A RELATION BETWEEN ANTIBODY AND NON-SPECIFIC γ-GLOBULIN PRODUCTION PREDICTABLE BY THE CLONAL SELECTION THEORY OF THE IMMUNE REACTION [1]

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That the secretion of antibodies in the blood of hyper-immunized animals is accompanied by a substantial increase in the non-specific γ-globulin[2] concentration was suggested by early work (for a review see Landsteiner (1945) p.146) but the experimental techniques employed did not permit a comparison of the rates of synthesis of the two types of proteins. More recently, Askonas and Humphrey (1958) have confirmed, by in vitro studies, that a stimulation of antibody synthesis is accompanied by an increased production of non specific γ-globulin.

If,as proposed by Burnet (1959), the specificity of antibodies is the result of somatic mutations, all cells of the clone affected by a given mutation may be expected to synthesize both antibody under the direction of the mutated gene and non-specific γ-globulin under the influence of the normal allele of

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2. Here non-specific refers to all γ-globulins (IgG) which do not react with the antigen used for immunization.

this gene. These two syntheses would thus be linked and the two types of proteins should be produced simultaneously in more or less equal quantities. The following experiments support such a prediction.

1) Fragments of spleen from rabbits immunized against the flagellae of Salmonella paratyphi B are cultured in vitro in the presence of ^{3}H-phenylalanine; during a period of 9 days, the fragments secrete labeled γ-globulins into the culture medium. These γ-globulins are separated from the medium by a preliminary precipitation of antibody with the flagellae of Salmonella followed by removal of all non-specific γ-globulins with an anti-rabbit γ-globulin antiserum. The total radioactivity of the antibody, measured at various times, is always approximately equal to the total radioactivity of the non-specific γ-globulins (Table I).

TABLE I

Incorporation of ^{3}H-phenylalanine into the non-specific γ-globulins and antibodies secreted in vitro by fragments of spleen from rabbits hyper-immunized with flagellae of Salmonella

	0	3 days	6 days	9 days
non-specific γ-globulin	25*	190	355	500
antibody	25	220	350	460

* Radioactivities are expressed in counts/min corresponding to the total quantity of γ-globulin secreted. ^{3}H-phenylalanine was injected at time 0. The culture technique of Corvazier et al.(1963) was employed.

2) Rabbits immunized with tobacco mosaic virus (TMV) are injected with ^{3}H-phenylalanine when the antibody titer of the serum has reached its maximum value and remains constant. The popliteal lymph nodes are removed 30 minutes later; at this time

the antibody synthesized after the injection of the labeled amino acid has not yet been liberated into the blood (Helmreich et al., 1961). The γ-globulins are prepared from a lymph node extract by chromatography on DEAE-cellulose and the antibody is separed from the non-specific γ-globulins by TMV precipitation. The radioactivity of the non-specific γ-globulin is approximately equal to that of the antibody (Table II).

TABLE II

In vivo incorporation of ^{3}H-phenylalanine into non-specific γ-globulins and anti-TMV antibodies produced in the popliteal lymph nodes

	Non-specific γ-globulins	Anti-TMV antibodies	Total protein of lymph node
Quantity found (µg)	603	457	-
Specific radioactivity counts/min/mg	5400 458	5088 542	1260 70

3) Rabbits immunized against TMV are injected with ^{3}H-phenylalanine at the same moment as in experiment 2. Serum samples taken at various times after injection of labeled phenylalanine show equal incorporation into anti-TMV antibodies and non-specific γ-globulins (Table III).

TABLE III

Incorporation of ^{3}H-phenylalanine into the non-specific γ-globulins and anti-TMV antibodies of serum (counts/min/ml)

	0h	2h	4h	7h	24h	26h	48h
non-specific γ-globulins	29	116	209	332	1420	1480	3180
antibodies	36	108	184	300	1210	1360	2600

^{3}H-phenylalanine was injected at time 0.

The same experiment is carried out on a series of rabbits which receive the ^{3}H-phenylalanine injection at varying times after the injection of TMV. When ^{3}H-phenylalanine injection is given during the first phase of antibody production where the concentration of antibody in the serum is increasing (up to about 20 days after the first injection of antigen), the ratio between the radioactivity incorporated into the non-specific γ-globulins and that incorporated into the antibodies decreases progressivly from a value of about 3 after 8 days to 1 after 20 days. The value of 1 is reached only when the system is at a steady state, i.e. when the antibody titer is relativly constant, and this value of 1 may be maintained as long as 7 months. At this time the antibody titer was still as high as 4 mg/ml (Table IV).

A progressive diminution in the ratio between the radioactivity incorporated into the non-specific γ-globulins

TABLE IV

Ratio between incorporation of ^{3}H-phenylalanine into non-specific γ-globulins and into anti-TMV antibodies at varying times after the beginning of immunization

Time after the first injection of antigen (days)	Antibody titer in serum (mg/ml)	Radioactivity of non-specific γ-globulins (counts/min) (a)	Radioactivity of antibodies (counts/min) (b)	a/b
8	0.78	14,200	4,338	3.3
		12,390	4,581	3
12	1.18	10,320	4,703	2.2
		9,360	4,551	2
30	8.25*	476	420	1.1
		473	386	1.2
		460	404	1.1

* This value is exceptionally high. In all other cases, the maximum antibody titer found was 4 mg/ml.

and that incorporated into antibody would be anticipated as the specific antibody produced is added in larger and larger quantities to the γ-globulins already being produced before the immunization. The very striking fact is that the limiting value should be 1.

It seems logical that this limiting value should be approached when the antibody producing system created by the immunization completely dominates, by its size and secretory power, that which existed in the animal before this immunization. If this is true, the best overal interpretation of our results appears to be that all antibody-producing cells are heterozygous with respect to the information necessary for determining antibody specificity and that the secretion of antibody is the result of the de-repression of 2 allelic structural genes which have the same operator. One of these genes would determine the structure of the antibody and the other, the structure of a non-specific γ-globulin.

The heterozygosity of antibody-producing cells with respect to the gene responsible for specificity is an obvious implication of Burnet's clonal selection theory. According to this theory, the specificity of an antibody is attributed to a somatic mutation. There is little chance that the same mutation would occur twice in the same cell. Thus the result presented above give *a posteriori* support to the clonal theory.

On the other hand the result presented here clearly exclude an informative theory as the basis of antibody specificity. It is impossible to understand how an antigen could affect the structure of only half of the γ-globulin molecules synthesized in its presence.

A detailed description of this work will appear elsewhere.

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