SUPPRESSION OF ANTIBODY FORMATION BY BONE MARROW T CELLS ACTIVATED BY HISTOCOMPATIBILITY ANTIGENS

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The suppressive action of bone marrow T cells activated by histocompatibility antigens on antibody formation was studied. The bone marrow of CBA mice was shown to contain thymus-dependent lymphocytes which, on hyperactivation by repeated transplantation into F_1 recipients, have a suppressive action on the development of the cooperative immune response to sheep's red cells. Preliminary treatment of the bone marrow cells with antithymocytic globulin and complement abolished the suppressive effect.

KEY WORDS: *bone marrow; suppressor T cells; antibody formation.*

Recent investigations have shown that the population of bone marrow cells contains, in addition to the B lymphocytes, which are precursors of the antibody-forming cells (AFCs), small numbers of other cells with the properties of T lymphocytes [1, 2]. However, because of the small number of thymus-dependent lymphocytes in the bone marrow, it is difficult to detect them, especially in mice. The difficulty of their identification is also due to the evident absence of the θ antigen, the most frequently detectable marker of T lymphocytes, on the surface of the bone marrow T cells [3]. The functional activity of the bone marrow T cells also has been inadequately studied. In particular, we do not know whether they possess a suppressive (regulatory) function.

EXPERIMENTAL METHOD

Experiments were carried out on CBA mice and (CBA × C57BL)F_1 hybrids. Bone marrow cells of CBA mice in a dose of $2 \cdot 10^7$ were injected intravenously 4-6 h after lethal irradiation (900 R) into CBA and (CBA × C57BL)F_1 mice. Seven days after transplantation of bone marrow the CBA (B_C mice) and (CBA × C57BL)F_1 (B_F mice) recipients were killed and their spleen cells removed and inoculated, in a dose of $1 \cdot 10^7$ together with $2 \cdot 10^7$ thymus cells of intact CBA mice and $2 \cdot 10^8$ sheep's red cells into secondary, lethally irradiated CBA or (CBA × C57B)F_1 recipients. Eight days later the number of AFCs were counted in the recipients' spleen by the local hemolysis-in-agar method [4]. In some experiments bone marrow cells of intact CBA donors or spleen cells of B mice, before injection into irradiated recipients, were incubated *in vitro* with antithymocytic globulins (ATG) and complement (guinea pig serum) for 45 min at 37°C. The recipients were irradiated with ^{137}Ce γ rays on the "Stebel' 3A" apparatus, with a dose rate of 900 R/min and a dose of $LD_{100/15}$, which is 900 R for CBA and (CBA × C57BL)F_1 mice. The results were subjected to statistical analysis with calculation of the geometric mean ($\bar{X}$) and the 95% ($P \leq 0.05$) confidence limits (I_{95}).

EXPERIMENTAL RESULTS

It will be clear from Table 1 that transplantation of spleen cells of B_C mice together with intact CBA thymocytes into irradiated CBA or (CBA × C57BL)F_1 recipients led to the accumulation of about equal numbers of AFCs (the differences were not statistically significant). Transplantation of spleen cells of B_F mice together with thymocytes of CBA mice into irradiated CBA recipients caused the formation of about the same number of AFCs as in the experi-

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TABLE 1. Suppression of AFC Formation in Spleen of Recipients Receiving Normal T Cells and Spleen Cells from B_C- and B_F- Mice

Donor of cells		Recipient	No. of recipients	Number of AFCs in spleen ($\bar{X}_{geom}$ and I_{95})
spleen	thymus			
B_c	CBA	CBA	16	8990 (7501 - 10 770)
B_c	CBA	(CBA×C57BL) F_1	15	7840 (6431 - 9557)
B_F	CBA	CBA	10	9177 (6466 - 13 002)
B_F	CBA	(CBA×C57BL) F_1	20	360 (290 - 447)

TABLE 2. Abolition of Suppression of AFC Formation by Antithymocytic Globulin

Cell donor		Recipients	Number of recipients	Number of AFCs is spleen ($\bar{X}_{geom}$ and I_{95})
spleen	thymus			
B_c	CBA	(CBA×C57BL) F_1	11	7120 (5896 - 8690)
B_F	CBA	CBA	8	5806 (3671 - 9183)
B_F	CBA	(CBA×C57BL) F_1	15	348 (263 - 457)
B_F*	CBA	(CBA×C57BL) F_1	18	4810 (2754 - 8137)
B_c†	CBA	(CBA×C57BL) F_1	9	7250 (5175 - 10 150)
B_F†	CBA	CBA	10	8120 (5826 - 11 301)
B_F†	CBA	(CBA×C57BL) F_1	14	6960 (4739 - 10 230)

*Spleen cells of B_F mice treated with ATC and complement before injection into secondary irradiated recipients.
†Bone marrow cells treated with AGC and complement before transplantation into primary irradiated recipients.

ments described above. If, however, spleen cells from B_F mice were injected together with CBA thymocytes into irradiated (CBA × C57BL)F_1 recipients considerable suppression of the immune response was observed. The number of AFCs, under these circumstances, was only 1/15 to 1/20 of that in the control experiments.

