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GENETICS OF ANTIBODIES[1,2]

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When George Beadle and Edward Tatum coined the one gene-one enzyme slogan in the 1940's, they started the process of focusing on the relationship between individual genes and specific proteins which led, over the next two decades, to the elucidation of the genetic code. From high school on we learn that each polypeptide chain is coded by a structural gene (cistron in the parlance of the molecular biologist-geneticist) or length of chromosomal DNA, which has a base sequence coding, triplet by triplet, for the exact amino acid sequence of a polypeptide chain. Regulators, operators, etc. all affect the functioning of the basic polypeptide-determining cistron, which either is or is not transcribed into the messenger-RNA, which is, in turn, always translated, if it can be translated at all, into the same protein [albeit a few soft spots in this dogma can be perceived (1)].

During this period of development of biochemical genetics, however, immunologists concerned with the genetics of synthesis of antibodies (which are proteins, after all, made of polypeptide chains) fought a difficult battle trying to make the basic "dogma" of genetics fit with the "facts" of antibody synthesis (2). As Cohn put the problem, geneticists have become reconciled to the Darwinian nature of the world, but immunologists are still flirting with Lamarckian concepts and explanations (3). Why? Perhaps because in part the immunologists have been unknowingly pushed into trying to climb a ladder without rungs. It has only been within the last two to three years that sufficient data on the primary structure of immunoglobulins has been accumulated to indicate that while the synthesis of a heterogeneous population of antibodies incorporates the one cistron-one polypeptide chain mechanism, it appears necessary to postulate further evolutionary development of the antibody synthesizing mechanism into what may be a qualitatively different system in order to explain the data (4, 5, 5a).

As we will discuss in detail in the following sections, immunoglobulin polypeptide chains are composed of a constant amino acid sequence region, which does not vary within a given antibody class, and a variable amino acid sequence region, which obeys some sequence restrictions but differs considerably from molecule to molecule within this class. All evidence so far ob-

[1] The survey of literature pertaining to this review was concluded in February 1968.

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tained, and it is rather convincing, leads us to conclude that variable and constant regions are synthesized as one polypeptide chain from the N terminal to the C terminal end just as other polypeptides studied (6). The constant region appears generally to be governed by all the laws of Mendel and molecular biology, and fits well into the one cistron-one polypeptide construct, but the variable region defies the simple genetic logic of the modern molecular biologist. Some of the mechanisms postulated for the generation of diversity of the variable region will be discussed later.

The mechanism of antibody "induction" is a most challenging problem in epigenetics and differentiation. The antibody-forming mechanism is a powerful evolutionary adaptation which provides a flexible protective mechanism against foreign substances, including invading organisms and malignant tumors of other animals. Virtually any protein, carbohydrate, complex biological macromolecule (or organic compound bound to these molecules) introduced into the tissues or body fluids acts as an antigen and sets in action a train of cellular differentiation events which culminates in the formation of cells specialized to produce and secrete antibodies specific for the original antigen. Although a vast array of different antibody molecules with different sequences is produced by the animal, each cell in the animal produces only a single molecular species of antibody. Since the population of antibody molecules elicited by an antigen evolves from less strongly binding (lower affinity) to more strongly binding (higher affinity) antibodies, the genetic changes can be seen in the Darwinian construct of cellular evolution within the population of antibody producing cells (or their precursors or both) with the antigen in some way providing the selective pressure (7, 8).

Does this evolutionary view imply somatic mutation of a few germline immunoglobulin genes (9) followed by somatic selection, or is it possible that mutation and selection during species evolution resulted in the storage of individual genes coding for the vast array of different antibody polypeptide chains in each vertebrate species' germline which are then selected by the antigen? If the latter alternative were true, antibody formation is simply a problem in gene regulation solved by evolution with a virtuosity which has an obvious parallel only in the mechanism of learning and adaptive behavior. Further, some prescient evolutionary principle would have to have been used to generate and store information in the germline for the production of specific antibodies to substances which have perhaps not yet existed and to guard against loss, by drift, of genes coding for antibodies which may have been called for once but not again for tens, hundreds, or thousands of generations.

Neither of the two alternatives, somatic mutation and selection, nor this sort of evolutionary principle, has any known precedent. It is the task of the present genetically sophisticated immunologists (or immunologically sophisticated geneticists) to set up means of testing critically these opposite hypotheses. In the hope of interesting geneticists in these exciting questions and introducing some recent findings in immunology into their thinking, we shall discuss several genetically relevant aspects of antibody formation: (*a*)

the structure and evolution of the immunoglobulins; (*b*) the classical immunogenetics of the constant regions of the immunoglobulin molecule; (*c*) the kinds of sequence variation found in the variable regions of immunoglobulin molecules, the possible genetic mechanisms for the generation of this diversity and the nature of the interaction of the antigen with this system; (*d*) the regulation of the expression of the classical Mendelian genes of immunoglobulins, including the fascinating and unique examples of allelic exclusion and suppression; and (*e*) the analysis of genetic differences in ability to respond to particular antigens, with a view towards identifying discrete biochemical steps in the antibody response.

Most of our examples will be chosen from work with mice, since recent reviews have covered the details in other species, and because the mouse has obvious advantages for genetic investigation in mammals.

Immunoglobulin Structure

A number of excellent reviews and symposium publications have recently appeared which should be consulted for more details on the very extensive structural and amino acid sequence information now available on immunoglobulins (3–5, 10–12a). A general summary of this information will be adequate for our purposes here. The archetype immunoglobulin γG (or IgG) found in all vertebrate species and usually comprising the majority of immunoglobulins in serum is a protein of about 150,000 molecular weight made up of a pair of identical light and a pair of identical heavy polypeptide chains held together by noncovalent hydrophobic bonds and covalent disulphide bridges (See Fig. 1). Each of the two L (light) chains

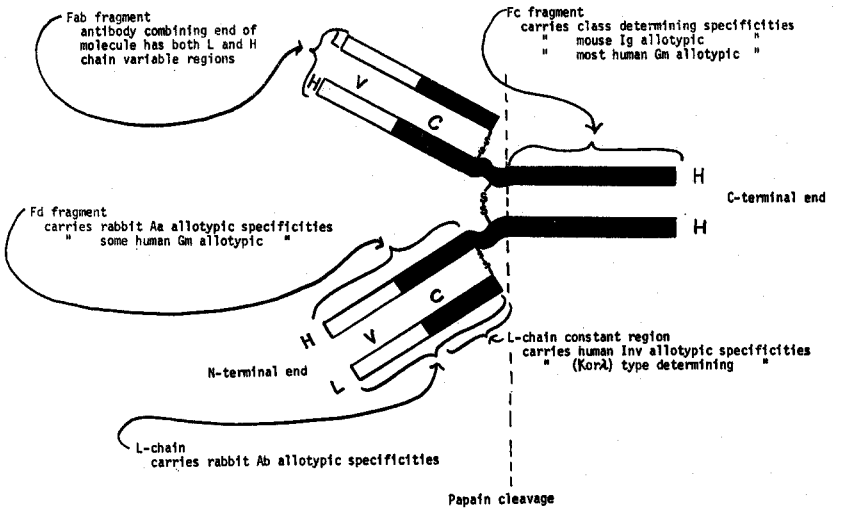

Fig. 1. γG immunoglobulin schematic structure.

(molecular weight of about 23,000) is bound to an H (heavy) chain of about 55,000 molecular weight. The disulphide bond between the two heavy chains can be selectively cleaved by reduction to give "half molecules." Each half molecule has intact one of the antibody combining sites. The two half molecules thus formed are identical in structure, combining specificity, and affinity (energy of binding). Each antibody combining site is formed from the N terminal end of an H chain in combination with an L chain. As shown in Figure 1, the enzyme papain cleaves the molecules at about the middle of the H chains. In the presence of a reducing agent such as cysteine or mercaptoethanol, which splits the disulphide bond, three fragments are produced, two identical Fab and one Fc. The antibody combining activity of the original molecule is all found to be associated with the Fab fragments.

The important question of whether the antibody combining activity is determined exclusively by the amino acid sequences of the chains in the Fab fragments or, as proposed by some of the early instructionist theories of antibody formation, chains of common amino acid sequence are somehow folded in complementarity with the antigen, has been definitively answered by recent experiments (13, 14). All secondary structure in the Fab fragments was effectively destroyed by reduction of all the inter- and intrachain disulphide bonds followed by dissolution in 8 *M* urea or 6 *M* guanidine. Upon removal of the denaturing agents and slow oxidation at very low protein concentration, extensive restoration of antibody activity was obtained. These results lend strong support to the idea that all the information necessary for the formation of antibody combining sites is present in the linear arrangements of amino acids in the polypeptide chains.

The Evolution of Immunoglobulins

Comparison of amino acid sequences, and, in particular, the location of intrachain disulphide bonds, in mouse and human light chains, human heavy chains, and the carboxyl terminal (Fc) half of rabbit heavy chains reveals a striking similarity in structure (12, 15–17). The light chains of mouse and man contain two intrachain disulphide loops, including about 60 amino acid residues, each approximately in the center of the variable and the constant regions. This pattern of one large intrachain disulphide loop in every 110 to 120 residues is repeated four times in the human heavy chain and twice in the rabbit Fc fragment and suggests the evolution of both light and heavy chain genes from an ancestral 110-residue precursor gene by a series of fused and detached gene duplications.

A corresponding similarity in amino acid sequences supports this view. There is significant homology (identical as well as similar amino acids at the same position, with allowance for small insertions and deletions) not only between the variable and constant region sequences of mouse and human light chains, but also between these sequences and the amino and carboxyl terminal halves of the rabbit Fc fragment, as well as between the

two halves of rabbit Fc themselves. Comparison of all six types of sequence shows a high degree of homology for the entire group.

