Immunoregulation and Autoimmunity

Volume Editors

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Paul Ehrlich (1854–1915)

'In the explanation of many disease phenomena, it will in the future be necessary to consider the possible failure of the internal regulation, as well as the action of directly injurious exogenous or endogenous substances.'

Paul Ehrlich (1901)

This volume is dedicated to the memory of *Paul Ehrlich* (1854–1915), an investigator of rare genius and foresight.

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Perspectives in Immunoregulation and Autoimmunity

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Studies on immunoregulation continue to occupy the forefront of immunologic research. There has also been a renaissance of interest in autoimmunity as evidenced by an international symposium on the topic held by the New York Academy of Sciences in mid-1985. In response to the continuing interest in both of these important and related fields of scientific endeavor, the present volume has been assembled by the editors to present representative reports of ongoing research by preeminent investigators whose laboratories serve as a focus for the latest development in immunoregulation and autoimmunity research.

Many articles in the present volume are based upon the basic tenets of *Jerne's* immune network hypothesis, which states that the antibody molecule's antigen-binding site (paratope) is encoded by variable region genes that have idiotopes as phenotypic markers. Each paratope recognizes idiotopes on a different antibody molecule. Interaction of idiotypes with anti-idiotypes to form the idiotype network represents a physiologic attribute of immune responsiveness. Idiotypy is known to be shared among the immunoglobulin classes that comprise antibodies produced in response to the same antigen. The idiotypic network of the immune system consists of interaction of idiotypes and anti-idiotypes involving free molecules as well as B and perhaps T lymphocyte receptors. Thus, it appears that idiotypes are central in immunoregulation involving autoantigens. It is anticipated that future studies will shed light on the extent of the idiotypic network in regulating responsiveness to autoantigens, which should provide improved understanding of autoimmune disease pathogenesis.

Flood, Chue and *Green* point to the ability of the immune response to distinguish self from non-self as central to the dogma of immunology. After

reviewing the intricate interactions that govern successful immunoregulation and problems of dysregulation of the immune response, they emphasize heretofore elusive events in the discrimination of self from non-self. Following a discussion of the context of self and the regulatory response, the authors provide a lucid explanation of immunoregulatory circuits and molecules citing specific examples from research data that underscore the principles they describe. The wealth of recent information on T cell subsets and the regulatory molecules that govern their interactions at various immunoregulatory levels of activity are presented in detail. The authors emphasize the significance of a maximal immune response to non-self while responding minimally to self. They discuss the effects of disruption in immunoregulation caused by an insufficient response to either self or non-self or of an excessive response to non-self or self. The ensuing discussion considers immune regulatory imbalances attributable to thermal injury, cancer, response to schistosomes and autoimmunity. Improved understanding of immunoregulatory circuits and interactions could lead to advances in immunotherapy.

Hausman, Sherr and *Dorf* describe a model system which they designed to investigate antigen-specific immunosuppression in mice. Their research has identified the roles of accessory cells as well as three separate subsets of T cells whose successful interactions are required for modulation of the immune response. This model system has enabled them to dissect mechanisms of immunoregulation and to develop a linear cascade of cellular interactions. To investigate suppressor T cell specificity, they employed the 4-hydroxy-3-nitrophenyl acetyl (NP) system, a hapten against which the antibody response has been well defined. Subjects targeted for discussion include inducer suppressor cells (Ts_1) and their factors, Ts_2 cells and factors, and Ts_3 cells and factors. They describe the role of gene products associated with H-2 and Igh complexes in governing cellular interactions associated with immunoregulation. Specific soluble suppressor factors formed by the distinct Ts cell subsets play significant roles in the cellular interactions of the NP suppressor cell pathway. The authors point out that not all experimental models of suppressor cell interactions fit into the NP suppressor cell scheme. But the use of this model has provided clarification of a significant model of the suppressor cell pathway.

