(field Nos. 88 and 104). They are part of the collection of Pompeian flora that I have deposited in the U.S. National Arboretum herbarium.

19. The Romans knew both the white poplar (*Populus alba* L.) and the black poplar (*Populus nigra* L.). I collected *Populus nigra* L. (field No. 87).
20. The individual coins are identified elsewhere (*3*, p. 69).
21. Trees were also found in the informally planted vineyard in the garden restaurant of Euxinus (region I, insula 11, entrance 10), which I excavated in 1964 [W. F. Jashemski, *Archaeology* **20**, 36 (1967)].
22. See L. Wittmack, *Beibl. Bot. Jahrb. No. 73* (1903), p. 38 for a study of the carbonized fruits and nuts that had been found in the excavations up to that time.
23. A study of all the plants known from the wall paintings, sculpture, mosaics, carbonized remains, and graffiti in the area destroyed by Vesuvius will be included in an appendix of a book on the gardens of the Pompeii area (W. F. Jashemski, in preparation; F. G. Meyer is collaborating on the appendix).
24. Pliny, *Naturalis historia*, book 17, sects. 164–166, 199–200.
25. See the report of the 1970 season (*4*) for a description and criticism of previous explanations of the vitis compluviata.
26. Varro, *De re rustica*, book 1, chap. 8, sect. 2.
27. Columella, *De re rustica*, book 4, chap. 17, sects. 3–6.
28. *Notizie degli scavi* (1923), p. 273.
29. *Corpus inscriptionum Latinarum*, vol. 4, inscriptions 6886–6887.
30. Pliny, *Naturalis historia*, book 18, sect. 110.
31. For a discussion of the "dry farming" techniques practiced in the Mediterranean area, see C. E. Stevens, in *The Cambridge Economic History of Europe*, M. M. Postan, Ed. (Cambridge Univ. Press, Cambridge, England, ed. 2, 1969), vol. 1, pp. 96–104.
32. For a description of this press, see A. Maiuri, *La Villa dei Misteri* (Istituto Poligrafico dello Stato, Rome, ed. 3, 1960), pp. 41–44.
33. For the marked effect of temperature on wine production, when expressed as heat summation ("sum of the mean daily temperature above 50° F" from April through October), see A. J. Winkler, *General Viticulture* (Univ. of California Press, Berkeley and Los Angeles, 1962), p. 58: "The base line is set at 50° [Fahrenheit] because there is almost no shoot growth below this temperature." The summation is expressed as degree-days. Naples has "4010 degree-days above 50° F" (p. 61, table 3), which would place it in climatic region 5 of Amerine's and Winkler's classification scheme of five climatic regions for wine production. Region 5 includes locations that have 4001 or more degree-days. Pompeii would be in the same region as Naples.
34. Martial, *Epigrams*, book 5, sect. 70, l. 3.
35. *Notizie degli scavi* (1900), p. 31.
36. *Corpus inscriptionem Latinarum*, vol. 10, inscription 1074; F. Mazois, *Les ruines de Pompéi* (Firmin-Didot, Paris, 1812), vol. 1; pp. 47, 51; pl. 30, fig. 1; pl. 31, fig. 3; pl. 32, fig. 3.
37. Pliny, *Naturalis historia*, book 8, sect. 210; Juvenal, *Satires*, book 1, ll. 140–141.
38. Pliny (*Naturalis historia*, book 28, sect. 265) gives horse flesh thoroughly boiled and taken in drink as a specific for the diseases of pigs.
39. The discovery that this large insula was planted makes me believe that other large open areas in the city may also have been planted and not used for commercial or other purposes. Several important areas were excavated in 1972, and in each I was able to recover the planting pattern and determine land use.
40. The excavations were conducted with the permission and generous cooperation of Professor Alfonso de Franciscis, superintendent of antiquities in Campania. Nicola Sicignano was foreman. I am also grateful to John R. McGrew, research plant pathologist, U.S. Department of Agriculture, Plant Science Research Division, Beltsville, Md., for invaluable counsel regarding the technical aspects of viticulture; to Frederick G. Meyer, research botanist in charge of the U.S. National Arboretum, for his generous help in identifying carbonized specimens and comparing them with contemporary specimens; and to Henry Setzer, mammalogist in charge of the African section at the Smithsonian Institution, through whose kindness the bones found in our excavations were examined and identified. All photographs, drawings, and statistical studies were made by Stanley A. Jashemski.

Antibody Structure and Molecular Immunology

Gerald M. Edelman

Some sciences are exciting because of their generality and some because of their predictive power. Immunology is particularly exciting, however, because it provokes unusual ideas, some of which are not easily come upon through other fields of study. Indeed, many immunologists believe that for this reason, immunology will have a great impact on other branches of biology and medicine. On an occasion such as this in which a very great honor is being bestowed, I feel all the more privileged to be able to talk about some of the fundamental ideas in immunology and particularly about their relationship to the structure of antibodies.

Work on the structure of antibodies has allied immunology to molecular biology in much the same way as previous work on hapten antigens allied immunology to chemistry. This structural work can be considered the first of the projects of molecular immunology, the task of which is to interpret the properties of the immune system in terms of molecular structures. In this lecture, I should like to discuss some of the implications of the structural analysis of antibodies. Rather than review the subject, which has been amply done (*1–3*), I shall emphasize several ideas that have emerged from the structural approach. Within the context of these ideas, I shall then consider the related but less well explored subject of antibodies on the surfaces of lymphoid cells, and describe some recently developed experimental efforts of my colleagues and myself to understand the molecular mechanisms by which the binding of antigens induces clonal proliferation of these cells.

Antibodies occupy a central place in the science of immunology for an obvious reason: they are the protein molecules responsible for the recognition of foreign molecules or antigens. It is, therefore, perhaps not a very penetrating insight to suppose that a study of their structure would be valuable to an understanding of immunity. But what has emerged from that study has resulted in both surprises and conceptual reformulations.

These reformulations provided a molecular basis for the selective theories of immunity first expounded by Niels Jerne (*4*) and MacFarlane Burnet (*5*) and therefore helped to bring about a virtual revolution of immunological thought. The fundamental idea of these theories is now the central dogma of modern immunology: molecular recognition of antigens occurs by selection among clones of cells already committed to producing the appropriate antibodies, each of different specificity (Fig. 1).

The results of many studies by cellular immunologists (*1*) strongly suggest that each cell makes antibodies of only one kind, that stimulation of cell division and antibody synthesis occurs after interaction of an antigen with receptor antibodies at the cell surface, and that the specificity of these antibodies is the same as that of the antibodies produced by daughter cells.

 The author is professor of biochemistry at Rockefeller University, New York 10021. This article is the lecture he delivered in Stockholm, Sweden, on 11 December 1972 when he received the Nobel Prize in Physiology or Medicine, a prize he shared with Professor Rodney R. Porter. It is published here with the permission of the Nobel Foundation and will also be included in the complete volume of *Les Prix Nobel en 1972* as well as in the series Nobel Lectures (in English) published by the Elsevier Publishing Company, Amsterdam and New York. Professor Porter's lecture appeared in the 18 May 1973 issue, page 713.

Several fundamental questions are raised by these conclusions and by the theory of clonal selection. How can a sufficient diversity of antibodies be synthesized by the lymphoid system? What is the mechanism by which the lymphocyte is stimulated after interaction with an antigen?

In the late 1950's, at the beginning of the intensive work on antibody structure, these questions were not so well defined. The classic work of Landsteiner on hapten antigens (*6*) had provided strong evidence that immunological specificity resulted from molecular complementarity between the determinant groups of the antigen molecule and the antigen-combining site of the antibody molecule. In addition, there was good evidence that most antibodies were bivalent (*7*) as well as some indication that antibodies of different classes existed (*8*). The physicochemical studies of Tiselius (*9*) had established that antibodies were proteins that were extraordinarily heterogeneous in charge. Moreover, a number of workers had shown the existence of heterogeneity in the binding constants of antibodies capable of binding a single hapten antigen (*10*). Despite the value of all of this information, however, little was known of the detailed chemical structure of antibodies or of what are now called the immunoglobulins.

