BACTERIOLOGICAL REVIEWS, June 1969, p. 159-171
Vol. 33, No. 2

Printed in U.S.A.

Immunoglobulins and Immunocytes[1]

JOHN J. CEBRA

Department of Biology, Johns Hopkins University, Baltimore, Maryland 21218

INTRODUCTION

Immunoglobulins are both polymorphic and polydisperse. That is, they can occur in many functionally and physico-chemically different molecular forms, which have been classified and typed. Molecules presently classed together are not homogeneous, but they show a micro-heterogeneity of amino acid sequence which is presumably related to their many known antibody specificities.

Figure 1 is a simplified schematic representation showing that all immunoglobulin (Ig) molecules are assembled of pairs of heavy and light polypeptide chains (14). Antigenic differences among heavy chains of human myeloma proteins—homogeneous immunoglobulins made by plasma cell tumors—were used to group these proteins into classes such as IgM, IgA, IgG, etc. (57). Differences among the light chains of these proteins detected serologically were used to divide immunoglobulins of each class into two main types, K and L (57). Presently, primary sequence data for certain portions of heavy and light chains characteristic of the classes and types are supplanting antigenic analyses as the principal criteria for grouping the immunoglobulins.

Genetically determined variants of a particular subclass of heavy chain or type of light chain are known to occur among the members of a species. By means of these markers, called allotypes, the close linkage of cistrons controlling heavy chains has been deduced from family studies and breeding experiments in man and mice, respectively (29, 35, 42). The cistrons determining synthesis of light chains appear to be not closely linked to the heavy-chain cistrons (17, 18).

Of concern in formulating mechanisms for the cellular control of Ig biosynthesis is the finding that individual Ig molecules appear to be homogeneous. That is, the heavy chains of a single molecule are always of the same subclass and the light chains of the same type, no matter how many pairs of these chains make it up. This homogeneity of single molecules even extends to the allotypes of heavy and light chains in animals

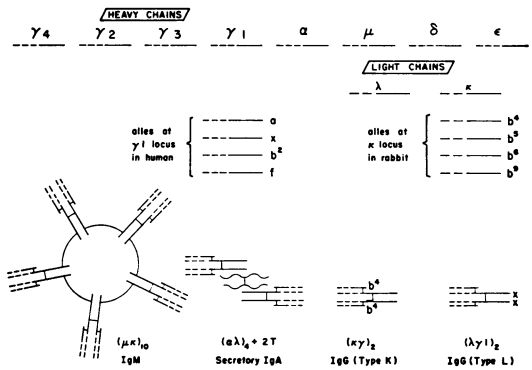


FIG. 1. *Schematic representation of some component polypeptide chains of immunoglobulins and some simple "stick" models indicating how a few of the polymorphic forms may be assembled. The diversity of classes and sublcasses of heavy chains and types of light chains shown has been established for human proteins. Examples of allotypic markers on subclasses of heavy chains and types of light chains are taken from human and rabbit proteins, respectively. The symmetry of complete molecules with respect to subclasses of heavy chain, types of light chain, and genetic markers is indicated. The marker "X" on human γ_1 chain stands for the antigenic sites "ax."*

heterozygous at the loci controlling both chains (16), as depicted at the bottom of Fig. 1.

The amino acid sequences characteristic of subclass, type, and allotype appear to be in sections of the Ig molecule which are constant for a particular polymorphic form. However, parts of both heavy and light chains appear to vary in a way which seems unrelated to poly-

[1] Eli Lilly Award Address, 1968.

morphic form but which could be the basis for antibody specificity. The variable sections are depicted as dashed lines in Fig. 1.

Over the past decade, many structural features of the several polymorphic forms of immunoglobulins have been established, and the terminal differentiation of lymphoid cells which synthesize these diverse molecules with different antibody specificities has been described. At present, considerable effort is now being directed toward positioning the antibody site in the molecule and correlating its sequence with specificity. Our laboratory has been concerned with several of these problems, and our own studies are probably typical of the many contributing to our present understanding of Ig structure and synthesis. Because the Eli Lilly Award this year recognizes immunochemical advances, I will describe three of our studies—at the gross molecular, fine molecular, and cellular levels, respectively—which illustrate many of the techniques that have been used productively by many immunochemists.

SECRETORY IGA

Gross Molecular Form

The first example concerns a secretory Ig from the rabbit which is homologous with the prototype human IgA protein. The kind, number, assembly, and cellular sites of synthesis of the polypeptide chains making up secretory IgA appear to be unique among immunoglobulins.

The rabbit secretory IgA was isolated from milk, on the basis of its size and charge properties, by gel filtration (Fig. 2), followed by cellulose ion-exchange chromatography (10). After establishing purity of this protein, it was rendered into its component polypeptide chains by reductive cleavage of all disulfide bridges in the dissociating solvent 6.7 M guanidine·HCl (11). The heavy polypeptide chain, α chain, could be separated from smaller chains by gel filtration in guanidine solvents (Fig. 3). The molecular weight (MW) of the intact protein was found to be about 385,000 whereas that of the α chain was 64,000 (11). From this data and from the overall recovery of α chains from the total bulk of IgA protein, the number of α chains per molecule was estimated at four or five. The yield of light chain was such that, if α and light chains were to be present in a simple whole-number ratio, its proportion relative to α chain was 1:1. Thus, the whole IgA molecule could accommodate only four pairs of α and light chains, or about 346,000 MW units, leaving about 40,000 units unaccounted for (11). The balance of the secretory IgA was found on disc-gel electrophoretic

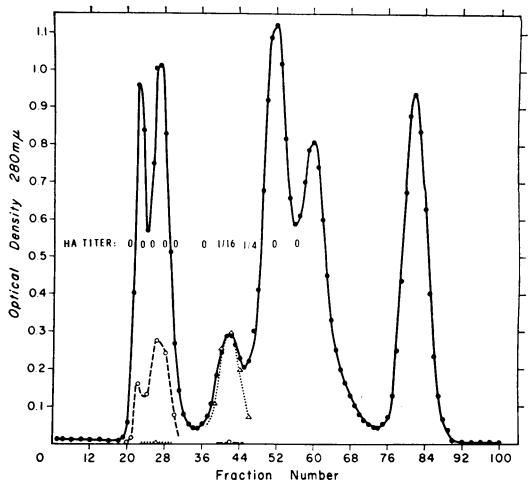


FIG. 2. *Gel filtration of clarified colostrum on Sephadex G-200 (10). Symbols: ●, optical density of the effluent; ○ and △, optical density of the dissolved precipitates obtained by adding 0.05 or 0.15 ml of selected fractions to 0.3 ml of anti-α or anti-γ chain, respectively.*

