immunology

A SCOPE® MONOGRAPH

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Published by THE UPJOHN COMPANY, *Kalamazoo, Michigan*

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Fig. 28. K. F. Austen, M.D., Harvard Medical School, Boston, Ma.

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Fig. 54, 61 and 65. John David, M.D., Harvard Medical School, Boston, Ma.

Figs. 55 and 56. Department of Dermatology, London Hospital Medical College, London, England.

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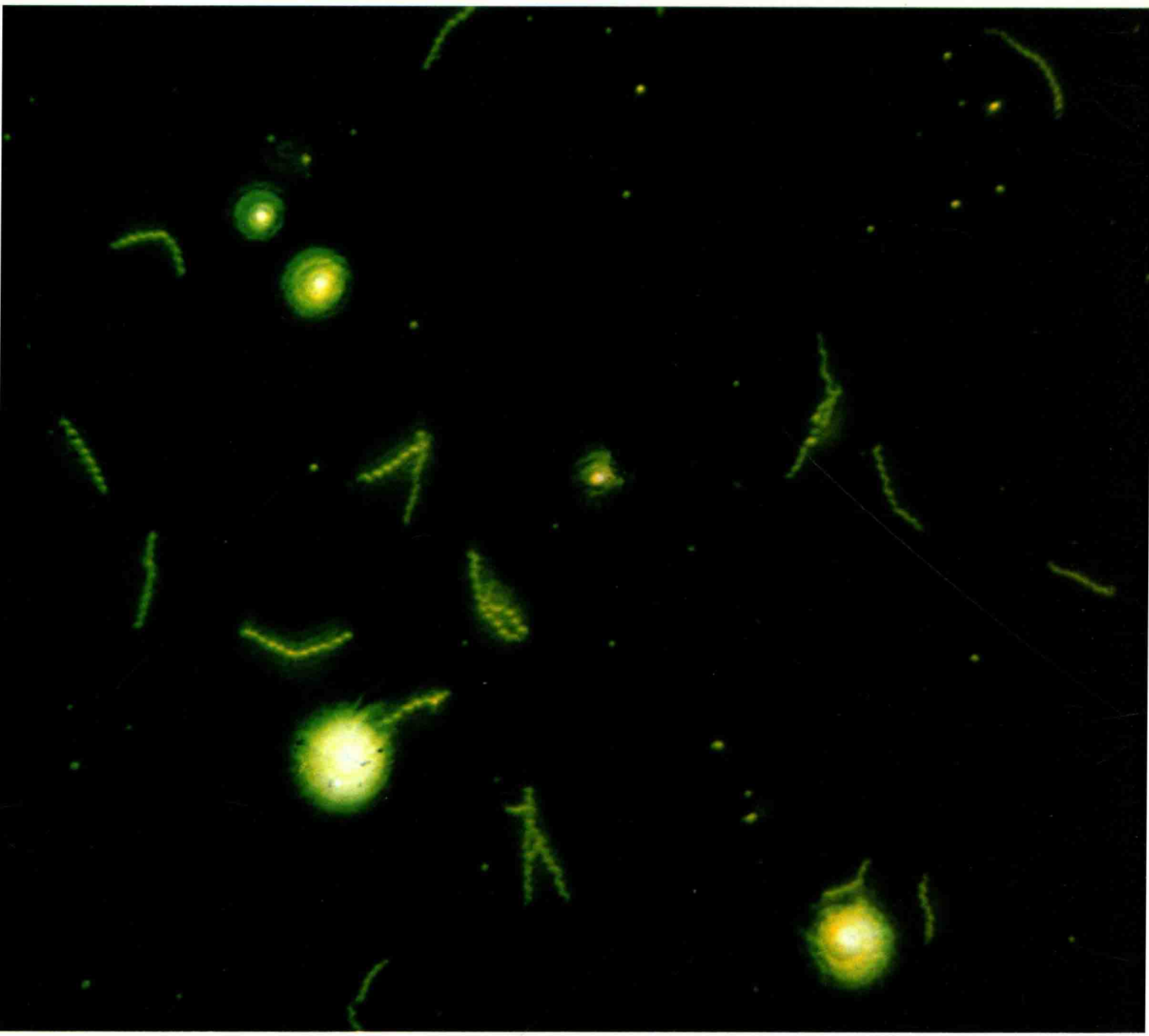
introduction

Ronald D. Guttmann, M.D., Guest Editor-in-Chief

The discipline of immunology has seen enormous progress and proliferation of important information during the last 25 years with a significant impact in the understanding of human disease and the alleviation of human suffering. Important practical problems still remain in the field of preventive medicine and immunization for as yet unconquered infectious diseases such as hepatitis, malaria, gonorrhea, meningococcemia and viral respiratory diseases. It is of the highest priority to obtain more information about the immune response to live vaccines in normal and ill individuals since killed vaccines are by nature limited. Further, there is pressing need for application of these techniques to areas of the world that have not yet reaped their benefits. In addition to the vast progress of microbiological immunology, there are two important subjects of medical research which have spurred the development of immunology as one of the most important disciplines of biological science. These two areas have been related to the clinical desire to carry out organ transplantations and to apply immunological knowledge towards the treatment of cancer.

