SYNTHESIS AND SECRETION OF ANTIBODIES AND NONSPECIFIC IMMUNOGLOBULINS IN VITRO AT DIFFERENT TEMPERATURES

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UDC 612.017.1.014.43-085.2

The synthesis of antibodies and nonspecific immunoglobulins in a suspension of spleen cells of hyperimmune rabbits and the synthesis of nonspecific immunoglobulins in a suspension of spleen cells of normal animals are increased on elevation of the temperature from 18° to 42°C; on a further increase of temperature to 46°C the intensity of synthesis falls sharply. Changes in the rate of synthesis of antibodies and nonspecific immunoglobulins on elevation of the temperature are almost identical. With a rise in temperature the rate of secretion of these proteins also increases, and as a rule the increase in the rate of secretion of nonspecific immunoglobulins by immune rabbits is greater than the increase in the rate of secretion of antibodies. Changes in the rate of secretion of nonspecific immunoglobulins in the spleen cells of unimmunized animals are very similar to changes in the rate of secretion of antibodies in immune rabbits. The possibility that antibodies and nonspecific immunoglobulins may be synthesized simultaneously in the same cell is discussed.

The effect of temperature on antibody synthesis has received very little study. Only one investigation could be found, in which the kinetics of appearance of cells synthesizing antibodies against sheep's erythrocytes was studied when the temperature was raised [7]. Nothing likewise is known of the effect of temperature on the synthesis of nonspecific immunoglobulins (IG). Nevertheless, information on these matters could be of great interest.

In the experiments described below the effect of temperature on synthesis and secretion of antibodies and IG in vitro was investigated.

EXPERIMENTAL METHOD

Suspensions of the spleen cells of unimmunized rabbits and suspensions prepared from spleens removed on the 4th day after repeated immunization of animals with horse γ-globulin were used in the experiment. The cells ($6 \cdot 10^7$–$12 \cdot 10^7$) were incubated in Eagle's medium (3 ml) containing glycine-C^{14} (1 μCi/ml) at 20-56°C [2].

At the end of incubation the samples were frozen and thawed three times, then clarified by centrifugation for 15 min at 15,000 rpm and at 0°C. The supernatant was used for determination of the total (intracellular and extracellular) content of antibodies (A_t) and of nonspecific immunoglobulin (IG_t).

In some experiments antibodies and nonspecific immunoglobulins were determined separately in the cells and culture medium. In these cases the cells were separated by centrifugation, washed twice or three times in cold 1% glycine solution in 0.9% NaCl, suspended in 3 ml of the same solution, and broken up by freezing and thawing. The mixture containing the disintegrated cells and the culture medium was clarified

Laboratory of Chemistry and Biosynthesis of Antibodies, Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR, O. V. Baroyan.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 72, No. 12, pp. 55-58, December, 1971. Original article submitted March 11, 1971.

TABLE 1. Synthesis of Antibodies and Nonspecific Immunoglobulins in Spleen Cells of Normal and Hyperimmune Rabbits at Different Temperatures (incubation time 8 h)

Expt. No.	Rabbit	Protein synthesized	Content of A_t and IG_t (pulses/min per sample) and ratio between them				
			22°	27°	32°	37°	42°
1	Normal	IG_t	20	47	84	116	102
2		IG_t	188	468	628	1 209	1 101
3	Hyperimmune	A_t	92	116	335	456	513
		IG_t	431	754	1 555	2 345	3 123
		A_t/IG_t	0,21	0,22	0,22	0,19	0,16
4	Hyperimmune	A_t	69	134	283	415	557
		IG_t	417	1 115	1 906	3 162	3 220
		A_t/IG_t	0,16	0,12	0,15	0,13	0,17

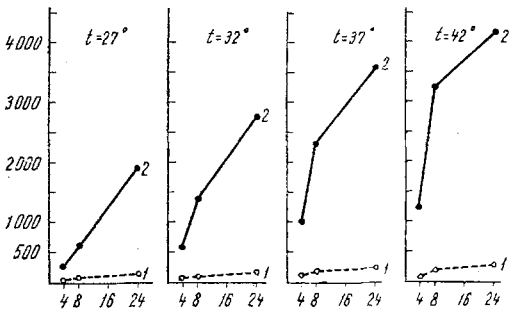

Fig. 1. Synthesis of antibodies and nonspecific immunoglobulins at different temperatures as functions of incubation time: 1) antibodies; 2) nonspecific immunoglobulins. Here and in Fig. 2: abscissa, incubation time (in h); ordinate, activity of C^{14} (pulses/min per sample).

by centrifugation and the supernatants were used for determination of the content of intracellular and secreted antibodies (A_c and A_s) and nonspecific immunoglobulins (IG_c and IG_s) from the increase in radioactivity on specific immunosorbents [1, 4].

EXPERIMENTAL RESULTS

The study of A_t and IG_t synthesis by spleen cells of hyperimmune rabbits (experiments of series I) showed that appreciable synthesis of both proteins takes place at 20-22°C, i.e., at a temperature very different from the animal's body temperature. With a further increase in temperature synthesis of both proteins increased, but within the range 37-42°C, as a rule, this increase was slower than within the range 22-37°C. On a further increase in temperature to 46 and 56°C, synthesis of immunoglobulins fell sharply.

The changes described above were also characteristic of IG_t synthesis in the suspension of spleen cells of unimmunized rabbits (Table 1).