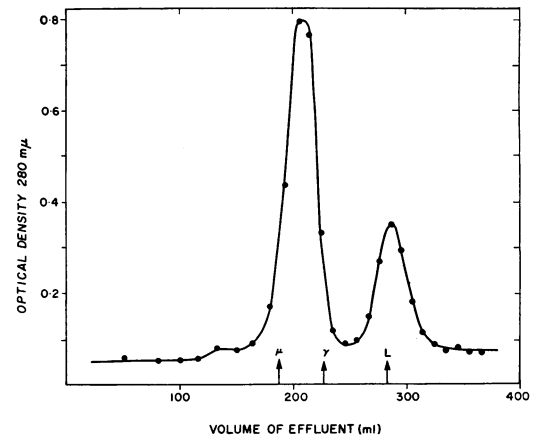


FIG. 3. *Gel filtration of totally reduced and alkylated colostral IgA in the presence of 5 M guanidine·HCl (11) on a column of Sephadex G-200. Positions of elution of the extensively reduced and alkylated polypeptide chains of other immunoglobulins are indicated by arrows as follows: μ, heavy chain of rabbit IgM; γ, heavy chain of rabbit IgG; L, light chain from either rabbit IgM or IgG.*

analysis of the pool of smaller, or "light chain-like," chains reductively dissociated from the molecule. This pool contains the usual charge polydisperse light chains found on all immunoglobulins and, in addition, a more homogeneous and more acidic component not found in IgG (Fig. 4). This "extra polypeptide," called "T

chain," is probably homologous to the "extra antigenic sites" found on human salivary IgA (28, 53) and dubbed "transport piece" (52). Most of the T-chains could be dissociated from the intact secretory IgA in 5 M guanidine·HCl without reduction and, presumably, were not covalently bound to the rest of the molecule (11). Figure 5 shows the separation of the T chains dissociated from the rest of the molecule and their recovery in "pool 2." This pool, when analyzed by disc gel electrophoresis (Fig. 4), was rich in T chain. After purification of T chain, its MW was found to be about 20,500 (Fig. 6). Thus, presumably, two T chains per molecule are required to account for the overall MW of the intact secretory IgA (46). Some of the characteristics of rabbit secretory IgA are as follows (10, 11, 46): sedimentation coefficient, 10.8*S*; extinction coefficient (0.1 N NaOH), 13.5; hexose content (1:1 ratio of galactose to mannose), 3.2%; hexosamine content, 3.2%; sulfhydral content, 0.75 mole/170,000 g; serum concentration (adult), 180 μg/ml. MW of secretory IgA is 385,000; α chain, 64,000; L chain, 22,500; T chain, 21,600. Figure 7 is a schematic

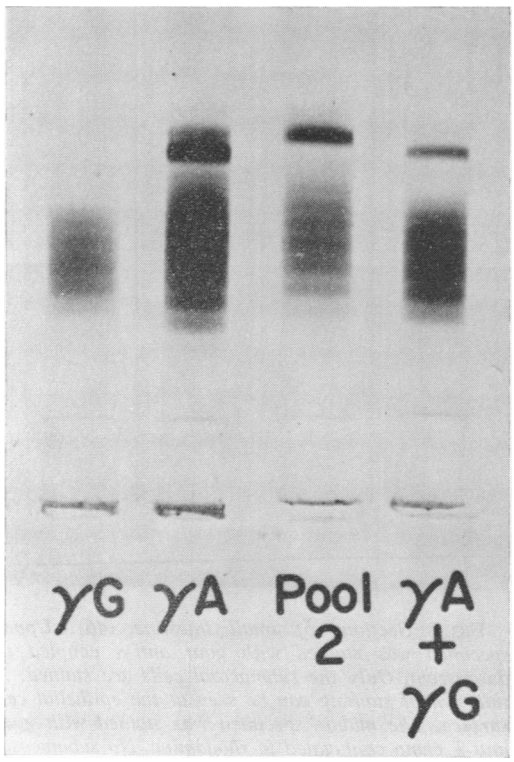


FIG. 4. *Disc electrophoresis in urea of light chains and light chainlike material derived from IgG and IgA after total reduction and carboxymethylation (11).*

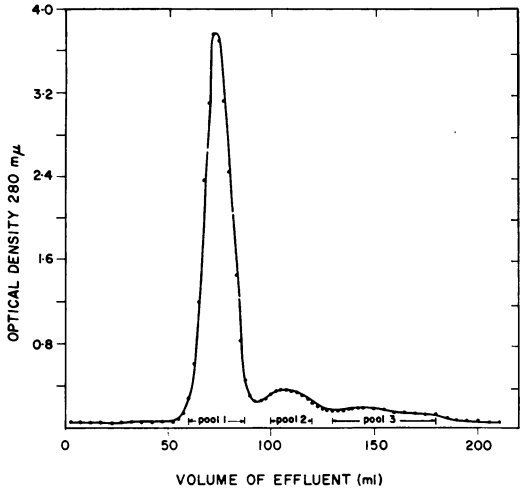


FIG. 5. *Gel-filtration elution pattern of colostral IgA dialyzed versus 5 M guanidine·HCl, 0.01 M in iodoacetamide, and passed through a column of Sephadex G-200 equilibrated with 5 M quanidine·HCl (11).*

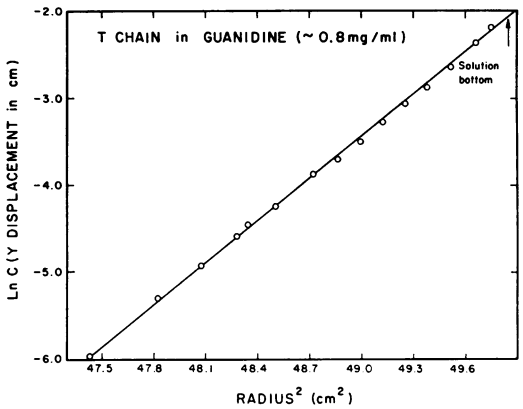
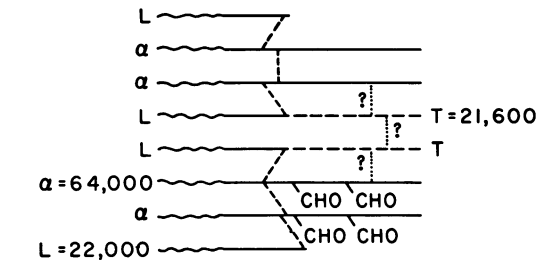


FIG. 6. *Plot of the logarithm of the vertical displacement from the base line of a fringe measured in centimeters versus distance from center of rotation squared (cm²), from a sedimentation equilibrium experiment in which 0.8 mg of purified T-chain per ml in 5 M guanidine·HCl was used (46).*

diagram of the molecule showing the number and possible arrangement of the chains. The T chain is shown noncovalently associating with the Fc portion of α chain only because this former component is not known to combine with any other Ig and because the class-specific region of Ig is generally localized in Fc. The location of carbohydrate moieties is based on analyses of human IgA (33). A suggestion that T chain may covalently bind to α chain secondarily through disulfide interchange reactions is represented by the dotted lines joining T and α chains in Fig. 7.



SECRETORY IMMUNOGLOBULIN-A

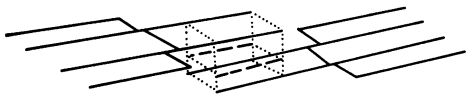


FIG. 7. *Proposed model of the colostral IgA (46). Dotted or broken lines in upper figure indicate positions of interchain disulfide bonds. CHO represents carbohydrate. T chain is represented by a line of long dashes.*

Cellular Localization

The extraordinary observation, made by using human salivary gland tissue, that "transport piece" seemed to be localized in epithelial cells lining glandular ducts, whereas the bulk of the IgA molecule was found in the underlying lymphoid cells (53), was confirmed and extended for the polypeptide subunits of rabbit secretory IgA (46). Double fluorescent antibody staining—with fluorescein labeled anti-α chain and anti-T chain tagged with the contrasting fluorochrome rhodamine—of rabbit intestines, salivary glands, mammary glands, and bronchi showed similar separate localization of α chain and T chain. The α chain was always found in the plasma cells underneath the wet epithelium lining the intestinal lumen or glandular ducts. The T chain was not detectable in these lymphoid cells, but it was clearly present in the overlying epithelial cells. Often, the border of the epithelial cells facing the duct or intestinal lumen stained for both α and T chain and, presumably, the intact secretory IgA was present here in secretions. Figure 8 illustrates the appearance of sections of intestines stained with the fluorescent antibody reagents which allowed the above conclusions. How the component polypeptide chains of this protein come together and associate—whether at the epithelial surface of the lumen after passage of the $[\alpha + L]_4$ portions between epithelial cells or within these cells upon passage of the lymphoid cell product through them—is a question presently under investigation. Justification of the term "transport piece" awaits its answer.