These stretches of sequence homology are in several cases greater than those noted in the evolution of vertebrate hemoglobins from myoglobin, an analogy that can be carried further when it is realized that the light chains (analogous to the alpha chains) are not linked to the heavy chains (analogous to the beta chains) whereas the genes for several closely related forms of heavy chain are tightly linked, as are the β, γ, and δ chains of hemoglobin. Thus they quite possibly arose by a series of tandem gene duplications.

To date, no striking differences have been found in the ability of primitive fish and mammals to respond to complex antigens, suggesting that the range of antibody variability has not changed radically during vertebrate evolution (18). No conclusive reports of antibody formation in invertebrates have been published.

Classes of Immunoglobulins

The immunoglobulins of each individual consist of several classes of molecules which are differentiated from each other by their H chains. The same types of L chains are found in all classes. Within a class the molecules all contain heavy chains with a constant amino acid sequence beginning at the C-terminal end of the H chain and extending approximately three-fourths of the length of the chain. The sequence at the N-terminal end varies between molecules of a given class. Thus the H chain is composed of a constant and a variable region. The evidence that the variable region is contiguous and about one-fourth of the chain length is not yet complete but this detail will soon be settled and need not concern us here (11).

Similarly, each L chain contains a constant and a variable region (12, 19). The C-terminal half (exactly one-half or almost so in all cases) is constant within an L chain type. (L chains are conventionally grouped as "types" and H chains as "classes".) The remaining half is variable from one molecule to the next. It is the variable region sequences of the two chains which give the molecule its particular antibody combining activity and the constant region sequences which determine to which class or type the molecule belongs.

Five immunoglobulin classes, based on H chain differences, have been defined in the mouse (20, 21). These are presented in Table I. Some of the classes contain just the basic subunit of two L and two H chains whereas others are higher molecular weight polymers of this subunit. Thus γM globulins can be separated from the various γG classes by gel filtration or sedimentation. The average charge on some classes differs sufficiently from that on others to allow separation by electrophoresis or ion exchange chromatography. Solubility differences in high or low salt concentrations also allow some separations to be made. Most recently, isopycnic density-gradient cen-

TABLE I

MOUSE IMMUNOGLOBULIN CLASSES

Class*	Locus	Number of† Alleles	Number of† Specificities	Biologic Activity (see 24)
γG_{2a} (γG)	Ig-1‡	8	11	Fixes complement, mediates cell lysis and fixes to tissues of other species and mediates local anaphylaxis.
γA	Ig-2	5	4	Secreted into milk, tears, intestinal lumen, nasal secretions.
γG_{2b} (γH)	Ig-3	6	7	Fixes complement, mediates cell lysis.
γG_1 (γF)	Ig-4	2	none§	Fixes to tissues of same species, mediates local anaphylaxis.
γM				Fixes complement, mediates cell lysis (more efficient than γG_{2a} and γG_{2b}).

* Potter-Lieberman notation given in parenthesis (30).

† For alleles, specificities and type strains of the four loci, Ig-1 through Ig-4, see tables III, IV and V and Fig. 4 respectively. Data presented here and in the referred tables combine our studies with those of Potter & Lieberman. Although these investigators do not define loci or alleles as such, their descriptions of determinants on immunoglobulins of standard inbred mouse strains allow us to compare their studies and ours and synthesize an inclusive system. The notation used here is consistent with the notation approved for the H-2 locus by the Committee for Standardized Genetics Nomenclature for Mice.

‡ Previous names for this locus are γG, MuA, Asa, Iga (29).

§ Alleles defined on the basis of electrophoretic mobility of Fc fragment.

trifugation has been shown to be a useful means of resolving some of the classes according to their buoyant densities, presumably reflecting differences in carbohydrate content (22). But often none of these methods, alone or in combination, allows a complete separation of the classes.

The definition and study of most immunoglobulin classes have been made possible mainly through the existence of pathological accidents of nature, the so-called myeloma proteins. These proteins are produced in man and mouse by plasma cell tumors. Each tumor is essentially a clone of immunoglobulin producing cells in which class and variable region are fixed, i.e. which makes a single protein species with a single amino acid sequence.

Myeloma proteins have been the source of most of the sequence information we now have. L chains produced by such tumors are frequently made in excess of the corresponding heavy chains and are secreted in large amounts in the urine. These urinary light chains, or Bence-Jones proteins, are a readily available source of light chains for sequence analysis and have been very extensively investigated.

As shown in Table I, each of the H chain classes has different biological activities correlated with different Fc portions of the H chains in these classes. The γM globulins, or 19S globulins, are highly efficient hemolytic or bacteriolytic antibodies by virtue of their complement binding capacities (23). Two of the γG immunoglobulin classes, γG_{2a} and γG_{2b} are similarly able to fix complement and cause the lysis of red blood cells and bacteria (24). Other classes, by virtue of their Fc fragments, are able to fix to skin of the same species in one case (class γG_1) or of another species (class γG_{2a}) and give an anaphylactic-type reaction when presented with the proper antigen (25). On the other hand, there is no readily apparent association of classes of immunoglobulins with particular antibody specificities. In general, most antigens elicit antibodies belonging to all classes (26).

H-Chain Genes

Genetic polymorphisms for a number of H and L chains in several species are known. These allelic differences were first found by serological methods and are usually referred to as "allotypes" (27). Although this term is not usually found in the genetic literature, it will be used here because of its currency among those interested in antibodies.

Recent reviews of allotypes in man (28), rabbits (29), and mice (30) permit us to be selective in our coverage of this area and concentrate on evidence relating to the genes for H chains in the mouse. (There are no known L-chain polymorphisms in mice.) Notation and nomenclature in a field which attracts the interest of immunologists and geneticists unfortunately seems to be difficult to standardize. This was particularly true in the early stages before the overall picture began to make sense. We believe that we have now passed into a second stage where some clarity has developed and the general outlines are agreed to by most workers and interested observers (29, 31, 32). In due course, a single easily understood notation will be established for the various species. For the moment, the unfortunate reader will be compelled to do some retranslating in reading different articles and reviews.

Four H-chain gene loci, Ig-1, Ig-2, Ig-3, and Ig-4, have been found in the mouse, corresponding to the H chains in the classes γG_{2a}, γA, γG_{2b} and γG_1 respectively (Table I) (33-36). The correspondences with Potter & Lieberman's notation (30) is given in the table. The alleles at Ig-1 through Ig-3 were first defined serologically through the use of antisera produced in mice of one inbred strain against one or another of the immunoglobulins of a second mouse strain. Such antisera, made in one member of a species

against antigens of another individual of the same species are called "alloantisera."

In some alloantisera, sufficient quantities of precipitating antibodies are present to allow detection of the reaction by immunodiffusion in agar. However, alloantisera often contain such small amounts of antibody that more sensitive detection methods must be used. Therefore, we turned to precipitation and inhibition of precipitation of I^{125}-labelled antigens for most of our analyses of allotypic differences.

Ig-1 locus.—Using this method we have defined 8 alleles at the Ig-1 locus displaying 11 distinct antigenic specificities on γG_{2a} immunoglobulins among the inbred strains of mice. Table II lists the inbred strains which carry each allele. A type strain which permanently defines the allele is italicized for each. That is, the allotype or Ig-1 allele is retained with the type strain if it is subsequently found that one or more strains in the particular allele group can be allotypically distinguished from the type strain. A new allele and a new type strain for the new allele group would then be designated (33).

In addition to these eight alleles in the inbred strains, Lieberman & Potter have found two other alleles among a considerable collection of sera from wild mice (37). Further examination of such wild mice may well turn up more alleles.

The Ig-1 specificities listed in Table III show that all the allelic Ig-1 allotypes are cross reacting and determine various combinations of relatively few specificities. The letter for each allele is a short notation for the entire set of specificities which actually define the allele. The numbered specificities are really symbolic representations of the cross reactions of the antigens determined by each allele, not necessarily separable antigenic determinants on the molecules. The presence of a particular specificity in two allelic types signifies that these strains have some structural site, or feature, in common which is absent from the Ig-1 locus-determined globulins (γG_{2a}) in mice not having that specificity.

For the Ig-1 antigens, the chemical nature of these structural sites is unknown. They might represent particular conformational arrangements of a few amino acids on the surface of the protein or they might be due directly to the sequences in a length of polypeptide chain. Two specificities present on the same molecule could be interpreted as being two physically separate determinants, or a single determinant eliciting a population of antibodies only part of which reacts to the second antigen. In man, allotypic differences detectible by serologic means have been shown to be correlated with specific amino acid substitutions (38–40). In the rabbit as well, certain allotypic specificities are associated with particular amino acids in certain positions of the relevant chains (41). The serological differences created by these amino acid substitutions may very well be conformational antigenic determinants. In fact, even a single amino acid change may result in multiple antigenic differences which would define several specificities. Although

TABLE II

DISTRIBUTION OF IG-1 ALLELES IN INBRED MOUSE STRAINS (33)

Ig-1[a]			Ig-1[b]		Ig-1[c]	Ig-1[d]	Ig-1[e]	Ig-1[f]	Ig-1[g]	Ig-1[h]
*BALB/cJ**			*C57BL/10J**		*DBA/2J**	*AKR/J**	*A/J**	*CE/J**	*RIII/J**	*SEA/Gn**
BALB/CGa	C58/J	PL/J	B10.D2(new)/Hz	SM/J	DBA/1J	AL/N	NZB/Bl	DE/J	DA/Hu	BDP/J
BUB/Bn	F/Ao	POLY1/Ao	B10.D2(old)/Hz	STA/Je	I/Ao		NZO/Bl	N/Ao	FZ/Di	BSL/Di
CBA/J	H-2G/Go	POLY2/Ao	C57BL/H	WB/Re	JB/Di		NZW	NH/N	STB/Je	P/J
CHI/Ao	JK/Bi	PRUNT/Ao	C57BL/Ka	WC/Re	RF/J					SEC/Gn
C3H/Hz	MA/J	ST/J	C57BL/6J	WH/Re	SWR/J					
C3H.SW/Hz	MA/MyJ	STR/N	H-2H/Go	WK/Re						
C57BR/cdJ	NZY/Bl	T6/H	H-2I/Go	58N/Sn						
C57L/J	PBR/Ao	129/RrGa	LP/J	101/Rl						
			SJL/J							

* Type strain for each allele is italicized.