It is apparent that by determining the ratio between A_t and IG_t synthesized at different temperatures some idea can be obtained of the relative changes in the rates of synthesis of these proteins. The results given in Table 1 show that the rates of synthesis of A_t and IG_t increase about equally with a rise of temperature.

If the cells were kept for 4 h at 20°C, and then for 20 h at 37°C, synthesis of A_t and IG_t was considerably greater than that taking place in samples incubated at 20°C for the whole 24 h, although it did not reach the characteristic values for incubation at 37°C.

The decrease in synthesis of A_t and IG_t at reduced temperatures was not due to its earlier termination. The lower the temperature, the longer the constant although a relatively low rate of synthesis was maintained (Fig. 1). It was constant for 8 h at all temperatures studied.

The decrease in synthesis of A_t and IG_t at high temperatures (46 and 56°C) was due to death of the cells.

In the experiments of series II, the values of A_c, A_s, IG_c, and IG_s were determined separately at different temperatures.

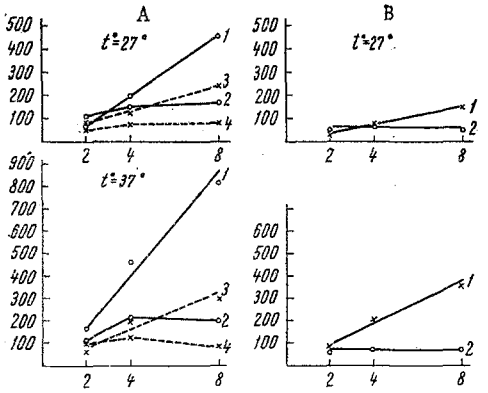

Fig. 2. Changes in content of antibodies and nonspecific immunoglobulins outside and inside spleen cells of hyperimmune (A) and normal (B) rabbits as a function of incubation time: 1) extracellular nonspecific immunoglobulins; 2) intracellular nonspecific immunoblobulins; 3) extracellular antibodies; 4) intracellular antibodies.

TABLE 2. Changes in Intracellular and Extracellular Content of Antibodies and Nonspecific Immunoglobulins in Spleen of Normal and Hyperimmune Rabbits in relation to Temperature (incubation time 8 h)

Rabbit	Temp., °C	Content (in pulses/min per sample)					
		A_S	A_C	IG_S	IG_C	A_S/A_C	IG_S/IG_C
Normal	20	—	—	73	115	—	0,64
	27	—	—	246	222	—	1,10
	32	—	—	333	295	—	1,10
	37	—	—	787	422	—	1,86
	42	—	—	740	361	—	2,65
Hyperimmune	22	26	43	161	256	0,60	0,63
	27	65	69	720	395	0,94	1,90
	32	156	127	1 423	483	1,20	3,00
	37	203	212	2 569	593	0,96	4,30
	42	346	211	2 739	481	1,60	5,70
Hyperimmune	27	132	274	1 151	408	0,48	2,82
	32	360	186	1 742	430	1,93	4,05
	37	379	248	2 389	473	1,53	5,05
	42	525	275	3 763	435	1,91	9,16

The results showed, first, how the content of these proteins changes at different times of incubation (1-8 h). The content of A_S and IG_S was found to increase roughly proportionally to the incubation time while the content of A_C and IG_C flattened out on a plateau after 2-4 h at 27-42°C (Fig. 2). Determinations made after incubation for 8 h thus reflected steady-state relationships between intracellular and secreted proteins. The results obtained in several such experiments are summarized in Table 2.

Evidently the values of A_C and IG_C are independent of temperature starting from 20°C (or in individual cases, with 32°C). Since the content of A_S and IG_S rose steadily with an increase of temperature, it is evident that not only the rates of synthesis, but also the rates of secretion of these proteins increased. As a rough approximation it can be taken that the ratio between the secreted and intracellular proteins reflects their rate of secretion. Determination of these ratios (Table 2) shows that the rate of secretion of IG rises faster with an increase of temperature than the rate of secretion of antibodies, and that it is much higher in the spleen cells of hyperimmune rabbits than in those of normal rabbits.

The study of the effect of temperature on synthesis of antibodies and IG in vitro thus showed that their synthesis can take place at 20°C but rises sharply with an increase in temperature to 42°C. The

increase in content of antibodies and IG in samples incubated at the higher temperatures in these experiments was probably due to their more rapid formation and not to an increase in the number of cells synthesizing these proteins. Comparison of the rates of synthesis of A_t and IG_t at the different temperatures shows that the rate of synthesis of the two proteins changes in an identical manner with elevation of the temperature. The fact that IG formation was definitely dependent upon the antigen, and the parallel changes in antibody and IG synthesis under the influence of chemical agents [2, 3, 6] suggested that their synthesis is localized in the same cells [5]. The results of the present investigation were in agreement with this hypothesis.

Raising the temperature also increases the rates of secretion of antibodies and IG, the effect on the latter being much stronger (Table 2). The possibility is not ruled out that antigen has a significant effect on the secretion of antibodies.

The rate of secretion of IG by spleen cells of normal rabbits and its dependence on temperature are very similar to those for antibodies in hyperimmune animals. The impression is obtained that "surplus" IG (synthesis of which is stimulated by antigen) differ in their nature from IG of unimmunized animals. The possible reasons for these differences have been discussed previously [5].

The author is grateful to A. E. Gurvich for discussing the work.

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