The apparent synthesis of different components of secretory IgA, a single protein, by two different cell types is certainly unusual. Ordinarily, a given protein is made entirely in a single cell. Thus, the burden is upon the cellular immunologist to show that the combination of α, light, and T chains is not a chance one. Arguments to

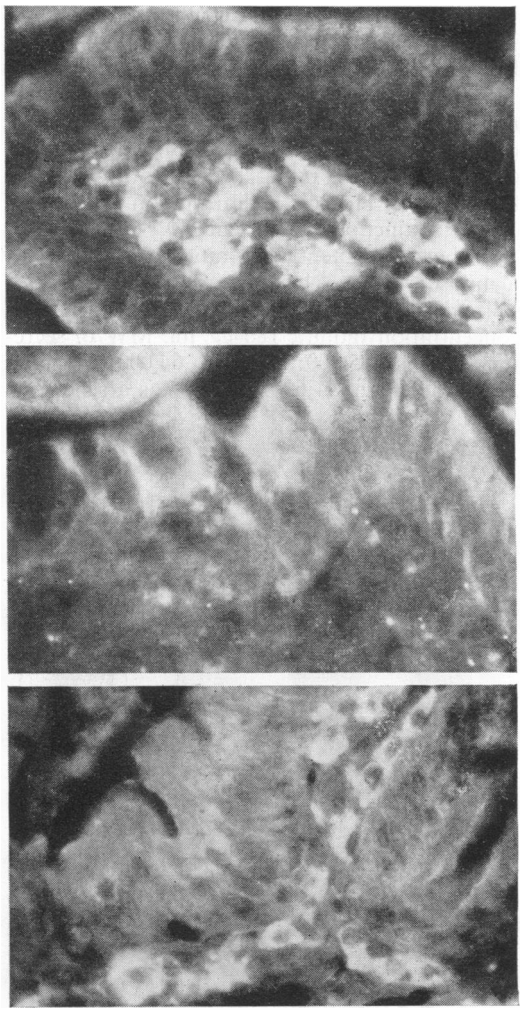


FIG. 8. *Sections of small intestine (46). Upper specimen was stained with goat anti-α coupled to fluorescein. Only the submucosal cells are stained. A thin rim of staining can be seen at the epithelial cell surface. The middle specimen was stained with goat anti-T chain conjugated to rhodamine. No submucosal cells are stained. The epithelial cells show strong fluorescence. The bottom section was stained with both the anti-T and anti-α fluorescent reagents. Both epithelial and submucosal cells appear fluorescent.*

this effect are not unequivocal and are as follows. (i) Association of T chain with other proteins of blood or secretions has not been detected and, therefore, its association with IgA seems relatively specific. (ii) Secretory IgA from different secretions of the same species all have T chain. (iii) All molecules of secretory IgA from a given fluid have a T-chain component. These generalizations (46) are based on immunological data, and the antisera used to obtain it are characterized by gel diffusion analyses partly shown in Fig. 9. Various secretions—milk, intestinal secretions, and tears—have an IgA containing T chain (Fig. 9, 10). By determining how much of several radio-labeled secretory IgA preparations was precipitable by both an anti-α chain and anti-T chain, the proportion of IgA molecules containing T chain could be estimated (46). From 86 to 92% of the molecules were directly precipitable with anti-α, and from 75 to 86% with anti-T (Table 1). Thus, most, if not all, IgA

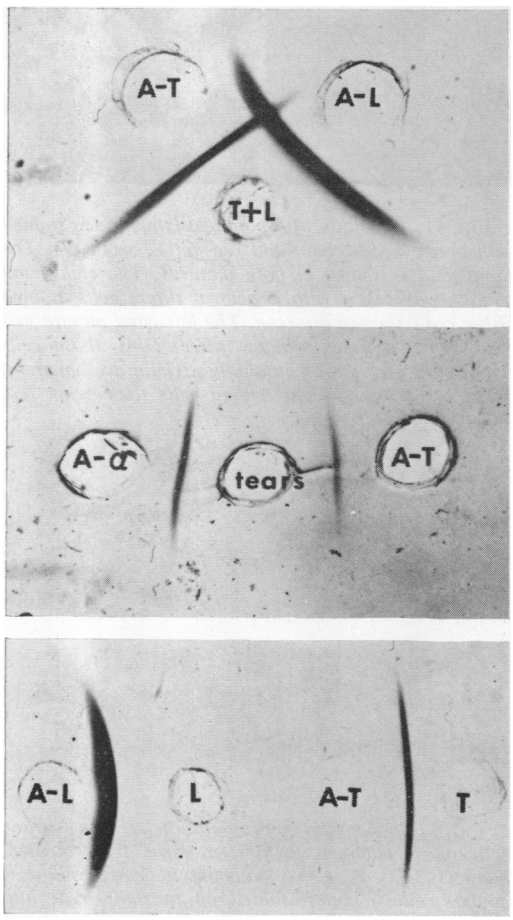


FIG. 9. *Analysis of the anti-T chain reagent by immunodiffusion (46). The non-cross reactivity of the goat anti-L chain and goat anti-T chain is shown. Symbols: T, T chain; L, L chain; A-T, anti-T chain; A-L, anti-L chain; A-α, anti-α chain.*

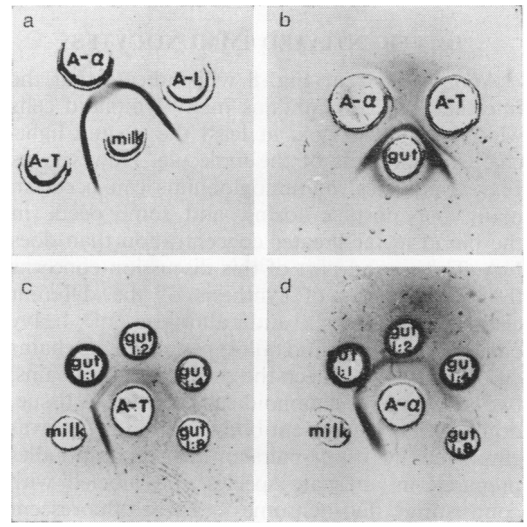


FIG. 10. *Immunodiffusion analysis of (a) 11S component isolated from rabbit milk (secretory IgA) showing a reaction of identity when tested with the goat anti-T, anti-α, and anti-L; (b) a gut perfusate showing secretory IgA that reacts both with anti-α and anti-T chain; (c) different dilutions of gut perfusate reacting with anti-T chain and (d) anti-α chain (46).*

TABLE 1. *Specific precipitation of three different radio-labeled preparations of secretory IgA by the use of excess anti-T chain or anti-α chain*[a]

Prepn	Amt of IgA	Anti-T		Anti-α	
		Amt	Counts/min precipitated	Amt	Counts/min precipitated
	μg	*ml*	%	*ml*	%
1	2	0.1	85		
	4	0.1	79	0.3	86
	8	0.1	81	0.3	86
	12	0.1	79	0.3	87
2	2	0.1	87		
	4	0.1	81	0.3	84
	8	0.1	86	0.3	92
	12	0.1	80	0.3	92
3	6	0.1	75	0.3	87
	12	0.1	77	0.3	63
	18	0.1	81		
	24			0.3	73
	36			0.3	67

[a] Data from reference 46.

molecules in milk have at least one T chain component. Further arguments for T chain being an integral part of secretory IgA await data on the biosynthesis of the molecule and the discovery of biologic functions of T chain.

