EXPERIMENTAL ANALYSIS OF GENESIS OF ANTIBODY-FORMING CELLS IN THE SPLEEN OF AN INTACT RECIPIENT AFTER TRANSPLANTATION OF IMMUNIZED DONOR CELLS

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UDC 612.017.12:612.6.02

It has been shown that if lymphoid cells of an immune donor are transplanted into a nonimmune recipient, various phenomena of artificial immunity may be reproduced in the recipient. The dominant role of lymphoid tissue in the formation of immunity to antigens of Salmonella typhi has been demonstrated by cytomorphological investigations [5, 7]. Further evidence of its importance is given by the fact that the transfer of lymphoid cells from immunized animals to intact animals transmits to the recipients the ability to produce O- and Vi-antibodies, immunologic memory, and specific protection against infection by typhoid microorganisms, i.e., the principal characteristics of artificial immunity to typhoid fever [3, 4, 6, 10]. However, the mechanism by which lymphoid cells exert their immunologic properties has not been explained. Immunization of mice with typhoid O-antigen is a convenient model for the study of the phenomenon of immunologic memory, for the first injection of this antigen induces practically no antibody formation in mice, while the second induces intensive antibody formation. Previous investigations [4, 10] have shown that after transplantation of a suspension of spleen cells (all of the fraction of small lymphocytes isolated from it) or of blood leukocytes from donors immunized once with O-antigen into recipients, the latter show the ability to respond to a first injection of O-antigen by a reaction of secondary type, i.e., immunologic memory has been transmitted to them.

The object of the present investigation was to study which cells—donor's or recipient's—form antibodies in the recipient's organism by a mechanism of secondary type. This problem is of great biological importance because it is bound up with the question of whether a method of transmission of immunologic

TABLE 1. Number of Antibody-Producing (ABP) Cells in Spleen of Mice Immunized with O-antigen of S. typhi

Line of mice	No. of mice	No. of injections of O-antigen	Number of ABP cells		Titer of O-antibodies in serum*
			per 10^6 spleen cells	per spleen	
CBA	16	—	0,6 —0,2–1,5†	193 104–616	1:28
	11	1	1,0 —0,1–2,2	217 —63–497	1:40
	14	2	86,0 52–121	23 200 17 140–29 260	1:4 036
F_1(CBA×C57BL)	10	—	0,5 0,2–0,8	75 43–131	1:19
	12	1	0,7 0,3–1,1	116 56–176	1:37
	12	2	181,0 67–295	49 650 20 190–79 110	1:6 109

*Four days after injection of antigen.

† Confidence interval with probability 0.95.

N. F. Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR; Moscow Research Institute of Epidemiology and Microbiology (Presented by Active Member of the Academy of Medical Sciences of the USSR, G. V. Vygodchikov). Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 64, No. 11, pp. 108-113, November, 1967. Original article submitted April 1, 1967.

TABLE 2. Number of ABP Cells in Spleen of Mice Receiving Lymphoid Cells

Recipients		CBA donors		Number of ABP Cells		Titer of O-antibodies in serum
line	number	immunization	cells	per 10^6 spleen cells	per spleen	
F_1 (CBA×C57BL)	10	+	Living	78,4 48,0 – 108,8	26 120 15 630 – 36 610	1:8 054
	6	+	Killed	1,3 –1 – 3,6	228 156 – 300	1:53
	11	–	Living	0,5 0,1 – 0,9	146 46 – 246	1:28
F_1 (CBA×A)	7	+	Living	57,6 48,6 – 86,6	12 354 9 471 – 15 237	1:3 708
	4	+	Killed	1,2 0,6 – 1,8	433 191 – 675	1:19
	6	–	Living	2,8 0 – 5,6	731 0 – 1 591	1:31
CBA	6	+	Living	106,5 19 – 193	30 190 11 340 – 49 000	1:6 124

