cells of the granulocyte series might represent a mechanism for the control of proliferating cells and that the special role of the Ia system on B lymphocytes and monocytes in the context of the immune response could be a phylogenetically more recent use of this system.

Judith A. Kapp (Jewish Hospital and Washington University School of Medicine) reported on the role of suppressor and helper T cells in immune responses to antigens under Ir gene control.

The synthetic polymer of L-glutamic acid^{60}-L-alanine^{30}-L-tyrosine^{10} (GAT) fails to stimulate development of GAT-specific antibody responses in nonresponder mice but does stimulate development of GAT-specific suppressor T cells that inhibit the development of normal anti-GAT placque forming cell (PFC) responses to GAT complexed to methylated bovine serum albumin (GAT-MBSA). Extracts from lymphoid cells of GAT-primed but not of control, nonresponder mice contain a T cell factor (GAT-TsF) that also specifically suppresses responses to GAT-MBSA by normal syngeneic mice. Kapp presented data to demonstrate: (1) that extracts from all GAT-primed nonresponder (H-2^{p,q,s}) mice tested contained GAT-TsF, (2) that non-H-2 genes did not restrict the production of GAT-TsF, (3) that all nonresponder strains of mice regardless of their non-H-2 genes were suppressed by GAT-TsF from all other strains bearing the nonresponder H-2^{p,q,s} haplotypes, (4) that suppression of GAT-MBSA responses by both syngeneic and allogeneic nonresponder spleen cells was mediated by a molecule encoded by the H-2 gene complex, and (5) that both syngeneic and allogeneic nonresponder mice were suppressed by purified GAT-TsF that lacked immunoreactive GAT. She also discussed the relationship of the genetics of GAT-TsF to other antigen-specific TsFs.

The next speaker, Edgar G. Engleman (Stanford University), discussed suppression of the mixed lymphocyte reaction (MLR) in man by T cells and T cell factors.

The failure of lymphocytes to proliferate in response to allogeneic cells in the MLR is an unusual event. The explanation for this phenomenon in two "unresponsive" individuals was sought.

Lymphocytes from an HLA-B12, DW4 homozygous man (MM), who received thymic irradiation shortly after birth, failed to respond in the MLR to almost all

allogeneic cells. They did respond normally, however, to the mitogens phytohemagglutinin (PHA) and concanavalin A (Con A). His lymphocytes suppressed responses in the MLR of DW4 persons but not in the MLRs of DW4 negative persons, regardless of their HLA-A or B antigens. This effect, which was radiosensitive, was due to a T cell.

Another individual (JH), a multiparous HLA-B7, DW2 homozygous woman, responded to most allogeneic cells but not to those of her husband (WH), who was HLA-Bw35, DW1 homozygous. When her T cells were added to the responder lymphocytes of HLA identical persons, their responses to her husband's allogeneic cells were suppressed by 60 to 95%. The responses of HLA nonidentical persons were not suppressed. More recently, her suppressor cells were separated from her responder cells, and suppression was shown to be specific for HLA-DW2 in the responders. Her suppressor cells differed from those of the HLA-B12, DW4 homozygous man mentioned above (MM) in that they were specific both for HLA-D in the responders and for determinants in the irradiated stimulators of WH.

In preliminary experiments, supernatants from cultures between MM and allogeneic cells or between JH and WH, were also suppressive of MLRs. Suppression was specific for HLA-D of the responder in both cases.

In summary, Engleman and his associates found that the failure of cells from two persons to respond in the MLRs was due to autosuppression by suppressor T lymphocytes. These cells (1) only inhibit the responses in the MLR of other persons who share identity of HLA-D, (2) may also be specific for the stimulator cell, (3) are functionally separable from responder T cells, and (4) may release soluble factors that mediate suppression.

The last speaker of this session, Stuart F. Schlossman (Harvard School of Medicine), talked about differentiation antigens on normal and leukemic lymphoid cells in man.

In man the precise dissection of the cellular mechanisms and interactions involved in the generation of the human response in vivo and in vitro has been facilitated in recent years by these interrelated areas: (1) The development of in vitro methods for the characterization and identification of human lymphocyte classes by cell surface markers, (2) the development of new techniques for the isolation of highly purified subsets of lymphocytes and monocytes, and (3) the development of in vitro techniques to discriminate both the functional properties and interactions of the isolated subsets of lymphocytes and macrophages. Schlossman's report described the preparation of heteroantisera and the recent fractionation of alloantisera that allowed the characterization of human helper, suppressor, and cytotoxic T cells.

The subject of the third session, with Norman Talal (University of California at San Francisco School of Medicine) as the chairman, was autoimmunity, and the opening paper was given by Toshikazu Shirai (Chiba University School of Medicine). He spoke on the differential effects of natural thymocytotoxic autoantibody (NTA) on suppressor and helper T cells.

