# ADVANCES IN

# Immunology

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FRANK J. DIXON HENRY G. KUNKEL

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# PREFACE

The progress of cellular immunology from descriptive phenomonology to the analysis of events in molecular and mechanistic terms is dependent in large part on the development of new technologies and new experimental systems. The five articles in this volume all deal to a greater or lesser extent with such developments and the new insights they have provided. They include (1) new methods for identifying major histocompatibility complex (MHC) determined leukocyte antigens and methods for the efficient detection and quantitation of immunoglobulins, (2) experimental activation of B cells and the use of lymphoid tumor cells to provide insights into normal cellular development and function, and (3) analysis of a hapten-induced immune response to reveal the intricacies of the regulatory systems controlling immune function.

In studying the development, functions, and regulatory interactions of the various kinds of lymphocytes, polyclonal activators of these cells have been invaluable. In the first chapter Waldmann and Broder review the use of polyclonal B cell activators in studying the regulation of human immunoglobulin synthesis, a field in which they have been prime movers. A thorough description of the various activators and their modes of action as well as the several techniques used to assay cellular activation is presented and critically evaluated. Since these agents differ in their actions, i.e., direct effect on B cells or action via stimulation of one or another regulatory cells, in total their use can provide the information necessary to define abnormalities in the function of both regulatory and immunoglobulin forming cells. From such studies have come clues suggesting that a variety of pathogenic mechanisms, some involving the B cells themselves and others affecting regulatory monocytes or T helper or T suppressor cells may be responsible for the disordered immunoglobulin synthesis in patients with immunodeficiency, autoimmunity, or malignancy.

Evidence of the dominant role played by the MHC in determining the immunologic potential and performance of an individual continues to accumulate. The MHC of man, the HLA system, controls two types of transplantation alloantigens (the HLA-A,B,C and the HLA-D/DR antigens) as well as some components of complement, immune response determinants, and susceptibility to a variety of diseases. The HLA-D/DR region is of particular interest since it appears to control early events of the immune response. In the second chapter Morling,

Jakobsen, Platz, Ryder, Svejgaard, and Thomsen review the biochemistry, genetics, and immunology of the HLA system and discuss the methods currently employed to define it. A new technique they have pioneered, i.e., primed lymphocyte typing (PLT) utilizing selected responder cells primed to homozygous typing cells, is presented and its advantages in defining D/DR and related antigens outlined. PLT has facilitated the typing of rare DR antigens, has permitted the detection of new DR antigens, and has led to the definition of a new set of DR-related antigens apparently coded for by genes located between the HLA-D/DR and GLO loci. Because of the versatility in application of PLT and recent technological refinements such as the development of monoclonal cell lines, this procedure should play a key role in the further unraveling of the D/DR region and its role in immunologic function.

Among the most useful reagents for the detection, quantitation, and isolation of immunoglobulins are the bacterial immunoglobulin receptor proteins particularly protein A of *Staphylococcus aureus*. In the third chapter Langone discusses this subject critically and in detail. The sources, methods of isolation, and the physicochemical properties of Staph A protein are described as well as its reactivity with the various Ig classes and subclasses from a variety of species. The biochemical characteristics of the interaction between Staph A and the Ig molecule are discussed thoroughly. Staph A also provides a versatile experimental tool for activation of humoral mediators of immunologic inflammation and stimulation of lymphocytes, both presumably via its interaction with the Fc portions of Ig heavy chains. Finally, the numerous analytical applications of Staph A protein to the measurement of a host of immunologic substances and events, i.e., Ig, antigen–antibody reactions, and immune complexes, to name a few, are described. This chapter provides important background and technical detail for anyone employing this valuable investigative tool.

The complexity of mechanisms regulating the immune response is being revealed primarily as a result of the exhaustive analyses of a few specific responses. In the fourth chapter Greene, Nelles, Sy, and Nisonoff present their findings concerning regulation of the response to the azobenzenearsonate hapten and compare them to related studies by others using different antigens. The suppressor control system emerging from these studies includes at least three separate sets of T suppressor cells interacting in large part via idiotype and antiidiotype events. The first cell ($Ts_1$) is activated by ligand and is idiotype positive, the second ($Ts_2$) is activated by $Ts_1$ or its factor and is antiidiotypic, the third ($Ts_3$) is activated by one or more factors from $Ts_2$ and is

idiotype positive. Although the interactions among the three sets of Ts cells have both $H_2$ and Igh restriction, the $Ts_3$ cell, once activated, appears to mediate its suppression in a relatively nonspecific mode. The possibility is raised of an extension of this suppressor system in which a parallel system initiated not by ligand but by antiidiotype might exist and cells of the two sets even interchange. Such a suppressor mechanism could operate both in humoral and in cellular immune responses.