Sercarz reviews the fundamental studies from his laboratory over the past several years on hierarchies of epitope preference and the problem of Ir gene control as these phenomena relate to immunoregulation. Complex

proteins are known to be nonimmunogenic in mice of selected MHC haplotypes. Beta-galactosidase was used to demonstrate that T suppressor as well as T helper cells were involved in the immune response to this large protein molecule. It was further shown that distinct domains (or peptides) on a protein molecule interacted with T suppressor cells exclusively. Further studies suggested that unresponsiveness might be attributable to suppressor T cell-inducing determinants and that other T helper cell-inducing epitopes might be present also on the molecule but not demonstrable prior to dissociation of suppressor from helper determinants. It was further suggested that individual T suppressor cells might antigen-bridge to T helper cell attracted to antigenic determinants over the molecular surface, thereby permitting inactivation of various target T helper cells resulting in the phenotype of Ir gene unresponsiveness. Both hierarchical and suppressive processes may explain the Ir gene effects of selected complex molecules such as lysozyme, beta-galactosidase and some random copolymers. *Sercarz* suggests that the response to antigen is a complex result of the hierarchy of T helper cell-inducing determinants and whether or not they may be suppressed considering the number and dominance of T suppressor cell-inducing determinants. He predicts that the antigen presentation T cell recognition system could have evolved in a way to prevent a multiplicity of determinants from competing for processing and presentation in the course of immunization. Further studies will be directed at the forces governing hierarchies within which regulator cells function.

Gately, Jenson and *Benjamin* discuss the role of cytokines, which include lymphokines and monokines, in T and B cell-mediated immunity. These soluble, biologically active substances are secreted in vitro by mitogen- or antigen-activated lymphoid cells. Improved methods of purification, recombinant DNA technology, and monoclonal antibodies have helped to define specific cytokines as well as the cell surface receptors with which they interact.

Two kinds of signals are requisite for activation of cytolytic T lymphocytes (CTL) from their inactive precursors. The first signal is generated by the interaction of antigen with CTL precursors, via antigen-specific receptors on their surfaces. Nonspecific helper factors, such as interleukin-2 (IL-2), may react with CTL precursors expressing surface receptors leading to formation of the second signal. T cell differentiation factor, which aids the differentiation of CTL from their precursors, acts in concert with IL-2, which causes cellular proliferation.

Regulation of immunoglobulin production by B lymphocytes also involves cytokines. A first step in B cell activation involves the cross-linking of surface immunoglobulins and B cell interaction with B cell stimulatory factor-provisional 1 (BSF-pl). IL-1 or IL-2 may augment these signals that lead to B cell proliferation. Once activated, B lymphocytes bind the distinct lymphokines BCDF I and BCDF II resulting in secretion of immunoglobulin.

In addition to advances in immunoregulation made possible through purifying cytokines to homogeneity and developing monoclonal antibodies against them and their cell receptors, future studies will be directed at cloning and expression of genes that encode still other cytokines against which monoclonal antibodies may be generated.

del Guercio and *Katz* point to the usefulness of categorizing peripheral blood lymphocytes as either regulatory cells or as effector cells to reflect their functional activities. Whereas, extensive studies of T cells and their subsets with respect to both phenotypic expression and functional activities have been pursued vigorously in the recent past, B lymphocytes have received less attention. In addition to merely forming immunoglobulins, B cells have been investigated for possible immunoregulatory activities, even though they are apparently less heterogeneous that the T cell system. *del Guercio* and *Katz* cite data concerning B cell lymphokines that may be associated with immunoregulation. These regulatory factors may be grouped into those produced exclusively by B cells versus those produced by B lymphocytes as well as by other lymphoid or nonlymphoid cells. The authors limit their discussion to regulatory molecules produced exclusively by B cells, which may be classified as either immunoglobulin or nonimmunoglobulin molecules. Immunoglobulins may exert immunoregulatory linker activities irrespective of their antigenic specificity. B cell-derived enhancing factor (BEF) represents a nonimmunoglobulin regulatory product that limits or inhibits T suppressor cell activation resulting in uninterrupted immunoglobulin production. Although produced in relatively low concentrations in the normal healthy individual, BEF causes a striking enhancement in the response to antigen when added at high concentrations to in vitro cultures. By contrast, B cells may exert a suppressive activity on immunoglobulin-secreting cells either through regulatory T cells or by affecting B cells directly. Nonimmunoglobulin-soluble factors are also able to mediate this suppressive effect. The authors suggest the possibility that the B lymphocyte-dependent regulatory circuit that is not immunoglobulin-mediated guarantees adequate synthesis of immunoglobulin molecules