One of the most remarkable abnormalities recognized in NZB mice is the progressive decline in certain T cell functions that is associated with a decrease in the number of recirculating T cells. Shirai and his associates reported that NZB mice produce autoantibody (natural thymocytotoxic autoantibody: NTA) that is specifically cytotoxic for thymocytes and peripheral T cells, and they suggested that the NTA contributes to the age-dependent decline of T cell functions that is closely related to the development of autoimmune phenomena. To assess the biological significance of NTA, Shirai and co-workers studied the distribution and content of NTA-reactive antigen among T cells through two different approaches: First, DNP-KLH-primed spleen cells from BALB/c mice were

treated with varying concentrations of NTA and complement (C), and were cultured with 0.1 μg/ml of DNP-KLH in the modified Mishell-Dutton culture system. It was found that although the treatment of DNP-KLH-primed spleen cells with NTA at a low dilution suppressed the anti-DNP secondary antibody response, the treatment at higher dilutions resulted in a significant enhancement of the in vitro antibody response. The results suggested that the enhancement of antibody response was due to the selective elimination of carrier-specific suppressor T cells that were more sensitive to NTA than helper T cells. Second, in order to learn whether certain T cells have a higher content of NTA-reactive antigen than others, these investigators stained the thymocytes and splenic T cells with NTA and fluorescinated rabbit antimouse Igs. The cells were subjected to the analysis with fluorescence-activated cell sorter. The fluorescence pattern indicated that there were two distinct populations of T cells that were clearly distinquishable by staining with NTA. That both populations had about the same size was determined by the size scattering pattern. The thymocytes and splenic T cells were treated with appropriate dilutions of NTA and complement; about 40% of the total cell number were killed and the peak with a higher fluorescence intensity disappeared.

Shirai and his associates have suggested that the difference in the content of NTA-reactive antigen between suppressor and helper T cells would account for the selective elimination of suppressor T cells in the above experiment, and that NTA has a selective role in the age-dependent decrease of certain T cell functions in NZB mice.

Alfred D. Steinberg (National Institutes of Health) talked on suppressor cell function and anti-T-cell antibodies in human and murine lupus.

New Zealand (NZB and NZB/NZW) mice and patients with systemic lupus erythematosus (SLE) have a defect in generation of suppressor function. In the mice, the defect appears to be in one of two cells that interact to bring about suppression. Suppressor cells can be activated, but they cannot carry out to completion the suppressive reaction because the second cell, perhaps a Ly/L3 precursor, is defective or present in inadequate numbers. Such cells are present in young mice, but are lost early in adult life. Both patients with SLE and NZB mice produce antibodies to T cells that in vitro and in vivo preferentially eliminate suppressor function. In SLE patients with such antibodies, the cells eliminated are brightly staining cells (SLE anti-T-cell serum + fluorescein isothiocyanate [FITC] anti-Ig) on the cell sorter. In NZB/NZW mice, administration of NTA from neonatal life leads to acceleration both in the loss of suppressor cells and in the autoimmune process. These observations suggest that anti-T-cell antibodies are important in exacerbating the T cell defects in New Zealand mice and patients with SLE.

Jane Morton-Siegel (University of Oregon Health Sciences Center) next spoke on stem cell abnormalities in NZB mice.

Several lines of evidence have pointed to the existence of a hemopoietic stem cell abnormality in the autoimmune strain of the New Zealand Black (NZB) mouse. Young adult NZB mice demonstrate very high radioresistance in terms of survival ($LD_{50(30)}$ = 964 rads), and in endogenous spleen colony formation (CFU) after sublethal x-ray exposure. NZB bone marrow radiation chimeras with the H-2d histocompatible BALB/c and DBA/2 strains also showed endogenous CFU formation that was elevated 10-fold or more. In the reverse situation, i.e., when marrow from a nonautoimmune donor was grafted into lethally irradiated NZB mice, high CFU formation was abrogated and chimeras showed normal donor-type

