ACTION OF DI-IODOBENZOTEPA ON FUNCTIONALLY DIFFERENT T-LYMPHOCYTE SUBPOPULATIONS

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Di-iodobenzotepa (DIB) was given per os daily for 3 days in a dose of 25 mg/kg to CBA mice. A decrease in the number of nucleated cells in the thymus by 74% and in the bone marrow by 29% was observed. Experiments with transplantation of lymphocytes from mice treated with DIB into intact or lethally irradiated (CBA × C57BL/6J)F_1 mice showed that DIB has no effect on the helper activity of T-lymphocytes but depresses the functional activity of B-lymphocytes and T-lymphocytes, inducing the graft versus host reaction, and of T-suppressor cells.

KEY WORDS: di-iodobenzotepa; T-helpers; T-suppressors.

Recent investigations have demonstrated the functional heterogeneity of T- and B-lymphocytes [1]. It is therefore interesting to seek preparations with selective action on functionally different lymphocyte subpopulations and on interaction between them.

The object of this investigation was to study the action of di-iodobenzotepa (DIB) on the functions of different T-lymphocyte populations, including T-helpers and T-suppressors. DIB (N-2,5-di-iodobenzoyl-N',N',N'', N''-diethylenetriamide of phosphoric acid) is a halogen-containing analog of benzotepa (benzoyl diethylenetriamide of phosphoric acid), a compound with high antitumor activity, belonging to the group of alkylating compounds and, in large doses, can cause depression of lympho- and myelopoiesis [3-6].

EXPERIMENTAL METHOD

CBA and (CBA × C57BL/6J)F_1 mice from the Stolbovaya nursery, Academy of Medical Sciences of the USSR, were used. DIB was given to the cell donors per os as a suspension in 0.5-1% starch solution, daily for 3 days in a dose of 25 mg/kg. The cells were isolated 24 h after the last injection of the compound. LD_{50} for mice was 500 mg/kg. Mice of the control groups received only starch solutions per os.

The cell recipients were irradiated with γ-rays in a dose of 850 R 4-24 h before intravenous inoculation of the cells. The dose rate was 124.9-114 R/min.

The action of DIB on the cooperative ability of T- or B-lymphocytes treated with the compound was investigated by the method of Mitchell and Miller [11]. Lethally irradiated mice were inoculated with 10^7 bone marrow cells and 2×10^7 syngeneic thymocytes isolated from mice treated with DIB or untreated mice, mixed with 2×10^8 sheep's red blood cells (SRBC). In some experiments, instead of intact bone marrow, bone marrow cells from mice treated with the compound were used. The number of hemolysin forming cells in the recipients' spleen was determined by Jerne's method [10] 7 days later.

To investigate the action of DIB on the ability of T-lymphocytes to induce the graft versus host reaction (GVHR) the model suggested by Blomgren and Andersson [7] was used. A dose of 10^5-2×10^7 thymus or spleen cells from the CBA mice aged 10-12 weeks, either intact or treated with DIB, was injected into (CBA × C57BL/6J)F_1 mice aged 4-6 weeks. On the 8th day after transplantation the intensity of the GVHR was estimated from the development of splenomegaly, assessed as the splenic index [13].

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TABLE 1. Effect of DIB on Helper Activity of T-Lymphocytes

Transplanted cells		Number of AFC in spleen M ± m			
bone marrow (10^7)	thymus (2×10^7)	experiment 1	experiment 2	experiment 3	experiment 4
Intact	Intact	1322 ±223 (6)	88 ±17 (10)	411 ±75 (10)	718 ±132 (6)
Intact	Treated	5678 ±958 (8)	433 ±106 (9)	1535 ±323 (14)	1939 ±203 (7)
Intact	Intact	1322 ±223 (6)	1491 ±271 (12)	2875 ±599 (13)	224 ±50 (10)
Treated	Intact	480 ±81 (6)	591 ±49 (14)	684 ±194 (13)	117 ±19 (9)

Legend. Number of mice in group shown in parentheses.