DIFFERENTIATED IMMUNOCYTES

Another look at Fig. 8 will indicate that the intestinal mucosa contains many lymphoid cells which synthesize IgA, at least the α- and light-chain components of the molecule. However, as Fig. 1 indicates, immunoglobulins can occur in many polymorphic forms, and some occur in the blood in far greater concentration than does IgA. The second part of this discussion concerns the cellular sites of synthesis of the different classes, subclasses, and allotypes of heavy chains and types and allotypes of light chains (*see* 5, 47). To detect these different Ig chains, two at a time, in lymphoid cells of various tissue, double fluorescent antibody staining (9) was employed by using pairs of reagent antibodies prepared in alternate species and labeled with contrasting fluorochromes. These fluorescent antibody reagents were each specific for a single kind of Ig chain. Examination of many hundreds of productive lymphoid cells—those containing sufficient immunoglobulin chains to be detectably stained—from lymph nodes and spleens of many humans indicated separate cellular localization of κ and λ light chains (2, 3). Thus, lymphoid cells appear to be differentiated with respect to the type of light chain they can make. A similar examination of lymph nodes, spleen, and intestinal mucosa from humans and rabbits, by the use of pairs of antiheavy-chain fluorescent reagents, showed that individual lymphoid elements contained either α, γ, or μ chain (3, 7). More than one class of heavy chain or one type of light chain was not commonly found in a given cell at a single time. Those few cells which did appear to contain mixtures of classes or types of Ig chains could have been artifacts of staining or could have represented cells caught at a time of transition from the synthesis of one polymorphic form of Ig to another. Figure 11 shows a section of a rabbit popliteal lymph node stained for μ and γ heavy chains. Many cells containing either μ or γ chain may occur interspersed in a great cluster of productive lymphoid elements.

By staining many sections of lymphoid tissue with different pairs of fluorescent antichain reagents, the relative distribution of cells containing each class of heavy chain and each type of light chain could be deduced (3, 6, 7). Figure 12 shows schematically this sort of data obtained for the spleen of one particular human, and Table 2 gives the frequencies of cells containing each heavy chain in the spleens of individual rabbits. In human lymph nodes and spleens, cells containing κ chain predominated over those containing λ chain by about 3:2. In these same tissues, the range of cells containing α heavy chain rather than γ chain was from 28 to 51%. In contrast, the relative number of cells containing each heavy chain in rabbit spleens or

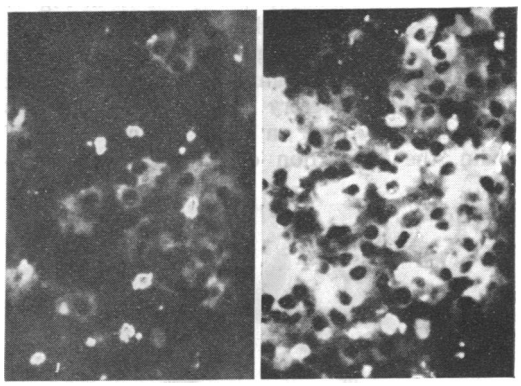


FIG. 11. *A section of a popliteal lymph node stained with green anti-μ chain and red anti-γ chain (7). The same field is shown in both pictures. The right-hand picture was taken with a neutral filter; cells stained either red or green are seen. The left hand picture was taken with a green window filter. Only those cells fluorescing green and containing μ chain are apparent. These were interspersed among cells fluorescing red, which are not visible here.*

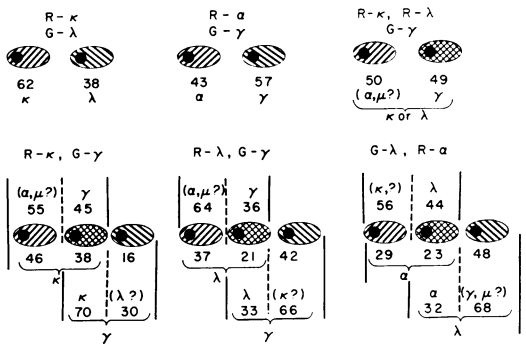


FIG. 12. *Schematic representation of the various cell counts obtained for spleen tissue from a single human (2, 3, 6). Cross hatching indicates yellow or doubly stained; lines slanted up to right, red; lines slanted up to left, green. Percentage of total fluorescing cells containing a given polypeptide chain is indicated. The fluorescent antibody reagents specific for a given polypeptide and used together for simultaneous staining are indicated as R-κ (rhodamine-labeled anti-κ chain), G-λ (fluorescein anti-λ chain), etc.*

TABLE 2. *Distribution of α, γ, and μ heavy chains and of allotypic markers Aa1 and Aa2 among rabbit spleen cells*[a]

Antibody	Relative no. of cells stained by each or both of the following reagents												Calculated distribution (%)			Avg distribution (%)		Relative serum concn (%)	
	G-α + R-γ			G-μ + R-γ			R-α + G-μ			G-A1 + R-A2									
	α	γ	α + γ	μ	γ	μ + γ	α	μ	α + μ	Aa1	Aa2	Mix	α	γ	μ	Aa1	Aa2	Aa1	Aa2
Anti-DNP #1[b]	7	93		25	75		22	78		72	28		7	67	26	70	29		
	10	90		32	68		23	77		72	27	1							
										65	34	1							
										71	28	1							
Anti-DNP #2	15	85		23	77		22	78		—[c]	—[c]		9	70	21				
				22	77	1	17	83											
Anti-DNP #3	12	88		29	70	1				81	19		9	64	27	84	16	80	20
										86	14								
Anti-*Salmonella* #1	5	95		13	87		29	71		81	19		6	80	14	81	19		
	6	94		16	83	1	30	70		79	21								
										79	21								
										86	14								
Anti-proteins #1				15	85					90	10					88	12	91	9
				12	88					86	14								
Anti-proteins #2				16	84		16	84		82	18		3	82	15	83	17	87	13
				13	87					84	16								

[a] Data from reference 7.
[b] DNP = 2,4-dinitrophenyl.
[c] Homozygote.

lymph nodes was usually about 3 to 9% for α chain, 14 to 27% for μ chain, and 64 to 82% for γ chain. By staining for all light chains and counter staining for individual heavy chains, and vice versa, one could deduce that normally productive lymphoid cells have both component chains needed for the assembly of an intact Ig, although they are restricted to the synthesis of one class of heavy chain and one type of light chain (3, 36). For man and the rabbit, the proportions of cells containing IgA, IgM, or IgG stand in the same order as do the relative serum concentrations of their products, estimated as 1.5, 6, and 92.5%, respectively, for rabbit Ig. Obviously, the steady-state levels of the different immunoglobulins reflect different dependences of synthetic and catabolic rate on concentration. Rates of degradation and escape from the vascular system certainly vary for the different immunoglobulins. The quantity of cells containing and, presumably, synthesizing each Ig probably reflects the number necessary to maintain a given steady-state level of their product. For types of Ig in man, K or L, the relationship between cells synthesizing each to serum concentrations is much closer, as shown in Table 3. Since both types of molecules within a given class are probably of equal influence in regulating the steady-state level of that class—the catabolic rate seemingly determined by the Fc portion of heavy chains—the size of the synthetic populations for each type would be expected to be accurately reflected by the concentrations of their products. Table 3 also makes another provocative point: the relative incidence of multiple myeloma of the different classes and types among the human population is close to the relative proportion of cells making the different polymorphic forms of Ig within normal humans. One interpretation of this is that lymphoid cells were already predifferentiated to make a given polymorphic form of Ig before oncogenesis. Thus, the incidence of myeloma of different sorts merely reflects the proportion of the different restricted cells equally at risk at the time a tumor developed (3). The restriction of lymphoid cells to the production, by each, of an apparently homogeneous product even extends

to the products of apparently allelic cistrons (6, 7, 36, 48). Tables 2 and 4 show that individual cells in rabbits heterozygous at the *a* locus, controlling alternate markers on heavy chains, or the *b* locus, controlling varying antigenic sites on light chains, only produce one or the other allelic product. The relationship between the proportion of cells containing each allelic product and the relative serum concentrations of these is very close in heterozygotes (7, 36).