activity. In all cases, immunologic hyperresponsiveness to sheep erythrocyte immunization and to stimulation by T cell mitogens as well as antinuclear antibody formation accompanied high endogenous CFU formation. Normal immune reactivity and the absence of autoantibodies accompanied low CFU counts. Transplantable spleen CFUs were somewhat elevated (two- to threefold) for 1- to 12-week-old NZB mice relative to the other strains. Transplantable marrow CFUs, however, were virtually identical for NZB, BALB/c, and DBA/2 mice of different ages. Endogenous CFU formation has been shown to reflect numbers of hemopoietic stem cells in DNA synthesis at the time of x-ray exposure; cells in this phase of the cycle are highly radioresistant. Morton-Siegel and her associates therefore have proposed an etiology for NZB immunologic dysfunction that is based on the thesis that stem cells in active cycle may constitute the physiologic state during which immune potential is expressed. The expanded population of antigen-reactive lymphocytes generated in the NZB mice from an augmented stem cell cycling fraction could thus account for immunologic hyperresponsiveness and the simultaneous development of autoantibodies in this strain. Attempts at regulation by introducing thymocytes from 3-week-old NZB or DBA/2 donors along with an inoculum of NZB bone marrow into lethally irradiated DBA/2 recipients were not successful. The spontaneous development of antinuclear antibodies was not altered. In contrast, coinjection of DBA marrow (5×10^6 cells) along with NZB marrow (2×10^6 cells) significantly diminished autoimmune conversion. This suppression may have been due to the reduced space available for NZB hematopoiesis or may have resulted from a control exerted by the normal DBA/2 component on the abnormal NZB component of the hematopoietic system. Because stem cells and T cells as well as B cells are hyperproliferative in the NZB strain, an abnormality in regulation common

to all components of the lymphocytic lineage should be considered. Whether this abnormality involves a suppressor cell, an endogenous regulator, or a receptor defect should be further explored.

The genetic aspects of autoimmune thyroid disease were discussed by Noel R. Rose (Wayne State University School of Medicine).

The obese strain (OS) chicken provides a striking example of genetically controlled autoimmune thyroiditis and hypothyroidism. Although the strain is not inbred, one can identify several of the main genetic controls. Of 12 blood-group markers identified in the OS strain, only one, the B blood group, is closely and consistently associated with the severity of disease and titer of autoantibody to thyroglobulin. The B locus is also the MHC of the chicken. Birds with the B^{15} haplotype have the most severe disease. B^{13} birds develop moderate disease, whereas B^{5} chickens without either of the other two haplotypes have the mildest disease. Despite this statistical association, some individual $B^{5}B^{5}$ birds have severe disease.

Three inbred substrains of the OS were developed (OSA, OSB, and OSC). In the OSA substrain, $B^{13}B^{13}$ homozygotes had the most severe thyroiditis at all ages studied, whereas $B^{5}B^{5}$ siblings had very little disease. Hybrid $B^{5}B^{13}$ chickens were intermediate. In the OSB subline, homozygous $B^{13}B^{13}$ and heterozygous $B^{5}B^{13}$ chickens were equally affected, but the $B^{5}B^{5}$ siblings had only mild disease. In the OSC substrain, young 4- to 5-week-old) $B^{5}B^{5}$ chickens had significantly milder disease than their $B^{5}B^{13}$ or $B^{13}B^{13}$ counterparts. However, this difference was not discernible in older (6- to 10-week-old) chickens. Thus, it became clear that, although the B genotype is one genetic element determining severity of autoimmune thyroiditis, other genetic, age-dependent, or environmental influences also play a role.

Some information on the possible mechanism of MHC influence on spontaneous thyroiditis in the OS chicken can be obtained from studies on induced autoimmune thyroiditis in the mouse. In this species it can be shown that the severity of disease is determined to a great extent by an MHC-linked immune response gene that can be located in the K or Ia subregions of the H-2 complex. This Ir gene expresses itself through the T cells since the severity of disease in irradiated, reconstituted mice depends upon whether the T cells are obtained from responder or nonresponder donors. B cells can be provided by responder or nonresponder donors.

Similarly, in the OS chickens, the immune response is regulated mainly by the thymus. Bursectomized birds have little if any disease. Thyroiditis develops in bursectomized OS chicks whether reconstituted with bursal cells of OS or normal, CS, origin. Bursal cells, therefore, do play a necessary role in autoimmune thyroiditis in OS chickens. However, passive transfer experiments with OS serum have shown that the disease can be transferred only to other members of the genetically susceptible OS; thyroid lesions do not develop in normal CS chickens after passive immunization with potent OS serum.

In addition to the B-gene-associated genetic control, OS chickens have two other abnormalities that may contribute to the severity of thyroiditis: a thymic disorder that seems to result in premature peripheralization of effector T cells, and an intrinsic abnormality in the thyroid gland, revealed by heightened iodide uptake and diminished output of thyroid hormone.

Autoantibodies to insulin receptors in diabetes were discussed by C. R. Kahn (National Institutes of Health).

The first step in insulin action is binding of insulin to a specific plasma membrane receptor. In several patients with insulin-resistant diabetes, Kahn and co-workers detected and characterized autoantibodies to the insulin receptor.

The antibodies were predominately IgG and polyclonal. They inhibited insulin binding to a variety of tissues from species as diverse as the North Atlantic hagfish and man. Antibodies obtained from different patients appeared to bind to different determinants on the receptor and to alter receptor function in different ways. Some antireceptor antibodies were capable of stimulating insulin-like effects on target tissues; others blocked insulin-stimulated effects. The insulin-like activity appeared to require bivalence and was lost in the monovalent Fab.