In the last chapter Abbas reviews the use of lymphoid tumor cells in the study of normal lymphocyte biology. The diversity of the lymphoid system makes it particularly difficult to isolate and analyze uniform populations of lymphocytes. Lymphoid tumor cells offer a particularly good source of large numbers of near monoclonal lymphocytes, which in many instances appear to be reasonable counterparts of lymphoid cells in one or another stage of development or function. B cell tumors have provided examples of pre-B cells, nonsecreting B cells, and secreting B cells as well as subjects for the study of antigen presentation by B cells. T cell tumors have provided sources of T regulatory factors and targets for analysis of T cell interactions. Fusion-derived lines have produced a variety of effector factors and models for regulatory cellular interactions. The potentials and the pitfalls associated with this experimental approach to the study of lymphocyte differentiation and regulation are carefully evaluated in the light of the author's considerable first hand experience, and the subject is put into excellent perspective.

The editors wish to thank the authors of these excellent reviews for the considerable care, time, and effort they have devoted to their preparation. Finally, it is a pleasure to acknowledge the cooperation and assistance of the publishers, who have done much to ensure the quality of this series of volumes.

FRANK J. DIXON
HENRY G. KUNKEL

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ADVANCES IN IMMUNOLOGY, VOL. 32

# Polyclonal B-Cell Activators in the Study of the Regulation of Immunoglobulin Synthesis in the Human System

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## I. Introduction

Many of the great advances in our understanding of the regulatory mechanisms that control human immunoglobulin synthesis have




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emerged from studies that have utilized polyclonal B-cell activators. These studies have been of value in defining the stages of differentiation of the cells of the B-cell series and in determining the role played by networks of immunoregulatory T cells and macrophages in the control of these maturational events. These studies have also brought to light new pathogenic mechanisms that underlie certain forms of primary immunodeficiency disease as well as autoimmune, malignant, and allergic disorders. Finally, they are providing the scientific basis for the development of new rational strategies for the treatment of these diseases.

The development of antibody-producing plasma cells from bone marrow stem-cell precursors involves a series of stages. The first identifiable stage involves a hierarchical rearrangement of the genes coding for the variable, diversity, and joining segments of the immunoglobulin heavy-chain genes (Sakano *et al.*, 1979; Early *et al.*, 1980). Following these initial rearrangements that affect the genes coding for heavy chains, there are rearrangements of the variable and joining segments of light-chain genes with kappa gene rearrangements preceding lambda ones (Hozumi and Tonegawa, 1976; Sakano *et al.*, 1979; Seidman *et al.*, 1979; Heiter *et al.*, 1980; Korsmeyer, 1981). After these rearrangements effective genes capable of inducing the synthesis of a particular heavy and a particular light chain are produced. As these changes progress at a DNA level, the stem cell matures into a pre-B cell with mu chains demonstrable in its cytoplasm and then into a B cell with surface markers characteristic of this cell (Cooper *et al.*, 1979). These markers include immunoglobulins acting as receptors for antigens. The union of appropriately presented antigen with surface immunoglobulin receptors triggers subsequent events, which include B-cell proliferation and terminal differentiation into antibody-synthesizing plasma cells. This process of B-cell maturation can also be activated in primates by the Epstein–Barr virus (Bird and Britton, 1979; Kirchner *et al.*, 1979; Rosen *et al.*, 1977) and by a series of plant and bacterial products termed polyclonal B-cell activators (Andersson *et al.*, 1972; Sultzer and Nilsson, 1972; Coutinho and Möller, 1973, 1975; Wu *et al.*, 1973; Waldmann *et al.*, 1974; Bona *et al.*, 1974; Dörries *et al.*, 1974; Möller, 1979). Many antigens and most, but not all, polyclonal activators require the presence of cooperating helper T cells and macrophages as well as the B cells to induce a maximal immunoglobulin response (Miller and Mitchell, 1968; Waldmann, 1977). More recently it has been recognized that certain monocytes and especially a network of T cells termed suppressor T cells act as negative regulators of B cells inhibiting the terminal matu-

ration of these B cells, thus inhibiting immunoglobulin synthesis (Gershon, 1974; Waldmann and Broder, 1977). It appears that the cells of the suppressor T-cell network emerge from the thymus as prosuppressor cells. These prosuppressor cells require an interaction with an inducer T cell as well as with an antigen or, alternatively, with a polyclonal activator to develop into final effectors of suppression (Waltenbaugh *et al.*, 1977; Feldman *et al.*, 1977; Tada *et al.*, 1978; Eardley *et al.*, 1978; Broder and Waldmann, 1978).

