THE ROLE OF NONSPECIFIC GLOBULINS IN THE BIOSYNTHESIS OF ANTIBODIES

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A comparison of the dynamics of change of separate protein serum fractions during immunization shows that the decrease in the concentration of albumin and the increase in the concentrations of the α- and β-globulin fractions take place, to a certain extent, in a definite proportion to each other, while the amount of γ-globulin does not depend on the amounts of other serum fractions [3, 4]. This brings up the question, as to what role does the change in the concentration of separate protein serum fractions play in the formation of antibodies.

The purpose of this work was to study the part played by nonspecific globulins in the biosynthesis of antibodies.

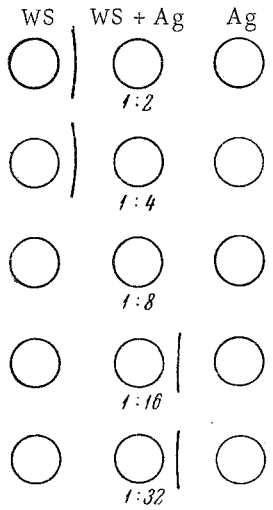
EXPERIMENTAL METHOD

Rabbits were injected subcutaneously simultaneously with the antigen (human γ-globulin) and with 5 ml of 1% homologous γ-globulin isolated by the rivanol-alcohol method [1].

Antibodies were determined by the ring precipitation test, complement fixation test (CFT) in 50% titer [2], quantitatively according to Heidelberger and Kendall [8], and by titrating antisera within the zone of equivalence. The last method has been developed by us. It is illustrated in the figure.

Three parallel rows of wells (6-8 per row) are made in an agar plate 8 mm in thickness. The wells are spaced 1 cm from each other vertically as well as horizontally.

The first row of wells are filled with the undiluted antiserum, and the third row with undiluted antigen. Specific precipitates are formed in the wells of the middle row, for which they are filled with equal volumes (of 0.1 to 0.2 ml) of undiluted antiserum and of antigen in different dilutions.



The formed precipitate falls to the bottom of the well, while the excess antigen or antibody diffuses into the agar and produces a line of precipitation on the corresponding side. There are no lines of precipitation opposite that well, in which the antigen and the antibody are present in equivalent amounts. The dilution of the antigen in this well is the antiserum titer within the zone of the equivalent ratio. This titer represents very well the quantitative antibody content of the serum.

EXPERIMENTAL RESULTS

The passive injection of homologous γ-globulin into healthy rabbits had no apparent effect on the biosynthesis of antibodies. When nonspecific γ-globulin was

Fig. 1. Titration of antiserum within the zone of equivalent ratios of the antigen and antibody. WS) Whole serum; Ag) undiluted antigen. Numbers show the dilutions of the antigen. The point of equivalence is found at antigen dilution of 1:8.

TABLE 1. Effect of Passively Injected Nonspecific γ-Globulin on the Biosynthesis of Antibodies in Healthy Rabbits and in Those Irradiated with a Dose of 1000 r

Healthy rabbits								Irradiated rabbits											
not injected with gamma-globulin				injected with gamma-globulin				not injected with gamma-globulin				injected with gamma-globulin and antigen into the same leg				injected with gamma-globulin and antigen into different legs			
time of appearance of antibodies		maximum amount of antibodies		time of appearance of antibodies		maximum amount of antibodies		time of appearance of antibodies		maximum amount of antibodies		time of appearance of antibodies		maximum amount of antibodies		time of appearance of antibodies		maximum amount of antibodies	
precipitation	CFT in 50% titer	titer in the zone of equivalence	according to Heidelberger	precipitation	CFT in 50% titer	titer in the zone of equivalence	according to Heidelberger	precipitation	CFT in 50% titer	titer in the zone of equivalence	according to Heidelberger	precipitation	CFT in 50% titer	titer in the zone of equivalence	according to Heidelberger	precipitation	CFT in 50% titer	titer in the zone of equivalence	according to Heidelberger
8	4	1:64	1 520	10	4	1:128	3 000	14	8	1:16	385	8	5	1:8	210	14	9	1:8	185
10	5	1:128	2 960	9	4	1:32	760	13	8	1:8	205	10	6	1:16	375	16	10	1:4	95
8	5	1:32	730	8	3	1:32	720	14	9	1:4	105	10	7	1:4	100	13	8	1:16	390
9	3	1:64	1 550	8	4	1:128	3 100	15	9	1:16	360	9	6	1:4	110	17	9	1:8	220
9	4	1:128	3 040	8	4	1:128	3 030	17	10	1:8	190	8	4	1:16	370	15	10	1:8	190
10	5	1:64	1 480	9	5	1:128	2 950	16	9	1:8	185	9	5	1:8	215	14	8	1:4	110
9	3	1:32	735	10	3	1:64	1 485	13	8	1:4	95	8	4	1:8	195	13	8	1:8	180
8	4	1:32	755	8	3	1:64	1 500	14	10	1:16	410	8	7	1:16	395	15	10	1:16	380
$M \pm m$	4,2 ±0,7		1 596 ±698		3,8 ±0,6		2 068 ±848		8,9 ±0,7		242±105		5,5 ±1,0		246±89		9,0 ±0,8		219±93

