A POSSIBLE GENETIC BASIS FOR SPECIFIC PATTERN IN ANTIBODY

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IN the original formulation of the clonal selection theory of immunity¹, it was suggested that the necessary range of immune patterns might arise if "at the appropriate stage of development a limited genetic determinant carrying the coding responsible for globulin pattern releases control in such a fashion that purely random arrangements are allowed" (ref. 1, p. 60). Since 1958-59 there have been great advances in the understanding of protein structure and particularly of the structure of immunoglobulins (see, for example, ref. 2). In addition, there has grown up in the past 10 years an intense interest in the evolution of chemical pattern in proteins which may well have a very important bearing on the antibody problem. It seems appropriate, therefore, at this time to attempt to rephrase the above quotation in the light of recent developments.

Structure of Immunoglobulin G Antibodies

It is generally accepted that an IgG antibody molecule is an elongated flexible structure of dimensions, according to Edelman and Gally³, of $240 \times 54 \times 19 \text{ \AA}$. It is symmetrical in character with specific combining sites at each end. The physical character of the combining sites is unknown, but it is accepted that they represent a configuration of parts of the peptide chains making up the macromolecule.

Appropriate treatment with proteolytic enzymes, reducing agents capable of splitting disulphide bonds, and detergents has provided a series of large fractions the interrelations of which are conventionally shown in Porter's well-known diagram⁴ (Fig. 1). This shows four chains held together by three (or five) disulphide linkages. The two light chains have a molecular weight of about 25,000 with 212 amino-acid residues⁵; the heavy chains with a molecular weight of $55,000 \pm$ would have ± 450 residues. Attached to the heavy chains is an oligosaccharide unit containing galactose, mannose, fucose, acetylhexosamine and sialic acid⁶.

In man, the light chains have a common character in all types of normal immunoglobulins and are found in most types of myeloma proteins. The Bence-Jones proteins found in the urine of myelomatosis patients appear to be identical with light chains⁷. There are two serological types of light chain characterized by antigens κ and λ .

Recent work from several laboratories is consistent with the finding first announced by Hilschmann and Craig⁸ that the light chains of myeloma proteins have two halves each comprising 106 residues which are strikingly different in character^{8,9}. Most work has been done with proteins the light chains of which are of κ type, but it seems clear that, apart from differences in the sequence

of the C-terminal region related to the L-K antigenic difference¹⁰, all light chains are made up in essentially the same fashion. The C-terminal half of κ -type light chains is identical in the three that have been investigated except for single residue replacements at three points. One of these, No. 189, is of special interest because replacement of leucine by valine is associated with a change in allotype from INVa to INVb (refs. 8 and 11). The N-terminal half, residues 1-106, differs strikingly from one myeloma κ protein to another. Milstein finds an indication in one that even the cysteine residues can be displaced. It is clear (a) that the C-terminal half is a typical polypeptide chain controlled by a stable cistron and subject only to rare mutational replacement of single residues, and (b) that the N-terminal half must have an entirely different type of genetic control. Yet, because this half is uniform in all myeloma protein molecules produced by the single clone of neoplastic cells, it is evident that there is firm genetic control during at least the productive phase of the disease. It is scarcely conceivable that the controlling sites in the genome are in the same cistron, and for ease of discussion we may speak of Ls (stable, C-terminal) and Lv (variable, N-terminal) sub-chains.

The heavy chain is not so amenable to investigation and no amino-acid residue sequence has been published. It is evident from the work of Meltzer *et al.*¹², Franklin *et al.*¹³, and Franklin¹⁴ that the crystallizable fraction Fc is of uniform structure apart from the presence or absence of two peptides which are correlated with the Gm+ or Gm- allotype antigens. Tryptic peptide maps of myeloma protein heavy chains show additional spots individual for each protein and therefore presumably carried on the Fd segment. In the comparable position in similar tests on normal IgG heavy chain there is much weak 'background', and this indicates that there is great heterogeneity in these peptides¹⁵. It seems certain, therefore, that the heavy chain, like the light chain, is composed of a variable Fd and a stable Fc segment. So far as I know, there is nothing to exclude the possibility that Fd and Lv are identical.

Nature of the Combining Site

Appropriate manipulation of fragments and recombined fragments of IgG antibody has shown:

(1) That half molecules containing one light and one heavy chain can bind specifically to antigen but have no precipitating power^{16,17}. They can be recombined from two antibodies to produce hybrid antibody molecules never found in Nature¹⁸.

(2) Separated light chains have only very little or no binding power for antigen (hapten). Separated heavy chains usually have definite binding power, but this is greatly increased when homologous light chain is recombined with it^{19,20}.

(3) Light chains recombine specifically with homologous heavy chains to re-create full biological activity²¹. According to Metzger and Mannik²², this is more striking if the corresponding antigen is included in the system.

