

QUANTITATIVE VARIATIONS IN L CHAIN TYPES IN GUINEA PIG ANTIHAPTEN ANTIBODIES*

BY VICTOR NUSSENZWEIG, M.D., AND BARUJ BENACERRAF, M.D.

*(From the Department of Pathology, New York University
School of Medicine, New York)*

(Received for publication 3 June 1966)

It is well known that mammalian immunoglobulins¹ consist of two types of polypeptide chains, H and L (1, 2). In human immunoglobulins, L chains were found to be of two distinct types (κ and λ) which differ widely in their amino acid composition and in the peptides which can be detected by fingerprint analysis (3). Two types of molecules (K or L) bearing either κ - or λ -types of L chains, in a 2/1 proportion are normally present within each class of human immunoglobulin (4, 5). We have recently found that guinea pig 7S immunoglobulins also consist of molecules bearing two different types of L chains (κ and λ) roughly in the same proportion as that found in human immunoglobulins (6). These two types of guinea pig L chains have distinct antigenic determinants and very similar molecular weights.

The question arises whether the genes which instruct the synthesis of these different types of L chains might not influence the final configuration of the antibody-combining site, as it is generally accepted that L chains in conjunction with H chains are involved in immunological specificity (7-12). If such an influence exists, individual antigenic determinants might induce the formation of antibody molecules in which the ratio of K molecules to L molecules deviates markedly from normal.

In order to study this problem, we chose to examine guinea pig antihapten antibodies. Such antibodies, rather than antiprotein or antipolysaccharide antibodies, were preferred because of the ease with which they can be specifically purified and because they constitute a better defined, though admittedly heterogeneous population. Guinea pig antihapten antibodies were also previously shown to be more homogeneous than nonspecific immunoglobulins or anti-protein antibodies in their L chain patterns in urea starch or acrylamide gel electrophoresis (7, 13, 14). The results obtained in the present study demonstrate that there is a relationship between the specificity of some antihapten

* Supported by United States Public Health Service Grant AI 04983 and the Health Research Council of the City of New York under contract No. I-138.

¹ Whenever possible a similar nomenclature to that suggested during the World Health Organization meeting on Nomenclature of Human Immunoglobulins has been used (*Bull. World Health Organ.*, 1964, 30, 447).

antibodies induced in guinea pigs and the relative proportion of K and L molecules they contain.

Material and Methods

Proteins.—Bovine gamma globulin (BGG), bovine serum albumin (BSA), and bovine fibronogen (BF) were obtained from the Armour Pharmaceutical Co., Kankakee, Illinois. Ovalbumin 5X cryst. (OVA) was purchased from Pentex, Inc., Kankakee, Illinois. Guinea pig serum albumin (GPA) was prepared by starch block electrophoresis as described (15). Guinea pig γ_2 -globulin was prepared as in reference 6. Poly-L-Lysine (PLL), with an average of 240 amino acid residues per molecule, was obtained from Pilot Chemical Co., Watertown, Massachusetts.

Preparation of Antigens.—Dinitrophenylated proteins (DNP) were prepared as previously described (16) by reacting 2,4-dinitrofluorobenzene with proteins at an alkaline pH, followed by extensive dialysis. Protein concentrations were determined by Kjeldahl analysis and the degree of substitution was calculated from the absorbency of the DNP derivative at 360 m μ by assuming a molar extinction coefficient of 17,530 for the DNP group. The following DNP conjugates were used for immunization: DNP₆₅-BGG, DNP₁₇-OVA, DNP₄₀-BSA, and DNP₁₄-PLL, the subscripts referring to the average number of hapten groups per molecule. Paraiodobenzenesulfonyl (pipsyl) conjugates to BGG and BSA were prepared as above using paraiodobenzenesulfonylchloride. The degree of conjugation was not determined, but a high number of groups per molecule was probably obtained by the coupling procedure employed. Arsanilic acid (AS) and dimethylaminonaphthalenesulfonyl (DMAN) conjugates to OVA and GPA were prepared as in reference 17.

Immunization of Guinea Pigs.—Hartley strain guinea pigs were injected with a solution containing 1 mg of antigen emulsified in an equal volume of complete, or, in a few instances, incomplete Freund's adjuvant (Difco Laboratories, Inc., Detroit) in the four foot-pads. During the 2nd and 3rd wk after the onset of immunization the animals were boosted intradermally each time with 0.2 to 0.4 mg of the antigen in saline. The animals were bled during the 4th wk.

Purification of Antibody.—Antibodies specific for the DNP determinant were isolated by the method of Farah et al. with DNP-BF (18) using the technique described in reference 16. Yields of 70% of the antibody present in the serum were obtained. Purification of anti-pipsyl antibodies was achieved by extraction of the washed specific precipitates (formed at the equivalence zone with pipsyl-BF) with pipsyl- ϵ -aminocaproic acid (2×10^{-3} M) in the presence of streptomycin (35 mg/ml), followed by extensive dialysis. Antibodies specific for the AS and DMAN haptens were obtained in a similar way (17). On immunoelectrophoresis all antibody preparations showed only γ_1 - and γ_2 -classes of immunoglobulins when developed with antisera against guinea pig immunoglobulins.