Note: Time of appearance of antibodies is in days; results of titration in dilutions of the antigen within the zone of equivalence; amount of antibody determined according to Heidelberger in mg of protein per 1 ml of blood serum.

TABLE 2. Dynamics of Accumulation of Passively Injected γ-Globulin in the Lymph Nodes and in Blood of Rabbits

Organs or tissues	1st day	2nd day	3rd day	4th day
Regional lymph node	1:300 / 750	1:100 / 250	1:40 / 100	1:10 / 25
Blood	1:50 / 125	1:100 / 250	1:100 / 250	1:50 / 125
Remote lymph node	0 / 0	1:20 / 50	1:30 / 75	1:20 / 50

Legend. The numerators show the titers of antibodies to Bact. paracoli, contained in the passively injected γ-globulin; the denominators show the number of micrograms of γ-globulin (the immunological unit of the γ-globulin preparation is equal to 2.5 μg of protein).

TABLE 3. The Effect of Passively Injected Homologous Albumin on the Concentration of Protein Fractions and on the Biosynthesis of Antibodies

Protein fractions	After intravenous injection of albumin				Without injection of albumin			
	initial	10 days	20 days	30 days	initial	10 days	20 days	30 days
Albumins	3,27 ±0,04	3,40 ±0,07	3,28 ±0,08	3,31 ±0,09	3,35 ±0,07	3,49 ±0,05	3,11 ±0,04	2,73 ±0,07
α-Globulin	0,97 ±0,04	0,90 ±0,08	1,04 ±0,04	1,08 ±0,06	0,98 ±0,04	0,97 ±0,05	1,11 ±0,04	1,53 ±0,03
β-Globulin	0,86 ±0,05	1,06 ±0,06	1,20 ±0,07	1,30 ±0,07	0,85 ±0,05	0,88 ±0,06	1,15 ±0,05	1,41 ±0,03
γ-Globulin	1,02 ±0,04	1,18 ±0,04	1,65 ±0,07	1,72 ±0,09	0,99 ±0,032	1,08 ±0,039	1,66 ±0,05	1,74 ±0,06
Antibodies	0	78	142	920	0	71	156	890

Note: Protein fractions are expressed in mg-%, antibodies in μg/ml.

injected into irradiated rabbits, in which the concentration of this serum fraction was sharply decreased in the regional lymphoid system [5], the inductive phase of the antibody synthesis became significantly shortened if the antigen and the γ-globulin were injected into the same leg. Thus, in control rabbits, which did not receive γ-globulin, the antibodies in the peripheral blood, according to CFT, appeared on the 8th-10th day following immunization, but according to the ring precipitation test only on the 13th-17th day. In experimental rabbits the antibodies in the peripheral blood were found on the 4th-7th and the 8th-10th days following immunization respectively. Moreover, the maximum amount of antibody, determined according to the method of Heidelberger and Kendall and by the method of equivalent titration, was found to be almost the same in animals of both groups (at the peak of antibody formation, according to Heidelberger 242 and 246 μg/ml respectively, and by the method of equivalent titration the titer was 8.74 in both cases, Table 1).

When the antigen and γ-globulin were injected into different legs there was no effect of γ-globulin on the inductive phase.

Most of the γ-globulin, labelled according to the method of Sosova [6], accumulated on the 1st day of the experiment within the regional lymph node, while during following days it could be detected in the more remote lymph nodes (Table 2).

The data obtained indicate that the inductive phase of the biosynthesis of antibodies terminates only at a certain definite concentration of nonspecific γ-globulins within the organs which produce immune proteins, during the first 1 or 2 days following the introduction of the antigen.

A passive injection of 1 ml of 1% homologous albumin (isolated according to the method of Kohn) per day, for 10-12 days, into immunized animals, almost completely normalized the content of α-, and to a lesser extent, of β-globulins. The injection of albumin had no effect on the concentration of γ-globulin and the biosynthesis of antibodies (Table 3). The incomplete normalization of the concentration of β-globulin was apparently related to the presence in this fraction of proteins which were immunologically and functionally identical with γ-globulin [7].

Our data show that there is no direct relation between immunogenesis and the increase in the concentration of α-globulins.

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