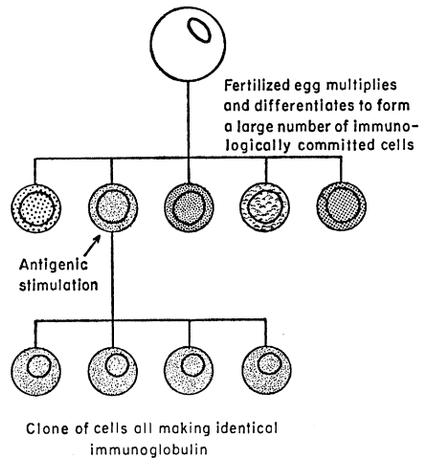

Fig. 1. A diagram illustrating the basic features of the clonal selection theory. The stippling and shading indicate that different cells have antibody receptors of different specificities, although the specificity of all receptors on a given cell is the same. Stimulation by an antigen results in clonal expansion (maturation, mitosis, and antibody production) of those cells having receptors complementary to the antigen.

Multichain Structure of Antibodies: Problems of Size and Heterogeneity

If the need for a structural analysis of antibodies was great, so were the experimental difficulties. Antibodies are very large proteins (molecular weight of 150,000 or greater), and they are extraordinarily heterogeneous. Two means were adopted around 1958 in an effort to avoid the first difficulty. Following the work of Petermann (*11*) and others, Rodney Porter (*12*) applied proteolytic enzymes, notably papain, to achieve a limited cleavage of the gamma globulin fraction of serum into fragments. He then successfully fractionated the digest, obtaining antigen-binding (Fab) and crystallizable (Fc) fragments. Subsequently, other enzymes such as pepsin were used in a similar fashion by Nisonoff *et al.* (*13*). I took another approach, in an attempt to cleave molecules of immunoglobulin G and immunoglobulin M into polypeptide chains by reduction of their disulfide bonds and exposure to dissociating solvents such as 6*M* urea (*14*). This procedure resulted in a significant drop in molecular weight, demonstrating that the immunoglobulin G molecule was a multichain structure rather than a single chain as had been believed before. Moreover, the chains obtained from both immunoglobulins had about the same size. The polypeptide chains (*15*) were of two kinds (now called light and heavy chains) but were obviously not the same as the fragments obtained by proteolytic cleavage, and therefore the results of the two cleavage procedures complemented each other. Ultracentrifugal analyses indicated that one of the polypeptide chains had a molecular weight in the vicinity of 20,000, a reasonable size for determination of the amino acid sequence by the methods available in the early 1960's.

Nevertheless, the main obstruction to a direct analysis of antibody structure was the chemical heterogeneity of antibodies and their antigen-binding fragments. Two challenging questions confronted those attempting chemical analyses of antibody molecules at that time. First, did the observed heterogeneity of antibodies reside only in the conformation of their polypeptide chains, as was then widely assumed, or did this heterogeneity reflect differences in the primary structures of these chains, as required implicitly by the clonal selection theory? Second, if the heterogeneity did imply a large population of molecules with different primary structures, how could one obtain the homogenous material needed for carrying out a detailed structural analysis?

These challenges were met simultaneously by taking advantage of an accident of nature rather than by direct physicochemical assault. It had been known that tumors of lymphoid cells called myelomas produced homogeneous serum proteins that resembled the normal heterogeneous immunoglobulins. In 1961, M. D. Poulik and I showed that the homogeneity of these proteins was reflected in the starch-gel electrophoretic patterns of their dissociated chains (*15*). Some patients with multiple myeloma excrete urinary proteins that are antigenically related to immunoglobulins but whose nature had remained obscure since their first description by Henry Bence Jones in 1847. These Bence Jones proteins were most interesting, for they could be readily obtained from the urine in large quantities, were homogeneous, and had low molecular weights. It seemed reasonable to suggest (*15*) that Bence Jones proteins represented one of the chains of the immunoglobulin molecule that was synthesized by the myeloma tumor but not incorporated into the homogeneous myeloma protein and was therefore excreted into the urine.

This hypothesis was corroborated one exciting afternoon when my student Joseph Gally and I (*16*) heated solutions of light chains isolated from our own serum immunoglobulins in the classical test for Bence Jones proteinuria. They behaved as Bence Jones proteins, the solution first becoming turbid, then clearing upon further heating. A comparison of light chains of myeloma proteins with Bence Jones proteins by starch-gel electrophoresis in urea (*16*) and by peptide mapping (*17*) confirmed the hypothesis (Fig. 2). Indeed, Berggård and I later found (*18*) that in normal urine there were counterparts to Bence Jones proteins that shared their properties but were chemically heterogeneous.

No physical means was known at the time that was capable of fractionating antibodies to yield homogenous proteins. It was possible, however, to prepare specifically reactive antibodies by using the antigen to form antigen-antibody aggregates and then dissociating the complex with free hapten. Although we knew that these specifically prepared antibodies were still heterogeneous in their electrophoretic proper-

Table 1. Human immunoglobulin (Ig) classes.

Class	Physiological properties	Heavy chain*	Light chain	Molecular formula†	Molecular weight ($\times 10^{-3}$) and sedimentation constant	Carbohydrate content (%)
IgG	Complement fixation; placental transfer	γ	κ or λ	$(\gamma_2\kappa_2)$ or $(\gamma_2\lambda_2)$	143–149; 6.7*S*	2.5
IgA	Localized protection in external secretions	α	κ or λ	$(\alpha_2\kappa_2)$ or $(\alpha_2\lambda_2)$	158–162; 6.8*S*–11.4*S*	5–10
IgM	Complement fixation; early immune response	μ	κ or λ	$(\mu_2\kappa_2)_5$ or $(\mu_2\lambda_2)_5$	800–950; 19.0*S*	5–10
IgD	Unknown	δ	κ or λ	$(\delta_2\kappa_2)$ or $(\delta_2\lambda_2)$	175–180; 6.6*S*	10
IgE	Reagin activity; mast cell fixation	ϵ	κ or λ	$(\epsilon_2\kappa_2)$ or $(\epsilon_2\lambda_2)$	185–190; 8.0*S*	12

* The class distinctive features of these chains are in their constant regions. † IgA can have additional unrelated chains called SC and J; J chains are also found in IgM.

ties, it seemed possible that antibodies to different haptens might show differences in their polypeptide chains. Baruj Benacerraf had prepared a collection of these antibodies, and together with our colleagues (*19*) we decided to compare their chains, using the same methods that we had used for Bence Jones proteins. The results were striking: purified antibodies showed from three to five sharp bands in the Bence Jones or light chain region and antibodies of different specificities showed different patterns. In sharp contrast, normal immunoglobulin showed a diffuse zone extending over the entire range of mobilities of these bands. These experiments showed not only that antibodies of different specificities were structurally different but also that their heterogeneity was limited.

The results of the experiments on Bence Jones proteins and purified antibodies had a number of significant implications. Because different Bence Jones proteins had different amino acid compositions, it was clear that immunoglobulins must vary in their primary structures. This deduction, confirmed later by Koshland (*20*) for specifically purified antibodies, lent strong support to selective theories of antibody formation. Moreover, it opened the possibility of beginning a direct analysis of the primary structure of an immunoglobulin molecule; for not only were the Bence Jones proteins composed of homogeneous light chains, but their subunit molecular weight was only 23,000. The first report by Hilschmann and Craig (*21*) on partial sequences of several different Bence Jones proteins indicated that the structural heterogeneity of the light chains was confined to the amino-terminal (variable) region, whereas the carboxyl-terminal half of the chain (the constant region) was the same in all chains of the same type. This finding was soon extended by studies of other Bence Jones proteins (*22*).