¹³¹I Labeling.—¹³¹I labeling was carried out as described in reference 6.

Specific Precipitation.—Specific precipitation of the iodinated protein was performed in the zone of antibody excess as described in reference 6. Rabbit antisera (Nos. 1 and 2) prepared against purified guinea pig L chains were used. These antisera were made specific for the λ -type of L chain by absorption with purified guinea pig anti-DNP₆₅-BGG antibodies, which contained only K molecules (6).

Separation of γ_1 - and γ_2 -Fractions.—Separation of γ_1 - and γ_2 -globulin fractions from purified antibodies was performed by ion exchange chromatography on DEAE-cellulose using the conditions described in reference 19. The purity of the eluted fractions was checked by immunoelectrophoresis and they were found to contain only γ_1 - or γ_2 -globulins, within the range of sensitivity of the test.

Geon Block Electrophoresis.—Separation of immunoglobulin fractions with different mobilities was carried out by zone electrophoresis on Geon block according to the technique described (20).

RESULTS

L Chain Contents of Purified Guinea Pig Antihapten Antibody.—Purified guinea pig antihapten antibodies of different specificities were individually labeled with I^{131} and precipitated with specific anti- λ -chain antisera. The percentages of labeled protein precipitated from these purified antibodies are sum-

TABLE I
Percentage of L Molecules in Purified Anti-DNP and Anti-Pipsyl Antibody Preparations Isolated from the Sera of Guinea Pigs Immunized with these Haptens Coupled to Different Carrier Proteins

Immunizing antigen			No. of sera	Per cent of L molecules in purified antibodies
Hapten	Carrier	No. hapten groups/molecule		
DNP	BGG	65	63	<i>mean</i> \pm 1 SD <1*
DNP	BGG	10	6	5 \pm 3.2
DNP	OVA	17	6	6 \pm 2.0
DNP	BSA	40	4	8.7 \pm 2.5
DNP	PLL ₂₄₀	14	4	17.3 \pm 3.9
Pipsyl	BGG		12	42.5 \pm 16.7
Pipsyl	BSA		5	39.4 \pm 24.3

* The percentages of L molecules in these antibody preparations were too low to be measured accurately by the method employed. The range of variation obtained in the individual samples was 0 to 1.5%. The antigen was incorporated in complete Freund's adjuvant (57 animals) or incomplete Freund's adjuvant (6 animals).

marized in Table I. In 63 preparations of anti-DNP₆₅-BGG antibodies isolated from individual sera of hyperimmunized animals less than 1% of L molecules were detected. In every preparation, however, trace amounts of L molecules do exist and can be shown to be present by double diffusion methods in agar. The same results were obtained irrespective of the use of complete or incomplete Freund's adjuvants.

When pipsyl-BGG was used as antigen the average composition of the antibody produced was quite different. In purified anti-pipsyl-BGG antibodies 42.5% (\pm 16.7) of the molecules were of the L type. In this case, marked differences in preparations from individual animals were noticed. In some as much as 75% of the antibody molecules had the λ -type of L chain while in others only 15 to 20% of L molecules were present.

The ratio of K/L molecules was also measured in a few preparations of AS-

OVA and DMAN-GPA antibodies. This ratio also differed from that found in normal γ_2 -globulin, but the number of samples studied was too small to draw definite conclusions.

These results are not due to some kind of selection resulting from the purification procedures. In the case of the anti-pipsyl antibodies 80 to 90% of the antibodies present in the serum were recovered. The recovery of anti-DNP antibodies was consistently 70%. In view of the somewhat lower recovery for these latter antibodies, the following experiment was done in order to demonstrate also a shift in the ratio of K/L molecules in the original guinea pig anti-DNP sera, similar to that found in purified anti-DNP antibodies. Aliquots from two different pools of anti-DNP₆₅-BGG sera were precipitated at the equivalence zone with DNP-BF. Both the supernatants of this precipitation reaction and the original antisera were submitted simultaneously to Geon block electrophoresis. Then, fractions with different mobilities were eluted from the block and separately labeled with I¹³¹. The amounts of total immunoglobulin and L molecules in these fractions were measured by specific precipitation. The results are shown in Figs. 1 and 2. The γ_2 -globulin fraction in the first serum pool consisted almost exclusively of anti-DNP antibodies and accordingly was found to contain almost only K molecules. The second serum pool showed a lower level of anti-DNP antibodies. Nevertheless in this case, also the presence of anti-DNP antibodies increased the proportion of K molecules in both γ_1 - and γ_2 -fractions. This was demonstrated by comparing the percentage of L molecules in fractions having the same electrophoretic mobility in the total serum and in the supernatant obtained after the precipitation of anti-DNP antibodies.

Influence of the Carrier and Degree of Conjugation.—In order to evaluate the influence of the carrier protein on the L chain composition of the antibody produced, several experiments were performed using DNP and pipsyl haptens coupled with several protein carriers. After purification of the corresponding antihapten antibodies the amounts of L molecules were measured. The results obtained are also shown in Table I. In the case of the pipsyl hapten, the change of carrier from BGG to BSA did not influence the proportion of L antibody molecules produced.