specific for *internal structures,* thereby regulating internal immune system activation.

Immunoglobulin molecules represent powerful regulators through both their V (idiotype) and C (isotope) regions. This regulatory circuit that is mediated by nonantigen-specific immunoglobulin responds to alterations in idiotype or isotype levels during an immune response.

Antigen-specific antibodies also represent powerful immunoregulatory agents, especially in situations involving acute alterations in the concentration of antigen. Following interaction with antigen, these antigen-specific immunoglobulins may exert either positive or negative influences on various types of immune reactions.

Hasek, Holan and *Hraba* present a classic description of immunologic tolerance from their special vantage point as pioneers in the field. By chronicling milestones in the development of immunologic tolerance, the authors develop its central role in immunoregulation.

In addition to the clonal deletion concept of *Burnet* in which an immunocompetent cell clone specific for the tolerated antigen is eliminated (a passive mechanism), active mechanisms may be operative also in establishing immunologic tolerance. If the function of a specific immunocompetent cell clone were blocked, it would persist during immunologic tolerance without the necessity for clonal deletion. Blocking factors present in the serum of tolerant animals may inhibit the cytotoxic action of their lymphocytes. Infectious tolerance represents a third active mechanism in which tolerance may be passively transferred with suppressor cells of tolerant animals.

The ability of adoptive lymphoid cell transfers to terminate some types of immunologic tolerance and the inability to identify suppressor cells to account for immunologic nonreactivity in selected tolerant, nonresponding animals constitute indirect evidence of clonal deletion in immunologically tolerant animals. The distinction between immunologic enhancement which describes prolonged survival of tumor allograft following pretreatment of the host with enhancing antibody and immunologic tolerance resulting from central inhibition of immunity by clonal deletion, is not as great as once believed since *Voisin* demonstrated that sera from mice rendered tolerant as neonates and carrying tolerated skin allografts could enhance allogeneic tumor growth. The enhancing activity of some sera might be associated with their content of anti-idiotypic (antireceptor) antibodies.

Blocking factors in the serum of tolerant mice were shown by *Hellstrom* et al. to inhibit the cytotoxic action of sensitized lymphocytes

against cells expressing tolerated antigens. Blocking serum factors have been associated also with neonatally induced tolerance. Investigators have disagreed concerning whether blocking factors were present in all tolerant animals. Some have concluded that blocking factors are related to incomplete tolerance whereas they may not be necessary for complete tolerance.

The description of suppressor cells in the early 1970s represented a major new mechanism operative in many types of immunologic tolerance. In addition to the most frequently described specific suppressor T cells, antigen-specific suppressor B cells, antigen-nonspecific suppressor macrophages and antigen-nonspecific suppressor T cells have also been identified.

Cellular interaction and cooperation in both humoral and cellular immune responses are reviewed in considerable detail. The authors outline the role of antigen-presenting cells (APC), T helper (T_h) lymphocytes, B cells and antibody-forming cells (AFC) as well as the suppressor cell circuit comprising T suppressor and contrasuppressor lymphocytes. Whether or not cytotoxic T cells or delayed-type hypersensitivity T cells mediate effector functions in cell-mediated reactions, their development is governed by regulatory circuits. Cell cooperation results either from direct interaction between cells or through informational molecules which they release. Nonantibody-soluble factors may be specific for antigen. The I-region of the major histocompatibility complex (MHC) codes for a product in such factors which is an I-A region product in the case of T helper cells and a I-J product associated with T suppressor cell factors. The role of histocompatibility antigens in cell cooperation and interaction were revealed by *Zinkernagel* and *Doherty* in 1974 to be MHC restricted. Non-MHC types of restrictions on cellular interactions such as idiotype-anti-idiotype interactions remain to be elucidated.