As a customary preliminary to trying to understand the mechanism for restriction of the synthetic potential of a cell, efforts were made to shift the proportions of lymphoid cells making various products by external agents. One successful way to influence the proportions of different Ig products is to administer antisera directed versus paternal allotypic markers to newborn rabbits heterozygous at the *a* or *b* locus. Usually, such offspring show a marked depression of the concentration of paternal-type molecules and a compensatory increase in Ig with maternal markers (37). Table 4 indicates that these "allotype-suppressed" animals have a corresponding rarity of cells making paternal-type molecules and a relative abundance of those with maternal markers (36). Another way to influence proportions of different polymorphic immunoglobulins produced is by deliberate antigenic challenge; the route, form of the immunogen, accompanying adjuvant, and other factors seem to exert an effect in a manner still obscure. Figure 13 shows the changes in the productive lymphon (the total population of cells making Ig) of the gut and spleen occurring after oral infection of rabbits with living *Trichinella* (15). Specimens of intestine taken 7 to 13 days after infection showed a relative increase in IgM cells in the mucosa. As the infection proceeded, the gut lymphon returned to a normal state, whereas the spleen and lymph nodes showed an unusual abundance of IgM cells (15). This kind of shift towards IgM production has been observed in other chronic infections.

The mechanism for restriction of lymphoid cells to the synthesis of a particular Ig is unknown. Certainly the restriction seems very strict, extending to subclasses of heavy chain (1) and, perhaps, to "idiotypes" of light chain (47). These many differentiable immunoglobulins all seem to be products of unique cistrons present in the genome of all cells of an individual. Thus, some mechanism for selective activation of cistrons, a mechanism to explain many examples of cell differentiation, must be sought. However, productive lymphoid cells also seem to be restricted to making Ig molecules with a single

TABLE 3. *Relative frequencies of κ versus λ chains and γ versus α chains*

Chain	Per cent serum concn[a]			Incidence of myeloma[b]	Cell count
	γG	γA	γM		
				%	%
Light					
κ	69	62	68	69	63
λ	31	38	32	31	37
Heavy					
γ[c]				64	57
α[d]				36	43

[a] Data from reference 21.
[b] Data from reference 34.
[c] Serum concentration was 76.2 (22).
[d] Serum concentration was 23.8 (22).

TABLE 4. *Distribution of allotype markers among spleen cells and among serum immunoglobulin molecules of normal and "immunosuppressed" rabbits*[a]

Animal	Allotype	Cell counts		Serum concn		Cell counts		Serum concn	
		a1	a2	a1	a2	b4	b5 + b negative	b4	b5
CM639B	$a^1a^2b^4b^4$	70	30	76	24				
CM639A	$a^1a^2b^4b^4$	80	20	79	21				
45FZ-6[b]	$a^1a^2b^4b^5$	91	9	90	10	90	10	87	13
45FZ-5	$a^1a^2b^4b^5$	88	12	91	9	60	40	66	34
45FZ-7	$a^1a^2b^4b^5$	88	12	91	9	74	26	79	21
45FZ-3	$a^1a^2b^4b^5$	83	17	87	13	60	40	69	31
C153[c]	$a^1a^2b^4b^5$	95	5	98	2				
C1941[b]	$a^1a^3b^4b^5$					94	6	94	6
C1942[b]	$a^1a^3b^4b^5$					96	4	93	7

[a] Data from reference 36.
[b] Suppressed for b5.
[c] Suppressed for a2.

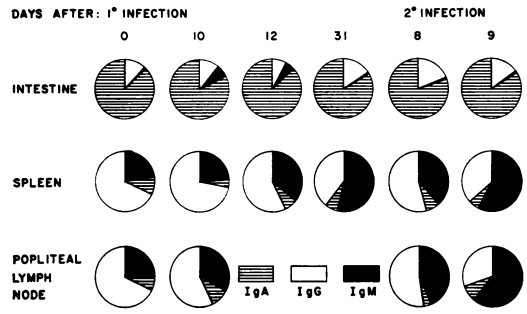


FIG. 13. *Changes in productive lymphoid cell populations after infection of rabbits with Trichinella (5, 15).*

kind of antibody site, a single specificity in serological terms (23, 27, 38, 45). Is there a special mechanism for introducing microheterogeneity into immunoglobulins of a given polymorphic form which acts on single cells of the immunocompetent animal, or is this just a finer case of selective activation of cistrons already present in all cells? A chemical understanding of the "antibody-combining site" may indicate the most likely possibility and may even help to precise the probable mechanism.

PRIMARY STRUCTURE OF IMMUNOGLOBULIN

The last part of the discussion will be concerned with efforts to localize the site of specificity in the antibody molecule and to describe the amino acid interchanges which may correlate with different activities. There are two main approaches to this problem. One can compare the sequences of a set of myeloma proteins of the same subclass, subtype, and allotype, each being a homogeneous protein with a unique primary structure. Sections of the molecules having identical sequences can be excluded from a direct role in precising antibody specificity. Since amino acid interchanges related to particular polymorphic forms should be minimized, sections showing sequence variability would be candidates for constituting the antibody site. The survey of many human light chains from myeloma patients that indicated a constant C-terminal and a variable N-terminal half of this polypeptide is a well known example of this approach (32, 41, 51). The association of selective binding activity for some common antigens with a few myeloma proteins (13, 19, 20) suggests that these homogeneous proteins may even provide sequence patterns which correlate with antibody specificity. The other approach to the chemical basis of antibody activities is to sequence, as far as possible, pooled normal immunoglobulin of a given type, subclass, and allotype. Those sections which permit assignation of a single sequence based on component peptides recovered in high yield presumably are constant in all molecules and are unrelated to specificity. Regions showing variability may be sequenced to the extent that major interchanges at given positions may be deduced. These may be involved in forming the site. An example of this approach is the considerable sequence data obtained for rabbit heavy chain which indicated a constant sequence throughout the Fc region extending through the C-terminal half of Fd and variable section at the N-terminal end of the chain (4, 31, 49). Some of these data are discussed below. With the preparation of more and more antibodies having uniform physicochemical properties (39, 44), it may become possible to correlate sequence patterns with specificity as an extension of the work with normal immunoglobulins.

The heavy chain prepared from specific antibodies retains one antigen-binding site, although its association constant is considerably lower than that of a site formed by a pair of heavy and light chains in the original molecule (54, 55). Thus, this chain, from normal rabbit IgG, was selected for the first sequence studies. Because of its length, some 450 residues, sections were obtained that have manageable numbers of residues. Hill and his co-workers used papain to digest whole IgG and obtained the C-terminal half of heavy chain, which they subsequently sequenced (30). A chemical cleavage was used to prepare more-or-less intact Fd from isolated heavy chain. Using cyanogen bromide to cleave at the five or six methionine residues in heavy chain, Givol and

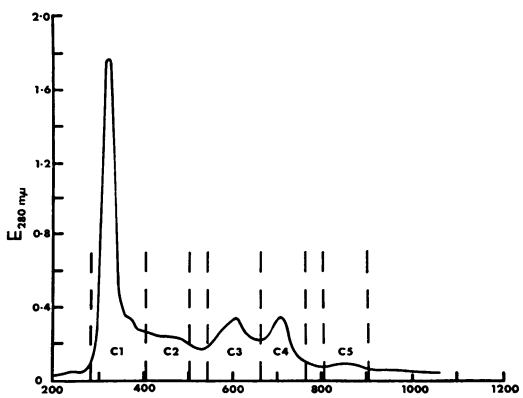


FIG. 14. *Separation by gel filtration of components in a cyanogen bromide digest of heavy chain from rabbit anti-DNP antibody (8). A digest of 2.8 μmoles of heavy chain was applied to a column of Sephadex G-100 in 1 N acetic acid.*