TABLE 3. Analysis of Origin of ABP Cells in Spleen of Different Groups of Mice

Line of mice	No. of mice	Receiving spleen cells of	No. of injections of O-antigen	No. of ABP cells per 10^6 spleen cells after treatment with serum		% of ABP cells with characteristics of		Absolute no. of ABP cells per spleen	
				normal	anti-R	D	R	D	R
CBA	14	–	2	76,0 39,4 – 112,6	75,3 40,7 – 109,9	101,6 87 – 115	0 –15 – 13	23 400	0
F_1 (CBA×C57BL)	11	–	2	135,5 101,5 – 169,5	3,3 1,7 – 4,9	2,4 1,2 – 3,6	97,6 96,4 – 98,8	1 600	48 050
F_1 (CBA×C57BL)	12	Immune CBA mice	1	100,7 71,2 – 130,2	61,9 26,4 – 92,4	61 41 – 81	39 19 – 59	16 020	10 100
F_1 (CBA×A)	7	Immune CBA mice	1	47 19,4 – 72,6	34,5 15,5 – 53,5	71,6 52,8 – 90,4	28,4 9,6 – 47,2	8 830	3 520

Note: D – donor, R – recipient.

information from cell to cell exists other than by division and formation of daughter cells with the same immunological properties as the material lymphoid cell. To solve this problem, transplantation of sensitized lymphocytes into unirradiated recipients is clearly desirable.

To determine the genetic constitution of antibody-forming cells in the organism of a recipient, natural antigenic cell "markers" may be used. It is convenient for this purpose to use as recipients hybrid mice of the first generation (F_1), and as donors mice of one of the parent lines. In the chimera thus obtained, the cells of donor and recipient may coexist for a long time and function normally. At the same time, the antigenic differences between the cells of donor and recipient make it possible to carry out discriminative analysis of the lymphoid cell population of the chimera [11, 16] in order to determine the genetic constitution of the antibody-forming cells.

EXPERIMENTAL METHOD

Donor mice of line CBA were immunized by a single intravenous injection of 10 μg O-antigen isolated by Boiven's method from S. typhi strain O-901. After 30-40 days, the suspension of spleen cells or the fraction of small lymphocytes isolated from it was transferred into intact recipients – first generation hybrids F_1 (CBA×C_{57}BL) or F_1 (CBA×A). The method of preparation of the cell suspensions was described earlier [4, 6]. Each recipient received 70×10^6 to 100×10^6 spleen cells intravenously. In control experi-

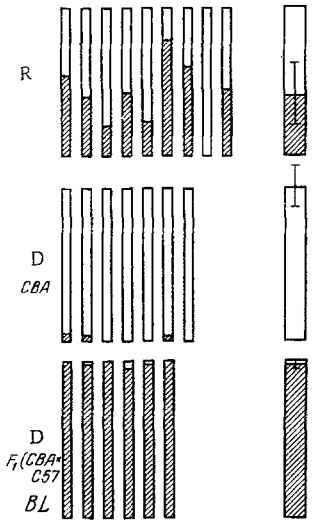

Fig. 1. Results of analysis of origin of cells producing O- antibodies in spleen of mice. $R-F_1$ (CBA × C57BL) receiving spleen cells of immune CBA mice and one injection of O-antigen. D_{CBA} and D_{F_1} – mice of corresponding lines receiving two injections of O-antigen. The shaded part of the columns represents cells of "recipient" origin and the unshaded part of the columns represents cells of "donor" origin. Narrow columns represent results for individual animals and wide columns give mean values.