TABLE III

THE IG-1 LOCUS*

Type Strain	Allele	Specificities†										
BALB/c	Ig-1^a	1(G7)	2(G8)	—	—	—	6	7	8	10(G1)	—	12(G6)
C57BL/10J	Ig-1^b	—	—	—	4	—	—	7	—	—	—	—
DBA/2J	Ig-1^c	—	2(G8)	3	—	—	—	7	—	—	—	—
AKR/J	Ig-1^d	1(G7)	2(G8)	—	—	5	—	7	—	—	—	12(G6)
A/J	Ig-1^e	1(G7)	2(G8)	—	—	5	6	7	8	—	—	12(G6)
CE/J	Ig-1^f	1(G7)	2(G8)	—	—	—	—	—	8	—	11	—
RIII/J	Ig-1^g	—	2(G8)	3	—	—	—	—	—	—	—	—
SEA/Gn	Ig-1^h	1(G7)	2(G8)	—	—	—	6	7	—	10(G1)	—	12(G6)

* Ig-1 determines γG_{2a} immunoglobulin H chains (33).

† Potter-Lieberman determinant names given in parentheses (30). See footnote †, Table I. Specificity 12 described only by Potter & Lieberman.

the possibility is considered unlikely, the participation of a carbohydrate group in the antigenic determinants of the Ig-1 system has not been eliminated.

Defining specificities.—The specificities and alleles have been defined by the use of inhibition of precipitation of labelled antigen. Purified normal γG_2 globulins of various mouse strains or γG_{2a} myeloma proteins have been trace-labelled with I^{125}. A small amount of labelled antigen is mixed with about a hundredfold excess of a test antigen in the form of unpurified whole serum, and an amount of specific alloantiserum is added which would nearly maximally precipitate the radioactive antigen in the absence of the test or inhibitor antigen. After a suitable incubation period, the tubes are centrifuged and the amount of precipitation is determined by the fraction of I^{125} removed from the supernatant. If the test antigen does not cross react with the labelled antigen there is no inhibition of precipitation. If, on the other hand, the test antigen is identical in its reaction with the antiserum to the labelled antigen there is complete inhibition of precipitation. In the case where the test antigen partially cross reacts with the labelled antigen, with the particular antiserum used, there is a partial inhibition of precipitation (*32*, 33).

A detailed exposition of how all these specificities in the Ig-1 system were defined would be too long for this review. However, to illustrate the process, we present a few examples of the rules that have been followed [full details have been published (*32*-34)]:

(*a*) a strain producing an alloantiserum has none of the specificities recognized by that antiserum; (*b*) the immunizing strain has all the specificities detectable by that antiserum regardless of the type of antigen used to detect the antibodies included in the antiserum; (*c*) however, the specificities detected in the immunizing strain by the antiserum made against it are not necessarily all the antigenic specificities present in the immunizing strain (i.e. antibodies may not be present to all the specificities); (*d*) the specificities detected by an antiserum when used with a labelled antigen from a strain other than the immunizing strain, represent the specificities detected by the antiserum minus those specificities which are present

in the immunizing strain and absent from the labelled antigen; (*e*) a test serum which completely inhibits has all the specificities detected by the antiserum in that assay, while a test serum which does not inhibit has none; (*f*) a test serum which partially inhibits a reaction has some but not all of the specificities detected in that assay; (*g*) two strains, which each partially inhibit in a given reaction, need not share any specificities with each other, but each shares some specificities with the labelled antigen strain and the immunizing strain; and (*h*) the number of specificities is always a minimum estimate of the number compatible with the results.

In the definition of specificities, the following notation is used: An I^{125}-labelled preparation of gamma globulin is indicated with an asterisk following the symbol of the strain from which it was prepared (for example, C3H*), while normal sera used in inhibition assays are listed by the strain symbols (e.g., C3H). The symbols C3H*-C57BL anti-C3H refer to the use of a labeled C3H gamma globulin preparation with a C57BL anti-C3H antiserum in an inhibition assay. The expression "C3H 1" means C3H has specificity one. The expression "C57BL -1" means C57BL does not have specificity one.

Several specificities are here defined:

(*i*) C3H*-C57BL anti-C3H precipitation is not completely inhibited by DBA. C3H therefore has at least one specificity not present in DBA (C3H 1; C57BL -1; DBA -1).

(*ii*) DBA*-C57BL anti-C3H precipitation is completely inhibited by AKR. Therefore C3H, DBA, and AKR share at least one specificity, 2 (C3H 2; C57BL -2; DBA 2; AKR 2).

(*iii*) AKR*-C57BL anti-C3H precipitation is not completely inhibited by DBA. Therefore AKR and C3H must share at least one specificity that is not present in DBA (AKR 1).

(*iv*) C3H*-C57BL anti-C3H precipitation is not completely inhibited by AKR. C3H therefore has at least one specificity not present in AKR (C3H 6; C57BL -6; AKR -6).

(*v*) Since C57BL anti-C3H recognized specificity 6, statement ii proves that DBA does not have 6 (DBA -6).

(*vi*) DBA*-C57BL anti-DBA precipitation is not completely inhibited by C3H or AKR. Therefore DBA has at least one specificity not present in either of these two strains (C3H -3; C57BL -3; DBA 3; AKR -3).

(*vii*) C3H anti-C57BL precipitates C57BL*, but not C3H*, DBA*, AKR*, or A/J*. C57BL therefore has at least one specificity not present in the latter four strains (C3H -4; C57BL 4; DBA -4; AKR -4; A/J -4).

(*viii*) C3H*-C57BL anti-C3H precipitation is completely inhibited by A/J. Therefore A/J has all specificities previously assigned to C3H (A/J 1, 2 and 6).

The complete notation for a specificity includes the locus; e.g., Ig-1.3 means specificity 3 of the Ig-1 locus. The specificities for each locus are designated in order of definition so that Ig-3.3, for example, has no particular relation to Ig-1.3. The specificities all represent serologically distinct reactivities. In the following sections we shall describe the specificities of other Ig loci.

Unassigned determinants.—It might be well to digress at this point to discuss the so-called unassigned determinants (specificities) of Potter &

Lieberman (30). By unassigned, these workers mean allotypic specificities which react with immunoglobulins of a strain but which they cannot assign to a particular immunoglobulin class. Potter & Lieberman have used precipitation of antigens by double diffusion in agar gels (also known as the Ouchterlony method) for allotype detection and have in general used whole sera as antigens rather than purified immunoglobulins of one class or another. In the cases where they used purified myeloma proteins as antigens, the specificities detected were assigned to the class to which the myeloma protein belonged. Where they could not use a purified myeloma protein as antigen, no class assignment was made. This restricted Potter & Lieberman to assignment of specificities to only a fraction of the immunoglobulin classes and genetic types known because plasma cell tumors, which produce the myeloma proteins, until fairly recently had been induced only in BALB/c mice and therefore most of the available myeloma proteins were of BALB/c origin. Recently, tumors originating in strains with other allotypes have been obtained, but these are still few in number. Potter & Lieberman used the following types, listed by our structural gene notations: Ig-1^a, Ig-2^a, Ig-2^b, and Ig-2^e, Ig-3^a and Ig-3^b. Specificities not found in these gene products were left unassigned.

We have been able to assign all specificities so far detected to one, or in a few cases two, class(es) of immunoglobulins by extension of the precipitation of labelled antigen method which we have described above. In this method all specificities can be associated with other specificities which can be related directly, or through one more step of association, to myeloma proteins of a particular class. By examination of Table III, it can be seen that every Ig-1 allele product has at least two specificities in common with the BALB/c allotype Ig-1^a. Since all the Ig-1 specificities have been found on proteins of the γG_{2a} class, we can state definitely that all the specificities in Table III are associated with γG_{2a} immunoglobulins of the various allelic types. We have also shown by sequential and mixed precipitations that all Ig-1^a specificities are on the same molecule (35). As will be seen later, one of these specificities, 9, is also present on immunoglobulins of the γG_{2b} class (42). We shall describe this in detail below.

Ig-2 locus.—The Ig-2 locus controls the heavy chain γA immunoglobulins in mice (35). By direct precipitation in agar, we demonstrated three alleles at this locus. Potter & Lieberman have found antisera which detect two other alleles (30). These five Ig-2 alleles are designated in Table IV. Since the strains which carry a particular Ig-2 allotype (allele) are either all or none of the strains which carry a particular Ig-1 allotype, the Ig-2 alleles have been designated by letters consistent with the Ig-1 notation. For example, all the Ig-1^a and Ig-1^h strains listed in Table 2 have the same Ig-2 allotype. No other strains tested have this allotype. Similarly all the strains in the Ig-1^c and Ig-1^g allele groups, and no others assigned to other Ig-1 allele groups, have the same Ig-2 allotype. We have used this remarkable association between alleles at Ig-1 and Ig-2 in the allele notation for

Ig-2. Thus, as seen in Table IV, the five so-far-defined Ig-2 alleles are called Ig-$2^{a,h}$, Ig-2^{b}, Ig-$2^{c,g}$, Ig-$2^{d,e}$ and Ig-2^{f}, thus showing in the allele name the Ig-1 allele groups of inbred strains in which these alleles are found. As more antiallotype sera become available, we expect it will be possible to divide the Ig-$2^{a,h}$, Ig-$2^{c,g}$, and the Ig-$2^{d,e}$ into two allele groups each. As will be seen below, the same system of allele designation is used for the other heavy chain loci in the mouse.