The majority of studies of immunoglobulin regulation *in vitro* in animal systems have examined the production of specific antibodies by immunocompetent cells stimulated by antigen *in vitro*. In contrast, the majority of studies in human systems have involved the use of polyclonal B-cell activators, since assays of antigen-induced specific antibody synthesis by the peripheral blood mononuclear cells *in vitro* in the absence of such activators have proved to be difficult in humans. Indeed, techniques that are antigen specific and that require no polyclonal activators, yet can be easily reproduced by many laboratories, are only just emerging. The studies with polyclonal activators have certain limitations, but they also have certain advantages over antigen-specific systems. In many cases they are not genetically restricted and thus permit the coculture of cells from unrelated individuals, a characteristic of great value in the analysis of monocyte function and of antigen nonspecific helper and suppressor T-cell activity from patients with disordered immunoglobulin synthesis. In addition, these antigen nonspecific approaches permit the definition of antigen nonspecific functions of clonal populations of regulatory cells, such as T-cell leukemias with retained helper or suppressor activity, without requiring that the particular antigen that is recognized by the antigen-specific T-cell receptor be identified. In addition, this approach has permitted the analysis of the activity of antigen-nonspecific helper and suppressor products produced and secreted by human mononuclear cells, cloned human T-cell lines, and human T-T-cell hybridomas.

In many of these studies of human cells pokeweed mitogen (PWM) has been utilized as the polyclonal B-cell activator. As noted below, the initiation of B-cell differentiation by PWM is a complex event requiring the presence of helper T cells and accessory cells. In addition, in certain circumstances PWM appears to activate prosuppressor T cells into effectors of suppression that inhibit the maturation of B cells. These characteristics are of value in many cases in understanding the cellular interactions involved in B-cell maturation, but in other cases these properties may make the interpretation of any disorders

observed with this system in health, and especially in disease, complex. For this reason many workers have studied other polyclonal activators of B cells, including those that differ from PWM in terms of their relative dependence on helper T cells and on accessory cells, and in terms of their propensity to activate prosuppressor cells into effectors of suppression (Bona *et al.*, 1974; Forsgren *et al.*, 1976; Bird and Britton, 1979; Möller, 1979; Montazeri *et al.*, 1980). In the present review, we will consider the different polyclonal B-cell activators that have been proposed in terms of these characteristics. We will emphasize our own experience in this area and will outline our views as to how the array of available polyclonal activators of human B cells can be used to develop rational approaches for categorizing disorders of human immunoglobulin synthesis as being due to intrinsic defects of B cells, as being due to disorders of helper T cells or accessory cells, or as being due to abnormalities of the suppressor cell network.

## II. Techniques Used to Assess the Polyclonal Activation of B Cells into Immunoglobulin- and Antibody-Producing Cells

A variety of *in vitro* techniques have been developed to study polyclonal activation of human B cells. These techniques include procedures that measure B-cell proliferation and those that are directed toward quantitating immunoglobulin production by B cells as assessed by a variety of methods. In some methods the proportion of cells with immunoglobulin in their cytoplasm is determined. In other approaches immunoglobulin- or antibody-secreting cells are enumerated. Alternatively, the quantity of immunoglobulin or antibody synthesized and secreted into the culture media is determined. One of the earliest approaches to the analysis of B-cell activation was the study of B-cell proliferation assessed by demonstrating blastic transformation of these cells or by quantitating their thymidine uptake after exposure to polyclonal activators (Douglas *et al.*, 1967; Phillips and Roitt, 1973; Greaves *et al.*, 1974; Janossy *et al.*, 1976; Brochier *et al.*, Montazeri *et al.*, 1980). Many materials that activate B cells directly or indirectly also stimulate T-cell proliferation; therefore procedures must be used to limit the cellular proliferation in the cultures to the B cells. For helper T cell-independent polyclonal activators, the proliferative responses of rigorously T-cell-depleted mononuclear cell populations may be examined. When activators that require the presence of inducer T cells for B-cell activation are being studied, any T cells added to the system must be irradiated or be treated with an agent such as mitomycin C to prevent their proliferation. Moreover, one

must recognize that proliferation per se does not measure the terminal differentiation of B cells into plasma cells.