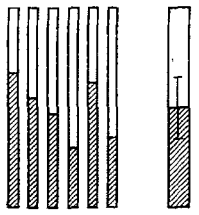

Fig. 2. Origin of cells producing O-antibodies in spleen of mice receiving small lymphocytes isolated from spleen of immune donors. Legend as in Fig. 1.

ments the recipient received spleen cells killed by freezing 3 times at −70° or living spleen cells of unsensitized CBA donors. A test injection of O-antigen (10 μg intravenously) was given to all recipients 24 h after the transfer. The animals were sacrificed 4 days after immunization and a discriminative analysis made of a suspension of spleen cells*. The cell suspension was treated in vitro in the presence of complement with line-specific serum against transplantation H-2 antigens of the recipient. To obtain such an antiserum, mice of line CBA were injected 4 times at intervals of 7 days with living spleen cells of C57BL or A mice, and blood was taken from them on the 6th-7th day after the last injection. Two portions, each containing 15×10^6 cells, were made from the test suspension of spleen cells, and to one of them was added 0.05 ml of anti-C57BL serum (or anti-A serum respectively), while to the other was added 0.05 ml of normal serum of CBA mice; 0.05 ml complement (whole normal rabbit serum) was added to both mixtures and their volume was made up to 0.5 ml with Hanks' solution, after which they were incubated for 45 min at 37°. The number of cells forming O-antibodies was then determined in both portions using the method of local hemolysis in agar described previously [9]. An essential modification of the method when working with mouse cells was that rabbit serum was used instead of guinea pig serum as complement.

The number of antibody-forming cells found in the portion treated with "anti-recipient" serum gave the number of cells of "donor" type. The difference between the number of antibody-forming cells in the portion treated with normal serum and in the portion treated with "anti-recipient" serum gave the number of cells of "recipient" type among the population of antibody-forming cells in the studied spleen. O-antibodies in the mouse serum were determined by the passive hemagglutination method [1].

EXPERIMENTAL RESULTS

It is clear from Table 1 that a single immunization of CBA or F_1 (CBA × C57BL) mice with O-antigen caused neither an increase in titer of antibodies in the serum nor a significant increase in the number of antibody-forming cells in the spleen. Conversely, a second immunization induced intensive antibody production, as shown by an increase in antibody titer in the serum (1:4000-1:6000) and also by a sharp (by 2 orders) increase in the number of antibody-producing cells in the spleen.

Recipient F_1 mice receiving spleen cells from sensitized CBA donors acquired immunologic memory and responded to the first injection of O-antigen by intensive antibody formation (Table 2). Many antibody-forming cells were found in the spleen of the recipients. Their mean number per whole spleen in F_1 (CBA × C57BL) recipients was 26120, and rather fewer – 12354 – in F_1 (CBA × A). Injection of O-antigen into recipients of killed spleen cells of sensitized CBA donors or living spleen cells of intact CBA donors did not lead to a marked increase in number of antibody-forming cells in the spleen.

* Technical details are discussed more fully elsewhere [11].

The results of discriminative analysis are summarized in Table 3. The first two lines demonstrate the high activity and specificity of the antiserum against C57BL antigens used ("anti-recipient" serum). After treatment with this serum, only 3.3 antibody-forming cells per 10^6 spleen cells remained in the suspension of spleen cells of immune F_1 (CBA × C57BL) mice (recipient line) compared with 135.5 after treatment with normal serum; the percentage of specific depression was thus 97.6. Cells of CBA mice (donor line) were found to be insensitive to the action of anti-recipient serum.

It is obvious that the population of antibody-forming cells in the spleen of non-chimera mice and mice immunized twice with O-antigen was homogeneous in its antigenic properties. No antibody-forming cells with C57BL characteristics were found in the CBA mice and, conversely, in the F_1 (CBA × C57BL) mice nearly all producing cells possessed these characteristics and died as a result of the action of antiserum against C57BL antigens.