Ig-3 locus.—Six alleles at the Ig-3 locus determining allotypic determinants in the γG_{2b} class of immunoglobulins have now been found (34). The set of alleles definable by observations in our laboratory (43) and in Potter's (30) are listed in Table V. In this table, we have also given Potter's determinant designations, the Ig-3 specificities which we have defined, and the type strains for each allele group of strains. The general description of

TABLE IV

THE IG-2 LOCUS*

Type Strains	Alleles	Specificities†			
BALB/cJ, SEA/Gn	Ig-$2^{a,h}$	—	2(A12)	3(A13)	4(A14)
C57BL/10J	Ig-2^{b}	—	—	—	—
DBA/2J, RIII/J	Ig-$2^{c,g}$	1	—	—	—
AKR/J, A/J	Ig-$2^{d,e}$	—	—	3(A13)	—
CE/J	Ig-2^{f}	—	—	—	4(A14)

* Ig-2 determines γA immunoglobulin H chains (35).

† Potter-Lieberman determinant names given in parentheses (30). See footnote†, Table I. Specificities 3 and 4 have been described only by Potter & Lieberman.

TABLE V

THE IG-3 LOCUS*

Type Strains	Alleles	Specificities†						
BALB/cJ, DBA/2J, SEA/Gn	Ig-$3^{a,c,h}$	1	2(H11)	—	4	7	8	—
C57BL/10J	Ig-3^{b}	—	—	—	4	7	8	9(H9)
AKR/J	Ig-3^{d}	1	—	3	—	7	8	—
A/J	Ig-3^{e}	1	—	3	—	7	—	—
CE/J	Ig-3^{f}	1	2(H11)	3	4	—	—	—
RIII/J	Ig-3^{g}	1	2(H11)	—	4?	—	—	—

* Ig-3 determines γG_{2b} immunoglobulin H chains (34).

† Potter-Lieberman determinant names are given in parentheses (30). See footnote †, Table I. Specificity 9 has been described only by Potter & Lieberman.

Ig-1 allotypes given above applies as well to the Ig-3 allotypes. All of them are cross reacting, within the locus system, and no specificities are unique to one allotype. It has been much more difficult in our laboratory, and apparently in Lieberman & Potter's as well, to obtain adequate antiallotype sera for the Ig-3 locus. We have no explanation for this difficulty. Nevertheless, we believe it likely that at least eight alleles will be distinguishable at Ig-3 as more antiallotype sera are made.

Ig-4 locus.—Although it has been the custom with immunoglobulins to resort to serology for characterization, allotypic differences need not be recognized only by serologic methods. Faced with the inability to raise alloantisera which reacted with γG_1 globulins, we turned to another classical method for demonstrating polymorphism at loci which determine protein structure: electrophoretic mobility. Using this method we can now define two alleles at the Ig-4 locus, one in BALB/c and the other in C57BL/6 (36, 44).

Rabbit antiserum for the γG_1 class was raised and rendered specific by absorption with immunoglobulins of the other classes. This antiserum was used to develop immunodiffusion patterns following electrophoretic separation in agar gels of γG_1 whole molecules or Fc fragments obtained by papain digestion. In Figure 2 the electrophoretic mobility difference between BALB/c and C57BL/6 is demonstrated both for whole molecules and the Fc fragments. The mobility differences are more clearly seen with the smaller and more rapidly migrating Fc fragments. This is to be expected since the variable regions of the H and L chains add considerably to the electrophoretic heterogeneity of the whole molecules of immunoglobulin of a given class.

All of the type strains and several strains from each allele group were tested for electrophoretic mobility differences in γG_1. Only the two mobility types shown in Figure 2 were seen. Thus, the Ig-4 locus has been defined and two alleles have been found. The two alleles are designated $Ig\text{-}4^{a,c,d,e,f,g,h}$ and $Ig\text{-}4^{b}$.

This method of recognizing allotypes is quite a simple one requiring small amounts of test serum and no prior purification of immunoglobulins from the test serum. It should find considerable applicability. The papain digestion is carried out on 0.1 ml aliquots of sera in the presence of a reducing agent and the reaction stopped with iodoacetamide. Five or ten microliters of fresh digest are then placed in the holes and electrophoresed for 60 to 90 min. The specific antiserum is then added to the trough and the line begins to be developed within several hours. The reaction is usually complete by 18 hr.

Linkage of the four heavy chain genes to one another.—Ig-1 and Ig-2 were shown by us and by Lieberman & Potter to be closely linked. As shown in Table VI, in the backcross $(C3H \times DBA/2)F_1 \times C57BL$ no recombinants were found in 149 progeny (35). Lieberman & Potter examined 1054 progeny from a backcross between $(BALB/c \times C57BL)F_1 \times C57BL$ and

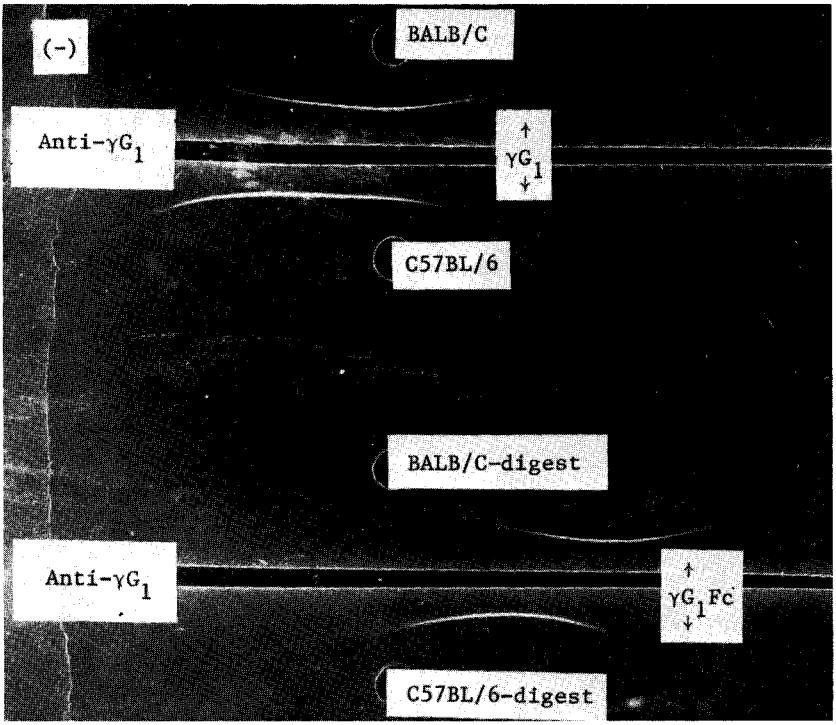

FIG. 2. Strain differences in electrophoretic mobility of whole γG_1 molecules and their Fc fragments (36).

also found no recombinants (30). Similar tests for linkage between Ig-1 and Ig-3 and between Ig-1 and Ig-4 have been carried out with no crossovers detected in some 600 animals tested. Thus, the genes Ig-1 through Ig-4 constitute a cluster of closely linked genes between which no crossovers have yet been found (44). Of course, since each allele seems to code for a particular combination of relatively few antigenic specificities, we may postulate that crossing-over occurred in the evolution of these alleles in analogy with the kinds of crossovers that are postulated to account for the present Rh alleles.

The low frequency of crossovers in mice is also found in man (45). In studying human allotypic specificities, Natvig & Kunkel (45) have recently found pedigrees showing direct evidence of crossovers. Recombination between specificities determined at 3 H chain loci in the Gm system were found at a frequency of less than one per 1000.

Earlier we said that there was an absolute positive association between alleles of the different Ig loci among inbred strains such that the eight Ig-1 alleles (each characteristic for a group of strains) are always associated with the same alleles for the other Ig loci. The minimum number of identi-

TABLE VI

LINKAGE OF IG-1 AND IG-2 (35)

Cross	Progeny	
(C3H × DBA/2)F_1 × C57BL	Parental Types	
	$\frac{\text{Ig-1}^a \quad \text{Ig-2}^a}{\text{Ig-1}^b \quad -}$	78
$\frac{\text{Ig-1}^a \quad \text{Ig-2}^a}{\text{Ig-1}^c \quad \text{Ig-2}^c} \times \frac{\text{Ig-1}^b \quad -}{\text{Ig-1}^b \quad -}$	$\frac{\text{Ig-1}^c \quad \text{Ig-2}^c}{\text{Ig-1}^b \quad -}$	71
	Recombinant Types	
	$\frac{\text{Ig-1}^a \quad \text{Ig-2}^c}{\text{Ig-1}^b \quad -}$	0
	$\frac{\text{Ig-1}^c \quad \text{Ig-2}^a}{\text{Ig-1}^b \quad -}$	0
	Total tested	149

fied alleles at the four loci listed in numerical order is 8, 5, 6 and 2. For independent loci at genetic equilibrium, 480 combinations of these alleles would be expected; only eight are known. Clearly, the inbred strains do not represent as many samples of such an equilibrium population as there are inbred strains, but independent samples are lost in the derivation of the laboratory mouse from wild mice (46). Mice captured in the wild remain behaviourly wild for decades and are difficult to breed with laboratory mice. It is conceivable that only a few pairs of mice gave rise to the non-inbred pet mice from which present day inbred strains derive. Only eight Ig chromosome types could then be a reflection of this small number of chromosomes originally introduced into laboratory mice, coupled with the known very low crossover rate (no crossovers detected in nearly 2000 backcross progeny). Of course, once inbreeding began, the opportunity for reassortment of alleles at the linked Ig loci was lost.

Present day wild mice may have different Ig chromosomes. In fact two Ig-1 alleles, not found in inbred strains, have already been detected in wild mice (37).

Linkage of the Ig region with other genetic markers.—Despite a fair number of two-point linkage tests which have been carried out with the readily available markers in the linkage-tester stocks of the Jackson Laboratories, as well as a fair number of other marker genes, no linkage of the Ig region with other genes has yet been found (30, 33, 47). The following genes listed by linkage group have been excluded from close linkage with the Ig genes:

I c,p; II d,se; III s,hr; IV Se; V A,Ra, Sd; VI Ca; VII Re; VIII b;

IX H-2, Ir-1; XI Mi^{wh}; XII ep, ru; XIII Lp; XIV sa; XV ax; XVI Va; XVIII Os, Ea-1; XX sex; unlinked Hc.

