IMMUNOLOGIC SPECIFICITY, NATURE, AND PROPERTIES OF SUPPRESSOR CELLS INDUCED IN MIXED LYMPHOCYTE CULTURES

B. D. Brondz and A. V. Karaulov

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After incubation of lymphocytes 1-5 days in allogeneic or syngeneic one-way mixed lymphocyte culture (MLC) suppressor cells blocking proliferation of 10 to 100 times as many syngeneic responding lymphocytes in fresh allogeneic MLC are generated. Neither "allogeneic" nor "syngeneic" suppressors have the properties either of B-cells or macrophages, both are resistant to mitomycin C, but their precursors are sensitive to cyclophosphamide. They differ in their properties both from T-killers and from T-suppressors induced in a lymphocyte monoculture. The "allogeneic" suppressors are heterogeneous T-cells as regards radiosensitivity and specificity. The formation of nonspecific T-suppressors is considerably reduced if embryonic calf serum in the medium is replaced by human or bovine serum. "Syngeneic" suppressors also are heterogeneous and differ from "allogeneic" in their greater resistance to anti-T-antibodies and irradiation, whereas their precursors are more resistant to hydrocortisone.

KEY WORDS: suppressors; mixed lymphocyte culture; activation of DNA synthesis.

The formation of suppressor cells is the earliest and most easily produced response to antigens and nonantigenic stimuli. T-suppressors are discovered under conditions of culture in which few T-killers are formed: by incubation of lymphocytes in monoculture for 3-5 days without mitogen in medium containing embryonic and calf serum (ECS) [10, 12], and also in allogeneic mixed lymphocyte culture (MLC) for 2 days [9] or 5-6 days, if thymocytes are used as reacting cells or if heat-killed lymphocytes are used as stimulators [15]. Depending on the conditions of induction in culture, T-suppressors differ in their sensitivity to γ-rays, mitomycin C (MC), hydrocortisone (HC), and cyclophosphamide (CP). They inhibit the generation of T-killers [3, 6, 8-10] or proliferation of T-cells [5] if transferred to fresh MLC, and also activity of T-helpers [11]. The action of T-suppressors, induced by alloantigens in MLC, as a rule is nonspecific [9-11], although sometimes some degree of specificity has been detected [3, 5, 10].

The object of this investigation was to study the nature and properties of suppressors induced in allogeneic and syngeneic MLC and to identify conditions of formation of specific T-suppressors.

EXPERIMENTAL METHOD

To induce suppressors in MLC a mixture of reacting spleen cells from B10.D2 ($H\text{-}2^d$) mice (abbreviated to D2), and stimulating spleen cells irradiated in a dose of 1500 rads, from either syngeneic (D2) or allogeneic C57BL/10 ($H\text{-}2^b$) mice (abbreviated to B10), were incubated in the ratio of 4:3 and in concentrations of 4 billion cells/ml in No. 3012 flasks (Falcon Plastics), using RPMI-1640 medium with the addition of 2 mM L-glutamine, 10 mM HEPES buffer, 10% ECS, $5 \cdot 10^{-5}$ M 2-mercaptoethanol, and antibiotics [13]. In some experiments the ECS was replaced by bovine serum (BS) or human serum (HS).

To determine the activity of suppressors, cells from MLC (normal D2 spleen cells in the control) were treated with MC in a dose of 50 µg/ml and added to the test MLC in the ratio of 1:10 or 1:100 to the reacting lymphocytes. The test MLC was set up in modification [1] of

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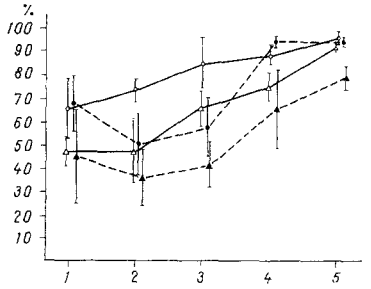

Fig. 1

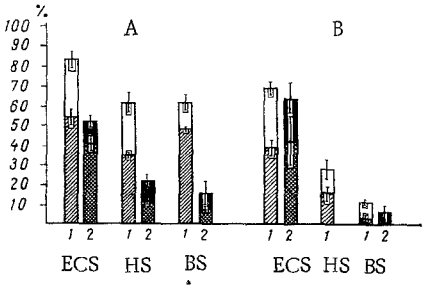

Fig. 2

Fig. 1. Dynamics of suppressor generation in MLC. Cells from allogeneic MLC D2 anti-B10 (continuous line) or syngeneic MLC D2 anti-D2 (broken line) treated with MC and added to test MLC D2 anti-B10 in the ratio of 1:10 (empty and filled circles) or 1:100 (empty and filled triangles) to the number of reacting lymphocytes. Abscissa, days of culture in 1st MLC; ordinate, II of DNA synthesis in test MLC.

