Immunity to Animal Parasites

Edited by

E. J. L. Soulsby

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IMMUNITY TO ANIMAL PARASITES

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PREFACE

The rapid development of immunology in the last decade has affected all areas of the biological sciences and not least the field of parasitology. The increased interest in parasitological problems by immunologists and in immunology by parasitologists has greatly assisted in placing studies of the immunology of parasitic infections on the firm bases of sound immunology and sound parasitology. A number of joint meetings of parasitologists and immunologists, arranged by various national and international agencies, have done much to foster the present healthy state of the subject, and this volume is, in part, a tribute to the foresightedness of the organizers of such meetings.

This volume represents the proceedings of the Fifth International Conference of the World Association for the Advancement of Veterinary Parasitology which was held in Mexico City in August, 1971, as a joint meeting with the Academie Nacionale de Medicina de Mexico. The invited papers of the conference, published in this volume, formed the core of the theme of the conference. They take an immunological look at the immunology of host–parasite relationships. The authors were asked for a critical appraisal of their subject areas and to place the knowledge of host–parasitic relationships into the context of modern immunology. It is increasingly possible to do this, and it is clear from the contributions to this volume that parasitologists have unfettered themselves from the mystique that immune responses to parasites are things apart from the immune responses to other antigens and infections. Nevertheless, by the very complexity of their structure, especially in the case of helminths and their developmental cycles, the responses to them are complex and multicomponent in nature. The contents of this volume

though illustrating this complexity also indicate the growing strength of the effort to analyze the complexity.

The cooperation and enthusiasm shown by all the authors were an indication of the excitement that pervades the field at this time. It is hoped their efforts in providing an up-to-date statement of the situation will serve as a point of departure for others, immunologists and parasitologists, who are drawn to the challenge of the work that lies ahead.

As with the other International Conferences of the WAAVP, the local flavor of the hospitality of our hosts gave an added dimension by which the theme of the conference will be remembered and which, unfortunately, cannot be translated in any tangible way in this volume.

I wish to express my thanks to the contributors for their wholehearted cooperation, including the observance of the editorial constraints placed on them for style, length of manuscript, and delivery of the finished product. To the staff of Academic Press I extend special thanks for their willingness and competence which have led to a smooth and speedy publication.

E. J. L. Soulsby

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I. Introduction

Jerne (1967) has defined cis and trans immunologists as persons approaching the mystery of antibody formation from the side of the antigen and the antibody, respectively. In a lecture in Copenhagen in 1968 Jerne gave the following account of the development of immunological concepts since 1900. With Ehrlich as the front figure since the 1890's, cis immunology was predominant during the first golden age of immunology. By 1910 the cis philosophy led to a stagnation which lasted to the late 1950's when the trans approach took over and opened the second golden age of immunology. Jerne (1967) has emphasized that this fully copes with a statement made by Francis Crick that "if you cannot study function, study structure." This review will reflect predominantly a trans approach and will review the pertinent data on the humoral immune response and the immunoglobulins of ruminants and swine.

Chemical studies of immunoglobulins started in 1845 when the so-called Bence-Jones proteins were first recognized (Bence-Jones, 1847). These proteins were encountered in the urine from a patient with *mollities ossium*, presumably multiple myeloma. It was found that the precipitate formed in acidified urine redissolved upon boiling. Bence-Jones proteins have been intensively examined in recent studies of immunoglobulins. The existence of Bence-Jones proteins and homogeneous populations of pathological immunoglobulins, myeloma proteins, and Waldenström macroglobulins has allowed detailed information to be accumulated about the structure, synthesis, and genetics of human immunoglobulins. This is also the reason there is a lack of information about immunoglobulins in species such as ungulates since pathological immunoglobulins are absent or extremely rare in these species.

II. Historical Aspects

The practical application of immunological principles goes back to variolation in China in the fifteenth century. Thus, specific resistance against infectious diseases was recognized centuries before the demonstration of circulating antibodies. The proof of humoral substances in serum as a basis for immunity was established in 1890 by von Behring and Kitasato. Working with tetanus and diphtheria toxins they named the protective serum substances *antitoxins*. In serum from nonimmunized animals Buchner (1893) recognized a heat labile factor that accomplished lysis of bacteria in cooperation with specific antibody. Bordet used the term *alexine* (Greek: to ward off) for this factor which was later named *complement* by Ehrlich (1900).