It is not quite 25 years since it was demonstrated that 1) "immunological tolerance" could be induced in neonatal laboratory animals, thus allowing grafts to be exchanged in adult life and 2) the equally important experiments showing that there were indeed tumor-specific transplantation antigens, thus opening the way for consideration of an immunological approach towards cancer. More recently, great interest has been generated to solve the fundamental problems of immunology by delineation of immunoglobulin structure and variability, the definition of phylogenetic and ontogenetic development of antibody formation and genetic diversity, and mechanisms of induction of the immune response. Cellular immunology has come into its own in the last decade due to the realization that lymphocytes bear a diverse spectrum of surface receptors for antigens. Furthermore, an appreciation is now possible of the complex cooperative events in the immune response involving thymus-derived lymphocytes, bone-marrow-derived lymphocytes, macrophages, and the effects of various immunoglobulins and receptor molecules that are all interrelated during the induction and effector phases of immunity. The deliberate manipulation of the immune response by immunosuppression as well as by immunostimulation is not well understood. Rapid progress will be made as we enlarge our basic understanding of the molecular and cellular events of immunology leading to more specific and rational approaches towards therapy.

Clinical organ transplantation and cancer immunology are new fields that seem to be progressing effectively and with optimism despite a highly empirical approach. While they develop it is likely that new biological insights will be revealed as well as a host of unforeseen problems of medical practice and ethics. Finally, the application of this very large aggregation of immunological information which is detailed in this text will undoubtedly have manifold future applications to other important clinical problems of autoimmune diseases, chronic illness associated with abnormal immune responses, and possibly even to certain biological processes involved in aging.



immunoglobulins

David Papermaster, M.D., Associate Professor of Pathology, Yale University School of Medicine

Antibodies are complex protein molecules produced by plasma cells and some lymphocytes. These cells can be found in numerous locations such as germinal centers of lymph nodes, follicles of the spleen, Peyer's patches of the intestinal tract, tonsils, adenoids, appendix and some cells which circulate in the blood and lymph. Antibodies are found in blood, lymph, and some are present in colostrum, saliva, the gastrointestinal and urinary tracts.

Antibodies are thought to combine with their antigens in a way that resembles the fitting of a key to a lock. All antibodies resemble each other in overall shape, yet each has unique regions that will make it fit to one antigen and not to another. This quality is termed specificity. An antibody specific for wart virus will not neutralize a polyoma virus (Fig. 1).

Constructing a molecule to combine with each antigen—It would be simplest, of course, to conceive a separate antibody structure for every antigen; in this case the problem of fitting an antibody and an antigen together would not differ basically from the bonding of an enzyme to its substrate. Thus immune cells —using means that are consistent with the known rules of inheritance and protein synthesis—could make a unique antibody for any antigen encountered. It is appropriate that antibodies are proteins because they can be made from any arrangement of 20 different amino acids into a long chain. Once the order of the amino acids in a protein is established, there is sufficient chemical force present to cause the protein to fold in a complicated but well-organized tangle back and forth upon itself; this folding creates hollows, clefts, and bumps to give the antibody its combining sites (Fig. 2). Since theoretically the order of amino acids is freely variable, a protein could be made that would eventually conform to any shape needed. However, cells do not vary their protein structures haphazardly. A very specific machinery for making proteins has evolved: each amino acid is coded in the