Antireceptor antibodies can also be used in the immunoprecipitation of solubilized insulin receptors or may be directly labeled with ^{125}I and used as a reagent for the detection and quantification of insulin receptors (the latter alternative eliminates the need to bind the hormone). Thus, these antibodies to the insulin receptor may be used in the definition of new disorders of this important membrane component.

The last speaker of this session, Jon M. Lindstrom (The Salk Institute of Biological Studies), spoke on the pathologic mechanism causing impairment of neuromuscular transmission in myasthenia gravis.

The muscular weakness characteristic of MG results from an impaired neuromuscular transmission caused by an autoimmune response to acetylcholine receptor (AChR). The AChRs are integral membrane proteins of the muscle postsynaptic membrane that participate in transmission of impulses from nerve to muscle. When the nerve impulse reaches the nerve ending, acetylcholine is released into the synaptic cleft. Binding of acetylcholine to AChRs causes an increase in cation permeability of the postsynaptic membrane, which, in turn, triggers an impulse that is propagated down the surface membrane of the muscle and results in contraction.

Immunization of animals with AChR protein purified from the electric organs of fish, with any of the four polypeptide chains composing the torpedo AChR molecule, or with AChR from syngeneic or other mammalian muscle, results in an autoimmune response to skeletal muscle AChRs. This is called "experimental autoimmune myasthenia gravis" (EAMG) because of the detailed similarities between animals immunized with AChR and patients with MG. This EAMG results from cross-reaction with the immunogen, rather than induced autostimulation by AChR in the immunized animal.

Studies on both EAMG and MG indicate that the autoimmune response to AChR is mediated primarily by antibody-dependent mechanisms, rather than by cellular immune mechanisms. Binding of antibodies to AChR has only a small effect on AChR function. The primary lesion in MG and EAMG is loss of AChR. This loss is accompanied by simplification of postsynaptic membrane structure. Two mechanisms are probably important in causing the observed loss of AChR. One is complement-mediated destruction. The other is antigenic modulation. It may be that complement-mediated destruction of the normal junctional architecture precedes efficient destruction of AChR by antigenic modulation. Treatment of MG patients by plasmaphoresis and immunosuppressive drug therapy results in reduced serum anti-AChR concentrations and marked clinical improvement.

The fourth session dealt with the regulatory effects of macrophages and other non-T, non-B hematopoietic cells. This session was chaired by Noel Warner (University of New Mexico School of Medicine).

The first speaker, Malcolm S. Moore (Sloan Kettering Institute), spoke on the regulatory role of phagocytic mononuclear cells in hematopoiesis.

In vitro clonal assay techniques in semisolid agar or methylcellulose have revealed the existence of hematopoietic progenitor cells committed to

granulocyte-macrophage (CFU-C) differentiation. Proliferation and differentiation of CFU-C depends on the presence of colony-stimulating factors (CSFs) that are specifically and continuously required for each stage of granulocytic and macrophage proliferation and differentiation. Peritoneal macrophages, particularly in induced exudates, are also capable of extensive proliferation in vitro in the presence of CSFs. Positive feedback control of CFU-C proliferation is suggested by the observation that monocytes and macrophages are a major source of CSFs and that increased CSF production is elicited by macrophage-activating agents. Limitation of positive feedback is in part mediated by a glycoprotein elaborated by mature granulocytes that suppresses monocyte-macrophage CSF production. An additional negative feedback control is provided by the inhibitory influence of prostaglandin E (PGE) that suppresses in vitro clonal proliferation of myeloid and lymphoid cells at concentrations of 10^{-7} to 10^{-8} M. Murine and human monocytes and macrophages are capable of elaborating suppressive concentrations of PGE, particularly after lipopolysaccharide (LPS) activation. The paradoxical situation of macrophage production of mutually opposing activities was resolved in studies showing that macrophages were responsive to the concentration of CSFs in their milieu and that their capacity to synthesize PGE was linearly related to the concentration of CSFs to which they were exposed. Studies with LPS-resistant C3H/HeJ mice showed that the PGE-inducing action of LPS was secondary to the acute induction of macrophage CSF production and that CSFs were directly capable of inducing PGE synthesis by phagocytic mononuclear cells. A parallel situation exists for B lymphocyte clonal proliferation in agar culture, which is dependent upon B cell mitogens present within the crude bactoagar or is provided exogenously by addition of LPS. Depletion of macrophages from murine spleen or lymph node dramatically decreases B lymphocyte cloning

efficiency, which is reconstituted by addition of low numbers of peritoneal macrophages. Increasing macrophage numbers progressively suppress B lymphocyte cloning due to elaboration of PGE. The combination of adherent cell-depleted lymph node B lymphocytes with varying numbers of peritoneal macrophages in the presence or absence of LPS and indomethacin, a potent inhibitor of PGE synthesis, has shown that mitogenic B lymphocyte proliferation depends on adherent cell production of a diffusible stimulatory factor. In vitro B cell proliferation is inhibited by adherent cell production of PGE that, in turn, is induced by macrophage-derived CSFs. Enhanced production of the latter activity is induced by agents such as LPS with dual activities both as direct B cell mitogens and as macrophage-activating agents. These studies suggest a central surveillance and regulation function of phagocytic mononuclear cells in the production of lymphomyeloid stimulatory factors and an opposing inhibitory activity identified as PGE.