(4) It is possible to investigate the combining site directly by using hapten either to shield the site from iodination, etc., "pair labelling"^{23,24}, or when appropriately modified to transfer tritium label to amino-acids in the site ("affinity labelling")²⁵. Results are consistent in showing

Fig. 1. A modification of the Porter diagram to illustrate the hypothesis of four "variable" sub-chains all of the same pattern

that both light and heavy chains are involved^{24,25}. The first group using pair labelling found most of the shielded residues in the light chain, while with affinity labelling both are involved.

(5) In a report which is in the course of being published, Doolittle and Singer²⁶ present evidence that similar peptides are labelled in both light and heavy chains. The peptides from the two chains concur in the average size of the labelled peptides, their hydrophobic quality and the range of heterogeneity.

There is a relatively simple hypothesis which satisfactorily covers all these findings and which could be tested experimentally. This is that the binding site is composed of a loop in which two identical chains Fd and Lv lie congruently, residue to residue, either parallel or in spiral relationship. This chain is controlled by a specific cistron *V* (variable) and is represented four times in the IgG molecule.

Fig. 2 suggests the disposition of such a duplex chain at one end of the IgG molecule. The primary requirement of a combining site is that it should present a specific adsorptive pattern that is constant in character and uniquely differentiable from the essentially random configurations of portions of other polypeptide chains which make up the surface of the macromolecule. The simplest unique quality that could be applicable to a wide variety of random patterns is that two (or more) strands of identical character should lie in parallel or helical contact with each residue matched with its equivalent residue on the other chain. Many more complex arrangements could be imagined, but the one suggested has the advantage that it can in principle be tested simply by establishing the sequences at the N-terminal end of the light chain and of the Fd section of the heavy chain with due regard to the fact that the N-terminal amino-acid is blocked on this chain²⁷.

In addition to the findings already noted, this hypothesis accounts for the symmetry and equivalence of the two combining sites, and for the similarity, sometimes very striking²⁸, of the specificity and avidity of IgM and IgG antibody when the change over from M to G occurs. It also requires a much smaller degree of phenotypic restriction than if the immune pattern is controlled by a number of separate genetic segments and leaves open an approach to the sub-cellular transfer of genetic (immunological) information if future experimental results should establish the occurrence of such processes.

Somatic Mutation as a Source of Diversity

The obvious implication of the hypothesis by which each combining site is composed of two identical chains varying widely from one IgG to another is that they are controlled by a single cistron of quite unusually labile character.

A rough calculation suggests that at birth a child may possess of the order of 3×10^{10} stem cells, thymocytes and lymphocytes which have undergone 32+ cell generations from the fertilized ovum. To ensure that at birth at least one amino-acid replacement from the initial pattern had occurred in all cells would require a mutation rate of

$1 : 10^4$ per nucleotide per cell generation. This is the same order of frequency as the phage mutation $T2r^+$ to $T2r$ (ref. 29). Even more relevant is the existence of 'hot spots' of high mutability in the phage T4 (ref. 30), and the fact that with bromuracil the mutability of a given locus may be raised to 10^4 times the spontaneous rate. The possibility of an unduly high mutation rate in a mammalian cistron cannot therefore be excluded *a priori*.

From an examination of the first accounts of amino-acid sequence in the Lv chain of three myeloma proteins, the results quite closely resemble what is found in comparative investigations of the structure of haemoglobin—including the relation of myoglobin and the individual globin chains (reviewed by Zuckerkandl and Pauling³¹) and of cytochrome *c* (reviewed by Margoliash and Smith³²). Perhaps the most important impression one gains from this work on species differences in proteins is the random character of the changes that have occurred in the course of evolution. The number of invariant groups in the globin chains, that is, residues constant in position through the whole of the available material, has now fallen to 11 in 150–160, and Jukes³³ has published a table indicating that the number of alternatives known for each site gave a distribution very close to a Poisson one with a mean of 2.1 alternatives per site. Similarly, the lengths of sequences which are invariant in the cytochrome *c* material from yeast to man also show a close approach to a Poisson distribution. In evolutionary changes the important matter is not the fact of mutation but the ability of a given mutation (*a*) to allow adequate function of the cells involved, and (*b*) to replace the previously existent sequence as that characteristic of the species. Only an infinitesimally small number of the mutations of protein sequence that must occur will ever find opportunity, through selective survival, genetic drift and other processes, to become a new standard for the species. Even so, it appears from the pattern of results that random processes predominate.

If it is legitimate to look for somatic mutation at an accelerated rate in cistron *V* for the source of immune patterns, one would expect, despite the fantastic difference in the time-scales involved, to find essentially similar random changes of amino-acid sequence occurring. Such random changes produced in one way or another are vital to any theory of clonal selection.

The present hypothesis of the origin of immune pattern has at least the virtue of demanding experimental tests. It should be found that the Fd and Lv chains from a given myeloma protein are identical or have considerable segments in common. Available information does not exclude the possibility that the variable portions of light and heavy chains are derived from distinct cistrons and, if there is no identity in pattern found experimentally, a more complex hypothesis will have to be sought. One would prefer, however, not to abandon the simple hypothesis solely on evidence from myeloma proteins, until it is more clearly established that they are structurally equivalent to monoclonal antibodies.