The response to the DNP hapten was, however, dependent on the protein carrier as well as on the degree of conjugation of the antigen. Highly and lowly derivatized DNP-BGG antigens induced different responses. Whereas anti-DNP₆₅-BGG contained very small amounts of L molecules, anti-DNP₁₀-BGG had $5\% \pm 3.2$ of L molecules. The same level of L molecules (5 to 10%) was found to be present in anti-DNP₄₀-BSA and anti-DNP₁₇-OVA antibodies. A much higher level ($17.3\% \pm 3.9$) of L molecules, approximately half that found in normal γ_2 -globulin, was found in anti-DNP₁₄-PLL antibodies.

Changes during the Course of Immunization.—In order to explore possible variation in the composition of antibodies produced in the course of immuniza-

tion the following experiment was performed. Guinea pigs were immunized with DNP₆₅-BGG and blood was collected at various times following the onset of immunization. Anti-DNP antibodies were purified from pooled samples and the percentages of L molecules measured. The results of two separate experi-

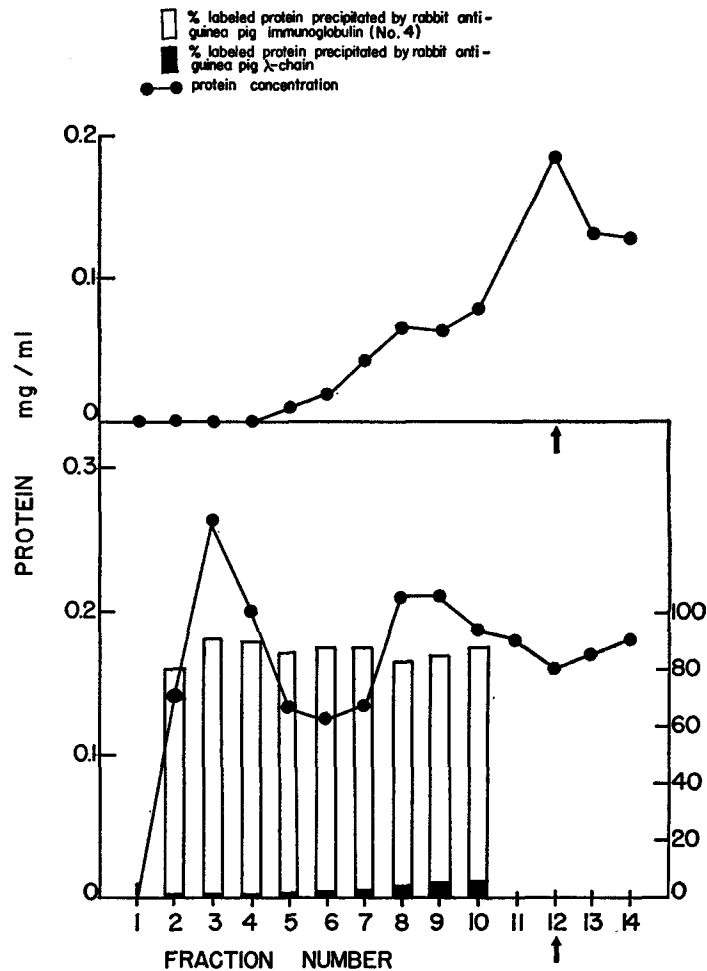


FIG. 1. Simultaneous Geon block electrophoresis of pool of guinea pig anti-DNP₆₅-BGG serum (bottom) and of the supernatant obtained after specific precipitation of anti-DNP antibodies from the same pool (top). After elution of the protein from successive $\frac{1}{2}$ -in. strips, the amounts of immunoglobulin and of L molecules were measured in each fraction from the native pool by specific precipitation. Note that the supernatant obtained after specific precipitation contains very little protein in the slower fractions (γ_2). The corresponding slow fractions eluted from the native pooled serum have much smaller proportions of L molecules than found in normal γ_2 -globulin. The anode is to the right of the figure.