The next speaker, Michael Bennett (Boston University), spoke on the immunobiological function of marrow-dependent cells.

Marrow-dependent cells are immunocytes; they include certain natural killer (NK) cells cytotoxic to lymphoma T cells, which must be induced to differentiate by an intact marrow microenvironment. M cells, which become functionally mature at 3 weeks of age, are depleted in adult mice treated with a bone-seeking isotope, ^{89}Sr. Such mice have aplastic marrow, and the spleen takes over stem cell function for the body. Conventional T cell, B cell, and macrophage functions of humoral and cellular immunity are intact, but ^{89}Sr-treated mice lose the ability to reject histoincompatible parental strain or allogeneic marrow grafts. The NK cells cytotoxic for YAC-1 and RI 1 lymphomas are depleted by ^{89}Sr treatment and mature functionally at 3 weeks of age. However, NK cells

themselves are heterogeneous, and some types are intact in mice treated with ^{89}Sr; these include NK cells induced by infection with viable BCG organisms, NK cells in spleens of mice 4 days after lethal irradiation doses, and NK cells cytotoxic for EL-4 cells of C57BL origin maintained in vitro. These latter cells are interesting because they mimic the genetics of marrow allograft reactivity in vitro, including Fl antiparent recognition and Ir-like gene control of the ability to lyse.

C57BL/10 and related strains of mice are good responders to most immunogenic allogeneic marrow grafts and have NK cells that lyse YAC-1 and RI 1 cells well. Such mice are also genetically resistant to the erythroleukemic and immunosuppressive effects of Friend virus complex (FV). Treatment of C57BL mice with ^{89}Sr abrogates this resistance completely, i.e., malignant erythropoiesis and suppression of antibody responses are observed. An in vitro system that mimics the genetics of in vivo immunosuppression involves the stimulation of spleen cells with T or B cell mitogens in cultures infected with FV. The FV suppresses mitogen responses indirectly by activating a suppressor T cell that in turn inhibits mitogen-responsive cells. Spleen cells from C57BL mice treated with ^{89}Sr are phenotypically susceptible to FV, and the spleens contain suppressor T cells. Thus, M cells of genetically resistant mice inhibit the generation or function of these suppressor cells.

Genetic analysis has indicated that a single autosomal gene, dominant for susceptibility, controls the resistance to FV in this in vitro system. The same, or a closely linked, gene controls resistance to suppression of antibody responses in vivo by FV. The gene, Fv-3, is not linked to H-2, Fv-1, Fv-2, or Ir-like genes regulating the ability to reject $H\text{-}2^b$ marrow grafts.

Subsequent studies have indicated that M cells inhibit growth of Listeria monocytogenes organisms during early stages of infection. Mice treated with ^{89}Sr are also extremely sensitive to encephalitis induced with herpes simplex-1 virus. The mechanisms for the increased susceptibility to such infections have not been determined, but loss of control over suppressor cells is a strong possibility. Mice treated with ^{89}Sr have a "plethora" of suppressor cells or their immediate precursors in lymphoid organs. Antibody responses to sheep erythrocytes in intact mice and in adoptive transfer systems are normal when ^{89}Sr-treated mice or their lymphoid cells are tested. However, both primary and secondary responses to sheep erythrocytes in vitro are suppressed by spleen cells from ^{89}Sr-treated mice. After 24 h of in vitro culture, spleen cells from such mice are suppressive in the adoptive transfer system in vivo. The cultured cells can also inhibit mitogen responses. Finally, spleens of ^{89}Sr-treated mice have suppressors for cell-mediated lympholysis reactions in vitro, whether the target cell is from a H-2 allogeneic or incompatible parental strain (hybrid resistance). The primary function of M cells in host defenses seems to be to prevent suppressor cells from limiting humoral and cellular immune responses to the challenging agent.

James N. Ihle (Frederick Cancer Research Center) then spoke on "null" cell reactivity associated with natural immunity to endogenous C-type viruses.