Fig. 2. Induction of specific and nonspecific suppressors by the use of different sera in the medium. Cells of 3-day allogeneic D2 anti-B10 (A) or syngeneic D2 anti-D2 (B) MLC cultured in ECS, HS, or BS, treated with MC, and mixed with reacting D2 lymphocytes in MLC test in ratio of 1:10 (unshaded and black columns) or 1:100 (shaded columns). Ordinate, II of DNA synthesis in 10 MLC stimulated by B10 (1) and DBA/2 cells (2).

the micromethod [7] in No. 3040 microdisks (Falcon Plastics), and thymidine-^{3}H was added 16 h before the end of culture. The index of inhibition (II) of DNA synthesis was calculated by the equation:

$$\frac{a-b}{a}\times 100,$$

where a and b are the numbers of counts per minute in the control and experiment respectively.

The characteristics of mouse and rabbit antisera against T-cells (anti-thy-1,2,* anti-Tt,† anti-Tb‡), against mouse B-cells (anti-Mls** and anti-Ig), † and also the conditions of treatment of the cells with these antisera, together with complement and carrageenin, and the method of carrying out adsorption on the disk was described previously [2].

EXPERIMENTAL RESULTS

It will be clear from Fig. 1 that suppressor cells were formed in both allogeneic and syngeneic MLC, starting from the first day of culture, and that they reached the maximum of their action by the 5th day. The greatest difference (by 1.5 times) between activity of the suppressors in the allogeneic and syngeneic MLC was found by the 3rd day. In subsequent experiments this was the only time of culture used.

The data in Table 1 show that suppressors formed in the allogeneic and syngeneic MLC were similar in several properties: They were resistant to MC and carrageenin, not adsorbed on the disk, not inactivated by antibodies against B-cells (anti-Mls and anti-Ig) in the presence of complement, but their precursors were equally sensitive to CP. These findings indicate that both types of suppressors do not belong to cells of the B-lymphocyte or macrophage type. At the same time, differences were seen between them: The "allogeneic" sup-

*Against θ-antigen.
†Exhausted serum against rabbit thymocytes, from Cederlane.
‡Exhausted serum against rabbit brain, from Cederlane.
**Minor lymphocyte stimulating locus.
††Immunoglobulin.

TABLE 1. Effect of Various Preparations and Procedures on Suppressor Activity of D2 Cells Induced in MLC by Allogeneic (B10) or Syngeneic(D2) Stimulators

Treatment of suppressors	Source of suppressors			
	allogenic MLC		syngeneic MLC	
	intact suppressors	treated suppressors	intact suppressors	treated suppressors
MC (50 μg/ml)	86,3±4,3	94,3±1,8	76±7,0	73±3,5 (4,0)
Carrageenan (400 μg/ml)	96±3,5	96±2,0 (0)	80,5±0,5	80,5±11,5 (0)
Adsorption on disk	83±0,5	80±1,0 (3,6)	55±5,0	59±10,0 (0)
2700 rads	85±6,0	38±3,5 (55)	67±4,2	59±4,4 (11,3)
NMS $^1/_3$+C	85±2,1	85±3,3 (0)	63±4,6	62±6,8 (1,0)
Anti-Thy:				
$^1/_3$+C	87±4,5	10±3,7 (89)	63±4,6	34±5,1 (46)
$^1/_9$+C	90±4,4	14±9,8 (84)	60±6,3	42±3,3 (34)
$^1/_{27}$+C	94±3,0	59±1,5 (37)	65±6,0	56±6,5 (15)
Anti-Tt $^1/_{10}$+C	94±3,0	12±11,5 (87)	65±6,0	29±2,5 (56)
Anti-Tb $^1/_{10}$+C	94±3,0	0 (100)	65±6,0	29±3,0 (56)
Anti-Mls $^1/_2$+C	94±3,0	84±6,0 (10,6)	65±6,0	66±0,5 (0)
Cp:				
50 mg/kg		87±2,0 (0)		61±4,0 (0)
100 mg/kg	86±2,0	0 (100)	55±3,0	9±7,0 (84)
HC (2,5 mg)		6±6,0 (93)		37±4,0 (32)