Structural localization of Ig determinants in mice.—All of the Ig specificities so far examined have been found to be associated with the Fc fragments obtained by papain digestion of the various immunoglobulin classes. Mishell & Fahey first showed by immunoelectrophoresis that the Ig-1a determinants were on the Fc fragments of normal γG_{2a} globulin (48). Subsequently we have found that the Fc fragments of each immunoglobulin class completely inhibit precipitation with antiallotype sera of the respective whole immunoglobulin molecules. A further demonstration that the Fc fragment contains the portion of the heavy chains which carries the allotypic determinants is shown in the next section. We have reason to hope that genetic (allotypic) differences in the Fd portions of the heavy chains will still be found. For example, in man, most of the antigenic determinants associated with the Gm allotypes are found on the Fc fragments, but at least two specificities are now known to be on the Fd fragments of the same molecules (49).

Genetically determined electrophoretic mobility differences in the mouse immunoglobulins.—As described in earlier sections, serological differences in γG_{2a}, γG_{2b} and γA globulin allowed us to define several alleles at each of the three loci concerned. Electrophoretic mobility differences in the γG_1 immunoglobins allowed us to define two alleles at the locus, Ig-4. Since serologic differences presumably reflect amino acid composition differences, it seemed likely that at least some serologically defined alleles would be detectable also by electrophoretic mobility differences. As shown in Figure 3, two alleles of Ig-1, three Ig-2 alleles and two Ig-3 alleles were found by immunoelectrophoretic analysis of the Fc fragments obtained by papain digestion of the molecules in unpurified whole sera. Rabbit antisera specific for each of the several classes was used for developing the immunoelectrophoretic pattern (44).

For each locus a complete association was found between a particular electrophoretic mobility type and one or more serologically defined alleles (allotypes), whether the testing was done on inbred strains or on progeny from segregating F2 and backcrosses (44). Examples of this are shown in Table VII.

Detection of allotypic antigens with heterologous antisera.—Although not of fundamental theoretical importance, the finding that the mouse allotypes which we have described can be detected not only with mouse antisera, which are sometimes difficult to obtain, but also with rabbit antisera to mouse globulins, may be of considerable help in exploiting the genetic and immunological uses of the mouse allotypes as markers (43). (Rabbits yield 25 to 50 ml of serum per bleeding; mice, 0.5 ml). This is an area which could use considerably more exploitation, but in the small amount of work so far done we have found rabbit sera which detect the Ig-1 specificities, 1.1, 1.2, and 1.5, and the Ig-3 specificity, 3.4.

Genetic Locus (Class)	Immunoelectrophoretic Arc of Fc Fragments (−) (+)		Ig Chromosome(s)*
Ig−1(γG_{2a})		fast	a, c, d, e, f, g, h
		slow	b
Ig−3(γG_{2b})		slow	a, b, c, f, g, h
		fast	d, e
Ig−2(γA)		slow	a, h
		intermediate	b
		fast	c, g
	not done		d, e, f
Ig−4(γG_1)		fast	a, c, d, e, f, g, h
		slow	b

* Defined by Ig−1 allele in type-strains: a=BALB/C, b=C57BL/10, c=DBA/2, d=AKR, e=A/J, f=CE, g=RIII, h=SEA.

FIG. 3. Electrophoretic mobility allotypes of Ig loci. The arcs are drawn to scale from immunoelectrophoretic patterns obtained from papain digests and class-specific antisera.

Common allotypic specificities on γG_{2a} and γG_{2b} immunoglobulins: evolutionary implications.—All of the allotypic specificities which we have so far described in this review are associated with only one of the various immunoglobulin classes. That is, they represent cross reactions between allelically differing proteins within each locus. However, three allotypic specificities have been found which are present both on γG_{2a} and γG_{2b} molecules (42). These γG2 common specificities, 1, 2 and 3, as we have called them, are found in the allele group type strains shown in Table VIII.

The evolutionary origin of common genetically polymorphic specificities on two different but closely related polypeptides poses an interesting puzzle. γG_{2a} and γG_{2b} probably arose by a gene duplication. Whether the duplication was followed by, or preceded by, mutational changes giving rise to the common specificities cannot be deduced from the present state. Each is possible. If duplication preceded mutation, then identical mutation(s) had to occur in both duplicated genes. If mutation leading to the polymorphism occurred first, then gene duplication had to occur on at least four different chromosomes, that is on four separate occasions, to give the four chromo-

TABLE VII

ASSOCIATION BETWEEN ELECTROPHORETIC MOBILITY ALLOTYPES AND SEROLOGIC ALLOTYPES (44)

Genetic Locus (class)	Cross*	Allotypes of Progeny	Fc Electrophoretic Mobility†		
			Fast	Fast-Slow	Slow
Ig-1 (γG_{2a})	$\frac{Ig\text{-}1^a}{Ig\text{-}1^b} \times \frac{Ig\text{-}1^a}{Ig\text{-}1^b}$ Fast/Slow × Fast/Slow	$Ig\text{-}1^a/Ig\text{-}1^a$	12	0	0
		$Ig\text{-}1^a/Ig\text{-}1^b$	0	12	0
		$Ig\text{-}1^b/Ig\text{-}1^b$	0	0	12
			Slow-inter		Fast-inter
Ig-2 (γA)	$\frac{Ig\text{-}2^a}{Ig\text{-}2^c} \times \frac{Ig\text{-}2^b}{Ig\text{-}2^b}$ Slow/Fast × Inter	$Ig\text{-}2^a/Ig\text{-}2^b$	17		0
		$Ig\text{-}2^c/Ig\text{-}2^b$	0		13
			Slow		Slow-Fast
Ig-3 (γG_{2b})	$\frac{Ig\text{-}3^c}{Ig\text{-}3^d} \times \frac{Ig\text{-}3^b}{Ig\text{-}3^b}$ Slow/Fast × Slow	$Ig\text{-}3^b/Ig\text{-}3^b$	16		0
		$Ig\text{-}3^c/Ig\text{-}3^b$	0		20

* Strains used in crosses:

γG_{2a} and γG_1—(C3H.SW/SnHz × C57BL/10SnHz)F_1 ⊗ ,
(BALB/cJ × C57BL/10J)F_1 ⊗

γA —(B10.D2/SnHz × DBA/2J)F_1 × C3H/HeJ

γG_{2b} —(DBA/2J × AKR/J)F_1 × C57BL/10J

† Relative electrophoretic mobilities within each class (i.e. fast, slow, intermediate) were scored independently of other classes with the aid of class specific heteroantisera. A cross for each class was chosen where the allotypic differences were associated with different electrophoretic mobilities of Fc fragments. From progeny of these crosses, a group of animals previously scored for allotype were chosen for mobility studies such that approximately equal numbers of each progeny genotype were included.

somal types defined by the combinations of γG2 common specificities shown in Table VIII.

In the rabbit allotypes on the Fd end of the heavy chains, a similar finding of common specificities in three H chain classes is known (50). There have been reports that the allotypes on γG and γM are identical in the rabbit (50), but the most recent evidence shows that they are not identical but crossreacting (51). That is, they share some but not all specificities. In the section on variable regions we shall return to this point. In both the rabbit and mouse examples, we can predict that lengths of amino acid se-

TABLE VIII

ALLOTYPIC SPECIFICITIES COMMON TO MOUSE γG_{2a} AND γG_{2b} IMMUNOGLOBULINS (42)

Type Strains	Allele	γG_2 Common Allotypic Specificities		
BALB/c	a	1	2	3
C57BL/10	b	1	–	–
DBA/2	c	1	2	–
AKR	d	–	2	3
A/J	e	–	2	3
CE	f	1	2	3
RIII	g	1	2	3
SEA	h	1	2	3

quences common to the classes involved will be found to have the amino acid substitutions responsible for these allotypic specificities. Indeed, in the mouse, we already have suggestive evidence that the genetic polymorphism is localized in that part of the Fc end of the H chain near the papain cleavage point. This is also the end which is probably identical in γG_{2a} and γG_{2b} (52).

L-CHAIN TYPES

Two types of L chains, kappa and lambda, are found in most higher vertebrate species. These types have different constant regions in the C terminal half of the respective chains. In addition, there are correlated differences in the N terminal halves (variable regions) of each type of chain. (See below.) When both types of chains are present in a species they are found associated randomly with the different H chains and therefore found in all the classes of immunoglobulins. Similarly they are not restricted, in general, to antibodies of any particular specificities. In man, all classes have about 60 per cent kappa and 40 per cent lambda chains (10, 26). In the mouse, the evidence so far available indicates that the vast majority of light chains are of the kappa type. However, lambda type chains have been found in several pathological γM proteins and one urinary Bence-Jones protein in the mouse (53).

VARIABLE REGION AND GENERATION OF DIVERSITY

As stated in the introduction, a real task for the geneticist looking at immunoglobulin synthesis is the invention of a theory consistent with modern molecular biology which can explain how the genome can code for immunoglobulin polypeptide chains so that they come out the same every time on one end (constant region) but different every time on the other (variable region). For detailed description, criticism, and reasons for preferences of the various speculations on the mechanism of the generation of

diversity of the variable region, we suggest the reader consult one or all of several excellent recent reviews and symposium volumes on the subject (3–5, 5a, 10). The facts with which the hypothesis on the variable region must be consistent follow. Space will not permit us to discuss these theories extensively, but we will give some outlines to serve as an introduction.