Various investigators have demonstrated a naturally occurring cytotoxic effector cell that lacks the classical surface markers theta and immunoglobulins for T and B cells. In his studies on the immune response in mice against genetically transmitted C-type viruses, Ihle observed that a "null" cell cytotoxicity is an integral component of the immune response against the virus. In particular, spleen cells from normal 6- to 10-week-old $(B6C3)F_1$ mice have

natural cytotoxic reactivity mediated by "null cells. The cytotoxic activity is specific for virus-infected target cells and can be blocked in vitro with purified gp71, the major envelope glycoprotein of the endogenous virus. In contrast, "null" cell cytotoxicity against virus-replicating target cells, detectable in NIH Swiss nude mice, is not abrogated by gp71, a fact that suggests this reactivity may have another immunologic specificity. In (B6C3)F_1 mice, "null" cell reactivity was also temporally associated with a "T" cell blastogenic response specific for gp71 and with the ability to induce, in vitro with gp71, cytotoxic "T" cells. Moreover, all these cellular reactivities preceded the appearance of antibody against the virus. These findings demonstrate that in some situations "null" cell reactivity is a normal, immunologically specific component of an immune response.

The next speaker, Alan S. Rosenthal (National Institutes of Health), spoke on macrophages in the expression of gene function in the immune response.

Macrophages are required for antigen-mediated lymphocyte proliferation in guinea pigs. In such systems, stimulation of T lymphocyte proliferation in vitro involves an initial uptake of soluble protein antigens by macrophages. This capacity to initiate antigen-specific clonal expansion is acquired by a temperature-dependent process that only requires brief exposure of the macrophage to antigen. This is followed by physical contact between macrophage and lymphocyte, a process known to be inhibited by drugs, such as cytochalasin, that interfere with microfilament function. Activation of the T lymphocyte to produce mediators or proliferate is restricted in that only antigen associated with syngeneic or semisyngeneic macrophages elicit such responses. Macrophages are also closely associated with lymphocytes in vitro when lymphoid cells from mice, rabbits, guinea pigs, or beings are cultured without relevant

antigen. This latter phenomenon suggests that macrophage-lymphocyte interaction may have additional biological functions, such as the maintenance of lymphocyte viability or the promotion of the functional maturation and differentiation of lymphocytes from lymphoid cell precursors.

The final speaker of the fourth session was Arthur Bankhurst (University of New Mexico), who spoke on the possible role of macrophage-monocyte populations in human-disease-associated immunodeficiencies.

The in vitro immunologic hyporesponsiveness associated with several human primary and secondary immunodeficiency diseases is caused by suppressor leukocyte subpopulations. Those human B cell immunodeficiency disorders include the following: the hypogammaglobulinemias accompanying common variagle immunodeficiency; thymomas and acute lymphoblastic leukemia; selective IgA deficiency; and the nonparaprotein hypogammaglobulinemia of multiple myeloma. Suppressors of leukocyte-caused human secondary T cell immunodeficiency is found in some patients with Hodgkin's disease (HD) and disseminated fungal infections. Also, leukocyte suppressors of peripheral lymphocyte cytotoxic reactions against tumor cells have been reported in patients with multiple myeloma and osteogenic sarcomas. Lymphocytes of monocytes have been identified as the suppressor cells in these diseases. Monocytes are considered to be the suppressor leukocytes in the secondary T cell immunodeficiencies and multiple myeloma, although a suppressor T cell may also be involved. The soluble suppressor material released from suppressor cells has not yet been identified.

Bankhurst and co-workers examined the role of a glass-adherent, prostaglandin-producing (PG-producing) suppressor cell in the hyporesponsiveness to PHA seen in HD. Addition of indomethacin, a PG synthetase inhibitor, to PHA cultures from 6 patients with HD resulted in a 182 $\pm$ 60% increase in

^{3}H-thymidine incorporation versus a 44 ± 18% (mean ± SD, $p < .001$) increase in 29 controls. Without indomethacin the mean response of the lymphocytes from patients with HD was 48% of that of lymphocytes from controls. With indomethacin it was 94% of the control value. In the PHA cultures, HD lymphocytes produced approximately four times as much prostaglandin E_2 (PGE_2) as normal lymphocytes ($p < 0.02$). Removal of glass-adherent cells also increased the response of HD lymphocytes but decreased the response of normal controls, so that there was no difference in the PHA response between patients with HD and normal controls. The PGE_2 production was also markedly decreased (80% or more) after glass-adherent cells were removed. A comprehensive battery of lymphocyte and monocyte markers revealed that the only significant cell population removed after glass-wool passage was that of the monocytes. When normal leukocyte suspensions were examined for the presence of cells with cytoplasmic PGE detectable by indirect immunofluorescence, only monocytes were so identified. When the normal leukocyte suspensions were double-labeled by latex ingestion and PGE immunofluorescence, it was found that 80% of the fluorescent cells contained ingested latex. Furthermore, the proportion of cells with nonspecific esterase or that were morphologically monocytes closely paralleled the percentage of cells with cytoplasmic PGE. Thus, it appears that a glass-adherent, PGE-producing suppressor cell, possibly a monocyte, is responsible for the hyporesponsiveness to PHA seen in some patients with HD.

