ROLE OF ANTIGEN-BINDING CELLS IN THE FORMATION OF PRODUCERS OF ANTIBODIES AND ANTIGEN-DEPENDENT NONSPECIFIC IMMUNOGLOBULINS

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KEY WORDS: antigen-binding cells; antibody-forming cells; cells forming antigen-dependent nonspecific immunoglobulins; antigens; receptors.

Introduction of an antigen into an animal not only induces the appearance of antibody-forming cells (AFC), but it also sharply increases the number of cells forming nonspecific immunoglobulins (NIGFC) [2, 14]. An even greater increase in the number of NIGFC, many times more than the number of AFC, is observed on induction of an immune response *in vitro* [8, 15]. Thus NIGFC production, like AFC formation, is an antigen-dependent process.

We know that AFC arise from cells which specifically bind antigen (antigen-binding cells — ABC) [14]. However, it is not yet clear what is the mechanism of triggering of NIGFC by the antigen, or what cells are precursors of antigen-dependent NIGFC. Since the number of ABC is many times greater than the number of AFC arising from them subsequently [3], the writers have postulated that most ABC take part in the formation of NIGFC.

The aim of this investigation was to test this hypothesis.

EXPERIMENTAL METHOD

Experiments were carried out on female C57BL/6 mice weighing 12-14 g, obtained from the "Stokbovaya" nursery, Academy of Medical Sciences of the USSR. Spleen cells from eight to ten mice were pooled and cultured (10^6 splenocytes in 0.2 ml of medium) for 4 days in 96-well panels (Linbro, England), by the method described in [5, 11]. Suspensions of normal splenocytes and suspensions of cells from which ABC were removed beforehand (exhausted cultures) were used in the experiments.

The method of rosette formation was used for exhaustion [3]. To 3-5 ml of the cell suspensions ($5 \cdot 10^6$ cells in 1 ml) 0.3-0.5 ml of a 20% suspension of SRBC or of donkey red blood cells (DRBC) was added. The mixture of cells was centrifuged for 3-4 min at 150-200g, and the light residue obtained was placed for 30-40 min in an ice bath. At the end of incubation the cells were carefully resuspended, centrifuged in a Ficoll—Verografin density gradient (d 1.08-1.09 g/cm^3) at 2800g for 20-30 min [13], the interphase was withdrawn, the red blood cells were lysed with distilled water, and the cells were washed with Eagle's medium 3 times in the cold.

To test the completeness of exhaustion the number of rosette-forming cells (RFC) was determined in the suspension. It was found that a single exhaustion was sufficient to reduce the number of RFC in the cell suspension from 0.46 ± 0.48% to 0.015 ± 0.015%.

Normal and exhausted suspensions were cultured without antigen, in the presence of specific antigen ($5 \cdot 10^6$ SRBC) or in the presence of nonspecific antigen: 10^6 hen's erythrocytes (HRBC), giving no cross reaction with SRBC at the level of either T cells or B cells, or DRBC, giving a cross reaction with SRBC at the T helper level [9].

At the end of incubation cells from 8 to 16 cultures were pooled, washed 3 times in the cold with Eagle's medium, after which the number of AFC [7] and of immunoglobulin-forming cells (IGFC) was determined [10]. The number of NIGFC was calculated as the difference between the number of IGFC and the number of AFC per 10^6 living cells.

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TABLE 1. Number of AFC and NIGFC in Normal and Exhausted Splenocyte Cultures Incubated in the Presence and Absence of Antigen

Antigen added to culture	Cultures of splenocytes					
	normal			exhausted		
	AFC to SRBC	AFC to HRBC	NIGFC	AFC to SRBC	AFC to HRBC	NIGFC
—	118 (90—155)	22 (20—25)	2296 (1741—3027)	109 (95—127)	11 (10—12)	1594 (1180—2148)
SRBC	1793 (1472—2184)	34 (28—41)	6269 (4960—7924)	205 (151—277)	41 (36—47)	2012 (1348—3003)
HRBC	61 (47—79)	309 (237—402)	7149 (5559—9194)	121 (117—125)	195 (168—225)	4571 (3548—5889)

Note. In each experiment 44 cultures were tested. Values of geometric means and standard deviations (in parentheses) are given.

