# ADVANCES IN
# Immunology

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HENRY G. KUNKEL FRANK J. DIXON

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# PREFACE

The interrelationship of different branches of biology is part of our evolutionary heritage, but it still frequently comes as a surprise to see how significant this is for scientific progress. Plants and bacteria do not have an immune system, at least in the sense that we know it in higher organisms, but they have a wide assortment of binding proteins that show many similarities to antibodies. They are proving of increasing utility in immunology as well as other branches of biology as is evident from the final chapter in this volume. In a very different direction, autoantibodies to nuclear constituents found in the serum of patients with systemic lupus erythematosus, and described in the chapter by Tan, are proving of great utility in basic experimental work on RNA processing after transcription. The autoantibodies are directed against important RNA–protein complexes that had only become apparent through their disease relationship in the human. These are two examples from a group of such interrelationships described in this volume.

The first chapter by Irwin Scher discusses the very interesting immune-deficient CBA/N mouse strain which has been shown to have an X-linked gene defect. A number of X-linked human immune deficiency diseases are known but the exact defect remains obscure. This mouse model promises to be of great utility in solving this question. At present the mouse strain has proven uniquely useful for studies of B cell differentiation since the defect appears to involve a subtype of B cells not recognizable in other ways. Some controversy remains about the relative position of the multiple alterations that have been described, but the significant aspects are well delineated by Dr. Scher.

The second chapter on T cell lymphokines by Altman and Katz summarizes in a very helpful fashion the vast literature on this subject. The advent of methodologies for obtaining cloned T cell lines has aided greatly in the distinction of different species of molecules. T cell hybridomas and cloned T cell lines stimulated by T cell growth factor which secrete single factors have proven particularly useful. T cell replacing factors (TRF) and antigen specific and nonspecific helper factors are discussed in special detail and their characterization as a number of specific proteins appears close at hand. In addition the work on immune interferons, suppressor factors, growth factors, and macrophage factors and their interrelationships is discussed in detail.

The potential usefulness of at least some of these isolated factors in clinical situations makes this an increasingly exciting field.

The third chapter is by Eng Tan and discusses the wide assortment of antibodies to nuclear constituents found in the serum of patients with systemic lupus erythematosus (SLE) and related conditions. He has been a pioneer in this field and much of the work stems from the efforts of his laboratory. The review is very timely because of the many new types of antibodies described recently and their potential clinical significance. It is remarkable how many interesting and unusual antibodies can be delineated. Some of them have direct significance especially with regard to the development of immune complexes which are involved in the severe renal complications of SLE. Perhaps the most interesting development is that many of these antibodies are quite specific for identifiable clinical syndromes even though it is difficult to see how they might be involved in the specific clinical manifestations. Further studies are required to understand these associations; at the moment they are useful for diagnosis. The antibodies to RNA–protein constituents appear of special interest at the present time as mentioned above.

The chapter by Cochrane and Griffin concerns an important host defense mechanism that has received insufficient emphasis in the past, the Hageman factor or contact system of plasma. In considerable part through the work of these authors, the component parts of this system have been dissected through the use of highly purified materials. Four primary proteins are involved, Hageman factor, prekallikrein, high-molecular-weight kininogen, and coagulation factor XI. This system clearly overlaps with both the coagulation and complement systems in various aspects of the inflammatory process. Components of the contact system are clearly involved in various lesions and diseases although their exact role requires further clarification. Vascular permeability, allergic reactions, joint inflammation, and bacteremic shock represent a few of the many processes implicated.

The final chapter of this volume, by Teodorescu and Mayer, concerns the interaction of bacteria with lymphocytes. This field is just beginning to develop and shows considerable promise for answering basic questions regarding cell–cell interactions as well as useful detection of lymphocyte populations. In some ways this work relates directly to the plant lectins which have proven of such value in lymphocyte studies and it is probable that bacterial lectins are responsible for many of the interactions described. A number of bacteria have been found which bind specifically to B lymphocytes or subpopulations of B cells; T lymphocyte subpopulations also bind specific bacteria. Ex-

periments to define the lymphocyte components reacting with specific bacteria are just beginning, but it is likely that at least in some instances known components reacting with monoclonal antibodies are involved. Definition of these lymphocyte components which should not be difficult may help us to understand the many cell–cell interactions involved in the immune system.