Most of the sequence data now available comes from analyzing individual L chain species produced by human and mouse plasma cell tumors (12, 19, 54–58). The tacit assumption that these proteins are random samples of normal L chain sequences has been only partially validated. The variation from L chain to L chain within an L-chain type, is found only in the N terminal half (v region). Several statements which describe the variation can be formulated:

(*a*) The v^{κ} region (variable region in κ chains) varies in length from 105 to 111 residues; thus, it is approximately the same length as the kappa constant region (c^{κ}, 107 residues) (55). (*b*) Some 50 to 60 per cent of the residues in v^{κ} do not vary; these residues are scattered throughout the v^{κ} region. (*c*) Of those residues which do vary, the range of amino acid substitutions found at any given residue is very restricted; in order of rapidly decreasing frequence, two, three, four, and five amino acids have been found at particular residue positions. (*d*) In terms of base changes (using the *E. coli* genetic code) needed to relate the known amino acid substitutions linearly, the largest number require only one base change to go from one amino acid to a second, a few require two base changes and very few require three. In the example in which there are three possible amino acids at a particular residue, by choosing the right residue as precursor only one base change will be required to go to the other two. Of course, when there are four or five amino acids at a particular position, no matter what amino acid is considered to be the precursor, at least two sequential substitutions at one base are required to go from this precursor to the fourth amino acid and three substitutions to go to the fifth amino acid. (*e*) Similar statements pertain to the sequences found for lambda type L chains. But v^{λ} is quite clearly different from v^{κ} although greater-than-random identities between v^{κ} and v^{λ} are observed (12, 16). (*f*) Much less data on the v regions of heavy chains is available, but what there is suggests that the variation follows the same rules. Further, separate sets of v sequences for at least mu and gamma (γM and γG heavy chains respectively) are suggested by the as yet scanty data (59). Both of these would differ from v^{κ} and v^{λ}, if these preliminary conclusions are confirmed. (*g*) Inspection of the some 35 v^{κ} partial sequences so far obtained reveals the possibility of division of the v^{κ} into three or four (sub)classes of sequences (3, 58, 60–62). The classification is based on the finding that a certain sequence in one part of a polypeptide is highly correlated with another sequence further down the chain. The substitutions within a class are considerably decreased from the substitutions within all the v^{κ} polypeptides. For example, two v^{κ} sequences of class I differ on average at only about 7/107 residues.

In addition to the facts presented above, there have been some commonly accepted although not rigorously proven views of how antigens act (these views are discussed in later sections): (*a*) Antigen does not influence the determination of the structure of the polypeptide chains; it acts to select either at the cellular or subcellular level among various sequences generated independently (63); (*b*) the unit for selection is the cell itself, which has been committed to produce a single immunoglobulin species with a single light and single heavy chain variable sequence; (*c*) antigen acts to select among these committed cells by causing those which react with it to proliferate and differentiate to clones of cells producing complementary antibodies (64, 65); and (*d*) antigen is not needed and is not present in the mature antibody-producing cells (plasma cells) (66, 67).

Some of the questions that the various models try to answer are:

(*a*) Does diversity arise by evolution in development of the species, or via somatic mutation in development of the individual? In other words, is there one copy or many varied copies of each v class of cistron (or ½ cistron) in the gamete's (haploid) chromosome set?

(*b*) Is every v region in the genome attached to a c, or are v and c carried as chromosomally separate genes?

(*c*) Is the v DNA base sequence (or set of sequences, not to prejudge the answer to question *a*) different for each c gene?

(*d*) Does the generation of diversity occur by the usual kinds of gene mutations (such as base substitutions and deletions), by ordinary or special crossing-over mechanisms, or are there mechanisms evolved especially for creating variation in the v region?

A priori a constant amino acid sequence suggests a single gene. The discovery of allotypes and the finding that in at least one case the allelic products differ only by one amino acid substitution (68), and probably only one base substitution, is taken as strong evidence for only one c gene for each class. The argument is that if there were many c genes, crossing-over in allotype heterozygotes would quickly lead to loss of the genetic polymorphism. If some absolute bar to crossovers is postulated and accepted for argument's sake, the conclusion is not changed because mutation would have the same effect and also would lead to divergence, i.e., generation of many different c regions.

The crossing-over argument cannot yet be used for deciding whether there are one or many germ-line v genes. However, more L and H chain sequences may reveal allotypes in the v regions which would then permit us to conclude that there is only one v gene per class. Without v region allotypes one could argue that the invariant portions of the v region are essential structural requirements for functional antibodies, but allotypes, as we argued for the c region, point to one v gene. There are already indications that allotypic differences in the N terminal quarter (v region) of the rabbit H chain exist (41, 69).

It is clear that v^{κ} and v^{λ} regions are distinguishable (12, 54). A v^{κ} se-

quence can be recognized as such without reference to the c^{κ} part of the sequence. The recent data, alrady mentioned, brought to our attention by L. Hood, are consistent with concluding that there are different v genes for each kind of c. The N terminal sequences of human γG heavy chains are variable but different from those of kappa, lambda and γM heavy chains (59). These H chain differences are based on very short sequences, and though quite suggestive of distinct v^{λ} and v^{μ} chains, we must await further sequence data before a final conclusion is reached.

There are also other data suggesting different v regions for the different H chain classes. Earlier we referred to Todd's recent finding that the rabbit Fd allotypes for the three H chain classes are cross reacting but not identical (51). Localization of the allotypic amino acid substitutions to the v regions would indicate different v regions. Indications that the sizes of γM combining sites are considerably smaller than γG combining sites for the antigens (70) also suggest different v regions for different classes.

If the evidence for one v per c in H chains is finally conclusive, there is no need to postulate separate v and c cistrons (4) and we can retain the concept of a single v-c gene for each class of chain. This would solve certain plaguing problems. For example, if v and c genes are chromosomally separate, some sort of translocation must put them together, since we know that the chain is made in one piece on one messenger (6, 71). Another mechanistic problem would be to devise translocation mechanisms specific for each v-c pair since each v is always associated with a particular c region.

If there are v-c genes, generation of diversity occurs only on half of each gene. What genetic diversifying mechanisms can be so specific? A recombination accelerator gene which increases intracistronic recombination in a single structural gene is known in *Neurospora* (72). A v-region specific DNase, combined with an error-prone repair enzyme, has also been proposed to explain the generation of diversity in a single v-c gene (73). Smithies has proposed several recombination theories (74). The most recent calls for two v regions (for each c gene) which recombine and thereby diversify the v and not c parts of a single (master) gene. Of course, confirmation of the demonstration of allotypes in the v region argues against any more than one v gene, even two. A reasonable argument for some sort of crossover mechanism is the greater ease of genetically determining the kind of variation without introducing unacceptable variations incompatible with function. Crossing over more easily maintains constant sequences (which are rather common in the variable regions) than would random mutations.

It should be clear from the foregoing comments that the available evidence does not permit a clear choice between single v-c genes for each class, or separate v and c genes. Whereas further allotype evidence may settle this point, this alone will not permit us to understand generation of diversity. The weight of evidence now available favors single v-c genes for each class, with the inescapable corollary that generation of diversity is a somatic process.

Cellular Genetics and Differentiation in the Immune Response

Regardless of the mechanism for creating many variable regions, it is clear that a specific antibody response requires a selective increase in the production of certain variable regions. The selective agent is antigen, apparently acting at precise stages in the differentiation of antibody producing cells. The cellular genetics of differentiation and the stage at which antigen acts will be discussed in the succeeding sections.

Allelic exclusion.—The evolution of the mechanism for the generation of diversity may be responsible for the immune system flaunting a general rule of cellular gene expression. Although the immunoglobulin loci are autosomal, individual cells producing immunoglobulins do not express both parental alleles. An antibody producing (plasma) cell in a heterozygote or homozygote produces only a single molecular species of immunoglobulin: a molecule with two H chains of the same class; two L chains of the same type; a single allotype of its H chains; a single allotype of its L chains; and a single variable region for each pair of chains (75–79). In other words, differentiation to antibody production involves not only the usual developmental commitment of a cell with respect to locus, but also the commitment to one of the two alleles at that (H chain, or L chain) locus with the exclusion from function of the other allele and the concomitant commitment to a particular H chain and a particular L chain variable region.

Allelic exclusion was first suggested after the finding that human myeloma proteins from individuals heterozygous for immunoglobulin structural genes do not have both parental allotypes, although the normal spectrum of immunoglobulins in the individual's blood has both allotypes represented (80). In humans, where H chain and L chain allotypes are known, no correlation was found between the parental H and L chain alleles expressed on the protein. In all well-studied cases, only one allotype of one locus for each chain has ever been found in a single myeloma protein (81). Studies with rabbits showed that normal immunoglobulins follow the same rules: by sequential precipitation of radioactively labelled globulins it was shown that the immunoglobulins of animals, heterozygous at a single locus, are a mixture of the immunoglobulins of the two parental homozygous types; hybrid molecules were not found. In the same study it was shown, however, that hybrid molecules could be formed *in vitro* by reduction of the intra H chain disulphide bond, mild acidification, and reneutralization. Thus, the absence of hybrid molecules *in vivo* is not due to a protein structural limitation on their formation (82, 83).

Suggestive as the protein results were, however, it required the demonstration that single plasma cells from normal heterozygous rabbit spleens always reacted only with one of the two anti-parental allotype sera with which they were treated, to establish firmly the existence of allelic exclusion at the cellular level (75).

The point in cellular differentiation at which the choice of the allele to

be used and the allele to be excluded is not recognized. It is possible, as with X-chromosome inactivation (84), that early in development one or the other of the chromosomes, or segments of chromosome, carrying H chain loci (and similarly for L chain loci) is irreversibly inactivated and that immunoglobulin mosaicism proceeds from this inactivation. Alternatively, there may be a stage in antibody production when the cell makes chains of each allotype, perhaps with different variable regions, and allelic exclusion occurs as part of the process of selection of the type of variable region to which the cell line will become committed.

A possible mechanism of allelic exclusion is that in the process of programming a variable region into the destiny of an antibody producing cell at the point of generation of a variable region, only one of the two c alleles can be used. Which c (class) locus is chosen may be an independent differentiational event, and which allele is chosen may be a random event. Once the choice is made, however, differentiation proceeds to the next step.