TABLE 2. Effect of Exhaustion of Splenocyte Cultures with Antigens Giving Cross Reactions at the T Helper Level on AFC and NIGFC Formation*

Cultures of splenocytes	Antigen added to culture	Number of AFC		Number of NIGFC
		SRBC	DRBC	
Normal	—	182** (172—193)	14 (10—20)	3193 (2563—3979)
	SRBC	1852 (1759—1950)	16 (12—22)	5588 (5127—6089)
	DRBC	126 (94—169)	341 (324—360)	6416 (6160—6684)
Exhausted with SRBC	—	153 (124—189)	31 (22—43)	3080 (2777—3416)
	SRBC	239 (199—287)	17 (14—21)	3299 (3276—3321)
	DRBC	371 (298—461)	230 (203—261)	6240 (6071—6414)
Normal	—	29 (29—30)	77 (68—88)	4773 (4548—5008)
	SRBC	618 (546—699)	63 (56—72)	10095 (8652—11779)
	DRBC	33 (29—38)	269 (264—274)	8533 (7811—9321)
Exhausted with DRBC	—	150 (130—173)	74 (68—80)	4367 (3739—5100)
	SRBC	1034 (1000—1069)	43 (30—62)	8676 (8174—9208)
	DRBC	119 (105—134)	66 (58—76)	4059 (4001—4117)

*Sixteen cultures tested in each experiment.
**Geometric means and standard deviations (in parentheses) are given.

EXPERIMENTAL RESULTS

When splenocytes were cultured under the conditions described above the survival rate of the cells after 4 days averaged 35-45%. No difference could be observed in the survival rate of cells in normal and exhausted cultures.

In suspensions cultured without antigen (normal and exhausted) a small increase in the number of AFC was usually discovered (109-118 AFC against SRBC and 11-22 AFC against HRBC). This was evidently due to the action of embryonic calf serum present in the medium.

Addition of SRBC or HRBC to normal splenocytes induced a distinct immune response in the cultures to homologous antigen: 1793 AFC to SRBC and 309 AFC to HRBC per 10^6 cells. The response to heterologous antigen under these circumstances remained at the background level, discovered in cultures not stimulated by antigen: 34 AFC to HRBC and 61 AFC to SRBC (Table 1).

Simultaneously with induction of the specific immune response a sharp increase was observed in the number of NIGFC (to 6269 per 10^6 cells compared with 2296 in cultures not stimulated by antigens). By contrast, addition of the specific antigen (SRBC) to the exhausted cultures virtually did not stimulate an immune response. On average 205 AFC to SRBC per 10^6 splenocytes were discovered in the cultures (compared with 1793 in the control), i.e., a few more than in cultures incubated without antigen (107 AFC to SRBC).

The absence of an increase in the number of NIGFC in the exhausted cultures, which did not exceed 2012 per 10^6 cells, i.e., it was virtually the same as the background level, which was 1594 per 10^6 cells (Table 1), was of the greatest interest.

To test whether removal of RFC leads to nonspecific elimination of the immune response, a foreign antigen (HRBC) was added to the exhausted cultures. It will be clear from Table 1 that HRBC evokes both a specific immune response (195 AFC) and a distinct increase in the number of NIGFC (4571) in the exhausted cultures. Admittedly, the figures given above are lower than values obtained in control cultures stimulated by HRBC (309 AFC and 7149 NIGFC per 10^6 cells). This was evidently the result of treatment of the cell suspensions.

The data described above are evidence that removal of ABC specific for SRBC leads not only to drastic inhibition of AFC formation, but also to complete suppression of formation of antigen-dependent NIGFC, and that these two processes are antigen-specific.

The importance of these results in principle is that they demonstrate dependence of formation of antigen-dependent NIGFC (and also AFC) on cells carrying receptors specific for the given antigen.

We know that such receptors are found both on B cells and on T cells [1]. Using antigens not giving cross reactions at either T cell or B cell level (SRBC and HRBC), we could not determine which cell population has a decisive influence on the formation of antigen-dependent NIGFC. For this purpose we studied how addition of SRBC and DRBC, which cross react only at the T helper level [9], to the cultures affects formation of AFC and NIGFC. In these experiments suspensions of splenocytes exhausted with SRBC or DRBC, respectively, were used.