Should the results show a consistent pattern in the variable segments it will become of first-rate importance to look for evidence as to how the variable pattern arose. There have been reports of myeloma proteins showing two spikes on electrophoresis, presumably indicating either a secondary mutation in the primary myeloma clone or a second primary mutation to neoplastic activity. The difference between the two patterns, both presumably derived from the pattern(s) characteristic of the fertilized ovum, should be illuminating. In any such investigation, the existence and extent of phenotypic restriction would be highly relevant. Since the Lyon phenomenon by which, in the female, one of the two X-chromosomes becomes non-functional in every cell is the only fully established example of phenotypic restriction at the somatic level, it is perhaps worth considering the possibility that the 'variable' cistron is carried on the X-chromosome. For

Fig. 2. A diagram to indicate the possible arrangement of a duplex polypeptide as combining site at the end of an IgG antibody. The cross-section of the molecule has been taken as $54 \text{ \AA} \times 19 \text{ \AA}$ and the average effective size of an amino-acid residue as $5 \text{ \AA}^2$.

this reason, a search for diclonal 'gammopathies' in males might be more rewarding than in females.

There seem to be at least three possible origins of diversity: (1) accelerated replacement of single nucleotide pairs as suggested here; (2) intra-cistronic inversions, duplications or deletions as suggested by Smithies; (3) a process equivalent to transformation in bacteria in which the replacing segment of DNA may come either from within the cell or from an active adjacent cell. When further variable chains have been examined for sequence it should become possible seriously to consider these alternatives.

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INTENSITY OF THE GEOMAGNETIC FIELD IN INDIA OVER THE PAST 4,000 YEARS

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BAKED bricks and pots acquire magnetization as they cool from the high temperatures prevailing inside kilns and hearths to normal temperatures. This effect can be detected later, when it is termed 'thermoremanent magnetization'. The magnetization is in the direction and proportional to the ambient magnetic field of the Earth at the time of firing. Folgheraiter¹ and Koenigsberger² suggested that such materials obtained from ancient (archaeological) sites could be used for studying the Earth's past magnetic field. Such an application had to await the development of a satisfactory technique for studying detailed variation in field-strength itself. This was achieved by Thellier *et al.*³, who compared ancient remanent magnetization in brick samples with that acquired when these samples were heated and cooled in the present magnetic field. This pioneering work led to the interesting observation that 2,000 years ago, the Earth's magnetic field in France was about 60 per cent stronger than it is at present.

In view of the importance of this result to geomagnetic studies and other allied fields, it was considered desirable to conduct similar studies in other areas. India has a geomagnetic latitude spread of 0°–25° N. and also has a large number of archaeological sites, many of which have recently been dated by the Radiocarbon Laboratory at this Institute. In view of these considerations, the present study was undertaken.

The method used was, in principle, the same as that of Thellier *et al.*³, who mainly used bricks for the purpose. The materials most commonly obtained from archaeological excavations are, however, pots and potsherds. In order to make use of these, a suitable technique had to be devised. Small pieces of pottery or brick were firmly embedded in plaster of Paris inside individual cylindrical porcelain containers (height, 4 cm; diameter, 4 cm). With their orientation thus fixed, the magnetic moment of the sample was measured by means of an astatic magnetometer (sensitivity, 10⁻⁵ gauss). Each sample was

first rotated below the magnetometer along the axis of the cylinder (Z component) and the directions of maximum (X-direction) and minimum (Y-direction) deflexion determined. Proper reference marks were then made and the deflexions along the X, Y and Z co-ordinates measured by the standard procedure which involved making a correction for sample inhomogeneity. The sample concerned was then heated to 300°–400° C with the X-direction aligned with the meridian, cooled and its magnetization remeasured. The sample was then reheated to the same temperature in reverse orientation, cooled and the magnetization measured again. These measurements enable us to obtain partial thermoremanent magnetization in ancient and laboratory fields by addition and subtraction of the vector co-ordinates X, Y and Z in the manner suggested by Thellier³. The sample was further heated to 675° C (which is the Curie point for haematite), cooled and its magnetization remeasured. This enabled us to deduce another value for the ratio of the ancient field strength to the present one.

The samples were heated in a non-inductive portable furnace. The heating elements were constructed from 22 gauge platinum wire, wound around quartz tubes with the lead wire of each element passing through the centre of the tube in a direction opposite to that of winding⁴. Ten such elements were mounted symmetrically inside a cement pipe (diameter, 22.5 cm; height, 13 cm). A thick copper vessel (diameter, 16.5 cm; height, 7.5 cm) was placed co-axially inside the furnace. Four samples were heated simultaneously inside this vessel in nitrogen atmosphere. A duplicate assembly made it possible to use the furnace to heat one set of samples while the other set cooled.

The precision of the experimental procedure was determined in the following way. A single pot was heated to 1,000° C in an oxidizing atmosphere for 2 h in order to destroy initial magnetization and to ensure that the constituent minerals remained stable during subsequent heatings, and then allowed to cool. In this way it acquired