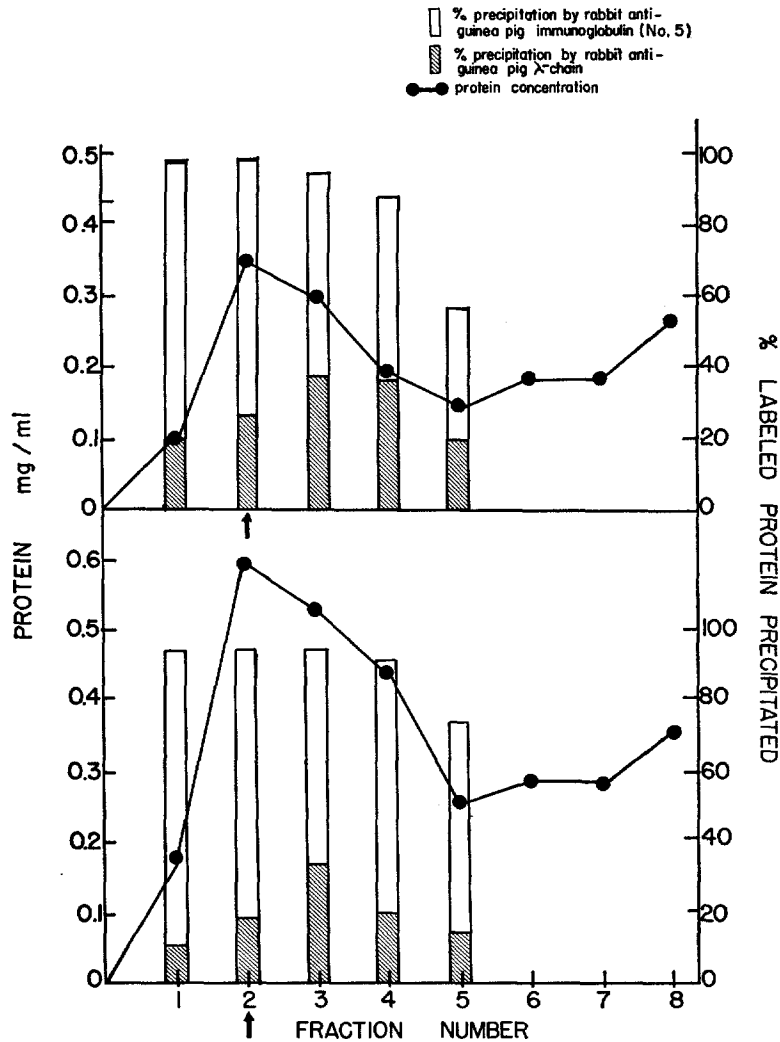


FIG. 2. Simultaneous Geon block electrophoresis of a pool of guinea pig anti-DNP₆₅-BGG serum (bottom) and of the supernatant obtained after specific precipitation of anti-DNP antibodies from the same pool (top). After elution of the protein from successive 1/2-in. strips, the amounts of immunoglobulin and of L molecules were measured in each fraction by specific precipitation. Note the lower percentages of L molecules in the native serum when compared to the supernatant obtained after precipitation of anti-DNP antibodies. The shift in the K/L ratio can be noticed in both γ_2 (fractions 1 and 2) and γ_1 -immunoglobulins (fractions 4 and 5). The predominance of γ_1 - and γ_2 -immunoglobulins in each of these fractions was determined by immunoelectroforetic analysis. The anode is to the right of the figure.

ments are shown in Table II. In both experiments at an early stage of immunization the anti-DNP₆₅-BGG antibodies contained appreciable amounts of L molecules (5 to 10%). When the serum antibody increased during the 3rd wk of immunization, almost only K molecules were produced. In all samples the immunoelectrophoretic analysis detected only the presence of γ_1 - and γ_2 -immunoglobulins.

κ - and λ -Types of L Chains in Different Classes of Immunoglobulins.— During the course of the immune response of guinea pigs to hapten conjugated proteins

TABLE II
Percentage of L Molecules in Guinea Pig Anti-DNP₆₅-BGG Antibodies Purified From Serum Samples Obtained at Various Times after the Onset of Immunization

Guinea pig group*	Time from onset of immunization	Antibody concentration in sera	L molecules in purified antibody
	days	mg/ml	%
I	9	0.25–0.35‡	6.2
I	16	3.7	<1.0
I	23	4.0	<1.0
I	30	5.5	<1.0
II	11	0.25–0.35‡	9.2
II	30		<1.0

* I, group of 10 animals; II, group of 20 animals.

‡ Calculated assuming that at the equivalence zone the ratio of antibody/antigen precipitated is from 5 to 7.

and complete adjuvant, two separate classes of immunoglobulin (γ_1 and γ_2) are produced. The question arises whether there is a relationship between the relative amounts of K and L molecules in these two separate classes of antibody produced in response to the same antigen. In order to answer this question several individual purified preparations of antihapten antibodies were studied; they contained both γ_1 - and γ_2 -immunoglobulins in variable proportions, as shown by immunoelectrophoretic analysis. These antibodies were separated into their γ_1 - and γ_2 -components by DEAE-cellulose chromatography. Each fraction was labeled with I¹³¹ and the amount of L molecules measured. The results shown in Table III and Fig. 3 demonstrate that within each sample the relative amounts of L molecules in both classes of immunoglobulins are remarkably similar.

DISCUSSION

Recent studies on the structure of Bence Jones proteins excreted in the urine of patients with multiple myeloma and which correspond to L polypeptide chains of immunoglobulins, have demonstrated that they have two distinct

TABLE III
Percentage of L Molecules in Purified γ_2 - and γ_1 -Fractions of Guinea Pig Antibody Preparations of Different Specificities Isolated from Individual Animals

Immunizing antigen*	Percentage of L molecules in purified antibody fractions	
	γ_2	γ_1
DNP-GPA	3.5	3.4
Pipsyl-BGG	52.0	82.8
Pipsyl-BGG	17.8	21.3
Pipsyl-BGG	42.0	40.8
As-OVA	19.8	22.6
As-OVA	3.3	3.1
DMAN-GPA	15.8	14.1
DMAN-GPA	23.2	24.7

* Both γ_1 - and γ_2 -fractions from 63 anti-DNP₆₅-BGG antibody preparations contained less than 1% of L molecules.

