ORIGIN OF EFFECTOR AND ROSETTE-FORMING CELLS DURING TRANSPLANTATION IMMUNITY IN MICE

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Immune lymphocytes destroying allogeneic target cells are eliminated in the presence of complement by antibodies against θ-antigen but not by antibodies against PC.1 and mouse γ-globulin (MGG). Conversely, rosette-forming cells reactive against the same H-2 antigens are not inactivated by anti-θ-antibodies, but are neutralized by antibodies against PC.1 and MGG. It is concluded that cytotoxic and rosette-forming cells reactive against the same H-2 antigens are two independent populations of T- and B-cells, respectively, and that their cooperation with other lymphoid populations is not required for destruction of target cells by effector T-lymphocytes.

The lymphoid organs contain at least two types of lymphocytes in various proportions: cells of thymus (T-cells) and bone-marrow (B-cells) origin [16]. The role of the T- and B-cells and the necessity for their interaction in various immunological phenomena are being intensively studied. The possibility cannot be ruled out that the B-cells, while not the effector lymphocytes of transplantation immunity, nevertheless facilitate the activity of the latter. Since H-2 antigens induce humoral antibody formation, they ought to react not only with the T-cells but also with the B-cells performing the function of precursors of antibody-forming cells in other systems.

In the present investigation the origin of the rosette-forming cells reactive against H-2 antigen [4, 14] was studied and compared with that of cytotoxic lymphocytes of the same specificity. For this purpose cytotoxic and rosette-forming lymphocytes were treated with antibodies against the antigenic markers of the T- and B-cells.

EXPERIMENTAL METHOD

Inbred mice of strains C57BL/10Sn, B10D2, BALB/cDe, C3H/Sn, and AKR were used at the age of 8-16 weeks. In order to immunize the mice with allogeneic tumors, cells of sarcomas induced by methylcholanthrene in the mice of the above strains were obtained by trypsinization and injected subcutaneously at five points in doses of $3 \cdot 10^7$-$4 \cdot 10^7$ cells per mouse [1]. From 8 to 15 days later cells of the regional lymph glands were washed three times and used in parallel tests to determine rosette-forming cells and the cytotoxic action on allogeneic target cells [1, 3].

Rosette-forming cells against H-2 antigens were determined by the direct method [14] as described fully previously [4]. A single lymphocyte to which at least three erythrocytes were attached was regarded as a rosette. Two Goryaev's chambers were examined (a total of $18 \cdot 10^3$ lymphocytes) and the number of rosettes per million lymphocytes was calculated.

Antisera against θ-antigen were obtained by immunizing AKR mice with C3H thymus [17], and antisera against PC.1-antigen by immunizing B10D2 mice with MOPC-21A plasmocytoma [18]. Rabbit antiserum against mouse γ-globulin (MGG) was obtained by O. M. Lezhneva by immunization with Freund's adjuvant into the popliteal lymph glands [12] and inactivated. It reacted with whole mouse serum in a titer

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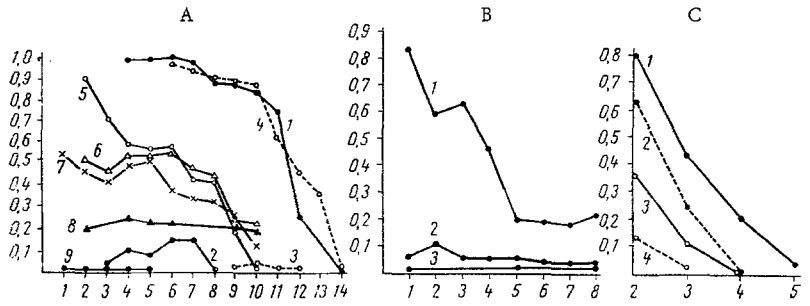

Fig. 1. Cytotoxic reaction of antisera with cells of various strains of mice: A) AKR serum against C3H thymus. Reaction with C3H (1, 3, 4) and AKR (2) thymocytes, with C3H (5), C57BL (6), BALB/c (7), B10D2 (8), and AKR (9) lymphocytes. Broken lines – serum treated with C3H brain (3) or C3H bone marrow (4); B) B10D2 anti-MOPC-21A serum. Reaction with MOPC-21A cells (1) and with cells of BALB/c (2) and B10D2 (3) lymph glands; C) reaction of rabbit immune serum against MGG (2, 4) and of normal rabbit serum (1,3) with C57BL lymphocytes; 1,2) original sera; 3,4) sera exhausted with NCH11 ascites sarcoma cells from C57BL mice. Abscissa, dilutions of sera ($\log_2$); ordinate, cytotoxic index.

TABLE 1. Inhibition of Cytotoxic Effect of Lymphocytes and Rosette Formation by Antibodies against Markers of T- and B-Cells

Treatment of lymphocytes*	Proportion of cells killed†‡(%)	No. living lymphocytes in cytotoxic effect exps (% · 10^6)	Cytotoxic effect† (in %)	No. of rosette-forming cells (· 10^6) (lymphocytes)‡	P**
Medium No. 199 (contol)	10,0	10—15 5—7,5	76,2 40,3	542,1±73,6	—
Normal mouse serum (1 : 8) + complem.	0	10—15 5—7,5	70,6 (7,4)†† 35,4 (12,1)	499,3±54,4 (7,9)[5]	>0,1
Anti-θ (1 : 8) + complement	37,3	10—15 5—7,5	0 (100) 0 (100)	535,7±67,1 (0)	>0,1
Anti-PC.1 (1 : 2) + complement	6,0	10—15 5—7,5	76,8 (0) 39,3 (0)	358,9±47,6 (28,1)	<0,05
Norm. rabbit serum (1 : 2) + complem.	5,1	10—15 5—7,5	73,5 (3,5) 37,0 (8,2)	540,0±70,8 (0,3)	>0,1
Anti-MGG rabbit anti-serum (1 : 2) + complement	24,8	10—15 5—7,5	67,4 (8,3) 31,6 (14,6)	186,6±58,0 (65,4)	<0,01

* $1.2 \cdot 10^8$ lymphocytes were treated with 2 ml serum for 40 min at 20°C, the serum was removed by centrifugation, the lymphocytes were treated with 2 ml complement (1 : 3) for 1 h at 37°C, washed twice, and counted, and the samples were equalized for the number of living cells.