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The Biochemistry and Pathophysiology of the Contact System of Plasma

CHARLES G. COCHRANE AND JOHN H. GRIFFIN



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# The CBA/N Mouse Strain: An Experimental Model Illustrating the Influence of the X-Chromosome on Immunity

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## I. Introduction

The influence of the X-chromosome on immune function has been evident for some years, based principally upon findings of X-linked immune deficiency diseases in man (Ayoub *et al.*, 1968; Rosen *et al.*, 1968). It was therefore of considerable interest when an inbred mouse strain was discovered which had an X-linked deficiency in its ability to respond to polysaccharide antigens (Amsbaugh *et al.*, 1972; Scher *et al.*, 1973a). Studies of this mouse strain have identified the gene, or closely linked group of genes, responsible for the immune defect and it has been named *xid* (Berning *et al.*, 1980). In this review I will attempt to summarize the known functional immune defects associated with *xid* and discuss the cellular basis for these defects. I further hope to demonstrate that the CBA/N strain has provided us with a powerful experimental model in which to study B-cell development, function, and heterogeneity.

## II. Influence of the X-Chromosome on Immunity

### A. Serum IgM Levels

The X-chromosomes of mice and men influence the immunological function of these species in a number of interesting ways. In addition to the well-known and profound effects illustrated by the various X-linked immunodeficiency diseases, the number of X-chromosomes of an individual directly influences the level of circulating IgM (Rhoades *et al.*, 1969; Wood *et al.*, 1970; Price *et al.*, 1974). These experiments, which were stimulated by observations that women have higher serum levels than men of IgM but not IgG or IgA (Butterworth *et al.*, 1967; Lichtman *et al.*, 1967), indicate that the mean serum IgM increases with an individual's X-chromosome complement, with XY = XO < XX = XXY < XXX. This phenomenon may help explain the higher titers of antibody directed against *Listeria monocytogenes* and *Escherichia coli* (presumably of the IgM class) (Gardner and Adinolfi, 1968) in normal females (XX), as compared to females with Turner's syndrome (XO) or normal males (Wood *et al.*, 1970), and the higher incidence of bacterial and viral infections in males (Washburn *et al.*, 1965; Thompson *et al.*, 1966; Asmar *et al.*, 1978; Goodman *et al.*, 1971). In studies of the influence of the murine X-chromosome on serum IgM levels, it was shown that the mean values of serum IgM were higher in female mice than in males of two different mouse

strains (Adinolfi *et al.*, 1978). However, in contrast to humans with Turner's syndrome (XO) who had lower mean serum IgM values than did normal XX females, the mean serum concentrations of IgM in XO mice were similar to that found in normal female mice.

### B. Immunodeficiency Diseases in Man

Bruton's X-linked agammaglobulinemia is a malady characterized by susceptibility to recurrent bacterial infections which appear in affected boys after 6 months of age. Children with this disease have very low serum IgA, G, and M levels and either lack or have very few surface Ig-bearing lymphocytes in their circulation or tissues (Good, 1973; Cooper *et al.*, 1975). It has been shown that children with this disease have cytoplasmic Ig-bearing pre-B cells in their bone marrow (Vogler *et al.*, 1976; Pearl *et al.*, 1978). This suggests that Bruton's X-linked agammaglobulinemia is the result of a developmental arrest of B lymphocytes at the level of pre-B cells. A few patients with X-linked inheritance of panhypogammaglobulinemia appear to have normal numbers of surface Ig-bearing B lymphocytes (Siegal *et al.*, 1971; Geha *et al.*, 1973; Litwin *et al.*, 1973). These groups, along with a family with two brothers and two maternal uncles, all of whom had an associated growth hormone deficiency (Fleisher *et al.*, 1980), may represent distinctive forms of X-linked immunodeficiencies with arrests of B-lymphocyte development at different stages (Cooper and Seligmann, 1977).

Patients with X-linked immunodeficiency with increased levels of serum IgM also have increased serum IgD and low or absent serum IgG or IgA (Rosen *et al.*, 1968). These patients have normal numbers of B lymphocytes bearing surface IgM, G, or A (Cooper and Seligmann, 1977) and are apparently deficient in their ability to switch from IgM to IgG or IgA production during an immune response. This deficiency may involve the mechanism for T-lymphocyte-dependent B-lymphocyte terminal differentiation.