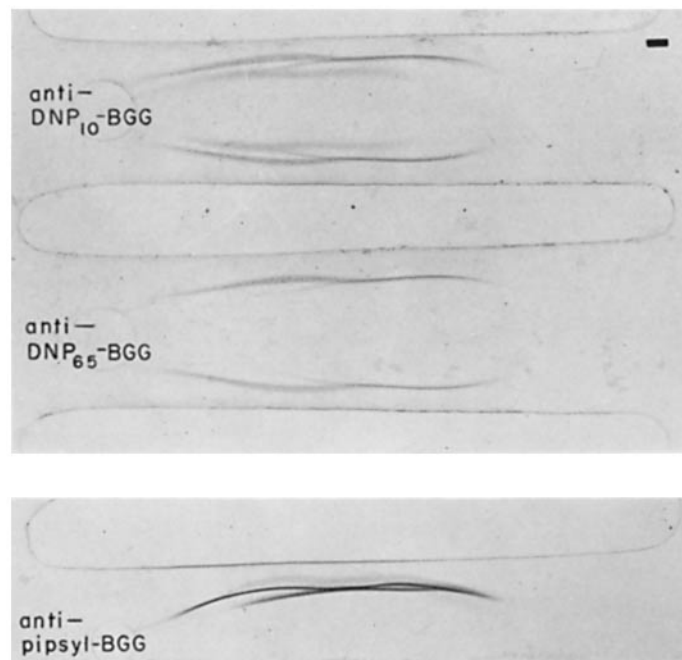


FIG. 3. Immunoelectrophoretic analysis of guinea pig purified antihapten antibodies isolated from the serum of animals immunized with DNP₁₀-BGG, DNP₆₅-BGG, or pipsyl-BGG, developed by rabbit antisera containing antibodies against both types of L chains. Note that only K molecules are detected in γ_2 - and γ_1 -anti-DNP₆₅-BGG antibodies. The intensity of the lines, corresponding to the L molecules, which are seen in anti-DNP₁₀-BGG and in anti-pipsyl-BGG are similar in both γ_1 - and γ_2 -fractions for each antibody preparation.

portions. One of them, containing the C terminal amino acid, has an amino acid sequence which is common to all Bence Jones proteins of the same type, while the other part of the chain contains sequences which are unique to each individual Bence Jones protein (21, 22). Two analogous structures, namely the variable and invariable parts of the L chain, have also been shown to be present in L chains isolated from mouse myelomas (23). The differences between L chain types correspond to changes in the amino acid sequences of their invariable portions. In this respect the invariable portion of each type of L chain corresponds, therefore, to a definite structure which is not subject to changes other than allotypic variations.

While it is considered most likely that changes in the variable portion of L chains are related to the very large number of different configurations that can be assumed by the antibody-combining site, no functional role has been attributed to the invariable portion. The present study, however, suggests that the structure of the constant portion of the L chain, is related to the conformation of the antibody site.

Previous studies on the structure of purified antibodies isolated from human sera have been concerned with the relationship between the type of L chain and antibody specificity. It was determined that the ratio of K/L molecules in purified anti-Rh antibodies, isoagglutinins, antithyroglobulin antibodies, anti-teichoic acid, and antidextran antibodies varied widely in different individuals and sometimes diverged markedly from the K/L ratio found in normal immunoglobulins (24).

It has been shown that many preparations of erythrocyte autoantibodies (25, 26) exhibit a very high percentage of molecules bearing a single L chain type. Moreover, the exclusive occurrence of the κ -chain type in every cold hemagglutinin isolated from sera of patients with chronic cold hemagglutinin syndrome has been reported (25, 27). These cold hemagglutinins are γ M-immunoglobulins and are electrophoretically more homogeneous than the γ M-globulin present in the same serum. They often have a particular serological specificity called anti-I. The absence of λ -chains in these cold hemagglutinins could be explained if their antibody specificity and (or) their peculiar temperature dependence for the reaction with the antigen were only compatible with the presence of κ -chains in the γ M-molecule (27).

In the present studies the antibody response in guinea pigs to different haptens has been analyzed. In every anti-DNP antibody preparation, irrespective of the protein carrier used for immunization, of the degree of conjugation of the antigen, or of the stage of immunization, a marked predominance of K molecules was demonstrated. When DNP₆₅-BGG was used as antigen less than 1% of the antibody molecules produced after a month of immunization were of the L type. When DNP₁₀-BGG, DNP₁₇-OVA, DNP₄₀-BSA were used as antigens a small amount of L antibody molecules were produced (5 to 10%). Anti-DNP₁₄-

PLL antibodies contained $17.3 \pm 3.9\%$ of L molecules. It was shown that these results were not due to the selection of a certain population of antibodies during the purification procedure. A high proportion of anti-DNP antibodies (70%) were actually recovered after purification. Moreover, a lower than normal percentage of L molecules was also demonstrated in aliquots of the same immune sera prior to isolation of the antibodies.

