It cannot be concluded from these results, it must be emphasized, that NIG producers, already present in the cell suspension, are stimulated to proliferate and differentiate (the number of NIGFC in normal and "immune" suspensions before the beginning of culture *in vitro* was 2027 and 3538-8707 per 10^6 living cells, respectively) or that cells not previously synthesizing NIG are activated under the influence of the antigen. The solution to this problem can be obtained only from experiments with cell cultures from which the "background" NIGFC have been removed beforehand.

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INCREASE IN NUMBER OF ANTIBODY- AND NONSPECIFIC IMMUNOGLOBULIN-FORMING CELLS IN MICE IMMUNIZED WITH T-DEPENDENT AND T-INDEPENDENT ANTIGENS

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KEY WORDS: T-dependent antigen; T-independent antigen; antibody-forming cells; cells forming nonspecific immunoglobulins; Vi-antigen; sheep's red blood cells.

It was shown previously that immunization of animals leads to an increase in the number not only of antibody-forming cells (AFC), but also of cells producing nonspecific immunoglobulins (NIGFC). The dynamics of the appearance of AFC and NIGFC has been found to differ [4, 10]. These results were obtained by the use of T-dependent antigens.

The object of this investigation was to study the dynamics of the increase in number of AFC and NIGFC after immunization of animals with T-independent antigen.

EXPERIMENTAL METHOD

Female BALB/c mice weighing 14-16 g were used. Sheep's red blood cells (SRBC) were used as T-dependent antigen and *Salmonella typhi* Vi-antigen as T-independent antigen. The mice received one or two intravenous injections of 500×10^6 SRBC or 1 μg of Vi-antigen with an interval of 2-4 weeks. The spleens were removed on the 1st-7th days after the 1st or 2nd immunization and cell suspensions were prepared from them. Spleen cells from unimmunized animals were used in the control experiments. Usually cell pools from three to five spleens were used.

AFC were counted by direct and indirect local hemolysis methods [7], using native SRBC or SRBC sensitized with Vi-antigen [3] as the test antigens and rabbit antisera against mouse IgC (to detect indirect AFC against SRBC and Vi-antigen) and against the μ-chains of mouse IgM (to detect AFC against Vi-antigen) as intensifying antisera.

Immunoglobulin-forming cells (IGFC) were determined with the aid of SRBC sensitized with rabbit antibodies against mouse IgG [9]. Antibodies against mouse IgG were isolated by affinity chromatography [2]. The number of NIGFC was

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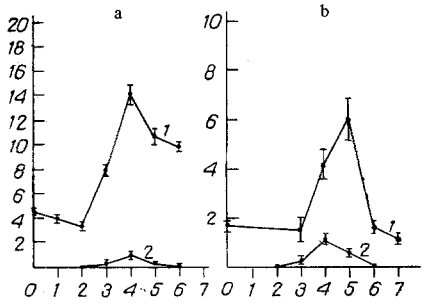

Fig. 1

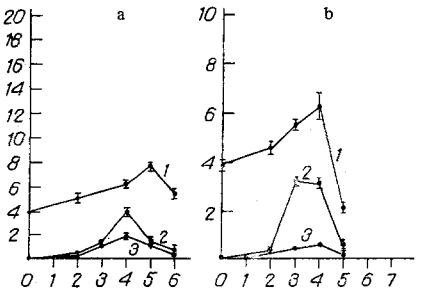

Fig. 2

Fig. 1. Dynamics of numbers of NIGFC (1) and IgM-AFC (2) during primary immune response to T-independent (a) and T-dependent (b) antigens. Here and in Fig. 2: abscissa, days after injection of antigen; ordinate, number of NIGFC and AFC (in thousands/10^6 cells).

Fig. 2. Dynamics of numbers of NIGFC (1), IgM-AFC (2), and IgG-AFC (3) during secondary immune response to T-independent (a) and T-dependent (b) antigens.

calculated as the difference between the numbers of IGFC and AFC per 10^6 cells.

The experimental results were subjected to statistical analysis.

EXPERIMENTAL RESULTS

In the experiments of series I the dynamics of the increase in the number of AFC and NIGFC was studied after primary immunization of mice with T-dependent and T-independent antigens. A single injection of Vi-antigen was shown to cause a rapid rise in the number of both AFC and NIGFC (Fig. 1) to reach a maximum in both cases on the 4th day after immunization. The NIGFC/AFC ratio at this period was 29:1. The mean doubling time (T_2) of the numbers of AFC and NIGFC, determined by the equation suggested previously [1], differed: 10.7 h for AFC and 58.6 h for NIGFC.