Although some work had also been done on the heavy chains of immunoglobulins, there was much less information on their structure. For instance, it was suspected but not known that they also had variable regions resembling those of light chains. Comparisons of heavy chains and light chains even at this early stage did, however, clarify the nature of another source of antibody heterogeneity, the existence of immunoglobulin classes (*23*).

Antibodies within a particular class have similar molecular weight, carbohydrate content, amino acid composition, and physiological functions (Table 1) but still are heterogeneous in net charge and antigen-binding affinity. Studies of classes in various animal species indicated that both the multichain structure and the heterogeneity are ubiquitous properties of immunoglobulins. The different classes apparently emerged during evolution (*24*) to carry out various physiologically important activities that have been named effector functions in order to distinguish them from the antigen-binding or recognition function. The various manifestations of humoral immune responses as well as their prophylactic, therapeutic, and pathological consequences can now be generally explained in terms of the properties of the particular class of antibody mediating that response. As a result of comparing their chain structure, it became clear that although immunoglobulins of all classes contain similar kinds of light chains (Table 1), the distinctive class character (*23*) is

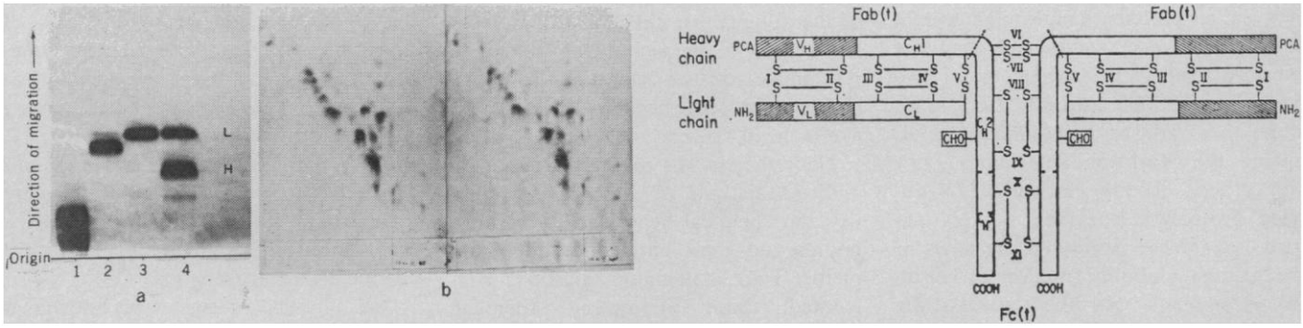

Fig. 2 (left). Comparisons of light chains isolated from serum immunoglobulin G myeloma proteins with urinary Bence Jones proteins from the same patient. (a) Starch-gel electrophoretic patterns in urea are shown for (1) serum myeloma globulin, (2) urinary Bence Jones protein, (3) Bence Jones protein reduced and alkylated, and (4) myeloma protein reduced and alkylated; L, light chain; H, heavy chain. (b) Tryptic hydrolyzates were analyzed by two-dimensional high-voltage electrophoresis. Pattern on left is urinary Bence Jones protein; that on right is of light chain isolated from the serum myeloma protein of the same patient. Fig. 3 (right). Overall arrangement of chains and disulfide bonds of the human γG_1 immunoglobulin, Eu. Half-cystinyl residues are numbered I to XI; I to V designate corresponding half-cystinyl residues in light and heavy chains; PCA, pyrrolidonecarboxylic acid; CHO, carbohydrate. Fab(t) and Fc(t) refer to fragments produced by trypsin, which cleaves the heavy chain as indicated by dashed lines above half-cystinyl residues VI. Variable regions, V_H and V_L, are homologous. The constant region of the heavy chain (C_H) is divided into three regions, C_H1, C_H2, and C_H3, that are homologous to each other and to the C region of the light chain. The variable regions carry out antigen-binding functions and the constant regions the effector function of the molecule.

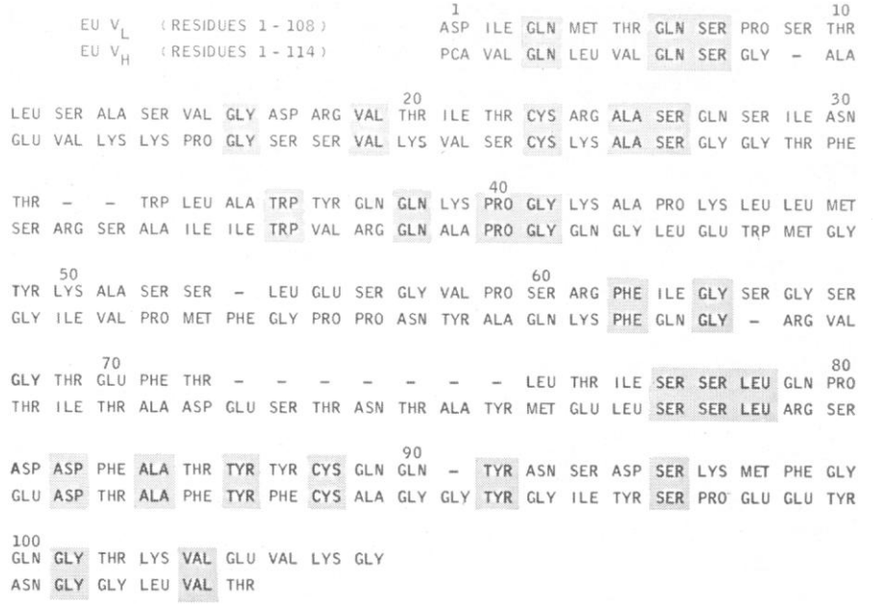

Fig. 4. Comparison of the amino acid sequences of the V_H and V_L regions of protein Eu. Identical residues are shaded. Deletions indicated by dashes are introduced to maximize the homology.

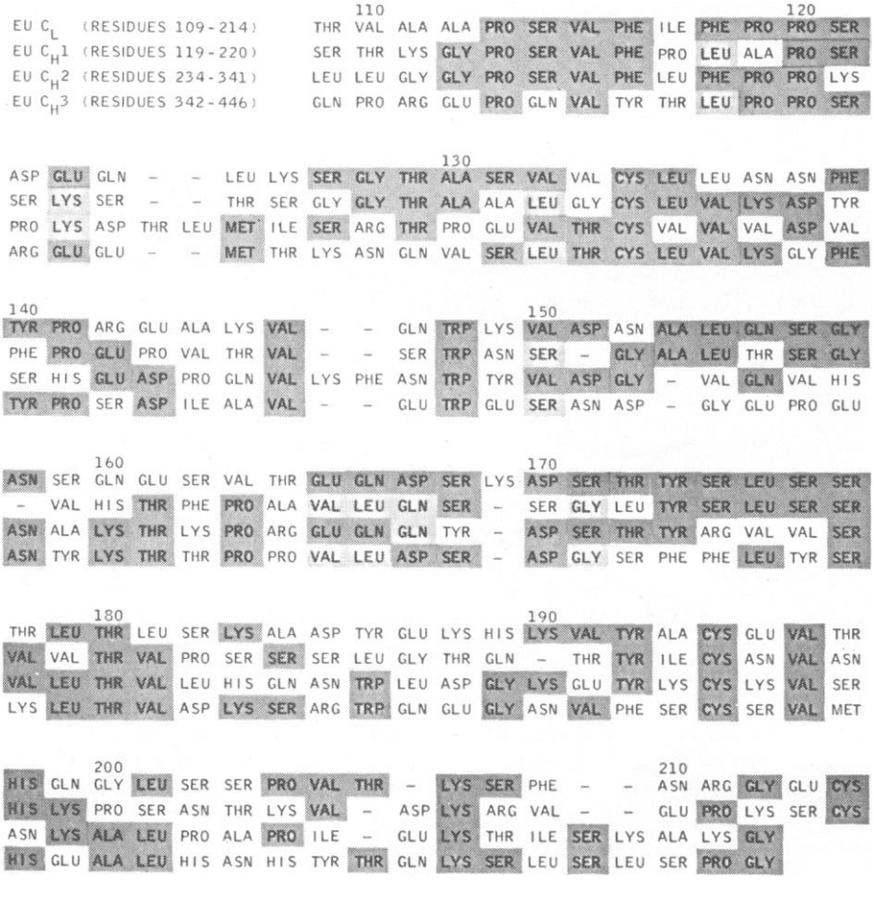

Fig. 5. Comparison of the amino acid sequences of C_L, C_H1, C_H2, and C_H3 regions. Deletions, indicated by dashes, have been introduced to maximize homologies. Identical residues are darkly shaded; both light and dark shadings are used to indicate identities which occur in pairs in the same position.

conferred by structural differences in the heavy chains, specifically in their constant regions, as I shall discuss later.