Marullo and *Strosberg* discuss the role of receptor-binding antibodies and anti-idiotypic antihormone antibodies in immune regulation. They begin with a consideration of *Jerne's* idiotypic network hypothesis which embraces the concept of natural autoantibodies. According to the idiotypic network hypothesis, each external epitope possesses an internal 'image' which may be present on cells or immunoglobulins or T cell receptors expressing idiotypic or anti-idiotypic determinants. Antireceptor and anti-idiotypic antihormone antibodies serve as useful tools to dissect regulatory mechanisms of self-recognition. The authors discuss antibodies raised experimentally against receptors with special consideration of models for autoantireceptor antibodies which may be implicated in human diseases.

Selected for special consideration are antibodies against nicotinic cholinergic receptors which are present in the blood sera of most myasthenia gravis patients; antibodies against beta-adrenergic catecholamine receptors in the blood sera of patients with Chagas' myocardiopathy; and autoantibodies to the insulin receptor in patients with insulin-resistant diabetes.

In their consideration of anti-idiotypic antibodies that recognize receptors involved in autoimmune diseases, the authors review anti-idiotypic anti-beta-adrenergic receptor antibodies with special reference to their own work involving antibodies against alprenolol, a potent beta-adrenergic catecholamine antagonist. Anti-idiotypic antibodies were raised by immunization of rabbits with antialprenolol antibodies which were used to study inhibition of binding of alprenolol to both hormone-specific antibodies and to beta-adrenergic receptors on cells of different types. Other subjects presented include anti-idiotypic antibodies inducing experimental myasthenia gravis; anti-idiotypic anti-insulin antibodies binding to the insulin receptor; and anti-idiotypic anti-TSH receptor antibodies. Anti-idiotypes specific for antihormone antibodies may bind to the hormone receptor. The authors present two structural models to explain this binding. In a discussion of mechanisms for the appearance of autoantibodies against receptors, evidence is cited to suggest the existence of autoantireceptor antibodies, that are either anti-idiotypic or against the receptor, before immunization. Under physiologic conditions, B cell clones that produce these antibodies are silent, producing only low affinity molecules specific for self antigens. How they are activated to proliferate and produce disease remains to be demonstrated. The authors present several mechanisms to explain such B cell proliferation. Other mechanisms which they consider include the concept of cross-reactive idiotypes.

Antigens must be associated with a Class II MHC component on an antigen-presenting cell suface in order to activate T cells. Since most hormone receptors are found on cells not expressing Class II MHC structures, it has been suggested that autoimmunity might follow derepression of genes that code for Class II antigens providing the potential for normal surface components to become autoantigenic.

The article concludes with the assertion that B cell activation alone is not sufficient to induce autoimmune disease by pathogenic autoantibodies. It is pointed out that autoantibodies must have a defined specificity, affinity and concentration to become pathogenic. If these latter conditions are not satisfied, there may be no adverse effect of circulating autoantibodies. The

authors discuss factors in addition to B cell activation that might be necessary for a pathogenic autoimmune reaction to be induced.

Boitard and *Bach* compare the relative roles of cell-mediated and humoral immunity in autoimmune diseases with the discovery that certain self antigens render key lymphocytes tolerant in contrast to the existence of autoreactive B lymphocytes. Much emphasis has been placed on humoral immunity at the expense of cell-mediated immunity as a principal effector mechanism in autoimmune disease development. With great advances made in understanding of experimental allergic encephalomyelitis (EAE) as well as the recent focus of research on autoreactive T lymphocyte clones, a new appreciation of the role of cell-mediated immunity in autoimmune diseases is being developed. It is anticipated that these T lymphocyte clones will permit studies on regulatory events that may be critical in initiating humoral and/or cellular mechanisms of autoimmune diseases. The authors emphasize the significance of T lymphocytes in certain organ-specific autoimmune diseases such as insulin-dependent diabetes mellitus (IDDM). The infilitration of target organs with lymphocytes, plasma cells and inflammatory cells suggests that an immune or autoimmune reaction may be developing in that tissue. The activated T lymphocytes infiltrating autoimmune disease tissues are principally suppressor-cytotoxic lymphocytes that express Class II MHC antigens.