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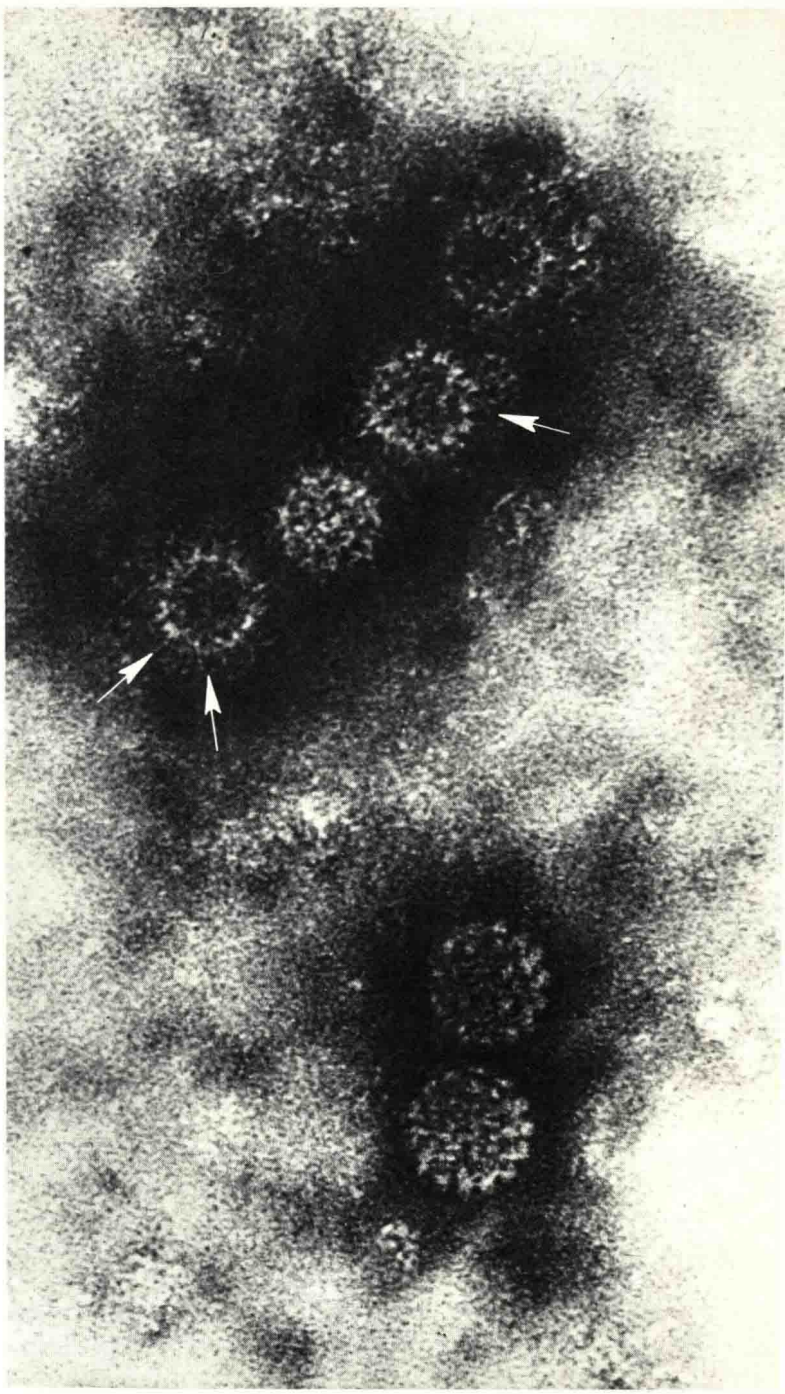

Fig. 1 *The specificity of antibody. Polyoma antiserum was added to a mixture of wart and polyoma virus.* Top: *the smaller, 450Å polyoma virus particles are coated with antibody (arrows).* Bottom: *the larger, 550Å wart virus remains free of antibody (X 450,000).*

genes of our chromosomes by a special three-letter code of DNA (deoxyribonucleic acid). A long array of these three-letter-code words (a gene) spells out the arrangement of the amino acids that will be linked together to form the protein chain. There is a one-to-one correspondence of the three-letter code and the amino acid's position in the protein. In order to make an infinite variety of proteins, an infinite variety of genes in the DNA would be required somewhere in the body to code for all the different protein antibodies we might ever need. This is the genetic dilemma that immunology has provided for so many years. It seemed unlikely that so many genes could be committed solely for the production of antibody molecules—especially when the total number of antigens could not be realistically defined. Current estimates of gene numbers committed to antibody synthesis still vary widely depending on the techniques used.

As if this complexity were not enough, there is an additional heterogeneity in the antibodies made to an antigen. For example, not all of the antibodies made against type A influenza virus have the same structure. If these antibodies are separated from all other antibodies in the blood of just one person and their protein structure determined, dozens of different antibodies will have been made; yet each will combine with influenza type A. This superimposed heterogeneity completely confused all attempts to analyze antibody structure because a purified antibody did not have a single amino acid sequence. Heterogeneity became an insurmountable obstacle until attention was turned to a curious group of proteins made by patients with multiple myeloma. The proteins found in the blood and urine of humans and some strains of mice with multiple myeloma had many similarities to the antibody molecules and one unique advantage for the protein chemists. All of the myeloma protein chain from one patient was homogenous; that is, all the protein chains of the myeloma protein from any one person had the same structure. This homogeneity of the myeloma proteins is reflected in their uniform charge so that they migrate as a single band during electrophoresis—the so-called "M" spot. About fifteen years ago researchers discovered that myeloma proteins consisted of four chains of protein hooked together by bridges between sulfur-containing