Addition of DRBC to cultures from which cells binding SRBC had been removed induced the formation of 230 AFC to DRBC and of 6240 NIGFC per 10^6 cells (instead of 341 and 6416, respectively) (Table 2). The reduction in the number of AFC to DRBC corresponded to the nonspecific reduction observed previously in the number of AFC to HRBC in cultures exhausted with SRBC (Table 1). Meanwhile, the reduction in the number of antigen-dependent NIGFC was not significant. Addition of SRBC to these cultures, just as in the previous experiments, induced neither any significant formation of AFC (only 239 per 10^6 cells compared with 1852 in the control) nor an increase in the number of antigen-dependent NIGFC (3299 compared with 5588, and with a background level of NIGFC of about 3000 per 10^6 splenocytes) (Table 2).

If the experiment was carried out in the opposite direction, and cells carrying specific receptors for DRBC were removed from the suspension of splenocytes, no decrease whatever was observed in AFC formation to SRBC, and the decrease in the number of NIGFC was not significant. Meanwhile, the response to DRBC, as regards both AFC and NIFGC, as might be expected, was sharply depressed (66 AFC to DRBC and 4059 NIGFC per 10^6 cells compared with 269 and 8533, respectively in the control) (Table 2).

The results of this series of experiments indicates that during exhaustion, mainly B cells carrying specific receptors for a particular antigen were removed. Otherwise no response would have been obtained to antigens cross-reacting at the T helper level. This agrees also with data in the literature, according to which, by the technique which we used it is possible to obtain only B-RFC [6]. At the same time, it will be evident that the final solution to this problem requires the use of separate T and B lymphocyte subpopulations.

The question of the nature of the receptors responsible for "triggering" cells forming nonspecific immunoglobulins by the antigen is an extremely interesting one. It can be tentatively suggested that incomplete products of immunoglobulin genes or products of incomplete genes, possessing definite affinity for the given antigen, are expressed on precursors of antigen-dependent NIGFC, so that the cells can receive the signal for differentiation during antigenic stimulation. However, the structural changes taking place in the immunoglobulin genes later differ from structural changes in precursors of the AFC, and for that reason they lead to the appearance of immunoglobulins with active centers that differ from those of antibodies. The presence of identical idiotypic determinants on antibodies and antigen-dependent nonspecific immunoglobulins [12] indicates a possible role of the latter in regulation of the immune response.

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IMMUNOLOGIC TYPING OF SPLENIC LYMPHOCYTES OF INTRAUTERINE HUMAN FETUSES USED AS DONORS OF PANCREATIC ISLET CELLS

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Free grafting of pancreatic islet tissue has been used in recent years for the treatment of patients with diabetes. A suspension of freshly isolated pancreatic tissue from cadavers of adults, children, and stillborn infants, and also pancreatic islet cells (PLC) from human fetuses, cultured beforehand, are used for this purpose [5, 6, 7]. This problem is under active investigation in the Soviet Union [2-5]. A matter of great importance for its further successful development is that of immunologic typing of fetal donors for the selection of PIC cultures antigenetically most compatible with the recipient.

The aim of the investigation described below was to study this problem.

EXPERIMENTAL METHOD

To determine the HLC phenotype of a potential donor of PIC, splenic lymphocytes of 52 human fetuses at the 18th-25th week of intrauterine development were used (spontaneous abortions, termination of pregnancy on medical grounds). Lymphocytes were isolated from splenic pulp by centrifugation in a Ficoll-Verografin density gradient. HLA antigens were detected by the two-stage NIH method, using a kit of anti-HLA-sera. Antigens of the A series: A1, A2, A3, A9, A10, A11, A19, A23, A24, A25, A26, A28, A29, A32; and antigens of the B series: B5, B7, B8, B12, B13, B14, B15, B16, B17, B18, B21, B22, B27, B35, B40, were determined. The time from removal of the spleen from the fetal cadaver to the beginning of typing was 3-8 h.

Cultures of human fetal PIC were obtained by the method described previously [1]. Growth of the culture was evaluated 3-8 days after seeding of the cells in 100-ml flasks, on the basis of cytophysiological data (morphologic study of the cultures, determination of the immunoreactive insulin concentration in the culture medium). In 7 cases the results of immunologic typing were compared with those of cytophysiological investigation of PIC cultures.

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