The Wiskott–Aldrich syndrome is a complex X-linked immunodeficiency disease with low serum IgM, normal serum IgG, and elevated serum IgA and IgE levels. Although T-lymphocyte function decreases in children with this disease as they age, delayed hypersensitivity reactions are normal early in life. The characteristic functional immune defect in these patients is their inability to mount antibody responses to polysaccharide antigens, whereas antibody responses to protein antigens are intact (Cooper *et al.*, 1968; Blaese *et al.*, 1968; Ayoub *et al.*, 1968). The primary immune defect in this condition is

unknown, as is the etiology for the associated thrombocytopenia and eczematoid dermatitis. However, the functional immune abnormalities in these patients suggest that they may have a defect in the development of a B-lymphocyte subline (Cooper and Seligmann, 1977).

An X-linked form of severe combined immunodeficiency disease has been described (Gitlin and Craig, 1963; Hoyer *et al.*, 1968). Children with this disease have variable degrees of lymphopenia, with small foci of lymphocytes in their lymph nodes and spleens. It is unclear if these patients have a primary B- or T-lymphocyte or stem cell defect, although it has been noted that three boys with this disease have normal numbers of circulating B-lymphocytes (Cooper and Seligmann, 1977).

A most interesting X-linked lymphoproliferative syndrome has been described (Purtilo *et al.*, 1975). Males with this syndrome appear well until they develop an Epstein–Barr virus infection. Thereafter, they may manifest a number of distinctive proliferative or nonproliferative clinical syndromes (Purtilo *et al.*, 1977), with different patients within the same kindred expressing different phenotypic forms of the disease. American Burkitt lymphoma, immunoblastic sarcoma of B cells, fatal infectious mononucleosis, or plasmacytoma are different proliferative forms of the syndrome, whereas acquired agammaglobulinemia, aplastic pancytopenia, or neutropenia are nonproliferative forms of the disease. The etiology of this syndrome is unclear, although it has been suggested that the variable phenotypic expression could have resulted from individual differences in the Epstein–Barr viral dose, duration of exposure, and/or age at the time of infection with the virus (Purtilo *et al.*, 1977). Recently, it has been shown that males with this disease have immune-deficient responses to the Epstein–Barr virus (Sakamoto *et al.*, 1980). The mechanism(s) responsible for this defect are unknown, but could involve defective T-cell killing of virus-infected B cells or an intrinsic defect in B-cell target cells.

### C. Immunodeficiency in Mice

Studies of X-linked immunodeficiency diseases in inbred mice began in the early 1970s, when it was shown that a mouse strain carried at the National Institutes of Health failed to make antibody responses to type III pneumococcal polysaccharide (S-III) (Amsbaugh *et al.*, 1972) or polyriboinosinic–polyribocytidilic acid [poly(I-C)] (Scher *et al.*, 1973a). CBA/N male and female mice, as well as $F_1$ males derived from matings of CBA/N females to males of immunologically

normal strains, were unresponsive to these antigens, whereas $F_1$ females from these crosses made excellent responses.

The CBA/N subline (originally referred to as CBA/HN) was derived from a CBA/Harwell line, beginning in 1966. All went well with the inbreeding until the seventh generation, when a breeding crisis occurred. However, the last pregnant female from this generation had a litter which included a single male and a number of females from which the CBA/N line was derived. At the time of the original studies of these mice, 15–25 generations of brother–sister matings had occurred (Amsbaugh *et al.*, 1972). The immunological defect of this strain was not apparent from their reproductive vigor or ability to exist in a routine laboratory environment, as these animals are normal in both regards.

The gene or group of closely linked genes responsible for the immune defect of CBA/N mice has been mapped on the X-chromosome and named X-linked immune deficiency (gene symbol *xid*) (Berning *et al.*, 1980). The two mutant genes used to map *xid* were tabby (*Ta*) and hypophosphatemia (*Hyp*), with the gene order and map distances in centimorgans being *Ta*—6.6 ± 1.8—*xid*—12.2 ± 2.3—*Hyp*.