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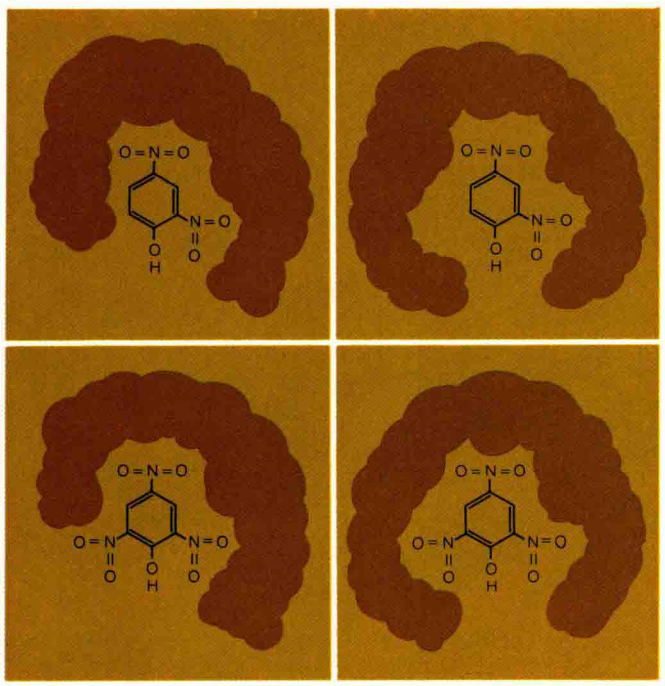

Fig. 2 *Two antibodies to DNP (dinitrophenol).* Upper left: *tightly fitting DNP antibody combining site;* Upper right: *loosely fitting DNP antibody combining site. Lower figures illustrate cross reactions with TNP (trinitrophenol).* Lower left: *nitro groups partially blocks entry into tight anti-DNP;* Lower right: *combining site has sufficient room to accomodate another NO_2 group.*

Fig. 3 *Schematic model of immunoglobulin molecule. This model is composed of two heavy and two light polypeptide chains and has two antigen binding sites (Fab) and a complement fixing site (Fc).*

Fig. 4 *Two disease states with abnormal protein urine excretion.*

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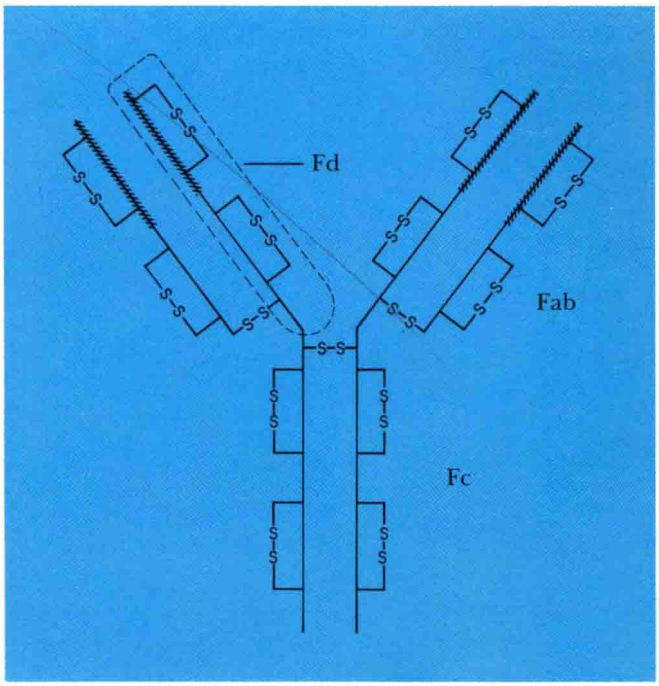

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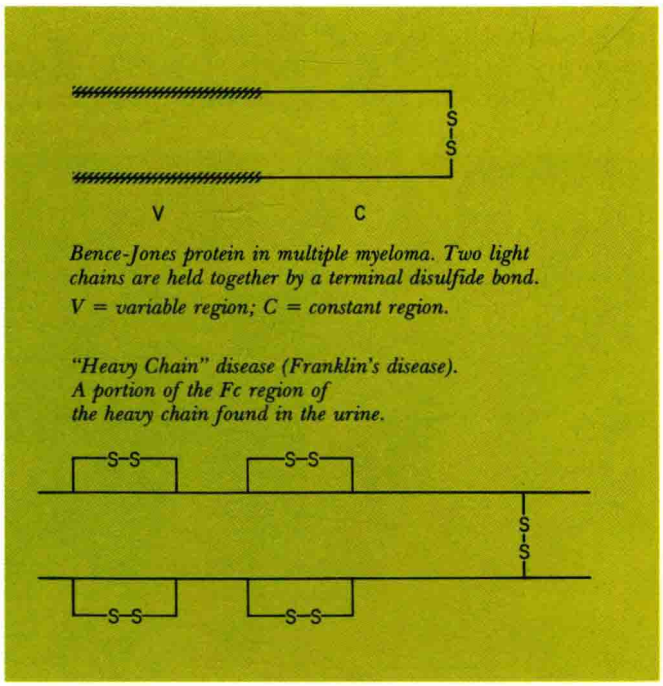

Bence-Jones protein in multiple myeloma. Two light chains are held together by a terminal disulfide bond. V = variable region; C = constant region.