Suppression of allotype production.—Perhaps answers to some of the questions raised in the last section will result from current studies on suppression of allotype production by antiallotype antisera. It has been shown, first with rabbits (85) and later with mice (86), that allotype heterozygous offspring born of mothers immunized to the paternal allotype are suppressed in the production of immunoglobulins carrying the paternal allotype. Production of the maternal allotype is not decreased. Thus, genetically a/b rabbits may be rendered phenotypically a/- for a period of weeks, months, or even years.

Homozygous mice are also suppressed by exposure to anti-allotype antibody (86). In this case, because mice receive so much immunoglobulin by nursing, it is necessary to transfer newborns, before they can suckle on their natural mother, to a foster mother of an allotype which will not react with the antiallotype serum. Transfer to a foster nurse making anti-allotype antibody results in suppression of allotype production, while transfer to non-immune foster nurses does not affect allotype production.

This suppression of homozygotes yields animals effectively agammaglobulinemic for the class of immunoglobulins which carry the allotype. For example, when Ig-1b is suppressed, homozygotes are suppressed for all γG_{2a}-immunoglobulin production. Thus, in distinction to heterozygotes which go phenotypically from a/b to a/-, homozygotes go phenotypically from b/b to -/-.

Suppression is not restricted to the neonatal period. Anti-allotype serum, injected as late as 21 days after birth, still causes demonstrable suppression of allotype production (44). By this age, mice have begun synthesis of allotypes, although they have little enough in circulation so that small amounts of injected anti-allotype antisera are not completely inactivated. The suppressing antiserum does not appear to attack the plasma cells, which are already committed to full scale allotype production, but, as reflected in the rate of increase of serum allotype levels, appears to interfere with the dif-

ferentiation of new cells into the plasma cell pool wherein immunoglobulin carrying the allotype is synthesized.

Thus, returning to the model for allelic exclusion, we picture the anti-allotype serum as reacting with the cell at a point after it has become committed to a unique immunoglobulin molecule but before it has met its antigen and been stimulated into the plasma cell differentiative pathway. At this point, an antigen-sensitive cell would have one or more of its immunoglobulin molecules placed on the surface of the cell in such a way that differentiation to plasma cells would occur on combination with the appropriate antigen. Combination with antiallotype antibody, however, effectively prevents an antigen-sensitive cell from differentiating to a plasma cell.

Evidence obtained on allelic exclusion and suppression, using indirect sheep-erythrocyte hemolysis (facilitated lysis) in gels, which enables identification of the class and allotype of specific antibodies produced by individual plasma cells (76, 86a, 87, 88), is consistent with this model. When heterozygous ($Ig\text{-}1^a/Ig\text{-}1^b$) animals, suppressed for Ig-1b, were immunized with sheep erythrocytes, no responding cells were found which produced antibody of the Ig-1b allotype. In the same animals, there appeared to be no difference in the number of cells producing Ig-1a antibody. That is, suppressed animals had one-half the number of responding cells of the γG_{2a} class (89). Thus, the antiallotype serum prevented the phenotypically Ig-1b sheep-erythrocyte antigen-sensitive cells from differentiating to a clone of antibody exporting (plasma) cells.

Whether the antigen-sensitive cell is already committed to one allele (i.e. that allelic exclusion has already occurred at this point) has been investigated recently by examining small pieces of spleens from sublethally irradiated $Ig\text{-}1^a/Ig\text{-}1^b$ heterozygous mice immunized with sheep erythrocytes. Some of these pieces had both Ig-1a and Ig-1b cells but others had only Ig-1a or Ig-1b antibody producing cells (90). It remains to be established that these latter pieces contain single clones, but if they do they presumably arise from individual antigen-sensitive cells which were already committed to a or b.

Genetic Control of the Immune Response to Specific Antigens

The immune response to a specific antigen is a phenotypic marker for an extremely complex process that begins with the initial introduction of antigen into the animal, and ends with the production either of sensitized cells or of circulating antibody directed against that antigen. Genetic control must be exerted at many steps in this process, and careful study of these genetic controls may uncover previously unsuspected steps.

In the present state of our knowledge, a precise classification of the different levels at which genetic controls could affect the immune response is impossible. We are, in fact, only at the stage of searching for genetic defects which will permit us to delineate these steps, or more or less where biochemical genetics was in 1940. These defects may be in immunoglobulin

structural genes, in other genes within the responding cells, in genes controlling the handling of antigen, or possibly in genes which are not directly concerned with the immune response but control processes leading to the destruction or sequestration of the antigen.

A large number of genetic differences in the immune response to specific antigen have been described without any attempt to analyze the mechanisms of gene action. We shall discuss in more detail those systems in which there has been some attempt to work out the nature of the genetic control. For the sake of convenience, the subject has been divided into genetic differences in the response to synthetic polypeptide antigens, and similar differences in response to more complex antigens, such as proteins, polysaccharides, and viruses.

Synthetic polypeptide antigens; the immune response of mice to branched synthetic polypeptides.—The ability of inbred strains of mice to make antibodies to branched, multichain, synthetic polypeptide antigens bearing a restricted range of antigenic determinants is a quantitative genetic trait (91). This trait appears to be governed to a large extent by a single autosomal locus, which can be designated Ir-1 (Immune response 1).

The antigens used in these studies are built upon backbones of poly-L-lysine, with side chains of poly-D,L-alanine, giving a branched polypeptide (A--L) which is not antigenic. Addition of short, random sequences of tyrosine and glutamic acid to the terminal amino groups of the poly-D,L-alanine side chains converts A--L into (T,G)-A--L, to which CBA mice respond poorly while C57 (C57Bl/various sublines) mice respond well. Substitution of histidine for tyrosine in the side chain terminal, to give (H,G)-A--L, leads to the opposite result; C57's respond poorly, and CBA's respond well (91, 92). When phenylalanine is substituted for tyrosine, to give (P,G)-A--L, both strains respond well (See Table IX). The F_1 hybrid (C57 × CBA) responds well to all three antigens. Backcross progeny segregate in response as a 1 : 1 mixture of F_1 and the respective homozygous parent animals.

There is no significant quantitative difference in distribution among the immunoglobulin classes of the antibodies produced to these antigens in dif-

TABLE IX*

RESPONSES OF CBA AND C57 MICE TO SEVERAL SYNTHETIC POLYPEPTIDE ANTIGENS

Antigen	Antibody response	
	CBA	C57
(T, G)-A—L	low	high
(H, G)-A—L	high	low
(P, G)-A—L	high	high

* Adapted from (92).

ferent strains of mice. In a segregating backcross population, no linkage was found between Ir-1 and the Ig region, indicating that response is not associated with the known structural genes coding for the heavy chains (93).

We have recently shown that the Ir-1 locus is closely linked to the major histocompatibility (H-2) locus and is thus localized in the IXth mouse-linkage group (94). As will be seen below, this surprising finding may have great importance in understanding the mechanisms of action of this gene. This linkage was initially suggested when C3H.SW mice responded well to (T,G)-A--L despite their being congenic with C3H, a low-responder strain. The C3H.SW strain (developed by Snell) differs from C3H only at the H-2 locus and some of the surrounding chromosome region. Linkage was established in a backcross test (see Table X) in which 14 of 15 (CBA × C57) F_1 × CBA mice which responded well to (T,G)-A--L carried the $H\text{-}2^b$ allele, while 22 of the 23 low-responders did not carry the $H\text{-}2^b$ allele. Similar linkage was found between the ability to respond to (H,G)-A--L and the $H\text{-}2^k$ allele. Of the three putative recombinant animals, only one has been fertile in a progeny test. Progeny testing of this animal shows that it was not, in fact, a recombinant. Further testing of backcross populations is required, but at the present there is no conclusive evidence that Ir-1 and H-2 are not identical.

The results of testing a large number of different inbred mouse strains of several different H-2 types for their ability to respond to these three syn-

TABLE X*

LINKAGE OF (T,G)-A--L RESPONSE TO $H\text{-}2^b$†

(CBA×C57) F_1×CBA	$H\text{-}2^b$ Positive	$H\text{-}2^b$ Negative
Responders		
8 (21–75%)†	8	0
7 (22–74%)	6	1
15	14	1
Nonresponders		
10 (2–12%)	0	10
13 (0%)	1	12
23	1	22

Total recombinants = 2/38

* Taken from (94).

† Antibody response is given in terms of "average percentage of antigen bound" in an antigen-binding capacity assay. The figures do not represent percentage animals responding.

thetic polypeptide antigens (Table XI) show a regular correlation between H-2 type and response. The variety (at least five) of patterns of response of different strains of mice to these antigens indicates either that there are

TABLE XI*

ANTIBODY RESPONSE OF INBRED MOUSE STRAINS TO A SERIES OF SYNTHETIC POLYPEPTIDE ANTIGENS

Strain‡	H-2 allele	(T,G)-A--L	(H,G)-A--L	(P, G)-A--L
A	a	low	high	high
A.BY	b	high	—	—
C57	b	high	low	high
C57BL/6	b	high	—	—
C57BL/10	b	high	—	—
C57L	b	high	—	—
D1.LP	b	high	low	high
C3H.SW	b	high	low	high
BALB/c	d	medium	low	low
C57BL/ks	d	medium	—	—
B10.D2	d	medium	—	—
DBA/2	d	medium	low	low
WH/Re	d	medium	—	—
CBA	k	low	high	high
C3H	k	low	high	high
C57Br/cd	k	low	—	—
C58	k	low	—	—
B10.BR	k	low	high	high
AKR	k	—	medium-high	medium-high
DBA/1	q	low	low	high
H-2^q linkage stock†	q	low	low	high
RIII	r	low	—	—
SJL	s	low	low	low
A.SW	s	low	low	low
WB/Re	w	low	—	—

* McDevitt, H.O. Unpublished data.

† Special H-2^q, tufted, short-tail linkage stock kindly supplied by Dr. Margaret Green.

‡ All strains were obtained from the Jackson Laboratories, except C57, which was originally obtained from the National Institute for Medical Research, Mill Hill, London.

multiple loci controlling the ability to respond to these antigens, or that there are multiple alleles (five or more) at a single locus, Ir-1.