In order to study the influence of the *xid* gene of CBA/N mice on the lipopolysaccharide (LPS) unresponsiveness of C3H/J mice (Sultzer, 1968; McGhee *et al.*, 1979; Watson and Riblet, 1975), CBA/N females were crossed to C3H/HeJ males and the resultant $F_1$ hybrid females were mated to C3H/HeJ males (Bona *et al.*, 1979, 1980). Approximately one-half of the backcross males derived from this cross expressed a more profound immunological defect than either of the parental strains. Thus, spleen cells from these mice were unresponsive to the proliferative actions of B-cell mitogens and they failed to give antibody responses to thymus-independent type 1 (TI-1) or TI-2 antigens. The synergistic defect in B-cell function of these backcross male mice was dependent upon the presence of the *xid* gene. However, the critical gene(s) from the C3H/HeJ strain was not the defective $LPS^d$ gene.

An interesting X-linked abnormality in the capacity of DBA/2Ha mice to respond to T cell-replacing factor (TRF) has been described (Tominaga *et al.*, 1980). B cells from (DBA/2Ha × BALB/c)$F_1$ male mice were incapable of responding to TRF, while their $F_1$ littermates gave excellent responses. This X-linked defect was associated with the inability of B cells from DBA/2Ha mice to absorb TRF activity, an action that B cells of other mice had. Thus, this defect appeared to be due to the absence of a TRF receptor on the B cells of DBA/2Ha mice.

## III. Immunological Defects in CBA/N Mice

### A. Thymic-Independent Immune Responses

#### *1. Characteristics of Types 1 and 2 Thymic-Independent Antigens*

Thymic-independent antigens consist of a group of substances sharing certain immunologic and physical properties, the most important being than they require little or no T-cell help in inducing antibody responses. Different authors have different criteria for including an antigen in this group; however, if an antigen is able to stimulate specific responses in *nu/nu* mice, in neonatally thymectomized, adult irradiated, and bone marrow-reconstituted mice, or in T cell-depleted cultures, it is usually considered TI. For example, the IgM responses to S-III (Howard *et al.*, 1971), LPS, polyvinylpyrrolidone (PVP) (Andersson and Blomgren, 1971), and polymerized flagellin (Feldmann and Basten, 1971) appear not to require T-cell help in certain mouse strains. Most TI antigens are large polymeric molecules that have a high density of repeating determinants or epitopes (Feldmann and Basten, 1971), are poor direct stimulators of T cells (Kruger and Gershon, 1972), are slowly degraded by biological system, and persist for long periods *in vivo* (Felton, 1949; Sela *et al.*, 1972). Continued interest in the properties of these antigens has led to the discovery of other TI antigens, such as haptenated derivatives of Ficoll (Sharon *et al.*, 1975; Mosier *et al.*, 1974), and these in turn have allowed investigators to divide TI antigens into two classes.

The distinction between TI-1 and TI-2 antigens was based upon their differential ability to induce responses in adult normal or adult CBA/N and immature normal mice (Table I) (Mosier *et al.*, 1977a,b). Earlier studies using S-III, poly(I-C), or dinitrophenyl (DNP) conjugates of Ficoll have suggested that the *xid* gene resulted in a global defect in the ability of mice to respond to TI antigens (Amsbaugh *et al.*, 1972; Scher *et al.*, 1973a, 1975a). However, when trinitrophenyl (TNP)-conjugated LPS was used to test the immune responsiveness of immune-defective mice, it was shown that they made responses which were only somewhat reduced when compared to those of normal mice at optimum concentrations of antigen (Cohen *et al.*, 1976; Mosier *et al.*, 1976). Furthermore, CBA/N mice also gave excellent responses to TNP–*Brucella abortus* (TNP-BA), an antigen, which like TNP-LPS, was shown to be TI in immune defectives as well as normals (Mond *et al.*, 1978). Additional studies demonstrated that antigens such as TNP-Ficoll, that were unable to induce responses in immune defec-

TABLE I
CHARACTERISTICS OF THYMUS-INDEPENDENT TYPES I AND II ANTIGENS[a]

| | Thymus-independent types | |
|---|---|---|
| | I | II |
| Induces antibody in CBA/N mice | + | − |
| Induces antibody in neonatal normal mice | + | − |
| Accessory cell dependent | − | + |
| Requirement for T cells | − | ± |
| Predominant class of antibody produced | IgM, $IgG_3$, $IgG_2$ | IgM, $IgG_3$ |

[a] Data compiled from Mosier *et al.* (1977a,b), Morrissey *et al.* (1981), Mond *et al.* (1980), and Slack *et al.* (1980).

tives, also failed to induce responses from immature normal mice (Mosier *et al.*, 1977a,b). By contrast, TNP-LPS and TNP-BA induced excellent responses in both immature normal or adult immune defectives. On the basis of these findings, TNP-Ficoll was designated a TI-2 antigen, whereas TNP-LPS and TNP-BA were designated TI-1 antigens (Table I).