Legend: 1. Cells from 3-day MLC treated with MC and mixed with reacting D2 lymphocytes in test MLC in the ratio of 1:2. 2. Numbers denote II (in %) of DNA synthesis (M±m, results of 2-5 experiments). 3. Suppression (in %) shown in parentheses. 4. C) Complement; NMS) normal mouse serum. 5. CP and HC were injected intraperitoneally into the mice 2 days before the experiment.

pressors were entirely T-cells (inactivated by anti-Thy-1- and anti-T-sera in the presence of complement), they were relatively radiosensitive (inactivated to the extent of 55% by γ-rays in a dose of 2700 rad), and their precursors were highly sensitive to HC. Conversely, the "syngeneic" suppressors were inactivated by anti-T-antibodies to the extent of only 50%, they were resistant to γ-rays, and their precursors resistant to HC (Table 1). About half of the "syngeneic" suppressors could therefore be classed as "zero" lymphocytes, radioresistant and resistant to HC.

To study the immunologic specificity of D2 anti-B10 suppressors they were added to a test MLC, stimulated by "foreign" DBA/2 (H-2^d) cells, the differences of which from the reacting D2 cells for the Mls locus were sufficient to activate DNA synthesis. It will be clear from Fig. 2 that the nonspecific component of suppression accounted for 63-75% of blocking of the response to B10 stimulators if the suppressors were incubated in medium with ECS, but this was reduced to 33 and 18-25% if HS and BS respectively were used instead of ECS. Activity of nonspecific "syngeneic" suppressors also was considerably reduced in this case (Fig. 2).

In view of data [6, 10] showing that specific T-suppressors are more radioresistant than nonspecific, an attempt was made to separate them by irradiation. However, it was found that if D2 anti-B10 suppressors were induced in ECS, their activity was reduced by half on irradiation in doses of 900 and 2700 rads, irrespective of which stimulators (B10 or DBA/2) were used in the test MLC (Fig. 3). Conversely, suppressors induced in HS were more radioresistant: Irradiation in a dose of 2700 rads reduced their activity only slightly (Fig. 3).

Culture of the lymphocytes in syngeneic or allogeneic MLC, even for 1 day, was thus sufficient to cause the appearance of highly active suppressors capable of blocking proliferation of 100 times as many lymphocytes as react in fresh MLC. The "syngeneic" suppressors differed from the "allogeneic" not only by their lower activity, but also by their lower sensitivity to anti-Thy-1- and anti-T-antibodies and to irradiation, and were less sensitive to HC. "Syngeneic" suppressors are evidently heterogeneous: About half of them are T-cells and about half are "zero" lymphocytes. They differ from T-suppressors induced in monoculture and blocking killer generation: In the latter case the suppressors are formed later, they are inactivated by MC and γ-rays in a dose of 1000 rads [2], and they are not generated from D2 lymphocytes [8].

Although all "allogeneic" suppressors are T-cells, differing in their properties from suppressors induced both in syngeneic MLC and in monoculture, they are likewise heterogeneous and can be divided into specific and nonspecific: The presence of ECS in the medium facili-

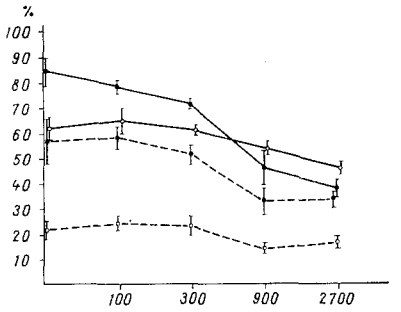

Fig. 3. Effect of irradiation on suppressors induced in MLC. Cells of 3-day D2 anti-B10 MLC cultured in ECS (filled circles) or HS (empty circles), treated with MC, irradiated, and added to test MLC in the ratio of 1:10 to reacting D2 lymphocytes. Abscissa, dose of irradiation (in rads); ordinate, II of DNA synthesis in test MLC, stimulated by B10 (continuous line) or DBA/2 (broken line) cells.

tates the formation of the latter, as well as of "syngeneic" suppressors. This action of ECS may be associated with polyclonal activation of T-cells [4] or with the presence of α-fetoprotein in the serum, inducing nonspecific T-suppressors [14]. Whatever the case, the use of HS or BS instead of ECS enables the specificity of T-suppressors to be significantly increased (Fig. 2), and their greater radioresistance in this case (see Fig. 3) is in agreement with other data [6, 10].

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