With the clarification of the nature of the heterogeneity of immunoglobulin chains and classes, attention could be turned to the problem of relating the structure and evolution of antibodies within a given class to their antigen-binding and effector functions. We chose to concentrate on immunoglobulin G, because this was the most prevalent class in mammals and the work on chain structure suggested that it would be sufficiently representative.

The Complete Covalent Structure and the Domain Hypothesis

An understanding of the chain structure and its relation to the proteolytic fragments (*25*) made feasible an attempt to determine the complete structure of an immunoglobulin G molecule. My colleagues and I started this project in 1965, and before it was completed in 1969 (*26*) seven of us had spent a good portion of our waking hours on the technical details. One of our main objectives was to provide a complete and definitive reference structure against which partial structures of other immunoglobulins could be compared. In particular, we wished to compare the detailed structure of a heavy chain and a light chain from the same molecule.

Another objective was to examine in detail the regional differentiation of the structure that had been evolved to carry out different physiological functions in the immune response. The work of Porter (*12*) had shown that the Fab fragment of immunoglobulin G was univalent and bound antigens whereas the Fc fragment did not. This provided an early hint that immunoglobulin molecules were organized into separate regions, each mediating different functions. In accord with selective theories of immunity, it was logical to suppose that variable regions from both the light and the heavy chains mediated the antigen-binding functions. Early evidence that some of the constant regions were concerned with physiologically significant effector functions was obtained by showing that Fc fragments would bind components of the complement system (*27*), a complex group of proteins responsible for immunologically induced cell lysis. A more detailed assignment of structure to function required a knowledge of the total structure.

Amino acid sequence analysis of the Fc region of normal rabbit γ chains (Table 1) by Hill *et al.* (*28*) demonstrated that the carboxyl-terminal portion of heavy chains was homogeneous. On the basis of internal homologies in this region, Hill *et al.* (*28*) and Singer and Doolittle (*29*) proposed the hypothesis that the genes for immunoglobulin chains evolved by duplication of a gene of sufficient size to specify a precursor protein of about 100 amino acids in length. Although direct confirmation of this hypothesis is obviously not possible, it was strongly supported by the results of our analysis (*26*) of the complete amino acid sequence and arrangement of the disulfide bonds of an entire immunoglobulin G myeloma protein.

Comparisons of the amino acid sequences of the heavy chain of this protein with others studied in Porter's laboratory (*30*) and by Bruce Cunningham and his colleagues in our laboratory (*31*) showed that heavy chains had variable (V_H) regions, that is, regions that differed from one another in the sequence of the 110 to 120 residues beginning with the amino terminus (Fig. 3).

Examination of the amino acid sequences (Figs. 4 and 5) allowed us to draw the following additional conclusions:

1) The variable (V) regions of light and heavy chains are homologous to each other, but they are not obviously homologous to the constant regions of these chains. Variable regions from the same molecule appear to be no more closely related than V regions from different molecules.

2) The constant (C) region of γ chains consists of three homology regions, C_H1, C_H2, and C_H3, each of which is closely homologous to the others and to the constant regions of the light chains.

3) Each V region and each C homology region contains one disulfide bond, with the result that the intrachain disulfide bonds are linearly and periodically distributed in the structure.

4) The region containing all of the interchain disulfide bonds is at the center of the linear sequence of the heavy chain and has no homologous counterpart in other portions of the heavy or light chains.

These conclusions prompted us to suggest that the molecule is folded in a congeries of compact domains (*26, 31*), each formed by separate V homology regions or C homology regions

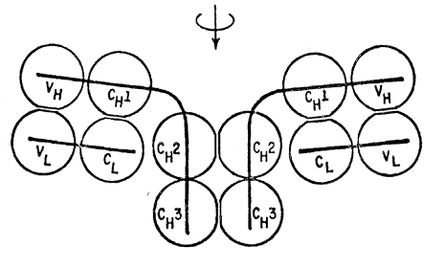

Fig. 6. The domain hypothesis. Diagrammatic arrangement of domains in the free immunoglobulin G molecule is shown. The arrow refers to a dyad axis of symmetry. Homology regions (Figs. 3 to 5) that constitute each domain are indicated: V_L and V_H are domains made up of variable homology regions; C_L, C_H1, C_H2, and C_H3 are domains made up of constant homology regions. Within each of these groups, domains are assumed to have similar three-dimensional structures, and each is assumed to contribute to an active site. The V domain sites contribute to antigen recognition functions and the C domain sites to effector functions.

(Fig. 6). In such an arrangement, each domain is stabilized by a single intrachain disulfide bond and is linked to neighboring domains by less tightly folded stretches of the polypeptide chains. A twofold pseudosymmetry axis relates the V_LC_L to the V_HC_H1 domains, and a true dyad axis through the disulfide bonds connecting the heavy chains relates the C_H2 and C_H3 domains. The tertiary structure within each of the homologous domains is assumed to be quite similar. Moreover, each domain is assumed to contribute to at least one active site mediating a function of the immunoglobulin molecule.

The last supposition is nicely demonstrated by the interaction of V region domains. The reconstitution of active antibody molecules by recombining their isolated heavy and light chains (*32*) as well as affinity-labeling experiments (*29*) confirmed our early hypothesis that the V regions of both heavy and light chains contributed to the antigen-combining sites. Moreover, the experiments of Haber (*33*) provided the first indication that Fab fragments of specific antibodies could be unfolded after reduction of their disulfide bonds and refolded in the absence of antigen to regain most of their antigen-binding activity. This clearly indicated that the information for the combining site was contained entirely in the amino acid sequences of the chains. That this information is contained completely in the variable regions is strikingly shown by the recent isolation of antigen-binding fragments consisting only of V_L and V_H (*34*). The chain recombination experiments suggested a hypothesis to account in part for antibody diversity: The various combinations of different heavy and light chains expressed in different lymphocytes allow the formation of a large number of different antigen-combining sites from a relatively small number of V regions.

One of the remaining structural tasks of molecular immunology is to obtain a direct picture of antigen-binding sites by x-ray crystallography of V domains at atomic resolution. Although crystals of the appropriate molecule or fragment yielding diffraction patterns that extend beyond Bragg spacings of 3.0 Å have not yet been obtained, it is likely that continued searching will provide them. The details of a particular antigen-antibody interaction revealed by such a study will be of enormous interest. For example, certain sequence positions of V regions are hypervariable (*35*) and are good candidates for direct contribution to the site. It will be particularly important to understand how the basic three-dimensional structure can accommodate so many amino acid substitutions. X-ray crystallographic work may also show in detail how the disulfide bonds in each of the V domains provide essential stability to the site (*26, 31, 36*).

The proposed similarities in tertiary structures among C domains have not been established, nor have the functions of the various C domains been fully determined. There is a suggestion that C_H2 may play a role in complement fixation (*37*). A good candidate for binding to the lymphocyte cell membrane is C_H3, the function of which may be concerned with the mechanism of lymphocyte-triggering after antigen is bound by V domains. The C_H3 domain has already been shown to bind to macrophage membranes (*38*), and there is now some evidence that lymphocytes can synthesize isolated domains (*39*) similar to C_H3 as separate molecules.