It soon became clear that the production of antibodies was not necessarily associated with immunity against disease. Thus, Ehrlich (1897) was able to block the hemagglutinating action of ricin with goat anti-ricin serum, Tchistovitch (1899) found that anti-eel serum neutralized the hemolytic action of normal eel serum on rabbit erythrocytes and Bordet (1898) demonstrated agglutinins against erythrocytes in serum from animals inoculated with defibrinated blood. The term *antibody* (Antikörper) was invented in 1900 by Ehrlich in his famous presentation of the "side chain theory" for the production of antibodies.

Gamma globulins were defined by Tiselius in 1937 as the slowest migrating electrophoretic group of serum proteins when electrophoresis was conducted at alkaline pH. Association of antibody activity with the gamma globulin fraction was demonstrated for the first time in 1939 by Tiselius and Kabat. Subsequent experiments revealed that antibody activity also resided in the electrophoretic beta region and Heremans in 1959 therefore proposed the "immunoglobulin concept." This concept was adopted by the World Health Organization in 1964 to designate proteins of animal origin with known antibody activity as well as other chemically related normal and pathological proteins (Cohen, 1965).

III. Nomenclature

For the human immunoglobulins (Rowe, 1970) a complete set of terminology has been adopted by the World Health Organization. The terminology used in this review will conform as closely as possible to the human nomenclature. The standard requirements for applying the human terminology to a particular immunoglobulin class of another species should be antigenic cross-reactivity between the Fc portion of the human immunoglobulin and the immunoglobulin of the other species. This standardization was recently proposed for the immunoglobulins of the domesticated Bovidae at a "Symposium on the Bovine Immune System." This symposium was held on November 18–20, 1970 at the Interstate Inn, College Park, Maryland.

The nomenclature and the main characteristics of the human immunoglobulins are summarized in Table I.

IV. Immunoglobulin Structure

A. The Four-Chain IgG Unit

Studies on the structure of IgG started in the 1940's when Peterman (1946) papain-digested human "gamma globulin" into fragments of

about one-half to one-quarter the size of the original molecule. R. R. Porter reported similar results in 1950 from studies on rabbit anti-ovalbumin. The distinction was then made between what is now known as the Fab and Fc fragments. The findings were further substantiated by R. R. Porter in 1958 and 1959 through studies employing highly purified crystalline papain. The rabbit 7 S IgG molecule was split into 2 Fab and 1 Fc fragments, each about one-third the size of the original molecule ($s_{20,w}$ 3.5) with molecular weights of 45,000 and 55,000, respectively (R. R. Porter, 1959, 1963).

A number of important biological properties are associated with the Fc fragment. Thus, the catabolic site is located on the Fc piece (Fahey and Robinson, 1963) as is the structure responsible for the persistence of IgG in the circulation (Spiegelberg and Weigle, 1965a,b). The sites involved in tissue affinity and complement fixation (Taranta and Franklin, 1961) reside in the Fc fragment and this fragment is essential for the transport of the IgG molecule across fetal membranes (Hartley, 1951; Brambell *et al.*, 1959, 1960).

Peptic digestion of 7 S rabbit IgG produces a 5 S $(Fab')_2$ fragment which upon reduction of a single and exceptionally labile disulfide bond yields two 3.5 S Fab' fragments (Nisonoff *et al.*, 1960, 1961). The human IgG subclasses are heterogeneous in their susceptibility to enzymatic digestion. Thus, IgG_2 is most resistant, IgG_3 most sensitive, and IgG_1 and IgG_4 intermediary sensitive (Virella and Parkhouse, 1971). Similarly, human secretory IgA and IgA_1 paraproteins have been found to be 3 to 4 times more resistant to pepsin digestion than IgA_2 paraproteins (Shuster, 1971).

The multichained structure of IgG was discovered in 1959 by Edelman. After reduction and alkylation of the interchain disulfide bonds, two types of polypeptide chains, light chains and heavy chains, could be isolated by cation exchange chromatography in 6 *M* urea (Edelman, 1959; Edelman and Poulik, 1961). However, the separated polypeptide chains were insoluble in aqueous media and had lost their biological activity. This problem was overcome by Fleischman *et al.* (1962, 1963) through reduction and alkylation in neutral aqueous media. The chains were separated by gel filtration on Sephadex G-75 or G-100 in 1 *N* propionic or acetic acid.