"Heavy Chain" disease (Franklin's disease). A portion of the Fc region of the heavy chain found in the urine.

amino acids (disulfide bonds). A pair of identical smaller chains—light chains—about 200 amino acids long—were linked to a pair of larger chains—heavy chains—about 450 amino acids long (Figs. 3 and 4).

Light and Heavy Antibody Molecule Chains—When the amino acid sequence of one light chain from a given myeloma protein was compared with the sequence of another myeloma protein, an important relationship evolved: namely, one half of the light chain—the half that contains the terminal carboxyl group—seemed to be the same from one light chain to the other; while the other half—the half containing the amino terminal end of the protein—had many different amino acids substituted from one end to the other. Such an arrangement demonstrates not only the high degree of variability but also the orderliness of the variation within this half of the light chain. Similar variation is found in the N-terminal half of the heavy chain.

Most current theories of protein synthesis contain the dogma "one gene, one polypeptide chain." A rigorous application of this dogma to the amino acid sequence data of light chains would require a gene of about 200-triplet code words linked together in a chromosome. One half of the gene—the part coding for the common half of the light chain—would then have to be the same time and again in every gene for a light chain. The other half would of course be different in every light-chain gene. However, as we have seen, the amino acid sequences in the variable half differ only slightly from one light chain to another at each position; these differences could have arisen during evolution by slight changes in the code words for the amino acid—a process called mutation.

Once a lymphoid cell has become committed to the production of an antibody to some unique antigen, it apparently makes that antibody and none other. Thus the lymphoid cell becomes committed to the

production of the products of only one of its light-chain genes and only one of its heavy-chain genes to make a completed immunoglobulin molecule. This process—the differentiation of the antibody-producing cell—is now actively being studied as a model for the differentiation of the other cell types in our bodies.

Antibody-Combining Site—Juxtaposition of the variable regions in the light (V_L) and heavy chains (V_H) as a result of the interaction between common regions of the light (C_L) and heavy (C_H) chains determines the folding of the immunoglobulin molecule and therefore the shape of the cleft that becomes the antibody-combining site. This hypothesis is drawn from data on myeloma proteins; so far, complete structural similarity has been shown, and some myeloma proteins actually exhibit antibody-like activity.

Antibody Functions

Antibody molecules are bivalent; that is, they have two combining sites. Bivalency is critical because it allows the antibody to act as a crosslink between two antigenic groups and clump them together. This clumping—termed agglutination—is thought to play a prominent role in clearing invaders from the bloodstream; it also forms the basis for many useful laboratory tests—such as blood grouping.

Antibody Fragments—The bivalent function of antibodies has been graphically visualized by the electron microscope (Figs. 5a and 5b). Protein digesting enzymes cleave the antibody molecule into three large pieces. Two of the pieces are identical and have the antibody-combining site. These have been termed the antibody-containing fragments, or Fab (Fig. 3). The Fab fragment is composed of the entire light chain and about one half of the heavy chains linked to each other by a crosslink disulfide bond. This half on the heavy chain is called the Fd fragment. The third fragment lacks a combining site, but has many of the functional sites, such as those determining complement fixation, catabolism and placental transport. This third fragment, which also tends to crystallize, has been termed the Fc fragment.

Immunoglobulin Classes

Besides the variation in each antibody molecule that determines its specificity for antigen, additional variation is present in the common region. These very slight

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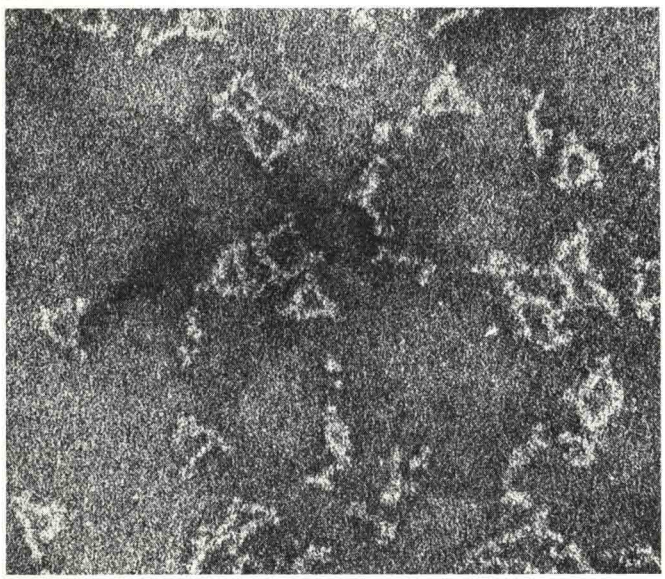