Although many details are still lacking, the gross structural aspects of the domain hypothesis have received direct support from x-ray crystallographic analyses of Fab fragments (*40*) and whole molecules (*41*), in which separate domains were clearly discerned. Indirect support for the hypothesis has also come from experiments on proteolytic cleavage of regions between domains (*34, 42*).

It is not completely obvious why the domain structure was so strictly preserved during evolution. One reasonable

hypothesis is that although there was a functional need for association of V and C domains in the same molecule, there was also a need to prevent allosteric interactions among the domains. Whatever the selective advantages of this arrangement, it is clear that immunoglobulin evolution by gene duplica- permitted the possibility of modular alteration of immunological function by addition or deletion of domains.

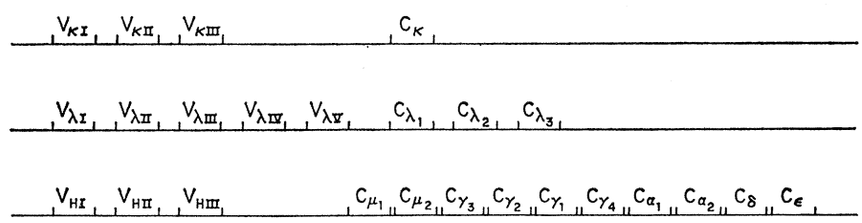

Fig. 7. A diagrammatic representation of the proposed arrangement in mammalian germ cells of antibody genes in three unlinked clusters termed translocons. Light chains κ and λ are each specified by different translocons, and heavy chains are specified by a third translocon. The exact number and arrangement of V and C genes within a translocon is not known. Each variable region subgroup (designated by a subscript corresponding to chain group and subgroup) must be coded by at least one separate germ-line V gene. The number of V genes within each subgroup is unknown, however, as is the origin of intrasubgroup diversity of V regions. A special event is required to link the information from a particular V gene to that of a given C gene. The properties of the classes and subclasses (Table 1) are conferred on the constant regions by C. genes.

Translocons: Proposed Units of Evolution and Genetic Function

The evolution by gene duplication of both the domain structure and the immunoglobulin classes raises several questions about the number and arrangement of the structural genes specifying immunoglobulins. Although time does not permit me to discuss this complex subject in detail, I should like to suggest how structural work has sharpened these questions.

According to the theory of clonal selection, it is necessary that there preexist in each individual a large number of different antibodies with the capacity to bind different antigens. One of the most satisfying conclusions that emerged from structural analysis is that the diversity of the V regions of antibody chains is sufficient to satisfy this requirement. This diversity arises at three levels of structural or genetic organization, two of which are now reasonably well understood:

1) Variable regions from both heavy and light chains contribute to the antigen-binding site, and therefore the number of possible antibodies may be as great as the product of the number of different V_L and V_H regions.

2) Analyses of the amino acid sequences of V regions of light chains by Hood *et al.* (*43*) and Milstein (*44*) and later of heavy chains from myeloma proteins (*30*, *31*) indicated that V regions fall into subgroups of sequences, which must be specified by separate genes or groups of genes. Within a subgroup, the amino acid replacements at a particular position are of a conservative type consistent with single base changes in codons of the structural genes. Variable regions of different subgroups differ much more from each other than do variable regions within a subgroup.

Although different V region subgroups are specified by a number of nonallelic genes (*44*), the analysis of genetic or allotypic markers suggests that C regions of a given immunoglobulin class are specified by no more than one or two genes. These allotypic markers, first described by Grubb (*45*) and Oudin (*46*), provide a means in addition to sequence analysis for understanding the genetic basis of immunoglobulin synthesis (*3*). Variable regions specified by a number of different genes can occur in chains each of which may have the same C region specified by a single gene. It therefore appears that each immunoglobulin chain is specified by two genes, a V gene and a C gene (*3*, *43*, *44*).

Work in a number of laboratories [reviewed in (*3*)] has shown that the genetic markers on the two types of light chains are not linked to those of the heavy chains or to each other. These findings and the conclusion that there are separate V and C genes led Gally and me to suggest (*3*) that immunoglobulins are specified by three unlinked gene clusters (Fig. 7). The clusters have been named translocons (*3*) to emphasize the fact that some mechanism must be provided to combine genetic information from V region loci with information from C region loci to make complete V-C structural genes. According to this hypothesis, the translocon is the basic unit of immunoglobulin evolution, different groups of immunoglobulin chains having arisen by duplication and various chromosomal rearrangements of a precursor gene cluster. Presumably, gene duplication during evolution also led to the appearance of V region subgroups within each translocon.

The key problem of the generation of immunoglobulin diversity has been converted by the work on chains and subgroups to the problem of the origin of sequence variations within each V region subgroup. It is still not known whether there is a germ-line gene for each V region within a subgroup, or whether each subgroup contains only a few genes (Fig. 7) and intrasubgroup variation arises by somatic genetic rearrangements of translocons within precursors of antibody-forming cells. At this time, therefore, we can conclude that only the basis but not the origin of diversity has been adequately explained by the work on structure. Although structural analysis of various immunoglobulin classes will continue to be important, it does not in itself seem likely to lead to an explanation of the origin of antibody diversity. What will probably be required are imaginative experiments on DNA, RNA, and their associated enzymes obtained from lymphoid cells at the proper stage of development.

In this abbreviated and necessarily incomplete account, I have attempted to show how structural work on immunoglobulins has provided a molecular basis for a number of central features of the theory of clonal selection. The work on humoral antibodies is just a beginning, however, for two great problems of molecular and cellular immunology remain to be solved. The first problem, the origin of intrasubgroup diversity, will undoubtedly receive great attention in the next few years. The second problem is concerned with triggering of the clonal expansion of lymphocytes after combination of their receptor antibodies with antigens and the quantitative description of the population dynamics of the responding cells. An adequate solution to this problem must also account for the phenomenon of specific immune tolerance as described by the original work of Medawar and his associates (*47*).

For the remainder of this lecture, I

shall turn my attention to some recent attempts that my colleagues and I have made to see whether these problems can be profitably studied using molecular approaches.

Lymphocyte Stimulation by Means of Lectins

The mechanisms of the cellular events underlying immune responses and immune tolerance remain a major challenge to theoretical and practical immunology (*47*, *48*). How does a given antigen induce clonal proliferation or immune tolerance in certain subpopulations of cells?

Cells reactive to a given antigen constitute a very small portion of the lymphocyte population and are difficult to study directly. Two means have been used to circumvent this difficulty: the application of molecules that can stimulate lymphocytes independent of their antigen-binding specificity, and fractionation of specific antigen-binding lymphocytes for studies of stimulation by antigens of known structure. Although the problem of lymphocyte stimulation is far from being solved, both of these approaches are valuable, particularly when used together.

Antigens are not the only means by which lymphocytes may be stimulated. Certain plant proteins called lectins can bind to glycoprotein receptors on the lymphocyte surface and induce blast transformation, mitosis, and immunoglobulin production [reviewed in (*49*)]. Different lectins have different specificities for cell surface glycoproteins and different molecular structures, although their mitogenic properties can be quite similar. In addition, they have a variety of effects on cell metabolism and transport. Such effects are independent of the antigen-binding specificity of the cell, and they may therefore be studied prior to specific cell fractionation.

The fact that antigens and lectins of different specificity and structure may stimulate lymphocytes suggests that the induction of mitosis is a property of membrane-associated structures that can respond to a variety of receptors. Triggering appears to be independent of the specificity of these receptors for their various ligands. To understand mitogenesis, it is therefore necessary to solve two problems. The first is to determine in molecular detail how the lectin binds to the cell surface and to compare it to the binding of antigens. The second is to determine how the binding induces metabolic changes necessary for the initiation of cell division. These changes are likely to include the production or release of a messenger, which is a final common pathway for the stimulation of the cell by a particular lectin or antigen.