Porter separated the Fd section (called C-1) from the fragmented Fc (25). The sections of heavy chain can be resolved by gel filtration as shown in Fig. 14, which depicts an elution profile of a digest of heavy chain from antidinitrophenyl antibody (8). Figure 15 gives the alignment of the CNBr sections. Since the N-terminal half of the chain, the C-1 section, contained about 245 residues, efforts were made to obtain smaller pieces of it. To minimize the number of tryptic peptides obtained from an enzymic digest of C-1 and to minimize the effect of possible heterogeneity on the separation of these peptides, the lysine residues were reversibly blocked. The totally reduced, carboxymethylated C-1 was reacted with *S*-ethylthiotrifluoracetate in 5 M guanidine. Tryptic cleavage would then be expected to occur only at the six arginine residues in substituted C-1. It was hoped that limited variation at some residue positions would not influence the separation of the component sections of C-1 and that these would be resolvable on the basis of the properties of their larger constant or homologous stretches of

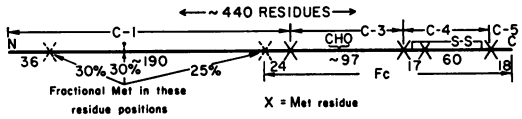


FIG. 15. *Schematic diagram of rabbit heavy chain showing the alignment of the sections of the chain produced by cyanogen bromide cleavage (4, 8).*

sequence. Table 5 lists the compositions of five arginine peptides and one homoserine peptide isolated from a tryptic digest of trifluoroacetylated C-1 by gel filtration and ion-exchange chromatography on substituted cellulose (8). Each of these peptides appears unique (not a variant of one of the others) and was isolated in 20 to 50% absolute yield. The compositions of all of the peptides can be summed, as shown in Table 5, to yield a composition which is very close to that of C-1 itself. The implication of the high recoveries of these peptides and the resemblance of the sum of their compositions to that of C-1 is that they account for most of the N-terminal half of heavy chain. Certainly one arginine peptide, of about 20 residues, remains unaccounted for, and those variants of C-1 with internal methionines (*see* Fig. 15) did not contribute those trifluoroacetylated arginine peptides which encompassed the variant methionine positions. Nevertheless, the set of peptides does represent the bulk of Fd and probably some of them contain the sequence variability which accounts for specificity. Peptides T2, T4, T3, and T1 have been isolated from specific antidinitrophenyl antibody in about the same yield as that from normal IgG (Table 6). Thus, this set of peptides is representative of Fd from active antibodies and cannot be considered to be constant sections from "blank" or "inactive" normal globulin.

To determine whether and how far the constant

TABLE 5. *Amino acid composition of principal tryptic peptides derived from trifluoroacetylated C-1*[a]

Amino acid	T1	T2	T3	T4	T5	T7	Sum of peptides	C-1 avg
Lysine	2.0	5.2	3.0				10	12
Histidine		1.0					1.0	1.6
Arginine	1.0		1.0	0.9	1.0	1.0	4.9	6.3
Aspartic acid	1.7	4.0	6.2	5.1			17	17
Threonine	5.3	7.8	9.3	3.0		1.0	26	30
Serine	4.2	9.8	7.2	1.1	1.8	1.0	25	28
Glutamic acid	2.0	2.9	4.4	5.9	3.0		18	15
Proline	2.5	12	7.7	2.1		0.9	25	22
Glycine	4.0	4.2	9.0		2.2		19	21
Alanine	3.0	3.1	3.9				10	14
Valine	2.0	7.9	8.2	6.2	1.1	0.7	26	23
Isoleucine	2.0	1.1	1.0	1.0			5.1	7.2
Leucine	2.6	5.0	7.3				15	17
Tyrosine	2.1	0.9	2.8	1.0			6.8	9.4
Phenylalanine	1.4	2.0	2.1	1.0		0.8	7.3	7.9
SCMC[b]	0.9	2.3	3.1	0.9			6.9	7.4
Homoserine		1.0					1.0	1.8
Tryptophan				2			2	4
Total residues	36.7	70.2	76.2	28.2	9.1	5.4	226	245

[a] Data from reference 8.
[b] S-carboxy-methyl-cysteine.

sequence obtained for Fc extended into the Fd region, sequence data were obtained for the two large, trifluoracetylated peptides T2 and T3 (12). Peptide T2, with 72 residues, could be assigned to the C-terminal end of C-1 because of its lack of arginine and its single homoserine residue. The sequence of its C-terminal 24 residues is identical with the beginning of Fc and, thus, T2 overlaps the papain cleavage point at the junction of Fc and Fd (Fig. 16; 24). A constant sequence continues into the Fd and, presumably, throughout peptide T2. The sequence and partial sequence of T3 (Fig. 17) indicates that this section of about 76 residues also has a constant sequence. Peptides T3 and T4 together probably account for most of the C-terminal half of Fd. Certainly they can be aligned with the C-terminal halves of rabbit Fc, human κ chain and human λ chain (4). In Fig. 18, the strong sequence homologies and identities among these regions of the IgG molecule are apparent. As schematized in Fig. 19, the sequence homologies of the second and fourth quarter of heavy chain and the second half of light chain support the hypothesis that genes for light and heavy chains originated from a single primitive cistron that gave rise to light and heavy chain cistrons by duplication and independent mutations (4, 30).

TABLE 6. *Comparison of compositions of tryptic peptides derived from C-1 of normal IgG and anti-DNP antibody*[a]

Amino acid	T2		T4	
	Normal	Anti-DNP	Normal	Anti-DNP
Lysine	5.2	5.2		
Histidine	1.0	0.8		
Arginine			0.9	0.9
Aspartic acid	4.0	4.0	5.1	4.6
Threonine	7.8	7.7	3.0	2.9
Serine	9.8	9.7	1.1	2.0
Glutamic acid	2.9	2.7	5.9	5.4
Proline	12	12	2.1	2.1
Glycine	4.2	4.0		1.5
Alanine	3.1	3.0		
Valine	7.9	8.1	6.2	5.8
Isoleucine	1.1	1.0	1.0	1.1
Leucine	5.0	4.8		
Tyrosine	0.9	0.9	1.0	0.7
Phenylalanine	2.0	2.1	1.0	1.7
SCMC[b]	2.3	2.0	0.9	0.4
Homoserine	1.0	1.1		

[a] Data from reference 8.
[b] S-carboxy-methyl-cysteine.