Early studies of different TI antigens, including polymerized flagellin (Diener *et al.*, 1970; Feldmann and Palmer, 1971), LPS (Lemke *et al.*, 1975; Yoshimaga *et al.*, 1972), TNP-Ficoll (Mosier *et al.*, 1974), and TNP-BA (Mond *et al.*, 1979b; Boswell *et al.*, 1980a), suggested that they were both thymus and macrophage independent. However, more recent studies of TNP-Ficoll have shown this antigen to be macrophage dependent (Chused *et al.*, 1976; Boswell *et al.*, 1980c). Indeed, removal of adherent cells on a Sephadex G-10 column resulted in a marked reduction or elimination in the *in vitro* responses of spleen cells to the TI-2 antigens TNP-Ficoll, TNP-dextran, and TNP-levan (Table II) (Morrissey *et al.*, 1981). Reconstitution of the responses to these antigens was achieved by the addition of irradiated $Ia^+$ splenic adherent cells. By contrast, control or G-10 passed cells responded equivalently to TNP-BA. Thus, although TI-1 and TI-2 antigens were not originally defined on the basis of their macrophage dependence, it now appears that this property may distinguish these two classes of TI antigens (Morrissey *et al.*, 1981).

As noted above, TNP-Ficoll was originally classified as a TI antigen on the basis of its ability to induce responses in *nu/nu* mice and in lethally irradiated mice reconstituted with anti-theta antibody (anti-

TABLE II
THE ROLE OF SPLENIC ADHERENT CELLS (SAC) IN THE STIMULATION OF TI-2 RESPONSES[a]

| Responding population | SAC | PFC/$10^6$ cultured cells | | | | |
|---|---|---|---|---|---|---|
| | | TNP-BA | TNP-Ficoll | TNP-dextran | TNP-levan | No antigen |
| Whole spleen | – | 308 | 120 | 206 | 308 | 22 |
| SAC depleted | – | 442 | 14 | 60 | 0 | 28 |
| SAC depleted | + | N.D. | 382 | 300 | 438 | 24 |

[a] Spleen cells from C57BL/10 mice were used as responding cells and were depleted of accessory cells by passage over a G-10 column. SAC were isolated, irradiated, and added back to G-10 passed spleen cells as noted. Optimum concentrations of TI-1 or TI-2 antigens were cultured with the whole spleen or SAC-depleted populations. The data are presented as the geometric mean of triplicate cultures (Morrissey *et al.*, 1981).

Thy) plus complement (C)-treated spleen cells (Sharon *et al.*, 1975). These findings were confirmed by other laboratories (Mosier *et al.*, 1974; Boswell *et al.*, 1980c). However, after extensive T-cell depletion by repeated anti-Thy + C treatment, the *in vitro* responses to TNP-Ficoll were significantly reduced or eliminated (Mond *et al.*, 1980). These responses could be restored by the addition of T cells to the cultures. By contrast, the *in vitro* responses to TNP-BA were occasionally diminished as a result of T-cell depletion, but never as drastically as the response to TNP-Ficoll. It was suggested, therefore, that TI-2 antigens required small numbers of T cells for optimal responses, whereas TI-1 antigens had either no or an even smaller requirement. Indeed, polyacrylamide beads which have been coupled to a low epitope density with TNP (Mond *et al.*, 1979b) and TNP-Dextran, which are considered to be TI-2 antigens, also require small numbers of T cells for optimal responses (Mond, personal communication). These findings, as well as those in which the response of adherent cell-depleted populations were analyzed, suggest that macrophages and small numbers of T cells are required for TI-2 but not TI-1 responses (Table I).

In addition to the apparent differences in the cellular requirements necessary for antibody responses to TI-1 and 2 antigens, recent data indicate that these antigens also induce different classes of antibodies in mice. Earlier studies had shown that the class of antibodies induced by groups A and C streptococcal carbohydrate, dextran, or phosphocholine were IgM and the rare $IgG_3$ subclass (Perlmutter