Similar results were obtained when the T-dependent antigen (SRBC) was used (Fig. 1). The curves showing the rise in the numbers of AFC and NIGFC were similar to those obtained in the experiments with Vi-antigen. The numbers of AFC and NIGFC reached a maximum on the 4th and 5th days after immunization; the NIGFC/AFC ratio was 7.4:1 (on the 4th day) and 24:1 (on the 5th day). The mean values of T_2 for AFC and NIGFC were 9.5 and 65 h, respectively.

The T-independent antigen, like the T-dependent antigen, thus stimulated the increase in the number of NIGFC. The absolute values of the number of NIGFC appearing in response to injection of Vi-antigen and SRBC were almost identical, although the specific immune response to the first antigen was less than half as strong (Fig. 1).

In the experiments of series II the dynamics of the increase in the number of AFC and NIGFC was studied during secondary immunization of mice with T-dependent and T-independent antigens. In these experiments cells forming IgM- and IgG-antibodies were determined as AFC. It will be clear from Fig. 2 that the number of AFC and of NIGFC increased exponentially following injection of both T-dependent and T-independent antigen. The number of AFC during immunization with Vi-antigen reached a maximum on the 4th day, and the number of NIGFC on the 5th day. The NIGFC/AFC ratio on the 4th and 5th days was 2.1:1 and 6.2:1, respectively. The mean value of T_2 for AFC in this case was 9.1 h and for NIGFC 144 h.

In response to SRBC the number of AFC reached a maximum on the 3rd-4th day and the number of NIGFC on the 4th day. The NIGFC/AFC ratio at these times was 2.9:1 and 3.3:1 (Fig. 2). The mean value of T_2 for AFC, just as in all previous cases, was much less than for NIGFC (11 and 129 h, respectively).

A common feature of both types of antigens was thus the much sharper increase in the number of AFC than of NIGFC during the secondary immune response. Compared with the first immunization the number of AFC increased by 3-6 times but the number of NIGFC increased by only 1.8 times. As a result, the NIGFC/AFC ratio fell in both cases from 24-29 for the primary response to 2.1-6.2 for the secondary response, in agreement with data in the literature [4].

Comparison of the values of T_2 for AFC and NIGFC after injection of T-dependent and T-independent antigens showed that the mean value of T_2 for AFC reached 9-11 h in both cases, in agreement with data in the literature [7, 11]. The mean value of T_2 for NIGFC in these same cases was 58-144 h, much longer than the generation time of lymphocytes [5, 8]. The reason may be the larger number of "background" NIGFC (4606 and 3395 cells). By extrapolating the curve of the rise in NIGFC to the zero level it can be calculated that the initial number of NIGFC precursors for both T-dependent

TABLE 1. Increase in Number of NIGFC during Simultaneous Immunization of Mice with T-Dependent and T-Independent Antigens (M ± m)

Series of experiments	Vi-antigen	Increase in number of NIGFC on 4th day after immunization	
		number of NIGFC per 10^6 cells	%
I	SRBC	1 416±493	0,14±0,05
	Vi-antigen	1 312±234	0,13±0,02
	SRBC + Vi-antigen	2 792±462	0,28±0,05
II	SRBC	6 258±298	0,63±0,05
	Vi-antigen	2 604±220	0,26±0,02
	SRBC + Vi-antigen	11 339±54	1,13±0,005
III	SRBC	10 019±416	1,00±0,04
	Vi-antigen	3 775±214	0,38±0,02
	SRBC + Vi-antigen	16 714±616	1,67±0,06

and T-independent antigens must be 380 and 132 cells respectively. In that case, T_2 for NIGFC is reduced to 15-32 h.

These experiments showed that the dynamics of the increase in the number of NIGFC following injection of T-independent antigen is similar to that following injection of T-dependent antigen, during both the primary and the secondary immune response. However, the decrease in the number of NIGFC after the maximum has been reached takes place much faster in the case of immunization with T-dependent antigen.

In the experiments of series III the aim was to determine whether the same or different cell populations are stimulated by T-dependent and T-independent antigens. Results obtained by De Vos-Cloetens et al. [6] point to the possibility of independent stimulation of different B cells by different antigens.

However, the workers cited used T-dependent antigens, and summation of the effect could be explained by the action of nonspecific stimulating T factors. Accordingly, it made sense to use a pair of antigens, one of which was T-independent.

SRBC and Vi-antigen were injected into the mice simultaneously into different caudal veins. The spleens were removed on the 4th day after immunization. Animals immunized with SRBC alone, with Vi-antigen alone, and unimmunized animals served as the controls. The results of determination of the number of AFC and NIGFC are given in Table 1. They show that injection of the two antigens led to summation of the number of NIGFC. This is evidence that T-dependent and T-independent antigens stimulate different populations of B-precursors of NIGFC. To elucidate the mechanism of induction of NIGFC formation under the influence of antigen experiments with separate populations of T and B cells are needed.

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