One of the important requirements for solving these problems is to know the complete structure of several different mitogenic lectins. This structural information is particularly useful in trying to understand the molecular transformation at the lymphocyte surface required for stimulation. With the knowledge of the three-dimensional structure of a lectin, various amino acid side chains at the surface of the molecule may be modified by group reagents that may also be used to change the valence of the molecule. The activities of the modified lectin derivatives may then be observed in various assays of their effects on cell surfaces and cell functions.

My colleagues and I (*50*) have recently determined both the amino acid sequence and three-dimensional structure of the lectin, concanavalin A (Con A) (Fig. 8). This lectin has specificity for glucopyranosides, mannopyranosides, and fructofuranosides and binds to glycoproteins and possibly glycolipids at a variety of cell surfaces. The purpose of our studies was to know the exact size and shape of the molecule, its valence, and the structure and distribution of its binding sites.

With this knowledge in hand, we have been attempting to modify the structure and determine the effects of that modification on various biological activities of the lymphocyte. So far, there are several findings suggesting that such alterations of the structure have distinct effects. Con A in free solution stimulates thymus-derived lymphocytes (T cells) but not bone marrow–derived

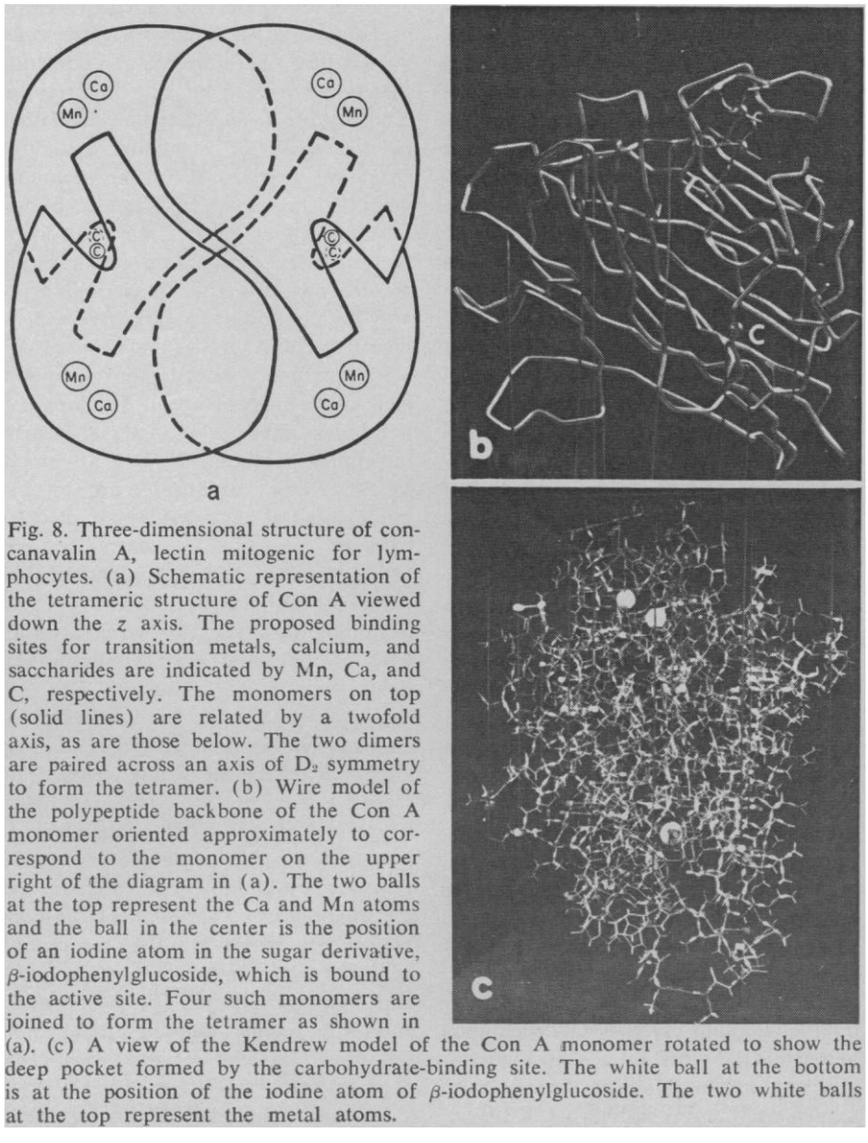

Fig. 8. Three-dimensional structure of concanavalin A, lectin mitogenic for lymphocytes. (a) Schematic representation of the tetrameric structure of Con A viewed down the z axis. The proposed binding sites for transition metals, calcium, and saccharides are indicated by Mn, Ca, and C, respectively. The monomers on top (solid lines) are related by a twofold axis, as are those below. The two dimers are paired across an axis of D_2 symmetry to form the tetramer. (b) Wire model of the polypeptide backbone of the Con A monomer oriented approximately to correspond to the monomer on the upper right of the diagram in (a). The two balls at the top represent the Ca and Mn atoms and the ball in the center is the position of an iodine atom in the sugar derivative, β-iodophenylglucoside, which is bound to the active site. Four such monomers are joined to form the tetramer as shown in (a). (c) A view of the Kendrew model of the Con A monomer rotated to show the deep pocket formed by the carbohydrate-binding site. The white ball at the bottom is at the position of the iodine atom of β-iodophenylglucoside. The two white balls at the top represent the metal atoms.

lymphocytes (B cells), and leads to increased uptake of thymidine and blast transformation. The curve of stimulation of T cells by native Con A shows a rising limb representing stimulation and a falling limb (Fig. 9), probably the result of cell death. The fact that the mitogenic effect and killing effect are dose-dependent suggests an analogy to stimulation and tolerance induction by antigens. When Con A is succinylated, it dissociates from a tetramer to a dimer without alteration of its carbohydrate-binding specificity. Although succinylated Con A is just as mitogenic as native Con A, the falling limb is not seen until much higher doses are reached.

Succinylation of Con A also alters another property of the lectin. At certain concentrations, the binding of Con A to the cell surface restricts the movement of immunoglobulin receptors (*51*). This suggests that it somehow changes the fluidity of the cell membrane, resulting in reduction of the relative mobility of these receptors. In contrast, succinylated Con A has no such effect, although it binds to lymphocytes to the same extent as does the native molecule. Both the abolition of the killing effect in mitogenic assays and the failure to alter immunoglobulin receptor mobility in B cells after succinylation of Con A may be the result of change in valence or of alteration in the surface charge of the molecule. Examination of other derivatives and localization of the substituted side chains in the three-dimensional structure will help to establish which is the major factor. Recent experiments suggest that the valence is probably the major factor, for addition of divalent antibodies against Con A to cells that had bound succinylated Con A resulted again in restriction of immunoglobulin receptor mobility.

Con A may also be modified by cross-linking several molecules. A striking effect is seen if the surface density of the Con A molecules presented to the lymphocyte is increased by cross-linking it at solid surfaces (*52*). Con A in free solution stimulates mouse T cells to an increased incorporation of radioactive thymidine but has no effect on B cells. When cross-linked at a solid surface, however, it stimulates mainly mouse B cells, although both T and B cells have approximately the same number of Con A receptors (*52*). Similar results have been obtained with other lectins (*53*). A reasonable interpretation of these phenomena (although not the only one) is that the lectin acts at the cell surface rather than inside the cell, that the presence of a high surface density of the mitogen is an important variable in exceeding the threshold for the lymphocyte stimulation, and that the threshold differs in the two kinds of lymphocytes.