5b

Fig. 5a *Rabbit anti-DNP IgG immunoglobulin saturated with a divalent DNP hapten (bis-N-DIP octamethylenediamine). Many of the antibody molecules are linked together to form wings with regular shapes and projections from each corner (X 500,000).*

Fig. 5b *Antibody–hapten complex of Fig. 5a after treatment with pepsin at pH 4.5 to digest the Fc fragment. Projections at the corners of the regular shapes have been detached and appear as small pieces (X 500,000).*

changes in the Fc region predominantly govern the biological behavior of each immunoglobulin in a general way. The variation in the common region has been clarified and systematized into a formal classification of immunoglobulins, see Figure 6.

IgG(γG)—This class of antibody has been previously known as γ_2-globulin, 7S γ-globulin and complement-fixing γ-globulin. In the human, this is the most common form of antibody molecule, comprising seventy percent of serum immunoglobulin. Its structure will be considered as a prototype for the other forms of immunoglobulin.

The term immunoglobulin has been abbreviated by the symbol Ig, and this class has therefore been termed IgG. Verbally it is more commonly referred to as gamma G (γG). The IgG molecule has the usual four-chain structure of two light chains and two heavy chains. Heavy and light chains may be used as antigens themselves and will induce the formation of antibodies to antigenic determinants—markers—present along the chain. Antibodies to light chains show that they fall into two antigenic groups; one termed kappa (κ), the other termed lambda (λ). The heavy chain has been called the gamma (γ) chain. Thus, the four chains of an IgG molecule can be diagrammatically represented as either $\gamma_2\kappa_2$, or $\gamma_2\lambda_2$. Actually it has become even a bit more complex because there are now at least four antigenically different kinds of γ-chain resulting from small genetic changes within the common region of the γ-chain.

The γ-chain is about 450 amino acids long, the light chain about 212 amino acids long, for a total molecular weight of about 150,000; this makes it one of the smallest immunoglobulins. This is also the reason it

6

Immunoglobulin Classification

Class:	*IgG*	*IgA*	*IgM*	*IgD*	*IgE*
Spoken name (pronounced gamma)	γG	γA	γM	γD	γE
Heavy chain name	γ (at least four gamma subclasses are known)	α (at least two alpha subclasses are known)	μ	δ	ε
Light chain name	λ or κ in all classes. (There are several λ and κ subclasses known.)				
Heavy chain molecular weight	50,000	64,500	70,000	67,000	72,500
Extra chains		J chain and secretory component	J chain		
% Carbohydrate	3%	7%	12%	13%	11%
Structure	$\gamma_2\lambda_2$ $\gamma_2\kappa_2$	$\alpha_2\lambda_2$* $\alpha_2\kappa_2$	$(\mu_2\lambda_2)_5$ $(\mu_2\kappa_2)_5$	$\delta_2\lambda_2$ $\delta_2\kappa_2$	$\epsilon_2\lambda_2$ $\epsilon_2\kappa_2$
Functions	Fixes complement, crosses placenta, 70% of human immunoglobulins, secondary response	Bodily secretions, immune response to pathogens entering by respiratory or gastrointestinal tracts, isohemagglutinins	Early antibody, common antibody to blood group substances, powerful agglutinin and hemolysin	?	Allergic responses sensitizes human mast cells for anaphylaxis
Normal serum concentration (Mg%)	700-1500	150-400	60-170	3.0	0.01-0.03

*IgA in serum tends to polymerize to form dimers and larger molecular weight polymers.

sediments fairly slowly in the ultracentrifuge and has been termed 7S γ-globulin.

In the common region of the γ-chain is a portion of the molecule which is altered after the Fab regions have combined with antigen. This alteration in shape activates a series of serum proteins called the complement system—a complex biological system which facilitates a number of immune reactions is to be described within a later section. These serum proteins assist the antibody molecule in agglutinating or lysing the antigen. The complement-activating site, also called the complement-fixing site, does not function until the antibody molecule has combined with antigen. This subtle change in shape of IgG, called a conformational change, protects against inadvertent activation of the complement components.

Role in Newborn—IgG is the major class of antibody that can cross the placenta from an immunized mother to provide the early forms of antibody protection for the newborn human child. None of the other classes of antibody appears to cross the placenta to any significant degree. If the mother has been previously sensitized to the Rh antigen and the fetus' cells carry that antigen, this form of immunoglobulin can cross the placenta to destroy the red cells of the fetus and cause erythroblastosis fetalis. Paradoxically, purified anti-Rh IgG can be administered to mothers before sensitization by their children's cells and prevent Rh sensitization. This may be a result of feed-back inhibition of immunoglobulin production by passively administered antibody.