It is suggested that depression of the immune response observed after transplantation of spleen cells of B_F mice with CBA thymocytes into (CBA × C57BL)F_1 recipients was due to the presence of T cells in the spleen of the B_F mice, having been introduced with the transplanted bone marrow cells. The ability of T cells, when activated by foreign histocompatibility antigens, to exert a suppressive action on the development of immune responses has been established [8-10]. In the light of these findings it can be accepted that T cells contained in the bone marrow of CBA mice, on transplantation into primary irradiated (CBA × C57BL)F_1 recipients, are activated by C57BL histocompatibility antigens. These "irradiated" T cells, when reactivated in the secondary, lethally irradiated (CBA × C57BL)F_1 recipients, have a suppressive action on AFC formation in the system of B_F cells + thymocytes → F_1. In the system of B_F cells + thymocytes → CBA no suppressive effect was observed, evidently because of the absence of reactivation of the irradiated T cells in the syngeneic secondary irradiated recipients.

To test this hypothesis spleen cells of B_F mice were treated with ATG and complement. As Table 2 shows, removal of the T cells from the suspension of spleen cells from B_F mice restored their ability to form AFCs when transplanted together with CBA thymocytes into irradiated (CBA × C57BL)F_1 recipients. After transplantation of bone marrow, contamination with T cells was evidently present in the spleen of the primary, lethally irradiated recipients, which contained mainly B cells. Since 7 days was too short a time for differentiation of T cells present in the spleen of the B_F and also, evidently, the B_C mice, from bone marrow precursors [6, 7], the T cells were evidently present in fact in the donor's bone marrow and were not contaminating cells as a result of transplantation.

To test this hypothesis cells of the donor's bone marrow were treated with ATG and complement before injection into the primary irradiated recipients. It will be clear from Table 2 that treatment of the CBA bone marrow used to obtain B_F mice with ATG and complement abolished suppression of AFC formation on transplantation of the spleen cells of these B_F mice together with thymocytes into irradiated (CBA × C57BL)F_1 recipients.

Consequently, treatment with ATG, eliminating T cells from the bone marrow, abolished suppression of the cooperative immune response in the system B_F + thymocytes → F_1. Suppression of the immune response on transplantation of cells of the parental genotype into F recipients is unconnected with the toxic effects of the graft versus host reaction [5].

It can accordingly be concluded from these results that cells of thymus origin (T lymphocytes) are present in bone marrow and, when hyperactivated by histocompatibility antigens, they acquire suppressive properties.

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COMPARATIVE ASSESSMENT OF THE PROPERTIES OF MACROPHAGE MIGRATION INHIBITING FACTOR AND OF INTERFERON

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Some functional properties of interferon and of a factor inhibiting migration of macrophages (MMIF), obtained by stimulation of human tonsillar lymphocytes by Newcastle disease virus (NDV) or streptolysin O, were investigated. Both interferon and MMIF were shown to inhibit migration of human tonsillar cells actively, but they differed in their antiviral activity and their sensitivity to heating to 56°C for 30 min. MMIF production reached its maximum later than interferon production. Stimulation of human tonsillar lymphocytes by NDV led to the production of a broader spectrum of mediators of hypersensitivity of delayed type than stimulation by streptolysin O.

KEY WORDS: *cellular immunity; interferon; macrophage migration inhibiting factor.*

Mediators of hypersensitivity of delayed type (HDT) have been studied widely in recent years. The suggestion has been made that some of them have a common chemical structure and functional properties [7, 8].

This paper describes the study of some properties of interferon, obtained by the action of Newcastle disease virus (NDV) on a culture of tonsillar lymphocytes, and a macrophage migration inhibiting factor (MMIF) obtained by treatment of a culture of human tonsillar lymphocytes with streptolysin O. The basis for the investigation was the observation that human tonsillar lymphocytes participate actively in HDT reactions [1, 3, 4, 9, 10].

EXPERIMENTAL METHOD

MMIF were obtained by cultivating human tonsillar cells with streptolysin O. The tonsillar tissue was cut into small pieces with scissors, filtered through Capron, and washed off with medium No. 199 containing 10% heat-inactivated calf serum, 100 units/ml of penicillin, and 60 μg/ml of streptomycin. Viable tonsillar cells (10^7) were grown in 2 ml of this medium containing 0.1 ml of a 5% solution of streptolysin O at 37°C for 12-30 h. The supernatant obtained after culture was dialyzed against physiological saline for 48 h and lyophilized [10].

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