The above data allowed R. R. Porter in 1962 to present his model for the IgG molecule (Fig. 1). The IgG molecule was postulated to consist of two light and two heavy polypeptide chains held together by disulfide bonds. This model has been able to cope with the findings of numerous investigations, including electron microscopic studies.

The IgA molecule also contains two heavy and two light polypeptide

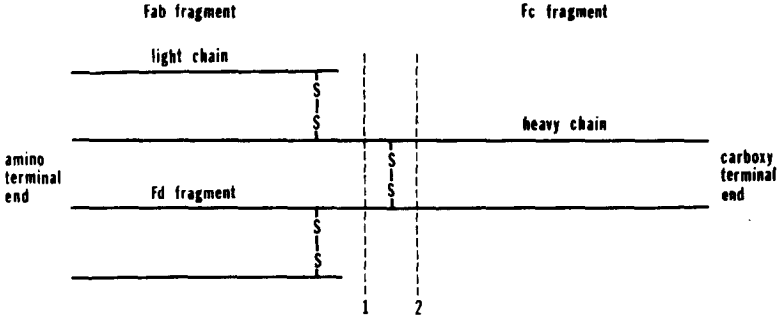

Fig. 1. Diagrammatic structure of IgG-immunoglobulin showing the terminology for polypeptide chains and enzymatic fragments. Dotted vertical lines indicate the site of papain cleavage (1) and the site of pepsin cleavage (2). After R. R. Porter, 1962.

chains held together in a molecular structure similar to IgG (cf. Butler, 1970). IgM consists of five building units similar in structure to the IgG molecule. These five units are held together in a spiderlike structure carrying ten antigen combining sites (Chesebro *et al.*, 1968). IgD and IgE exist in IgG-like monomer units with sedimentation coefficients of 7–8 S and 8 S, respectively (cf. Butler, 1970).

B. Primary Structure and Antigenic Determinants

Intensive sequence studies of human and mouse Bence-Jones and myeloma proteins have revealed that the amino terminal half of the light chains varies considerably from kappa chain to kappa chain, and from lambda chain to lambda chain, while the carboxy terminal half is essentially invariable within the kappa and lambda entities, respectively (Putnam *et al.*, 1967). The recognition of these two antigenic subclasses of light chains was made in 1956 by Korngold and Lipari (1956a,b) studying Bence-Jones proteins. It was subsequently found (Mannik and Kunkel, 1962; Fahey, 1963a,b; Migita and Putnam, 1963) that each immunoglobulin class is made up of molecules carrying either kappa or lambda chains, but never both.

The class specific antigenic determinants reside exclusively in the heavy polypeptide chains (Franklin and Stanworth, 1961). In man five types, gamma, alpha, mu, delta, and epsilon, of heavy polypeptide chains have been identified. These chains belong to the IgG, IgA, IgM, IgD, and IgE classes, respectively. Also the heavy polypeptide chains contain a variable NH_4-terminal region which is about the length of the light chain variable region, while the constant COOH-terminal region is ap-

proximately 3 times as long as the constant region of the light chain (Köhler *et al.*, 1970). The sequence of the constant portion of the heavy chain is class-specific, whereas antigenic subgroups common to alpha, gamma, and mu chains (Seligmann *et al.*, 1966; Todd *et al.*, 1967) have been found to be paralleled by sequence homologies on the variable part of these chains (Köhler *et al.*, 1970; Wang *et al.*, 1970). This is in contrast to light chains where the variable region is characteristic of the kappa and lambda chains, respectively. On the basis of differences in the variable portion, three and five antigenic subgroups have been identified within the kappa and lambda chains, respectively (cf. Rowe, 1970).

Although monoclonal gamma-M and gamma-G both had kappa-1 chains with extensive variable region sequence homologies, the sequence of the variable regions of the mu and gamma chains was only 30% identical (Putnam and Köhler, 1969). In this case, then, the sequence difference between the light and heavy chains of the same molecule was of the same magnitude as between light and heavy chains of different molecules (Köhler *et al.*, 1970).