-Ser-Val-Pro(Ser,Thr,Val)Ser-Glx(Ser,Thr,Asx,Ser,Pro,Cys,Val)(Thr,

His,Ala,Ala,Asp,Val)Thr-Lys-Val-Asp-Lys-Thr-Val-Ala-Pro-Ser-

-Thr-Cys-Ser-Lys(Thr,Pro)Cys-Pro-Pro-Pro-Glu-Leu-Leu-Gly-

-Gly-Pro-Ser-Val-Phe-Ile-Phe-Lys-Pro-Pro-Pro-Lys-Asp-Thr-Leu-Met

FIG. 16. *Partial sequence of tryptic peptide T2 from C-1 (12).*

-Asx-Leu(Thr,Gly,Gly,Val,Leu)Val-Gly-Gln-Pro(Thr,Ser,Ser)Lys-

-Ala-Pro-Ser-Val-Phe-Pro-Leu-Ala-Pro-Cys-Cys-Gly-Asp-Pro-

-Thr-Ser-Ser-Thr-Val-Thr-Leu-Gly-Cys-Leu-Val-Lys-Gly-Ser-Leu

(Thr,Thr,Glx,Pro,Asx,Val,Val,Tyr)Gly(Gly,Pro,Thr,Thr,Asx,Leu,Val)Arg

FIG. 17. *Partial sequence of tryptic peptide T3 from C-1 (12).*

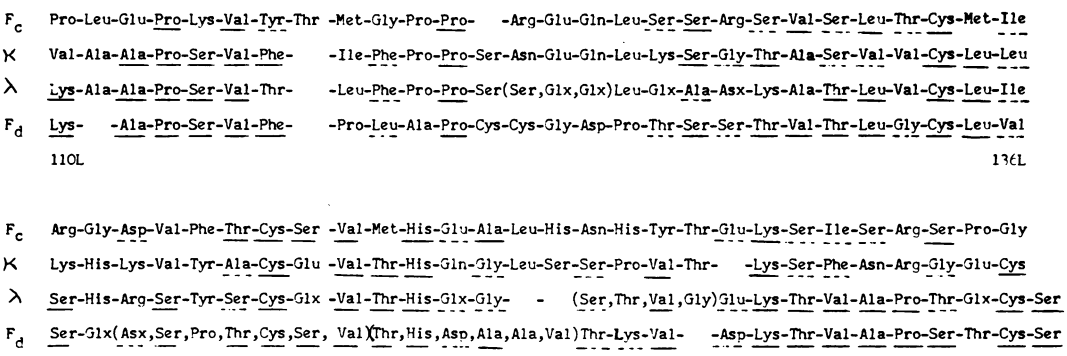


FIG. 18. *Homologies in sequence among rabbit Fc and Fd (γ chain) and human κ and λ chains.*

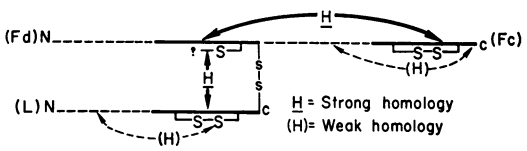


FIG. 19. *Reoccurrence of homologous regions within the IgG molecule (4).*

What of the balance of the Fd, the N-terminal half, and the variability one would expect in normal heavy chains which are mixtures of many antibodies? Peptides T5, T1, and T4 account for much of this region. Peptide T5 represents the N-terminal end of heavy chain and is included within the N-terminal 35 residues isolated from those variants having a methionine at position 35 (49, 56). This N-terminal peptide does show a limited number of replacements at certain positions such as 2-, 3-, 9-, 23-, and 35-N in its sequence (49). Peptide T1 also seems to show sequence variability, and no sensible sequence has yet been deduced (26). Thus, the N-terminal half of Fd seems to consist of a set of homologous sections, which can be isolated as T5, T1, T4, etc., and it probably has a basic sequence with replacements at some, but not all, positions. The sequence, then, becomes constant in the C-terminal half of Fd.

Where, then, should one place the sequence variability related to antibody specificity? One possibility is that there is a highly variable section in a definite place in the heavy chain. This section could be represented by such a mixture of different peptides that it could easily have been missed in our isolation procedures. Although one arginine peptide is not accounted for, our data would indicate that this missed variable section could only be on the order of 20 residues in length, for a larger missing section would be difficult to accommodate in C-1 along with the peptides already isolated. Another possibility is that there can be limited variation in a fraction of the residue positions distributed throughout the N-terminal halves of both Fd and light chain. The qualitative pattern of amino acids at only 5 to 10 residue positions of a possible 40 varying ones may determine a particular antibody specificity (4). The latter explanation seems best in keeping with the data from light chains from myeloma patients (32, 41, 51). However, a considerable number of residue positions known to vary may be related to gross polymorphism and not directly to antibody specificity. For instance, many variants at the N-terminal end of Bence-Jones proteins and rabbit heavy chains may be related to their subtype or allotype, or both (40, 43, 50). At the moment, ability to discriminate between subtype, allotype, and specificity-associated variability is rather poor. The recent finding that affinity label marks the rabbit T-1 peptide from Fd may indicate that variation pertinent to specificity occurs in this section (24). At the moment, attempts to describe more completely the genetically controlled immunoglobulin polymorphism in the outbred population of human donors of myeloma proteins, searches for more myelomas with antibody activity, and the use of inbred animal donors of both normal immunoglobulins and antibodies with uniform physico-chemical properties appear to offer the best procedures for more accurately describing the antibody site.

LITERATURE CITED

1. Bernier, G. M., R. E. Ballieux, K. T. Tominaga, and F. W. Putnam. 1967. Heavy chain subclasses of human γG-globulin. Serum distribution and cellular localization. J. Exp. Med. **125**:303–318.
2. Bernier, G. M., and J. J. Cebra. 1964. Polypeptide chains of human gamma-globulin: cellular localization by fluorescent antibody. Science **144**:1590–1591.
3. Bernier, G. M., and J. J. Cebra. 1965. Frequency distribution of α, γ, κ, and λ polypeptide chains in human lymphoid tissues. J. Immunol. **95**:246–253.
4. Cebra, J. J. 1967. Common peptides comprising the N-terminal half of heavy chain from rabbit IgG and specific antibodies, Cold Spring Harbor Symp. Quant. Biol. **32**:65–73.
5. Cebra, J. J. 1968. Lymphoid cells differentiated with respect to variety of their immunoglobulin product, p. 69–83. *In* K. B. Warren (ed.), Differentiation and immunology. Symp. Int. Soc. Cell Biol., vol. 7. Academic Press Inc., New York.
6. Cebra, J., and G. M. Bernier. 1967. Quantitative relationships among lymphoid cells differentiated with respect to class of heavy chain, type of light chain, and allotypic markers, p. 65–76. *In* R. T. Smith, P. A. Miescher, and R. A. Good (ed.), Ontogeny of immunity. University of Florida Press, Gainesville.
7. Cebra, J. J., J. E. Colberg, and S. Dray. 1966. Rabbit lymphoid cells differentiated with respect to α, γ, and μ-heavy polypeptide chains and to allotypic markers Aa1 and Aa2. J. Exp. Med. **123**:547–558.
8. Cebra, J. J., D. Givol, and R. R. Porter. 1968. Common peptides from the N-terminal half of heavy chain of immunoglobulin G from normal rabbit serum and a specific antibody. Biochem. J. **107**:69–77.
9. Cebra, J. J., and G. Goldstein. 1965. Chromatographic purification of tetramethylrhodamine immune globulin conjugates and their use in the cellular localization of rabbit γ-globulin polypeptide chains. J. Immunol. **95**:230–245.
10. Cebra, J. J., and J. B. Robbins. 1966. γA-immunoglobulin from rabbit colostrum. J. Immunol. **97**:12–24.
11. Cebra, J. J., and P. A. Small, Jr. 1967. Polypeptide chain structure of rabbit immunoglobulins. III. Secretory γA-immunoglobulin from colostrum. Biochemistry **6**:503–512.
12. Cebra, J. J., L. A. Steiner, and R. R. Porter. 1968. The partial sequence of two large peptides from the N-terminal half of heavy chains from normal rabbit immunoglobulin G. Biochem. J. **107**:79–88.
13. Cohn, M. 1967. Natural history of the myeloma. Cold Spring Harbor Symp. Quant. Biol. **32**:211–221.
14. Cohen, S., and C. Milstein. 1967. Structure and activity of immunoglobulins. Advan. Immunol. **7**:1–89.
15. Crandall, R. B., J. J. Cebra, and C. A. Crandall. 1967. The