The demonstration of immunoglobulin in the cytoplasm of mononuclear cells cultured in the presence of polyclonal B-cell activators has also been used to define agents capable of activating B cells into immunoglobulin-synthesizing cells (Cooper *et al.*, 1971; Wu *et al.*, 1973). This has usually been studied using antibodies labeled with fluorochromes to determine the proportion of cultured cells with immunoglobulin in their cytoplasm at the end of the culture period. However, in other cases the uptake of radiolabeled precursors into cytoplasmic immunoglobulins (Geha *et al.*, 1974; Choi, 1977; Geha, 1979) or the quantitation of cellular immunoglobulin content by sensitive radioimmunoassays has been used in these analyses (Korsmeyer *et al.*, 1981). The approach using antibodies labeled with fluorochromes is relatively easy to establish but has certain limitations. It is a tedious procedure that is less sensitive than other approaches that measure immunoglobulin synthesis. It has a subjective component in the analysis of the proportion of positive cells and numerous potential causes of false positive results exist. It should be noted that the proportion of cells in a culture assayed as immunoglobulin-containing cells by this procedure usually far exceeds the proportion identified as immunoglobulin-secreting cells by the reverse hemolytic plaque technique discussed below. Despite these limitations this technique has been used successfully to obtain important information concerning B-cell activation and to define the nature of disorders in patients with abnormalities of immunoglobulin production (Wu *et al.*, 1973).

The Jerne hemolysis in gel plaque assay has been modified to determine the number of B cells that mature on stimulation into immunoglobulin-secreting cells by a so-called reverse hemolytic plaque assay or into specific antibody-secreting cells by direct plaque-forming-cell assays. The reverse hemolytic plaque assay may be used to determine the total number of cells in a culture producing and secreting immunoglobulin or, by modification, the number of cells secreting immunoglobulins of a particular isotype (Eby *et al.*, 1975; Gronowicz *et al.*, 1976; Friedman *et al.*, 1976; Ginsburg *et al.*, 1978; Kirchner *et al.*, 1979). Typically, in this procedure the lymphocytes obtained at the termination of the culture period are mixed with erythrocytes coated with protein A or with an appropriate antibody that is directed to human immunoglobulins. These cell mixtures are then incorporated into an agar layer. After a brief incubation period the plaques are generally developed by the sequential addition of antisera to human im-

munoglobulin of the isotype being examined and then complement. The reverse hemolytic plaque assay is a sensitive method that permits the determination of the proportion of cells producing immunoglobulin of a particular class rather than a particular antigenic specificity at a particular time point in the culture. In an alternative approach the antigen-specific antibody response of B cells polyclonally activated *in vitro* can be assessed with a direct plaque-assay system using sheep red cells or other erythrocyte targets (Fauci and Pratt, 1976a; Fauci and Ballieux, 1979; Fauci, 1979). This approach has the advantage of measuring the portion of a polyclonal immune response that is directed toward a specific antigen, but it yields relatively small numbers of plaques when compared to the reverse plaque assay and may not yield any plaques when the cells of some normal individuals are utilized.

Another approach used to analyze B-cell activation, the one that we have used most frequently in our own studies, is to quantitate the immunoglobulins or antibodies synthesized and secreted into the culture media by mononuclear cells stimulated by polyclonal activators (Waldmann *et al.*, 1974; Platts-Mills and Ishizaka, 1975; Stevens and Saxon, 1978). In this procedure the starting cell population is extensively washed, and the immunoglobulins synthesized and secreted into the medium by the mononuclear cells activated *in vitro* are then quantitated by sensitive double-antibody radioimmunoassay procedures (Waldmann *et al.*, 1974) or by ELISA procedures (Kelly *et al.*, 1979). Alternatively, specific antibody that is secreted by such cells can be measured by solid-phase radioimmunoassay or enzyme-linked immunoassay approaches (Stevens and Saxon, 1978). The limitations of these approaches are that they are more difficult to establish than are certain of the procedures discussed above and that they cannot be performed in the presence of human serum or other biological materials containing the human immunoglobulin molecules being studied. In addition, they cannot be used when the polyclonal B-cell activators being studied catabolize or bind the secreted human immunoglobulins. This latter problem comes into play when agents such as staphylococcal protein A or staphylococcal organisms of Cowan strain I, which produce protein A, are being studied, since these agents bind most IgG and certain IgM molecules.

The procedures involving assays of secreted immunoglobulins do, however, have certain significant advantages when compared to other approaches. They are not subjective, and they are very sensitive. They permit the quantitative analysis of the product of small numbers of immunoglobulin-secreting cells. This feature is quite important when