Extensive interspecies homologies have been identified in the amino acid sequence of kappa chains from man, mouse, and rabbit (Doolittle and Astrin, 1967; Titani *et al.*, 1967) and of human kappa chains and the Fc fragment from rabbit IgG (Hill *et al.*, 1966). On the basis of these findings Titani *et al.* (1967) have suggested a common ancestry of light and heavy polypeptide chains of the IgG molecule.

The kappa and lambda light polypeptide chains have been identified in a broad variety of animals, but the ratio between the two classes of chains varies considerably from species to species (Hood *et al.*, 1967). For IgG the normal ratio in man is 2:1, but 1:1, 1:8, and 1:8 in swine, ox, and sheep, respectively. Kappa chains are completely lacking in the horse.

The kappa:lambda ratio for normal human immunoglobulins is similar to the kappa:lambda ratio for the incidence of patients with multiple myeloma and Waldenström macroglobulinemia (Laurell and Singurowicz, 1967). This may be interpreted to indicate that the kappa and lambda producing cells mutate and start to produce M-components in proportion to their normal occurrence (Laurell and Singurowicz, 1967).

A variety of antibodies in man [Rh antibodies, isoagglutinins (anti-A and anti-B), thyroglobulin antibodies, dextran antibodies, and teichoic acid antibodies] have been demonstrated to be expressed in both kappa and lambda type immunoglobulins, but the kappa:lambda ratio in purified antibody preparations varies widely in different individuals and sometimes diverges markedly from the typical 2:1 kappa:lambda ratio

(Mannik and Kunkel, 1963). Gamma-M cold agglutinins have been found to be either kappa or lambda type (Mannik and Kunkel, 1963). Similar observations have been made in the guinea pig (Nussenzweig and Benacerraf, 1966) and rabbit (Knight *et al.*, 1967). Human myeloma proteins (gamma-G_1, lambda) with antibody activity were identified by Eisen *et al.* (1967), and several similar findings have been made in mice (cf. Yamada *et al.*, 1970).

Four human gamma-G subclasses have been identified corresponding to the gamma-1, gamma-2, gamma-3, and gamma-4 chains (Grey and Kunkel, 1964). Similarly three IgG subclasses are present in mice (Fahey *et al.*, 1964b). At least two IgA (Kunkel and Prendergast, 1966; Vaerman and Heremans, 1966; Feinstein and Franklin, 1966) and two IgM (Harboe *et al.*, 1965) heavy chain subclasses are known in man. The relative abundance of the gamma-G subclasses may be seen in Table I. Cases of hypogammaglobulinemia with drastically altered IgG subclass abundance have been reported (Virella *et al.*, 1970; Yount *et al.*, 1970). All the gamma-G subclasses are present in the electrophoretically fast migrating human IgG entity, while only gamma-G_1, gamma-G_2 and gamma-G_3 are represented among the electrophoretically slow migrating gamma-G molecules (Skvaril and Morell, 1970).

C. Allotypes

Genetically determined intraspecies antigenic differences among immunoglobulins were first observed in rabbits by Oudin (1956). While the class- and subclass-specific antigenic determinants are common to all individuals of a given species, the various allotypic markers are only present in a fraction of the species members.

In man two sets of allotypic markers, *Gm* and *InV*, have been identified. The *Gm* (genetic marker; Grubb, 1969) markers are exclusively located on the gamma chains (Franklin *et al.*, 1962) while the *InV* factors are properties of the kappa chains (Franklin *et al.*, 1962; Harboe *et al.*, 1962). Thus *Gm* types are only present within the IgG class while the *InV* factors are encountered in all immunoglobulins of the kappa light chain type. Twenty-three *Gm* factors have been identified (Grubb, 1969). Each individual may possess several of the *Gm* factors, but a particular IgG molecule carries only a single *Gm* determinant. Several *Gm* markers have been identified on the gamma-1 and gamma-3 chains, respectively, only one on gamma-2, and so far none on gamma-4 chains (Natvig *et al.*, 1967). Gm^1 and Gm^5 are mutually exclusive alleles in Caucasians (Grubb, 1969). However, in the Negro Gm^1 and Gm^5 may occur together on the same IgG molecule (Steinberg *et al.*, 1960), and