L. E. Benjamini (University of California at Davis School of Medicine) convened the fifth session, on the manipulation of immunoregulation. The first speaker was Gideon Goldstein (Ortho Pharmaceutic Corporation, Raritan, New Jersey), who discussed immunoregulation by thymopoietin and other peptides. Thymopoietin is a 49 amino acid polypeptide chain (with a molecular weight of

5,500) that is secreted by epithelial cells of the thymus. A synthetic pentapeptide sequence within thymopoietin has full biological activity. Thymopoietin is a classical hormone in that it is secreted by one tissue (epithelial cells of the thymus) and acts on other cells (cells of the immune system). Thymopoietin regulates the induction of early thymocyte differentiation, but it also circulates in the serum to act upon and regulate the reactivity of the immune system. Thymopoietin can be demonstrated to affect the LY 123 subclass of cells in mice as shown in adult thymectomized mice. Paradoxically, thymopoietin injections lessen morbidity and mortality in NZB mice, wherein there is defective suppressor function, yet act to improve the quality of the immune response in aged mice, wherein there is defective helper function. Thus, thymopoietin appears to act as a homeostatic regulatory hormone for the immune system. Studies delineating thymopoietin's mechanisms of action, which can be best explained in terms of network pertubations in the immune system, should provide interesting data.

Autoimmunity and anti-idiotypes was the subject of the next talk, which was presented by Hans Wigzell (Uppsala, Sweden.

It is now well established that the immune system is endowed with the ability to react against several self-constituents, including the antigen-binding areas of the individual's own antibodies. The antigenic determinants on such variable regions of immunoglobulin molecules are called idiotypic. T and B lymphocytes reactive against the same antigenic groups do express receptors for that antigen and with shared idiotypic determinants. It is possible to eliminate such idiotype-positive lymphocytes with anti-idiotypic antibodies in the presence of complement, and data exist that suggest anti-idiotypic T cells may also exist. Viewing the auto-anti-idiotypic immune model as one

with possible future usefulness as a modulator of the immune response in adult immunocompetent individuals, Wigzell and his associates studied the transplantation immune reactions against major histocompatibility antigens in such a context. In mice and rats they induced auto-anti-idiotypic immunity measured at both the humoral and cellular level with specificity for receptors with reactivity against certain histocompatibility antigens. Such auto-anti-idiotypic immunity could be achieved either with immunization via purified idiotypic receptors or with immunization via antigen-specific T lymphoblasts generated from contact with alloantigens in primary MLC reactions. The consequences of such auto-anti-idiotypic immune reactions could be shown to result in selective elimination of those cells with specific reactivity against the relevant alloantigens as measured by MLC, CTL, or GvH reactions. However, significant variation did exist between the reduction as measured in the different assays; MLC reduction was the easiest to induce. Transplantation tolerance, as measured by permanent survival, normally was not achieved with the procedure, although a three- to fourfold specific increase in skin graft survival was noted.

Clonal interaction of complementary idiotypes was discussed by Hans Kohler (University of Chicago), who focused his presentation on the concepts of complementary idiotypes, defined by mutual affinity and exquisite specificity, in the context of recent findings on their occurrence, restriction, and functional requirements.

The response of BALB/c mice to phosphorylcholine (PC) is predominantly of the HOPC-8 idiotype. Previous studies demonstrated that the BALB/c mouse has the potential to produce anti-H8 idiotypic antibody. Isologous BALB/c and homologous A/J anti-H8 were compared by the criteria of idiotypic

specificity, clonal restrictions, and isotype composition. Both anti-idiotypic antibodies were restricted by isoelectrofocusing and plaque-inhibition analysis and by the G1 subclass. Furthermore, the Fc portion of anti-H8 was essential for inducement of idiotype suppression in vitro.

Measurement of the amount of H8 and anti-H8 in sera from normal BALB/c neonates revealed the presence of anti-H8 antibody immediately after birth; it disappeared by day 5. On day 5, the level of H8 idiotype began to rise; it reached adult levels after 28 days. The possible rate of this early anti-H8 as a regulator in the development of clones is supported by the fact that the amount of idiotype present during ontogeny affects the development of clones. When neonatal liver cells were transferred into normal lethally irradiated syngeneic recipients, the clonal dominance of H8 in the response to PC did not appear. However, when H8-idiotype-suppressed recipients were used, clonal dominance to PC was restored. Kohler et al. have proposed a model for the role, during ontogeny, of interacting complementary idiotypes that regulate the expression of clones and thus determine the clonal profile of the adult response.

The next discussion was by Robert J. Scibienski (University of California at Davis School of Medicine) who talked on antigenic modification as an approach to immune manipulation.