A completely different picture was observed in the chimeras—the F_1 (CBA × C57BL) mice receiving injections of spleen cells of immune CBA donors and one injection of O-antigen. The population of antibody-producing cells in the spleen of these recipients was heterogeneous and consisted of cells with characteristics of "donor" and of "recipient" types. Treatment of a suspension of chimera spleen cells with "anti-recipient" spleen caused a decrease in the number of antibody-producing cells (61.9 per 10^6 spleen cells compared with 100.7 in the control), but not their complete disappearance. Consequently, among the cells producing O-antibodies by the secondary response type of mechanism in the spleen of the chimeras, a mean number of 61% of cells possessed characteristics of "donor" antigenic type (not subjected to the action of "anti-recipient" serum) and 39% possessed characteristics of "recipient" type (dying after treatment with "anti-recipient" serum). Similar results were obtained in experiments in which the donors were immune CBA mice and the recipients were intact F_1 (CBA × A) hybrids. The population of cells producing O-antibodies in the spleen of the chimera recipients was 71.6% of cells of "donor" type and 28.4% of cells of "recipient" type.

The chimerism of the antibody-forming cells in the spleen of the recipient mice was a regular feature found in the great majority of animals. For instance, of the 9 recipient mice used in the experiment, the results of which are illustrated in Fig. 1, only in one animal were all antibody-forming cells of the "donor" type; in the rest the population of producer cells was mixed and the percentage of cells of "recipient" type varied from 21 to 75. The same phenomenon was observed in experiments in which F_1 (CBA × C57BL) recipients were injected with a suspension of small lymphocytes isolated from the spleen of immune CBA donors and then injected with O-antigen. Many cells producing O-antibodies appeared in the spleen of the recipients of the small lymphocytes (on the average 63.7 per 10^6 spleen cells and 22080 per whole spleen). Discriminative analysis showed (Fig. 2) that in this case also the population of antibody-forming cells was heterogeneous and consisted of cells of "donor" (mean 51.3%) and "recipient" (48.7%) origin. Chimerism of the producer cells of "recipient" type varied in individual animals from 29 to 67.

The following conclusions may be drawn from the facts described above. Cells in the recipients' spleen forming antibodies by the secondary response type are heterogeneous and show characteristics of varied genetic origin. Some antibody-producing cells have characteristics of "donor" origin. These cells evidently arise by transformation of transplanted cells of an immune donor or are their direct progeny. The direct participation of donor cells in the immune response of a chimera recipient is particularly interesting in the case of transplantation of immune small lymphocytes. The results of these experiments show directly that small lymphocytes are not only the carriers of immunologic memory, as has been shown previously [4, 10, 13, 15, 20], but are also the direct precursors of cells producing antibodies during the secondary response; the possibility that this may be so has been denied by some authors. Besides cells of "donor" type, among the antibody-forming cells of the recipient's spleen cells are constantly present with characteristics of "recipient" origin, although the lymphoid cells of the recipient are in contact with O-antigen for the first time and cannot respond by marked antibody formation.

A number of hypotheses may be submitted to explain the appearance of cells of "recipient" type among the population of producer cells. The hypothesis of sensitization of recipient cells by O-antigen possibly present in the suspension of spleen cells of the immune donor is ruled out because of the negative results of experiments in which a killed suspension of the same cells was injected, and also because of the short interval (24 h) between transplantation of the cells and the test injection of antigen. The possibility that donor cells may acquire the antigenic properties of recipient cells cannot be completely ruled out, although there is no experimental evidence to support it. Explanation of the observed phenomenon by

hybridization of cells of donors and recipients is unconvincing, because such hybridization is a relatively rare phenomenon, and a long time is necessary before a significant number of hybrid cells can accumulate [17, 18].

The most probable hypothesis, although it has not yet been verified, is that a mechanism of transmission of immunologic information from cells of an immune donor to cells of an unimmune recipient exists as a result of which the cells become capable of responding to contact with antigen by a secondary reaction of antibody formation. Transmission of immunologic information by lymphocytes to other cells has been postulated previously [8]. The possibility that modified immunologic reactivity can be conveyed by the action of RNA on lymphoid cells in vitro has been demonstrated by other workers [2, 12, 14, 19]. The possibility is not ruled out that in this particular case we have an analogous process, one taking place in physiological conditions in the organism. However, this conclusion is hypothetical in character and requires further experimental verification.

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