Following consideration of the effects of thymectomy versus bursectomy in animal models of autoimmune diseases, the authors review the *experimentum crucis* to ascertain whether antibodies in serum or specifically sensitized lymphoid cells are able to mediate an autoimmune disease. Genetically inbred animals that have been thymectomized and lethally irradiated serve as recipients in immune reconstitution experiments. Whereas, antibodies against acetylcholine receptors have been shown to have a pathogenic role in inducing myasthenia gravis, the passive transfer of serum did not transfer insulin-dependent diabetes mellitus in rats, lupus in mice, or EAE in most animal models. Of course, such experiments shed no light on the role of regulatory T lymphocytes in assisting B lymphocytes. The classic model in which cell transfer experiments demonstrated the critical role of cell-mediated immunity in autoimmune disease was EAE. The passive transfer of lymphoid cells has been shown to induce insulitis in the diabetes-susceptible BB rat.

The clear role of antibodies in selected human autoimmune diseases has been revealed by the transfer from mother to fetus of antibodies mediating such diseases as myasthenia gravis, Graves' disease, thrombocy-

topenic purpura, hemolytic anemia and congenital heart block in newborns from patients with lupus.

Studies of effector mechanisms in autoimmune diseases reveal that regulatory T lymphocytes initiate B and T cell-mediated effector mechanisms. Autoantibodies with defined functional activities include those that react with the blood elements or those specific for intrinsic factor or for coagulation factor or against cellular receptors. In some instances, the autoantibodies may be specific for a functional molecule such as immunoglobulin or complement. Cytotoxic T lymphocytes comprise a major effector mechanism of cell-mediated cytotoxicity. The role of cytotoxic T lymphocytes in inducing target tissue cytotoxicity in autoimmune diseases is supported by many in vitro observations reported by the authors.

In a section on autoreactive T lymphocytes, the authors recount the differences between T and B lymphocyte tolerance. This is followed by a section on autoantigenic binding cells in which physiologic tolerance to thyroglobulin and myelin basic protein in experimental animal models is reviewed. Autoreactive T cell lines and T cell clones have been employed to passively transfer such diseases as EAE and experimental thyroiditis.

Manipulation of T cells to prevent the development of autoimmune diseases has been accomplished with traditional drugs such as corticosteroids, azathioprine and cyclophosphamide, which lead to nonspecific suppression of the immune response; irradiation, which is effective against radiosensitive helper T lymphocytes with a less certain action on suppressor T cells; antilymphocyte sera (ALS); monoclonal antibodies against T lymphocytes; and Cyclosporin A (cyclosporine), a noncytotoxic drug which has been used not only to facilitate survival of renal allotransplants in man but has been tested in animal models of autoimmune diseases, including EAE, experimental thyroiditis, and adjuvant arthritis, among others. It has proved useful in preventing selected spontaneous autoimmune diseases. Human autoimmune diseases in which cyclosporine has been shown effective include uveitis, systemic lupus erythematosus and insulin-dependent diabetes mellitus. Selected autoimmune diseases have also been treated with thymic hormones. The goal of immunotherapy is to delete autoantigen-specific clones responsible for autoimmune injury of a target organ.

Kang and *Kohler* consider the immune network of *Jerne* as a self-recognizing system. Antibody molecules possess several idiotypic determinants in addition to the antigen binding site termed a paratope. Thus, idiotopes and paratopes are constantly recognizing each other under a