Reduction and alkylation of the four disulfide bonds of chicken egg white lysozyme (CL) in the presence of 6 M urea results in complete denaturation of the molecule. This derivative (CMCL) totally lacks cross-reactivity with the native protein at the humoral level. In contrast, T cells with specificity for CL or CMCL react with both forms of the antigen in a variety of assays. This cross-reactivity is evident at the level of delayed-type hypersensitivity,

tolerance, helper cell activity, and Ir gene function. Further denaturation of the CMCL molecule, including modification of tyrosine, tryptophan, histidine, and lysine residues, has no significant effect on its cross-reaction with native CL at the helper T cell level. Therefore, it may be possible to preferentially induce cell-mediated immunity to a variety of protein antigens.

One can also manipulate the immune response to CL by coupling the antigen to bacterial LPS. The modified immunogen (CL-LPS) induces a very quick, intense primary anti-CL antibody response that is thymus-dependent (as shown by a number of criteria). This thymus dependence notwithstanding, CL-LPS is capable of inducing a vigorous response in congenitally athymic nude mice. This anti-CL-LPS response of nude mice is sensitive to treatment with antilymphocyte serum, a fact that suggests it may involve some form of T cell function.

The CL-LPS is also capable of inducing anti-CL antibody responses in animals that normally are specifically unresponsive to lysozyme. Thus, mice with either an acquired or a genetic inability to respond to lysozyme in Freund's complete adjuvant nevertheless respond vigorously to the lysozyme adjunct of CL-LPS. In the case of genetically unresponsive mice, CL-LPS induces a response despite the apparent presence of lysozyme-specific suppressor cells. These results indicate that by coupling a protein antigen to LPS one can overcome a number of the factors that normally are refractory to immune responsiveness.

The conference terminated with a presentation, by Dr. Eli Sercarz (University of California at Los Angeles School of Medicine), on the regulatory role of key antigenic determinants. Recent work describing the interaction of T cell subpopulations engaged in regulation of the immune response has emphasized the delicate balance of the opposing cell types as a function of their

relative numbers and activity. Sercarz's talk, however, was concerned with the presence of rare suppressive antigenic determinants on protein macromolecules and their influential effect on the potential T cell response directed against the other epitopes on the antigen.

Sercarz described two systems. In the first, the response to ß-galactosidase (GZ), Sercarz and co-workers located an antigenic determinant on a peptide, "CB-2," comprising 9% of the GZ molecule, which addresses itself primarily to suppressor T cells. The sequential waves of help and suppression induced by the native macromolecule can be conceived of as reflecting regulatory interactions among T cell subsets, each of which apparently is restricted to combination with different determinants on the antigenic molecule. That, at 7 days, the suppressive action induced by CB-2 was due to Ly-2+ suppressor T cells was shown in experiments in which the suppressor cells were titrated into target helpers. At 30 days, the spleen cells from CB-2-primed mice were still unresponsive, but for a different reason; they were no longer suppressive, and the unresponsiveness was no longer Ly-2-dependent. This is remarkable because CB-2 priming managed to prevent non-CB-2-related helper cells from expressing themselves without the mouse ever having been exposed to other helper-inducing regions of GZ. Sercarz hypothesized that a common idiotypy between major helper and suppressor cell T receptors might explain this result.

In the second system, the H-2 controlled response to the lysozymes, evidence was presented that the nonresponsive H-2^b animal has all the cooperating cellular elements necessary for mounting an antibody response towards HEL. Various experimental lines converge to suggest that suppressor cells directed against a single determinant on HEL (chicken lysozyme) can subvert the helper mechanisms and thus result in the lack of obvious antibody responsiveness.

The suppressor determinant can be assigned to the N-terminal portion of chicken lysozyme containing the critical phenylalanine residue at position 3. By removing 25% of the molecule, containing this presumptive suppressor determinant, it could be demonstrated that the remaining large peptide induced a vigorous T cell proliferative response.

In each of these diverse systems, it appeared that the T cell repertoires of helper and suppressor subpopulations were distinct. Whether this situation was a result of distinct generators of diversity, macrophage presentation, or idiotypic network control could not be determined.

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The conference opened with a general discussion of the regulation of immune responses by suppressor T cells and soluble suppressor factors. The remainder of the first session dealt with the role of antigen-presentation and feedback inhibition in regulation of immune responses. The second session dealt with the immunoregulatory effects of the major histocompatibility complex and I-region gene products. The influences of regulator cells (T-helper and T-suppressor) in autoimmunity was also discussed in
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detail. The fourth session dealt with the role of macrophages and other non-T and non-B lymphocytes in the regulation of the immune response. The final session discussed the regulation of immunity by modification of antigen structure and the regulatory role of key antigenic determinants and peptides.