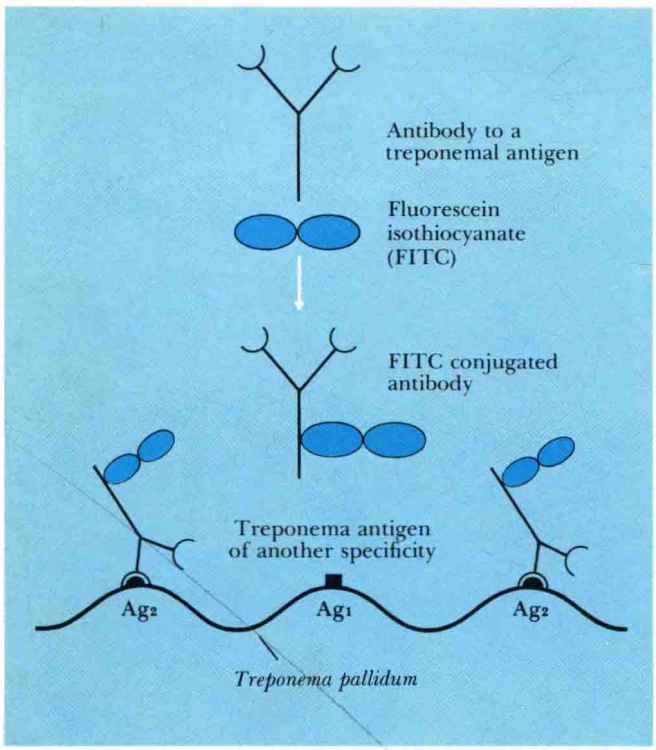
IgA (γA)—This class of antibody, found predominantly in saliva and the secretions of the gastrointestinal tract and respiratory tract, was formerly called β_2A globulin. It is composed of four chains, two light and two heavy. The light chains are also of the κ or λ type; however, the heavy chains are larger than the γ-chains of IgG, and have been termed α-chains, so that the four-chain structure of the IgA molecule can be summarized as either $\alpha_2\kappa_2$ or $\alpha_2\lambda_2$. Like all other immunoglobulins, the IgA molecule is made by cells of the lymphoid series—namely, the lymphocyte and plasma cell; however, the anatomic distribution of centers for greatest production of the IgA proteins lies next to the various lumina of the body—such as the parotid gland, along the gastrointestinal tract in Peyer's patches, and beneath the bronchial mucosa. After the synthesis of the IgA protein, additional proteins are attached (J chain) possibly by an epithelial cell as the IgA molecule is transported across the mucosa and into the lumen. An additional small chain, secretory component (SC) is also attached during this transport. Thus, only a small portion of the IgA antibody response is reflected in the blood.

About 10% of the weight of the IgA molecule is composed of carbohydrate, in contrast to the IgG molecule, which is only 1% or 2% carbohydrate. The role of the carbohydrate in all immunoglobulins is not clear, but is thought to govern their secretion by the cells that produce them. The carbohydrate may also increase the binding of the Fc regions of the heavy chain to the surfaces of some cells. Certain persons have an inability to make this immunoglobulin and yet appear to be in good health. This is a marked contrast to those who have agammaglobulinemia of the IgG type which expresses itself as a severe deficit in immune response.

Mucosal Absorption—The exact function of the IgA molecule is not clear, although it may provide more efficient immune protection against pathogens that invade the host through the respiratory or gastrointestinal tract. Recent exploitation of this postulate has led to the development of influenza vaccines that are administered as a nasal spray, rather than as an intramuscular injection. The immune response to this form of vaccine administration, while expressed very little in the serum as circulating anti-influenza IgG or IgA molecules, is highly effective in protecting against subsequent inoculation of virulent influenza virus through the natural respiratory route. The potential

IgE: The Special Immunoglobulin—Probably the most important immunoglobulin in effecting acute hypersensitivity or allergic reactions is immunoglobulin E (IgE). Its reaction with antigen may result in a chain of acute pharmacologic reactions which are in some way related to the fact that the Fc portion of the IgE molecule has an affinity for surface receptors on mast cells and basophiles. The pharmacological mediators associated with immediate hypersensitivity are: 1) Histamine which may cause capillary vasodilatation, increases in capillary permeability and bronchoconstriction; 2) slow reacting substance (SRS) which can cause prolonged smooth muscle contraction. Urticaria is a common form of local anaphylaxis; while antigen on reaching the circulation and binding with its appropriate antibody may give rise to the more serious generalized anaphylactic reaction characterized by generalized bronchoconstriction and hypotension. The rationale for specific immunotherapy in diseases of acute allergy is the prevention of antigen fixation on immunoglobulin E and the subsequent release of the various pharmacologic mediators of acute allergy. This may be partly accomplished by production of "blocking" antibodies of the IgG class with combining specificity comparable to the allergenic IgE. Competition by these relatively harmless antibodies for antigen eliminates it before IgE sites on sensitized mast cells are encountered.