† Mean results of three experiments with C57BL anti-B10D2 lymphocytes and BALB/c anti-C57BL lymphocytes.

‡ Mean data for 13 mice ± standard error.

** Significance of inhibition of rosette formation.

‡ Inhibition of cytotoxic effect and of rosette formation shown in parentheses (in % of control). Control for normal mouse and rabbit serum was medium No. 199, and for immune mouse and rabbit sera the corresponding normal mouse and normal rabbit sera.

of 1:128 and formed one precipitation band in agar. After removal of normal antibodies against mouse cells from the rabbit immune serum by treatment with cells of NCH11 ascites sarcoma from C57BL mice, the titer of antibodies against MGG was reduced by not more than one dilution.

The cytotoxic reaction in vitro to detect the antibodies was carried out by a modified [2] method [11] using guinea pig serum in a dilution of 1:3 as complement. For the reaction with mouse thymocytes the complement was first treated with agar to remove its nonspecific toxicity [8]. The antisera were treated with half the volume of residues of homogenates or cells, previously washed five times, for 1 h at 4°C followed by clarification of the serum.

EXPERIMENTAL RESULTS

The curves in Fig. 1A show that antibodies against θ-antigen of C3H mice killed about 100% of the C3H thymocytes but not more than 14% of the AKR thymocytes in the presence of antiserum in dilutions up to 1:64. The percentage of C3H thymocytes killed by the antibodies fell, gradually at first and then steeply, so that the titer of the serum corresponding to a cytotoxic index (CI) of 0.36 was 1:8192 (curve 1). The presence of anti-θ-antibodies in the serum was confirmed by the fact that its cytotoxic activity was neutralized by C3H brain tissue (curve 3) but not by C3H bone marrow, which does not contain θ-antigen (curve 4). In the reaction with lymph gland cells the same serum killed 70% of the C3H cells, 45-55% of the BALB/c and C57BL cells, and about 20% of the B10D2 cells (curves 5-8) but did not damage the AKR lymphocytes (curve 9).

Serum against PC.1 antigen killed more than 80% of cells of the MOPC-21A plasmocytoma in a dilution of 1:2, with a sharp decrease in CI in subsequent dilutions. In the reaction with BALB/c lymphocytes CI did not exceed 0.1, and there was no reaction with B10D2 lymphocytes (Fig. 1B).

Immune rabbit serum against MGG, exhausted with mouse sarcoma, killed about 35% of C57BL and BALB/c lymph gland cells, but exhausted normal rabbit serum killed not more than 11% of these same cells (Fig. 1C).

Further experiments showed that preliminary treatment of the lymphocytes with anti-θ or anti-MGG antibodies in the presence of complement neutralized the subsequent cytotoxic effect of the same antibodies and strengthened the cytotoxic action of heterologous antibodies by comparison with the original cytotoxic activity. The results indicate that under the experimental conditions used, antibodies against θ-antigen and against MGG killed virtually all the cells with the corresponding surface marker and that lymphocytes carrying each of these two markers constitute nonoverlapping cell populations.

The results of a study of the effect of antibodies against markers of the T- and B-cells on the cytotoxic and rosette-forming activity of lymphocytes of the same mice immune against H-2 antigens are shown in Table 1. Control treatment was carried out with normal mouse serum or normal rabbit serum in the presence of complement and also with medium No. 199. The results in Table 1 show that after treatment of the immune lymphocytes with anti-θ antibodies 34-42% of the cells died, and this led to complete neutralization of the cytotoxic action of the living lymphocytes of the target cells. Conversely, antibodies against PC.1 and MGG, like normal sera, did not significantly modify the cytotoxic effect of the lymphocytes.

In the same experiments treatment with anti-θ antibodies and complement had no effect on the number of rosette-forming cells, whereas rosette formation was inhibited by 65.4% by antibodies against MGG and by 28.1% by antibodies against PC.1. These two antisera killed 23-26 and 0-12% of the lymph gland cells respectively. Treatment with normal mouse or rabbit serum was ineffective in this case also.

The effector lymphocytes destroying the allogeneic target cells are thus exclusively T-cells, in agreement with data in the literature [6,13]. Since removal of the B-cells from the population does not reduce the cytotoxic activity of immune lymphocytes, it must be supposed that the T-cells are not only essential, but they are also adequate for the complete cytotoxic effect. The same conclusion was drawn by Golstein et al., who used other methods to eliminate the B-cells [9], and whose results were published after this investigation had been completed. The B-cells are unnecessary not only for the effector function of ready-made immune lymphocytes, but also for their induction during allografting [5, 7, 9].

The results of the present investigation confirm our view that effector lymphocytes and rosette-forming cells reactive against H-2 antigens are two independent populations [4]. Just as in most other immunological systems [10, 18] rosette-forming cells reactive against H-2 antigens are B-lymphocytes. It must be

assumed that rosette-forming lymphocytes and cells synthesizing antibodies against H-2 antigens, and detectable by the plaque method in agar [15], belong to the same class of lymphocytes and differ from each other in their degree of maturity. In this connection only 28% of rosette-forming cells were inhibited by antibodies against PC.1, which is the marker of mature plasma cells [18].

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