The reason for the differences in the response of guinea pigs to the DNP-hapten which occur with variations in the carrier protein is not well understood. Other influences of carrier protein on the immune response have been previously observed (28, 29). It was shown, for instance, that DNP-BGG is a better DNP antigen for guinea pigs than DNP-BSA or DNP-OVA if the serum levels of anti-DNP antibodies and their intrinsic association constants are taken into consideration. Furthermore, the response of guinea pigs to DNP-PLL seems to be considerably poorer than to DNP-BGG, DNP-BSA, or DNP-OVA as shown by 5 to 10 times lower levels of anti-DNP antibodies in their serum.

The increased production of K antibody molecules by guinea pigs to DNP antigens does not seem to be related directly to the electrical charge of the antigen, as recently observed by Sela et al. (30) with rabbit antibodies. We have previously shown (6) that K molecules are more positively charged than L molecules. If the same relationship between the charge of the antigen and the charge of the antibody that was found by those investigators were to prevail in this case, we would expect that the more acidic antigens would induce the formation of higher proportions of K antibody molecules. The opposite was actually found to be true in several instances: DNP₁₇-OVA and DNP₄₀-BSA migrate faster than DNP₆₅-BGG when electrophoresis is performed at pH 8.6, but a higher percentage of L molecules was found in antibodies induced by DNP₁₇-OVA and DNP₄₀-BSA. Moreover, although pipsyl-BSA and pipsyl-BGG have very different net charges, the antihapten antibodies in this case resembled each other with respect to their relative amount of L molecules.²

Our results can be interpreted as indicating that K molecules are more adequate for forming anti-DNP antibody sites than L molecules and that a selective mechanism involving the choice of the L chain type is operative at the cellular level during immunization. This interpretation is supported by the experiments in which the type of L chain in early and late anti-DNP antibodies was investigated. The results showed clearly that anti-DNP antibodies produced about 10 days after the onset of immunization contain higher levels of L

² It is also pertinent to point out that no clear differences were found either in the electrophoretic mobilities of anti-DNP antibody isolated from the sera of animals immunized with DNP₆₅-BGG, DNP₄₀-BSA, DNP₁₇-OVA, and DNP₁₄-PLL in spite of the markedly different electric charge of these antigens. On the other hand, we have shown previously that anti-AS antibodies are consistently faster than anti-DNP antibodies when electrophoresis is performed at pH 8.6 (31). AS-GPA, the antigen used for immunization is, however, more negatively charged than DNP₆₅-BGG.

molecules than the anti-DNP antibodies produced 1 wk later. This decrease in the percentage of L molecules occurs during the period when the serum level of antibodies increases approximately tenfold. It is known that the average intrinsic association constant of rabbit anti-DNP antibodies increases during the immunization (32). It is probable that the antibody population in guinea pig sera obtained from early bleedings, which contains a higher level of L molecules, has a lower affinity for the DNP hapten than the population of antibodies produced later. The direct demonstration that in anti-DNP antibodies L molecules may differ from K molecules in their intrinsic association constants has actually been obtained (33).

The disparity between the proportion of K and L molecules synthesized after antigenic stimulation and the proportion present in normal immunoglobulins was also observed in the pipsyl haptenic system. In contrast to the observations in the DNP system, anti-pipsyl antibodies contain a higher proportion of L molecules than found in normal γ_2 -globulins. In some individual anti-pipsyl preparations more than 70% of the antibody population was found to be of the L type. In this case no difference was detected in the response of guinea pigs to the pipsyl hapten coupled to two different protein carriers, BSA and BGG. A certain degree of heterogeneity, however, was noticed in the individual response of guinea pigs to both pipsyl BGG and BSA. While no satisfactory explanation can be given to account for this observation, it is possible that genetic factors are involved because random bred guinea pigs were used in these experiments.

Our studies show also that the relationship between the amounts of K and L molecules is remarkably constant in both γ_1 - and γ_2 -immunoglobulins isolated from the same individual guinea pig antihapten antibodies. In every anti-DNP antibody preparation from animals immunized with DNP₆₆-BGG both classes of immunoglobulins were present in variable amounts but only K molecules were detected. In anti-pipsyl antibodies increased amounts of L molecules were detected in both γ_1 - and γ_2 -immunoglobulins. These experiments demonstrate that a given antigen stimulates the synthesis of populations of these two immunoglobulin classes with very similar if not identical proportions of the two L chain types. They indicate also that the control of the synthesis of the type of L chain of an immunoglobulin is not linked to the control of the class of immunoglobulin synthesized. This could be the result of the parallel differentiation of individual cells capable of producing antibodies of identical specificities but of different classes or of the existence of a common cellular precursor which is only differentiated with respect to the L chain (and perhaps the Fd fragment) it produces and which is capable of giving rise to cells synthesizing the same L chain but different classes of H chains according to nonspecific factors affecting the immune response. A degree of independence of the regulatory mechanisms which govern the synthesis of L and H chains has been recently reported in studies on the structure of mouse myeloma proteins produced by several pairs