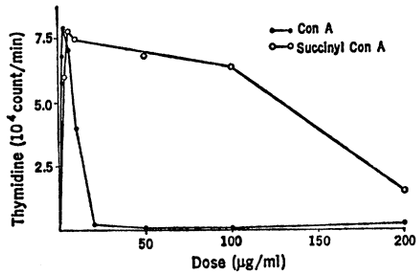

Fig. 9. Stimulation of uptake of radioactive thymidine by mouse spleen cells after addition of concanavalin A and succinylated concanavalin A in increasing doses.

Alteration of the structure and function of various lectins appears to be a promising means of analyzing the mechanism of lymphocyte stimulation. One intriguing hypothesis is that cross-linkage of the proper subsets of glycoprotein receptors by lectins is essentially equivalent in inducing cell transformation to cross-linkage of immunoglobulin receptors in the lymphocyte membrane by multivalent antigens. The central effector function of receptor antibodies, triggering of clonal proliferation, may turn out to be specifically related to the mode of anchorage of the antibody molecule to the cell membrane. The mode of attachment of antibody and lectin receptors to membrane-associated structures and their perturbation by cross-linkage at the cell surface may be similar and have similar effects despite the difference in their specificities and molecular structures.

Antibodies on the Surfaces of Antigen-Binding Cells

The most direct attack on the problem of lymphocyte stimulation is to explore the effects of antigens of known molecular geometry on specifically purified populations of lymphocytes. For this and other reasons, it is necessary to develop methods for the specific fractionation of antigen-binding cells.

In carrying out this task it is important both theoretically and operationally to discriminate between antigen-binding and antigen-reactive cells. In clonal selection, the phenotypic expression of the immunoglobulin genes is mediated in the animal by somatic division of precommitted cells (Fig. 10). The pioneering work of Nossal and Mäkela and later of Ada and Nossal [reviewed in (*48*)] clearly showed that each cell makes antibodies of a single specificity and that there are different populations of specific antigen-binding cells. An animal is capable of responding specifically to an enormous number of antigens to which it is usually never exposed, and it therefore must contain

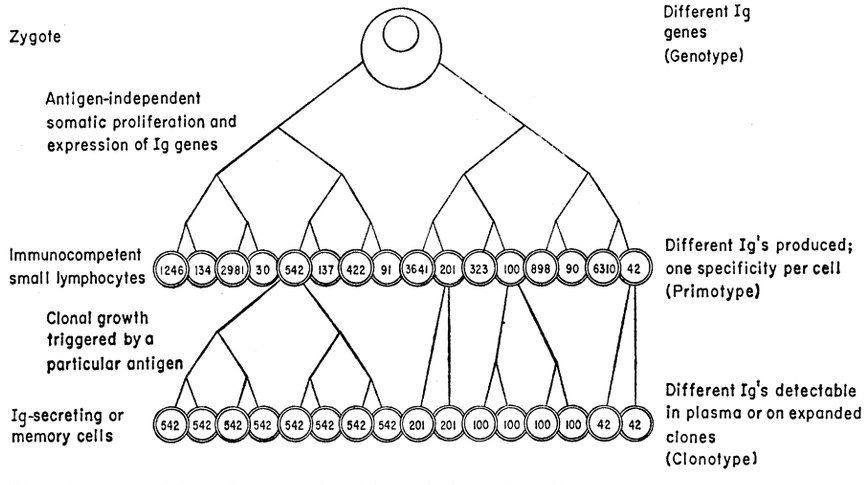

Fig. 10. A model of the somatic differentiation of antibody-producing cells according to the clonal selection theory. The number of immunoglobulin genes may increase during somatic growth so that in the immunologically mature animal, different lymphoid cells are formed, each committed to the synthesis of a structurally distinct receptor antibody (indicated by an Arabic numeral). A small proportion of these cells proliferate upon antigenic stimulation to form different clones of cells, each clone producing a different antibody. This model represents bone marrow–derived (B) cells but with minor modifications it is also applicable to thymus-derived (T) cells.

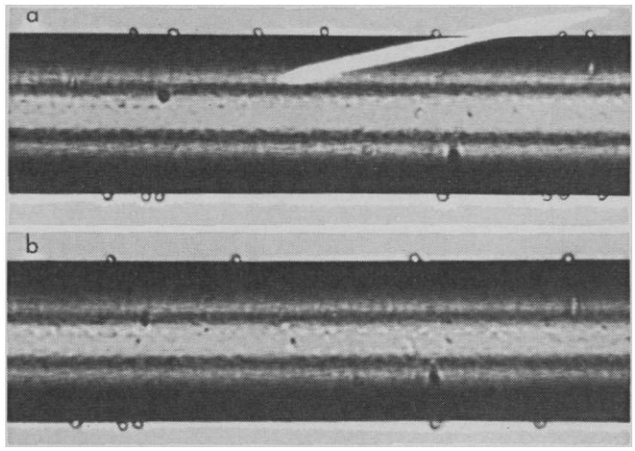

Fig. 11. Lymphoid cells from the mouse spleen bound by their antigen-specific receptors to a nylon fiber to which dinitrophenyl bovine serum albumin has been coupled. Treatment of bound cells in (a) with antiserum to the T cell surface antigen θ and with serum complement destroys the T cells, leaving B cells in (b) still viable and attached (Table 2) (× 175).

genetic information for synthesizing a much larger number of different immunoglobulin molecules than actually appear in detectable amounts in the bloodstream. In other words, the immunoglobulin molecules whose properties we can examine may represent only a minor fraction of those for which genetic information is available.

One may distinguish two levels of expresssion in the synthesis of immunoglobulins that I have termed for convenience the primotype and the clonotype (*3*). The primotype consists of the sum total of structurally different immunoglobulin molecules or receptor antibodies generated within an organism during its lifetime. The number of different molecules in the primotype is probably orders of magnitude greater than the number of different effective antigenic determinants to which the animal is ever exposed (Fig. 10). The clonotype consists of those different immunoglobulin molecules synthesized as a result of antigenic stimulation and clonal expansion. These molecules can be detected and classified according to antigen-binding specificity, class, antigenic determinants, primary structure, allotype, or a variety of other experimentally measurable molecular properties. As a class, the clonotype is smaller than the primotype and is wholly contained within it (Fig. 10).

Although a view of the clonotype is afforded by the analysis of humoral antibodies, we know very little about the primotype. It is therefore important to attempt to fractionate the cells of the immune system according to the specificity of their antigen-binding receptors (*54*). We have been attempting to approach this problem of the specific fractionation of lymphocytes using nylon fibers to which antigens have been covalently coupled (*55, 56*). The derivatized fibers are strung tautly in a tissue culture dish so that cells shaken in suspension may collide with them. Some of the cells colliding with the fibers are specifically bound to the covalently coupled antigens by means of their surface receptors. Bound cells may be counted microscopically in situ by focusing on the edge of the fiber (Fig. 11). After unbound cells are washed away, the specifically bound cells may be removed by plucking the fibers and shearing the cells quantitatively from their sites of attachment. The removed cells retain their viability provided that the tissue culture medium contains serum.

Derivatized nylon fibers have the capacity to bind both T cells and B cells (*57*) according to the specificity of their receptors for a given antigen (*58*) (Fig. 11 and Table 2). About 60 percent of spleen cells specifically isolated are B cells, and the remainder are T cells. By the use of appropriate antisera to cell surface receptors (Table 2), the cells of each type can be counted on the fibers, and most of the cells of one type or the other may then be destroyed by the subsequent addition of serum complement. In this way, one can obtain populations of either T or B cells that are highly enriched in their capacity to bind a given antigen (Fig. 11).

Cells of either kind may be further fractionated according to the relative affinity of their receptors. This can be accomplished by prior addition of a chosen concentration of free antigen, which serves to inhibit specific attachment of subpopulations of cells to the antigen-derivatized fibers by binding to their receptors. As defined by this technique, cells capable of binding specifically to a particular antigen constitute as much as 1 percent of a mouse spleen cell population. Very few of these original antigen-binding cells appear to increase in number after immunization, however, and the cells that do respond are those having receptors of higher relative affinities (*55*).