The remarkable association between the H-2 allele and pattern of response is consistent with close linkage between Ir-1 and H-2 since the inbred mouse strains are a small sample from a population not in genetic equilibrium, or with identity of Ir-1 and all or part of H-2. Since the H-2 locus is large and complex, identity does not necessarily mean that the observed genetic differences in immune response are due to the presence or absence of particular H-2 specificities. However, the possibility must be considered that a cell's own complement of surface antigens may predetermine the type of exogenous antigens with which it may interact. An effect of this sort could conceivably be exerted upon any cell involved in the immune response.

The localization of Ir-1 in the IXth mouse-linkage group not only places it near H-2 but also near the genes controlling the thymus-leukemia antigen and serum substance, in short, in a chromosome region which controls many "self" antigens (95). This raises the possibility that this genetic control is, in fact, a form of "cross-tolerance" in which the animal or strain which responds poorly to a particular antigen does so because it shares antigenic determinants on its own self-antigens with the particular synthetic polypeptide antigen. This possibility is ruled out for several reasons: (*a*) the F_1 of a cross between low-responder and high-responder animals responds well, although it would be expected to respond poorly since it would contain the self-antigens of both parental strains; (*b*) C57 anti-CBA ($H\text{-}2^b$ anti-$H\text{-}2^k$) antisera do not bind (T,G)-A--L; and (*c*) when embryonic (fetal liver) potentially immunocompetent cells are transferred from C57 embryos into lethally irradiated CBA recipients, the recipients are subsequently capable of responding well to (T,G-A--L (96). Since the embryonic C57 cells would have been rendered tolerant of CBA antigens, this effectively rules out the possibility that cross-tolerance is the mechanism of action of the Ir-1 locus.

The fact that the response of strains carrying different Ir-1 alleles is specific for the amino acid composition of the side chain termini (i.e., is structure-specific), is compatible with an effect of Ir-1 on the structure of the variable region of specific antibodies, but is equally compatible with effects on other steps in the immune response, or steps unrelated to antibody formation. We have tentatively concluded from the following cell transfer studies that the mechanism of action of the Ir-1 locus is directly related to the process of antibody formation rather than due to prior antigen destruction or sequestration.

When normal spleen cells were transferred from a (C3H × C57B1/6) F_1 high-responder strain into C3H recipients, lethally irradiated to avoid immune reactions against the transferred cells, the recipient animals were then capable of responding well to (T,G)-A--L. The genetic difference between high-responder and low-responder strains thus appears to be a property of one of the cell types contained in the mixed spleen cell population.

Studies are currently in progress utilizing purified cell populations and Ig region allotype markers to determine the cell type which transfers a particular Ir-1 phenotype and to determine the source of the antibody in the recipient animals, which are cellular chimeras for both the Ir-1 and the H-2 loci.

In summary, the evidence so far obtained is compatible either with an effect of Ir-1 on the processing of antigen, on the selection of specific antibody producing cells, or on the structure of the variable regions. Further understanding of the mechanism of action of the Ir-1 locus may come from cell transfer studies utilizing allotype markers, and studies of the affinity of antibodies produced against these synthetic polypepetide antigens in different strains of mice.

Synthetic polypeptide antigens; the immune response of mice to linear synthetic polypeptides.—Linear polymers of a single amino acid and random copolymers of two amino acids are not immunogenic in mice, but copolymers of three amino acids are immunogenic in most strains. It has recently been shown that a random copolymer of glutamic acid and lysine which contains only 5 per cent alanine (GLA_5) induced an immune response in 47 per cent of random-bred Swiss mice (97). The results of matings between responder mice and between nonresponder mice indicated that the ability of mice to respond to GLA_5 is governed by a single autosomal dominant gene.

All C3H and BALB/c mice respond to GLA_5, but no C57B1/6, A, or CBA mice do so. All Swiss mice and all of the above inbred strains respond to GLA_{10}, a random linear copolymer similar to GLA_5 but containing 10 per cent alanine. The mechanism by which this gene affects the immune response to GLA_5 is not yet known. However, the pattern of response of several inbred strains of mice already indicates that this gene is not Ir-1, since C3H and CBA strains have the same response to the branched polypeptides discussed in the previous section, but differ in their ability to respond to GLA_5. It is therefore likely that a second gene affecting the same or different steps in the immune response has been identified.

Synthetic polypeptide antigens; the immune response of guinea pigs to poly-L-lysine. The immune response of guinea pigs to poly-L-lysine (PLL) and hapten conjugates of poly-L-lysine (H-PLL) is governed by a single autosomal dominant gene, the PLL gene (98). The step in the immune response that this gene affects is not known, but a systematic study being carried out by Benacerraf and his colleagues has already narrowed the possibilities.

Responders (including all strain-2 guinea pigs) make antibodies against any hapten, such as the dinitrophenyl group, conjugated to PLL, while nonresponders (including all strain-13 guinea pigs) fail to do so (99). Similar results are found with G,L (a linear copolymer of L-glutamic acid and L-lysine), although antibodies to hapten-PLL and G,L do not cross-react. Nonresponder animals also fail to respond to a variety of linear and branched polypeptide antigens containing lysine (100). This suggests that

the PLL gene is not directly concerned with the determination of the antibody combining sites (variable regions) but acts either at a step concerned with antigen handling, or possibly via a mechanism not generally related to the process of antibody formation, e.g., the presence, or absence, of an enzyme hydrolyzing PLL and related polymers, although such an enzymatic difference has not yet been detected (101).

Subsequent experiments support this interpretation. Although genetic nonresponders are incapable of immune response to PLL or its hapten conjugates (H-PLL), they respond quite well to H-PLL when complexed with negatively charged (acetylated) foreign albumins (102). The anti-H-PLL antibodies produced in this case apparently have the same specificity for H-PLL as those produced by responder guinea pigs immunized with H-PLL alone. However, the entire immune response is not the same in the two situations. Genetic responders immunized with H-PLL alone produce not only circulating antibodies but also cell-bound immunity (delayed hypersensitivity) to the conjugate and lymphocytes which are capable of being stimulated to synthesize DNA by incubation with the H-PLL conjugate. On the other hand, genetic nonresponders immunized with H-PLL-albumin complexes, although they make circulating antibodies, do not develop delayed hypersensitivity to the H-PLL conjugate or produce lymphocytes which can be stimulated to synthesize DNA by exposure to this conjugate (102, 103).

These differences suggest that in nonresponders the H-PLL acts only as a hapten added onto the acetylated albumin and not as an independent antigen. This interpretation is strengthened by the finding that in nonresponders, previously made immunologically tolerant (unresponsive) to the acetylated albumin, no antibodies are made either to the albumin or to the H-PLL conjugate (103). It is possible that the antibody made in nonresponders to H-PLL complexed with foreign albumin is actually a qualitatively different antibody, despite the fact that all tests for specificity to date indicate that it is the same as that made in responder guinea pigs.

Cell transfer experiments have provided more conclusive results (104). In these experiments, lymph node cells from responders immunized with H-PLL, or H-PLL complexed with a negatively charged foreign albumin, were injected into random-bred Hartley-strain guinea pigs. One hour later the animals were skin tested, for delayed hypersensitivity, with H-PLL. The recipient animals were subsequently immunized with H-PLL to determine whether they were genetic responders. Delayed hypersensitivity to H-PLL conjugates could be transferred successfully only by means of immunized cells from genetic responder guinea pigs, and in almost all cases only to genetic responder guinea pigs. The failure to transfer this cell-bound immune reaction to nonresponders was not due to early destruction of the transferred lymph node cells, since almost all genetic nonresponders, as well as the genetic responders, had delayed hypersensitivity reactions to the complexing albumin.

This result indicates that in nonresponders PLL antigens are either

destroyed, bound, or cannot react with the transferred, previously immunized, responder lymph-node cells due to the lack of an essential processing step. Thus, the PLL gene acts in some way concerned with the handling of poly-L-lysine antigens.

Complex antigens.—Several studies have indicated that the immune response to a wide variety of complex antigens, such as diphtheria antitoxin, insulin, bovine serum albumin, viruses, and red cells, is under some form of genetic control (105, 106, 107, refs. in 91). The great number of antigens for which a genetic difference in immune response has been demonstrated suggests that many genes will be found to interact in immune systems. Thus, genetic differences in capacity to make antibodies are not restricted to synthetic antigens but may be important in defense against natural antigens, for example infectious agents. It has been known for many years that it is possible to breed guinea pigs for the ability to respond or not to respond to diphtheria toxoid and in recent years similar findings have been described for the ability of rabbits and mice to respond to bovine serum albumin (106). In these studies, three to five generations of selective breeding were required to obtain populations with a uniform response or lack of response, a result which suggests that more than one gene was involved.

The existence of genetic differences in immune responses to a wide variety of specific antigens suggests that: (*a*) there are a large number of genes which are specific for particular antigens and which affect the immune response at a similar step; or (*b*) there are genes which affect the immune response at a large number of different steps; or (*c*) there are genes which affect the structure of particular variable regions and result in the ability or inability to synthesize antibodies of a particular specificity; or (*d*) all of the above may be true. Unfortunately, there is very little evidence available to decide among these possibilities. One system which may involve a qualitatively different antibody response under gene control has been under study (105): two inbred strains of guinea pigs and their F_1 and F_2 hybrids apparently show differences in antibodies made to particular antigenic sites on insulin molecules. Both genetic and immunological complexities, due in part at least to the assay system employed, make interpretations of the mechanisms of gene action difficult.

Concluding Comment

Concluding sections of reviews are frequently devoted to lacy speculations which wear less well than the supporting fabric. We have instead laced our speculations through the data sections in the hope that placing them there may enable the reader to see them for what they are worth. This leaves us little for a parting word. We end with the hope that we have conveyed the idea that immunology is a fertile field for the application of genetic principles and for the development of new genetic theory.

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