of plasma-cell tumor lines originating from the same primary host (34). It has been demonstrated, using fingerprinting and serological techniques, that the serum and urinary proteins produced by each pair of related transplant lines contained the same L chain variant. The differences between these pairs of cell lines appeared to be due to alterations in the regulatory mechanism of their H chain synthesis. The results obtained suggested that these plasma-cell lines originated from a common precursor cell in which the precise L chain structure had already been determined (34). Furthermore, it is known that in the rabbit the genes controlling allotypes on L chains are not linked to genes controlling allotypes on H chains (35, 36). There appears also to be a dissociation of the InV allotypes, present on L chains, from Gm allotypes, present on H chains in human IgG (37). Therefore, the regulatory mechanisms controlling the synthesis of H chain types and L chains are not the same.

SUMMARY

In guinea pig purified antihapten antibodies, the proportion of molecules bearing the κ - or λ -type of L chains (K or L molecules) may diverge markedly from that found in normal γ_2 -globulins. This has been evaluated by precipitation of I^{125} -labeled antibody preparations using a specific anti- λ -chain antiserum. Anti-DNP antibodies isolated 3 wk after immunization of guinea pigs with DNP₆₅-BGG antibodies, contain less than 1% of L molecules, while in pipsyl antibodies, isolated from the sera of animals immunized with pipsyl-BGG, the proportion of L molecules is significantly greater than in normal γ_2 -globulins.

Anti-DNP antibodies produced against conjugates of this hapten with carriers other than BGG (BSA, OVA, or poly-L-lysine) or with BGG with a small number of DNP groups (DNP₁₀-BGG) contained a greater proportion of λ -chain bearing molecules than anti-DNP antibodies isolated from late sera of guinea pigs immunized with highly conjugated DNP₆₅-BGG. An increased percentage of L molecules was detected in preparations of anti-DNP₆₅-BGG antibodies isolated early (10 days), when compared to those isolated later during the course of immunization. However, the level of L molecules in all these anti-DNP antibody preparations was always considerably below that present in normal γ_2 -globulin. The relative amounts of L molecules in distinct immunoglobulin families (γ_1 and γ_2) in antibody preparations isolated from individual animals was remarkably similar.

BIBLIOGRAPHY

1. Edelman, G. M., and Poulik, M. D., Studies on structural units of the γ -globulins, *J. Exp. Med.*, 1961, **113**, 861.
2. Cohen, S., and Porter, R. R., Structure and biological activity of immunoglobulins, *Advances Immunol.*, 1964, **4**, 287.

3. Schwartz, J. H., and Edelman, G. M., Comparisons of Bence Jones proteins and L polypeptide chains of myeloma globulins after hydrolysis with trypsin, *J. Exp. Med.*, 1963, **118**, 41.
4. Mannik, M., and Kunkel, H. G., Two major types of normal 7S γ -globulin, *J. Exp. Med.*, 1963, **117**, 213.
5. Fahey, J. L., Two types of 6.6S γ -globulins, β_2 A-globulins, and 18S γ_1 -macroglobulins in normal serum and γ -microglobulins in normal urine, *J. Immunol.*, 1963, **91**, 438.
6. Nussenzweig, V., Lamm, M. E., and Benacerraf, B., Presence of two types of L polypeptide chains in guinea pig 7S immunoglobulins, *J. Exp. Med.*, 1966, **124**, 787.
7. Edelman, G. M., Benacerraf, B., Ovary, Z., and Poulik, M. D., Structural differences among antibodies of different specificities, *Proc. Nat. Acad. Sc. U. S.*, 1961, **41**, 1751.
8. Edelman, G. M., Olins, D. E., Gally, J. A., and Zinder, N. D., Reconstitution of immunologic activity by interaction of polypeptide chains of antibodies, *Proc. Nat. Acad. Sc. U. S.*, 1963, **50**, 753.
9. Franěk, F., Kotýnec, O., Šymek, L., and Zikán, J., S-sulphonate and anti-dinitrophenyl antibodies. Some specific features of the interaction between the heavy and light subunits, *in* Molecular and Cellular Basis of Antibody Formation, (J. Sterzl, editor) Prague, Publishing house of the Czechoslovak Academy of Sciences, 1964.
10. Roholt, O. A., Onoue, K., and Pressman, D., Specific combination of H and L chains of rabbit γ -globulins, *Proc. Nat. Acad. Sc. U. S.*, 1964, **51**, 173.
11. Nisonoff, A., and Hong, R., Heterogeneity in the complementation of polypeptide subunits of a purified antibody isolated from an individual rabbit, *J. Immunol.*, 1966, **96**, 622.
12. Lamm, M. E., Nussenzweig, V., and Benacerraf, B., Isolation of purified H and L polypeptide chains from guinea pig γ_2 -immunoglobulin after mild reduction, *Immunology*, 1966, **10**, 309.
13. Edelman, G. M., Benacerraf, B., and Ovary, Z., Structure and specificity of guinea pig 7S antibodies, *J. Exp. Med.*, 1963, **118**, 229.
14. Nussenzweig, V., and Benacerraf, B., Electrophoretic patterns at acid and alkaline pH of reduced guinea pig 7S gamma globulin and antihapten antibodies of different specificities, *Internat. Arch. Allergy and Appl. Immunol.*, 1965, **27**, 193.
15. Kunkel, H. G., Zone electrophoresis, *Methods Biochem. Analy.*, 1954, **1**, 141.
16. Eisen, H. N., Preparation of purified anti-2,4-dinitrophenyl antibodies, *Methods Med. Research*, 1964, **10**, 94.
17. Thorbecke, G. J., Benacerraf, B., and Ovary, Z., Antigenic relationship between two types of 7S guinea pig γ -globulins, *J. Immunol.*, 1963, **91**, 670.
18. Farah, F. S., Kern, M., and Eisen, H. N., Preparation and some properties of purified antibody specific for the 2,4-dinitrophenyl group, *J. Exp. Med.*, 1960, **12**, 1195.
19. Oettgen, H. F., Binaghi, R. A., and Benacerraf, B., Hexose content of guinea pig γ_1 and γ_2 immunoglobulins, *Proc. Soc. Exp. Biol. and Med.*, 1965, **118**, 336.