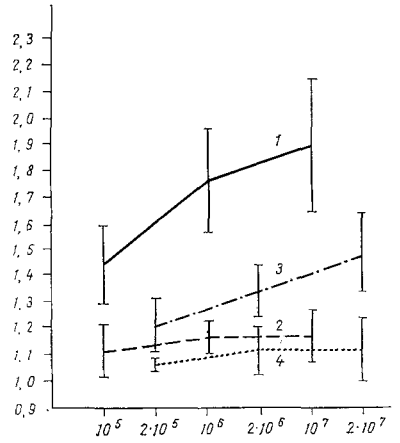

Fig. 1

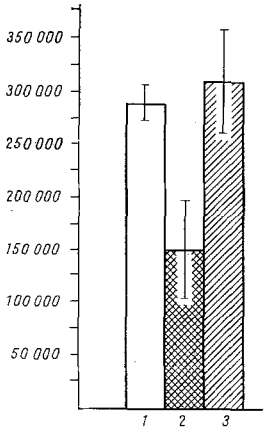

Fig. 2

Fig. 1. Effect of DIB on ability of spleen and thymus cells to induce GVHR. 1) Intact spleen cells; 2) spleen cells treated with DIB; 3) intact thymus cells; 4) thymus cells treated with DIB. Ordinate, splenic index, abscissa, number of transplated cells.

Fig. 2. Effect of DIB on formation of T-suppressor lymphocytes. 1) Intact F_1 mice; 2) mice receiving 5×10^7 spleen cells of intact CBA mice; 3) mice receiving 5×10^7 spleen cells of DBA mice treated with DIB. Ordinate, number of AFC in spleen.

The effect of DIB on T-suppressor formation was studied by Möller's method [12]. Splenocytes (5×10^7) of CBA mice treated with DIB were injected intravenously into (CBA × C57BL/6J)F_1 mice. After 7-8 days the recipients were given an injection of 5×10^8 SRBC and the number of hemolysin-forming cells in the animals' spleen was counted 5 days later by Jerne's method. F_1 mice receiving splenocytes of intact mice mixed with SRBC or SRBC alone served as controls.

EXPERIMENTAL RESULTS

The effect of DIB on helper activity of T-lymphocytes, as reflected in their ability to cooperate with B-lymphocytes, is shown in Table 1. After transplantation of bone marrow cells of mice treated with the compound, mixed with intact thymocytes, into irradiated recipients the number of antibody-forming cells (AFC) was reduced on average (based on the results of four experiments) by 64% compared with transplantation of the same doses of intact cells. However, when the recipients were injected with intact bone marrow cells mixed with thymus cells isolated from mice receiving DIB, the number of AFC was increased on average by 3.9 times. When the results are analyzed it must be remembered that when mice are treated with the compound in these doses the number of nucleated cells in their thymus falls from $(267.3 \pm 18.1) \times 10^6$ to (68.4 ±

12.1) × 10^6, i.e., it is reduced by 76%. The number of nucleated bone marrow cells, counted per femur, was reduced only a little: From (21.5 ± 3.3) × 10^6 to (15.5 ± 1.3) × 10^6, i.e., by 29%. This shows that DIB depresses the functional activity of B-lymphocytes and, despite exhaustion of the thymus, it does not affect the helper function of the thymocytes. The action of DIB on the thymus resembles the action of hydrocortisone. Just as after injection of hydrocortisone (125 mg/kg) leads to exhaustion but does not affect the helper activity of the T-lymphocytes [8]. However, unlike hydrocortisone, which does not affect ability of thymocytes or spleen cells to induce to GVHR [7, 9], DIB led to the virtually total loss of this function of the lymphocytes (Fig. 1).

The action of DIB on T-suppressor formation is illustrated in Fig. 2. Clearly DIB abolished T-suppressor formation. In the spleen of F_1 mice receiving splenocytes from CBA mice treated with DIB, mixed with SRBC, the same number of AFC were found as in the spleen of F_1 mice receiving erythrocytic antigens only. Meanwhile transplantation of intact splenocytes containing T-suppressors, mixed with SRBC, into F_1 mice reduced the number of AFC by half.

Although DIB has no effect on the helper function of T-lymphocytes, it thus depresses functional activity of B-lymphocytes, of T-lymphocytes inducing the GVHR, and of T-suppressor cells.

The relative selectivity of action of DIB on T-suppressors which, as has been shown [2], are activated during tumor growth, may be evidence that one of the mechanisms lying at the basis of the antitumor action of DIB is depression of the activity or elimination of this particular subpopulation of T-lymphocytes.

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