7a



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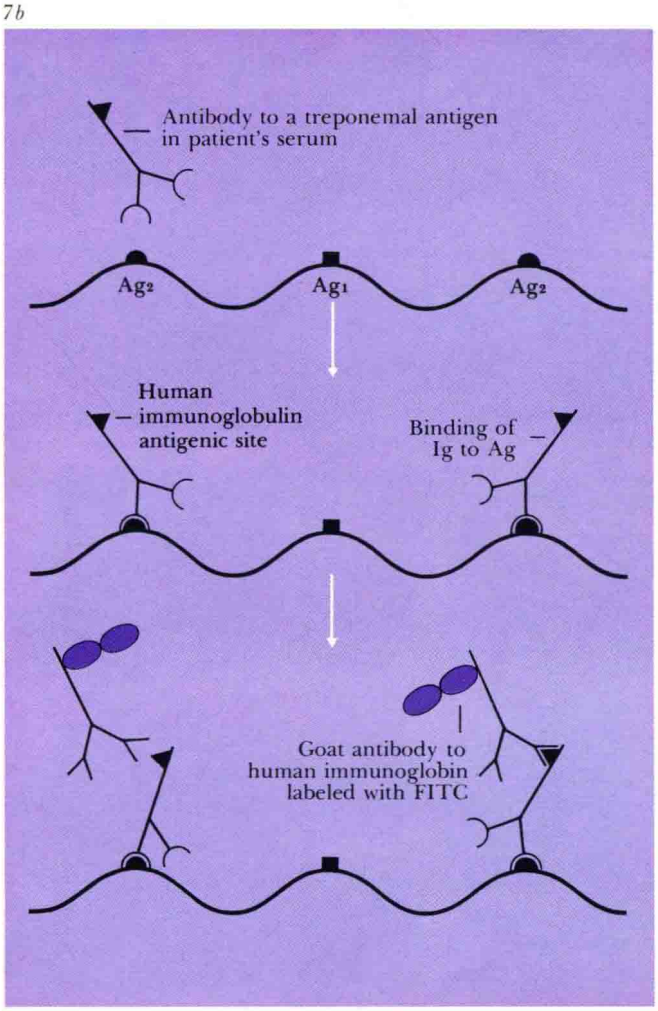

for such immunization through the nasal passages, though just beginning, may offer an important mode of immunotherapy. The effectiveness of the oral polio vaccines may also result partially from this form of immune response.

IgM(γM)—Largest of the immunoglobulins (molecular weight = about 900,000), IgM has the basic four-chain structure of two light and two heavy chains. The heavy chain in this case in termed the μ-chain. Thus, the basic structure can be summarized $\mu_2\kappa_2$, or $\mu_2\lambda_2$. It now appears that five of these four-chain units are joined together in a large molecule composed of 20 chains, held together by additional disulfide bonds near the Fc end of the μ-chain. The μ-chain is the largest of all the immunoglobulin's heavy chains, having a molecular weight of about 70,000 in contrast to a γ-chain with a molecular weight of about 50,000. About 10% of the μ-chain's weight is carbohydrate, resembling IgA in this respect. The comparative relationships of chain structure, chemical composition and size are summarized in Figure 6. Because of their large size, IgM molecules sediment rapidly in the ultracentrifuge and have been called 19S macroglobulins. Some IgM antibodies fix complement, but most do not participate in the classic allergic reactions.

IgM antibodies are the first to be formed after immunization, and the level of IgM antibody usually falls rapidly after the onset of IgG antibody synthesis. The molecule tends to stay within the vascular space, crossing the capillary with difficulty but not crossing the placenta to any significant degree. The IgM molecule appears to be from 700 to 1,000 times as efficient as IgG in agglutinating a red cell or bacterium—probably because of the many combining sites present on one IgM molecule.

When a myeloma affects the cells making IgM molecules (Waldenstrom's disease) the concentration of this large protein in the blood can rise to extraordinary levels and increase the blood viscosity so that spontaneous microthrombi and hemorrhages occur. This unusual complication can be temporarily relieved by plasmapheresis—removal of a unit of blood, separations of the cells from the plasma and return of the cells to the patient—thus lowering the IgM level until the abnormal synthesis again restores the protein.

Relationship to ABO Blood Groups—For some reason carbohydrate antigens—such as the A or B group substances—elicit the prolonged production of IgM antibodies rather than the usual switchover to the IgG

Fig. 7a *Direct fluorescent antibody technique. After labeling a specific antibody with FITC, it can be reacted with its antigen and can be identified microscopically.*

Fig. 7b *Indirect fluorescent antibody technique. Serum containing antibody is reacted with the specimen. Bound antibody is then detected with FITC labeled goat anti-human Ig.*