Whether these populations correspond to the primotype and clonotype remains to be determined. It is significant, however, that fiber-binding cells do not include plaque-forming (*59*) cells, and it is therefore possible to fractionate antigen-binding cells from cells that are already actively secreting antibodies. Recent experiments indicate that the antigen-binding cells isolated by this method may be transferred to irradiated animals to reconstitute a response to the antigen used to isolate them. This suggests that the antigen-specific population of cells removed from the fibers contains precursors of plaque-forming cells.

We have been rather encouraged by these findings, for the various methods of cell fractionation appear to have promise not only in determining the specificity and range of T and B cell receptors for antigens but also in analyzing the population dynamics of T and

Table 2. Characterization of mouse lymphoid cells fractionated according to their antigen-binding specificities. Nylon fibers were derivatized with hapten conjugates of bovine serum albumin, and mice were immunized with either the dinitrophenyl (DNP) or tosyl hapten coupled to hemocyanin. Inhibition of binding was achieved by addition of hapten-protein conjugates (250 μg/ml) or rabbit antiserum against mouse immunoglobulin (Anti-Ig) (250 μg/ml) to the cell suspension. High-avidity cells are defined as those which are prevented from binding by concentrations of DNP–bovine serum albumin of less than 4 μg/ml in the cell suspensions. Cells inhibited by higher concentrations are defined as low-avidity cells. Virtually complete inhibition occurs at concentrations of homologous hapten greater than 100 μg/ml.

Immunization	Cells bound to fiber (cm^{-1})	Inhibition of binding (%) by DNP	Tosyl	Anti-Ig	High-avidity cells (cm^{-1})	Low-avidity cells (cm^{-1})	T cells (%)	B cells (%)
None	1200	90	1	85	< 100	1200	41	59
DNP	4000	95	2	93	2800	1200	39	56
None	800	5	75	73			43	54
Tosyl	2000	10	87	90				

B cells in both adult and developing animals. Now that fractionated populations of lymphocytes specific for particular antigens are available, it should be possible to determine the connection between lectin-induced and antigen-induced changes by comparing responses to both agents on the same cells.

Although many experiments remain to be done in this area of the molecular immunology of the cell surface, continued analysis of the mitogenic mechanism should undoubtedly clarify the problems of immune induction and tolerance. The results obtained with lymphocytes may also have general significance, however, and bear upon the nature of cell division in normal and tumor cells as well as upon growth control and cell-cell interactions in developmental biology. Immunology can be expected to play a double role in these areas of study, for it will be a tool as well as a model system of central importance.

Conclusion

Immunology has been and is a curiously reflexive science, generating its own tools for understanding, such as antibodies to antibody molecules themselves. While this approach is a powerful one, a fundamental understanding of immunological problems requires chemical analysis. The determination of the molecular structure of antibodies is a persuasive example and its virtual completion has allied immunology to molecular biology in a very satisfying way:

1) The heterogeneity of antibodies and complexity of immunoglobulin classes have been rationalized in a fashion consistent with selective theories of immunity.

2) The structural basis for differentiation of the biological activity of antibodies into antigen-binding and effector functions has been made clear.

3) The detailed analysis of antibody primary structure has provided a basis for studying the molecular genetics of the immune response, particularly the origin of diversity and the commitment of each cell to the synthesis of one kind of antibody.

4) A general framework has been provided for studying antibodies at the cell surface, opening several molecular approaches for analyzing stimulation and cell triggering.

5) Finally, it is perhaps not too extravagant to suggest that the extensions of the ideas and methods of molecular immunology to fields such as developmental biology has been facilitated. In this sense, immunology provides an essential tool as well as a model with distinct advantages: dissociable cells with unique gene products of known structure; the capacity to induce specific cloned cell lines for in vitro analysis; the means to fractionate cells according to their state of differentiation and binding specificity, allowing quantitative studies of their selection, interaction, and population dynamics.

Whether or not the immune response turns out to be a uniquely useful model, we can expect that continued work by molecular and cellular immunologists will solve the major problems of the origin of diversity and the induction of antibody synthesis and tolerance. In view of the intimate connection of these problems with problems of gene expression and cellular regulation, their solution should bring valuable insights to other important areas of eukaryotic biology and again transform immunology both as a discipline and as an increasingly important branch of medicine.

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60. By its very nature, science is a communal enterprise. I am deeply aware of the essential contributions to this work made by my many colleagues and friends throughout the last 15 years. This occasion recalls the daily life we have shared with warmth and affection as well as the personal debt of gratitude that I owe them. I am equally cognizant of the fact that the knowledge of antibody structure was developed by many laboratories and researchers throughout the world. Not all of this work has been cited, for specific recognition here runs the risk of an unintentional omission; reference may be made to the reviews cited.
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Science, Technology, and Some Dilemmas of Advocacy

Social implications of biological research in developmental disabilities are considered.

Margaret Adams

Advocacy is a time-honored concept that originated in the law, permeated medicine by way of the physician-advocate, and is now accepted as an explicit function of social work (*1*). Within social work, the terms "advocacy" and "advocate" ["one who pleads, intercedes, or speaks for another" (*2*)] are used to denote the actions and role that social workers are committed to when the human, moral, civil, and legal rights of their clients are transgressed by individuals, groups, or social institutions. This article presents some facets of advocacy that are now confronting social workers as a result of recent dramatic advances in the medical sciences and the impact these advances have upon the lives of individuals—in this instance, upon the mentally handicapped and their families. I attempt to explore major points at issue that can arise between social workers and research scientists, especially those working in the biological sciences. I also suggest areas of common concern that can be exploited to develop a constructive dialogue between the two professional groups instead of the mutual disparagement, suspicion, and even paranoia that sometimes color the thinking of both, to the detriment of cooperative effort and a more sophisticated understanding of the complex nature of the problems of mental handicap. To illustrate simply: the research scientist must keep in mind that an anomaly in, say, the chemical behavior of a neurone terminates in a badly damaged child who belongs to a distraught family; equally, the social worker, who is dealing with their immediate distress and future anxiety, must realize that this chemical misbehavior may derive from an aberrant gene, which could manifest itself in the tragedy of a second affected fetus unless there is scientific intervention in the shape of amniocentesis and genetic counseling (*3*).

Research and Social Priorities

The crucial areas in which science and social work are apt to overlap and work at cross-purposes are (i) future gains versus immediate relief, (ii) prevention versus supportive help, (iii) common good versus individual good, all of which impinge upon most of social work's cherished tenets and firmly entrenched methods of working. Consider, first, the different perspectives on the time factor—namely, reasonably certain, prompt relief as against predictable future gains. Social workers, with their orientation toward problem-solving, crisis intervention, and the pressing problems of the individual's adjustment to his social milieu, sometimes find it hard to accept the value of experiments that can do nothing for the damaged child and besieged family, even though they may save future families from the tragedy that their clients are experiencing. But if social workers are to keep pace with the march of crucial developments and retain the professional respect of their scientific colleagues, they must try to identify, at least in part, with these long-term goals, even though their primary allegiance is to the present client.

This area of concern is very closely tied in with another—public health versus individual treatment—which raises many issues. For example, given that it is desirable to reduce the incidence of defective children and that research technology has provided mechanisms for identifying at-risk parents, how should we react to a proposal for screening for Tay-Sachs disease the population known to be at high risk—namely, Jewish men and women of Ashkenazi origin? Although this is a physically harmless public health measure of unquestionably benign intent, it also contains a psychologically disruptive element: anxiety about ethnic discrimination. Because of their long history of persecution, all members of this group, particularly recent immigrants from Europe who carry inherited and firsthand memories of genocide, are potentially sensitive to discrimination. For people of African descent, screening for sickle cell anemia may have similar implications, which are reinforced by realistic fears of adverse discrimination in respect to employment, life insurance, and so forth (*4*).

The third issue, personal welfare versus the common good, presents a constant conflict to social workers,

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