relative proportions of IgG-, IgA- and IgM-containing cells in rabbit tissues during experimental Trichinosis. Immunology 12:147–158.
16. Dray, S., and A. Nisonoff. 1964. Relationship of genetic control of allotypic specificities to the structure and biosynthesis of rabbit γ-globulin, p. 175–190. *In* J. Sterzl (ed.), Molecular and cellular basis of antibody formation. Czechoslovak Academy of Sciences Press, Prague.
17. Dray, S., G. O. Young, and L. Gerald. 1963. Immunochemical identification and genetics of rabbit γ-globulin allotypes. J. Immunol. 91:403–415.
18. Dubiski, S., J. Rapacz, and A. Dubiska. 1962. Heredity of rabbit gamma-globulin iso-antigens. Acta Genet. Statist. Med. 12:136–155.
19. Eisen, H. N., J. R. Little, C. K. Osterland, and E. S. Simms. 1967. A myeloma protein with antibody activity. Cold Spring Harbor Symp. Quant. Biol. 32:75–81.
20. Eisen, H. N., Simms, E. S., and M. Potter. 1968. Mouse myeloma proteins with antihapten antibody activity. The protein produced by plasma cell tumor MOPC-315. Biochemistry 7:4126–4134.
21. Fahey, J. L. 1963. Two types of 6.6*S* γ-globulins, B_2A-globulins, and 18*S* γ_1-macroglobulins in normal serum and γ-microglobulins in normal urine. J. Immunol. 91:438–447.
22. Fahey, J. L., and M. E. Lawrence. 1963. Quantitative determination of 6.6*S* γ-globulins, B_{2A}-globulins and γ_1-macroglobulins in human serum. J. Immunol. 91:597–603.
23. Gershon, H., S. Bauminger, M. Sela, and M. Feldman. 1968. Studies on the competence of single cells to produce antibodies of two specificities. J. Exp. Med. 128:223–233.
24. Givol, D., and F. DeLorenzo. 1968. The position of various cleavages of rabbit immunoglobulin G. J. Biol. Chem. 243:1886–1891.
25. Givol, D., and R. R. Porter. 1965. The C-terminal peptide of the heavy chain of the rabbit immunoglobulin IgG. Biochem. J. 97:32–34c.
26. Givol, D., and S. Shaltiel. 1968. Isolation of a tryptic peptide from the antibody-combining site. Israel J. Chem. 6:108.
27. Green, I., P. Vassali, V. Nussenzweig, and B. Bennacerraf. 1967. Specificity of the antibodies produced by single cells following immunization with antigens bearing two types of antigenic determinants. J. Exp. Med. 125:511–526.
28. Hanson, L. A. 1961. Comparative immunological studies of the immune globulins of human milk and of blood serum. Int. Arch. Allergy Appl. Immunol. 18:241–267.
29. Herzenberg, L. A. 1964. A chromosome region for gamma 2a and beta 2A globulin H chain isoantigens in the mouse. Cold Spring Harbor Symp. Quant. Biol. 29:455–462.
30 Hill, R. L., R. Delaney, R. E. Fellows, Jr., and H. E. Lebovitz. 1966. The evolutionary origins of the immunoglobulins. Proc. Nat. Acad. Sci. U.S.A. 56:1762–1769.
31. Hill, R. L., H. E. Lebovitz, R. E. Fellows, Jr., and R. Delaney. 1967. The evolution of immunoglobulins as reflected by the amino acid sequence studies of rabbit Fc fragment, p. 109–127. *In* J. Killander (ed.), Gamma globulins: structure and control of biosynthesis. Nobel Symp. 3. Almquist and Wiksell, Stockholm.
32. Hilschmann, N., and L. C. Craig. 1965. Amino acid sequence studies with Bence-Jones proteins. Proc. Nat. Acad. Sci. U.S.A. 53:1403–1409.
33. Ko, A., J. R. Clamp, G. Dawson, and J. Cebra. 1967. The presence of an oligosaccharide unit in the region of an interchain disulfide bond on the heavy chain of an IgA myeloma globulin. Biochem. J. 105:35–36.
34. Korngold, L. 1961. Abnormal plasma components and their significance in disease. Ann. N.Y. Acad. Sci. 94:110–130.
35. Lieberman, R., S. Dray, and M. Potter. 1965. Linkage in control of allotypic specificities on two different γG-immunoglobulins. Science 148:640–642.
36. Lummus, Z., J. J. Cebra, and R. Mage. 1967. Correspondence of the relative cellular distribution and serum concentration of allelic allotype markers in normal and "allotype-suppressed" heterozygous rabbits. J. Immunol. 99:737–743.
37. Mage, R., and S. Dray. 1965. Persistent altered phenotypic expression of allelic γG-immunoglobulin allotypes in heterozygous rabbits exposed to isoantibodies in fetal and neonatal life. J. Immunol. 95:525–535.
38. Mäkelä, O. 1967. Cellular heterogeneity in the production of antihapten antibody. J. Exp. Med. 126:159–170.
39. Miller, E. J., C. K. Osterland, J. M. Davie, and R. M. Krause. 1967. Electrophoretic analysis of polypeptide chains isolated from antibodies in the serum of immunized rabbits. J. Immunol. 98:710–715.
40. Milstein, C. 1967. Linked groups of residues in immunoglobulin chains. Science 216:330–332.
41. Milstein, C., B. Frangione, and J. R. L. Pink. 1967. Studies on the variability of immunoglobulin sequence. Cold Spring Harbor Symp. Quant. Biol. 32:31–36.
42. Natvig, J. B., H. G. Kunkel, and S. D. Litwin. 1967. Genetic markers of the heavy chain subgroups of human γG-globulins. Cold Spring Harbor Symp. Quant. Biol. 32:173–180.
43. Niall, H. D., and P. Edman. 1967. Two structurally distinct classes of kappa chains in human immunoglobulins. Nature 216:262–263.
44. Nisonoff, A., S. Zappacosta, and R. Jureziz. 1967. Properties of crystallized rabbit anti-*p*-azobenzoate antibody. Cold Spring Harbor Symp. Quant. Biol. 32:89–93.
45. Nossal, G. J. V., and O. Mäkelä. 1961. Kinetic studies on the incidence of cells appearing to form two antibodies. J. Immunol. 88:604–612.
46. O'Daly, J. A., and J. J. Cebra. 1968. Structure and cellular localization of secretory IgA. Protides Biol. Fluids Proc. Colloq. Bruges 16:205–219.
47. Pernis, B. 1968. Relationships between the heterogeneity of immunoglobulins and the differentiation of plasma cells. Cold Spring Harbor Symp. Quant. Biol. 32:333–341.
48. Pernis, B., G. Chiappino, A. S. Kelus, and P. G. H. Gell. 1965. Cellular localization of immunoglobulins with different allotypic specificities in rabbit lymphoid tissues. J. Exp. Med. 122:853–876.
49. Porter, R. R. 1967. The structure of the heavy chain of immunoglobulin and its relevance to the nature of the antibody combining site. Biochem. J. 105:417–426.
50. Prahl, J. W., and R. R. Porter. 1968. Allotype-related sequence variation of the heavy chain of rabbit immunoglobulin G. Biochem. J. 107:753–763.
51. Putnam, F. W., K. Titani, M. Wikler, and T. Shinoda. 1967. Structure and evolution of kappa and lambda light chains. Cold Spring Harbor Symp. Quant. Biol. 32:9–28.
52. South, M. A., M. D. Cooper, F. A. Wollheim, R. Hong, and R. A. Good. 1966. The IgA system. I. Studies of the transport and immunochemistry of IgA in the saliva. J. Exp. Med. 123:615–627.
53. Tomasi, T. B., E. M. Tan, A. Soloman, and R. A. Prendergast. 1965. Characteristics of an immune system common to certain external secretions. J. Exp. Med. 121:101–125.
54. Utsumi, S., and F. Karush. 1964. The subunits of purified rabbit antibody. Biochemistry 3:1329–1338.
55. Weir, R. C., and R. R. Porter. 1966. The antigen-binding capacity of the peptide chains of horse antibodies. Biochem. J. 100:69–72.
56. Wilkinson, J. M., E. M. Press, and R. R. Porter. 1966. The N-terminal sequence of the heavy chain of rabbit immunoglobulin IgG. Biochem. J. 100:303–308.
57. World Health Organization. 1964. Nomenclature for human immunoglobulins. Bull. World Health Organ. 30:447–450.