20. Müller-Eberhard, H. J., and Kunkel, H. G., The carbohydrate of gamma-globulin and myeloma proteins, *J. Exp. Med.*, 1956, **104**, 253.
21. Hilschmann, N., and Craig, L. C., Amino acid sequence studies with Bence-Jones proteins, *Proc. Nat. Acad. Sc. U. S.*, 1965, **53**, 1403.
22. Titani, K., Whitley, Jr., E., Avogardo, L., and Putnam, F. W., Immunoglobulin structures: partial amino acid sequence of a Bence-Jones protein, *Science*, 1965, **149**, 1090.
23. Potter, M., Dreyer, W. J., Kuff, E. L., and McIntire, K. R., Heritable variation in Bence-Jones protein structure in an inbred strain of mice, *J. Mol. Biol.*, 1964, **8**, 814.
24. Mannik, M., and Kunkel, H. G., Localization of antibodies in group I and group II γ -globulins, *J. Exp. Med.*, 1963, **118**, 817.
25. Franklin, E. C., and Fudenberg, H. H., Antigenic heterogeneity of human Rh antibodies, rheumatoid factors, and cold agglutinins, *Arch. Biochem. and Biophysics*, 1964, **104**, 433.
26. Leddy, J. P., and Bakemeier, R. F., Structural aspects of human erythrocyte autoantibodies, I. L Chain types and electrophoretic dispersion, *J. Exp. Med.*, 1964, **121**, 1.
27. Harboe, M., VanFurth, R., Schubotho, H., Lind, K., and Evans, R. S., Exclusive occurrence of κ -chains in isolated cold haemagglutinins, *Scand. J. Haemat.* 1964, **2**, 259.
28. Siskind, G. W., Paul, W. E., and Benacerraf, B., Studies on the effect of the carrier molecule on antihapten antibody synthesis. I. Effect of carrier on the nature of the antibody synthesized, *J. Exp. Med.*, 1966, **123**, 673.
29. Paul, W. E., Siskind, G. W., and Benacerraf, B., Studies on the effect of the carrier molecule on antihapten antibody synthesis. II. Carrier specificity of anti-2,4-dinitrophenyl-poly-L-lysine antibodies, *J. Exp. Med.*, 1966, **123**, 689.
30. Sela, M., and Mozes, E., Dependence of the chemical nature of antibodies on the net electrical charge of antigens, *Proc. Nat. Acad. Sc. U. S.*, 1966, **55**, 445.
31. Nussenzweig, V., and Benacerraf, B., Differences in the electrophoretic mobilities of guinea pig 7S antibodies of different specificities, *J. Exp. Med.*, 1964, **119**, 409.
32. Eisen, H. N., and Siskind, G. W., Variations in affinities of antibody during the immune response, *Biochemistry*, 1964, **3**, 996.
33. Nussenzweig, V., in preparation.
34. Potter, M., and Kuff, E. L., Disorders in the differentiation of protein secretion in neoplastic plasma cells, *J. Mol. Biol.*, 1964, **9**, 537.
35. Dray, S., Young, G. O., and Gerald, L., Immunochemical identification and genetics of rabbit γ -globulin allotypes, *J. Immunol.*, 1963, **91**, 403.
36. Dray, S., and Nisonoff, A., Relationship of genetic control of allotypic specificities to the structure and biosynthesis of rabbit γ -globulin, in *Molecular and Cellular Basis of Antibody Formation*, (J. Sterzl, editor), Prague Publishing house of the Czechoslovak Academy of Sciences, 1964.
37. Steinberg, A. G., Progress in the study of genetically determined human gamma globulin types (the Gm and InV groups), *Progr. Med. Genetics*, 1962, **2**, 1.