MONOCLONAL ANTIBODIES AS A MODEL OF "UNIVERSAL" BONE MARROW

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Intensive radiochemotherapy followed by bone marrow transplantation is an effective method of treatment of hemoblastoses in man. This method has limitations connected with the need to choose histocompatible donor and recipient in order to minimize the risk of development of transplantation sickness. Although several immunodepressive agents capable of suppressing the graft versus host reaction (GVHR) exist, this problem is still far from being solved.

The use of monoclonal antibodies (McAb) has created fresh prospects for preventing the development of GVHR [5-7]. However, a problem has arisen in the choice of optimal McAb and the optimal method of treatment of the donor's bone marrow.

The aim of this investigation was to create a model of the prevention of development of transplantation sickness with the aid of ICO-10 McAb, directed against the Thy-1 antigen [1].

EXPERIMENTAL METHOD

Experiments were carried out on male BALB/c, (CBA × C57BL/6j)F_1 hybrid, and B10RIII mice. Bone marrow was obtained from the long bones of BALB/c mice and kept in medium 199.

The hybrid mice serving as recipients of bone marrow were irradiated on a ^{137}Cs γ-radiation system in a dose of 10 Gy.

Transplantation was carried out on the day of irradiation of the mice by intravenous injection of bone marrow cells in doses of 3.2×10^8-5.0×10^9/kg body weight.

Ascites fluid from BALB/c mice carrying an ICO-10 hybridoma, with a titer of 1:10,000, served as the McAb.

Serum from rabbits aged 1.5 months, kept in the frozen state, was used as complement.

The bone marrow cells were treated for 0.5 h with ICO-10 and for 1 h in the presence of complement at 37°C in optimal dilutions of 1:100 and 1:4 respectively, yielding the maximal cytotoxic index.

The indirect immunofluorescence test (IIFT) was carried out by the method described previously [1].

Alloantigens were detected on the erythrocytes of the experimental animals by a micromodification of the indirect hemagglutination test (IHAT) [2], using alloimmune mouse sera against antigens $H\text{-}2^k$, $H\text{-}2^d$. The rabbit serum against mouse IgG was generously provided by A. F. Étkin.

To carry out the mixed lymphocyte culture (MLC) test a suspension of spleen cells from donors and recipients of the bone marrow containing 10^6/ml of responding cells and 0.5×10^6/ml of irradiated stimulators' cells in a total volume of 200 µl was used [4].

The karyologic investigations were conducted in the usual way [3].

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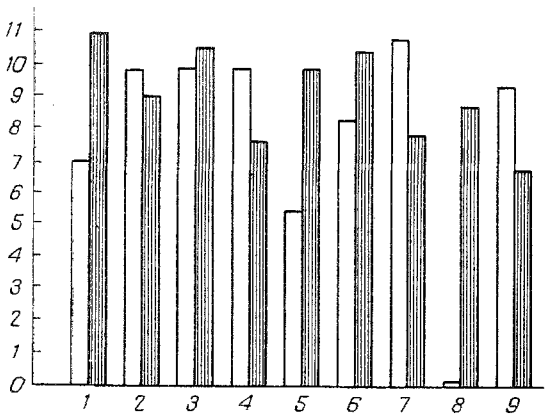

Fig. 1. Expression of H-2^d antigen on erythrocytes of experimental (CBA × C57BL/6j)F_1 mice. Abscissa: 1-7) Nos. of experimental mice, 8, 9) control (CBA × C57BL/6j)F_1 and BALB/c mice respectively; ordinate, $\log_2$ of dilution of alloimmune sera. Unshaded columns — index of intensity of reaction to H-2^d antigen, shaded columns — to H-2^k antigen.

TABLE 1. Proliferative Response of Splenocytes of Mice Receiving Bone Marrow in MLC Test

Responding	Stimulator cells	Number of counts per minute (M ± m)	Index of stimulation	p
F_1—PKM	F_1—PKM	1660±164,5		
F_1—PKM	F_1	1787,2±68,14	1,08	>0,05
F_1—PKM	BALB/c	1695,3±63,6	1,02	>0,05
F_1—PKM	B10RIII	1623,8±70,05	0,98	>0,05
F_1	F_1	576,7±24,8		
F_1	BALB/c	1144,3±82,4	1,98	<0,001
F_1	B10RIII	985,0±42,48	1,69	<0,001
F_1	F_1—PKM	698,7±43,52	1,16	>0,05
BALB/c	BALB/c	540,8±39,0		
BALB/c	F_1	1331,0±48,54	2,46	<0,001
BALB/c	B10RIII	793,0±30,6	1,47	<0,01
BALB/c	F_1—PKM	586,8±25,71	1,08	>0,05
B10RIII	B10RIII	401,3±41,5		
B10RIII	BALB/c	881,5±57,65	2,20	<0,001
B10RIII	F_1	692,3±60,44	1,73	<0,01
B10RIII	F_1—PKM	640,5±41,2	1,60	<0,01

Legend. F_1 denotes (CBA × C57BL/6j)F_1 mice; RBM denotes recipients of bone marrow.

EXPERIMENTAL RESULTS

Bone marrow of BALB/c mice was treated once and twice, when a decrease in the cell concentration was observed from 1.8×10^7 to 7.8×10^6/ml and from 3.3×10^7/ml to 1.2×10^7/ml respectively.

After a single treatment, the development of GVHR was observed in nine of the 10 (CBA × C57BL/6j)F_1 recipients, with death during 1 month after transplantation.

In the group of mice protected by allogeneic bone marrow, the development of a GVHR was not observed after two treatments and the state of all 10 animals remained good during the period of observation (over 4 months). No T cells were found in the peripheral blood of these animals 2 months after transplantation in the IIFT using ICO-10 McAb and McAb against Thy-1,2 antigens. In the IHAT to detect alloantigens on erythrocytes of the experimental mice, antigens characteristic of both donors and recipients of the bone marrow were found (Fig. 1).

The MLC test revealed no response of splenic lymphocytes of this group of mice to antigens of (CBA × C57BL/6j)F_1 and BALB/c mice (Table 1). It must be pointed out that 4 months after investigation of bone marrow of recipients Nos. 1 and 5, no cells of the female karyotype were found in them. In some of the mice investigated, which were protected by bone marrow, total atrophy of the thymus was observed after two treatments.

The investigation thus showed that treatment of allogeneic bone marrow twice with McAb completely eliminated T cells, thereby preventing the development of the GVHR. Only after treatment of the bone marrow twice with McAb and complement was 100% protection of the irradiated mice obtained. The clinical state of the treated mice remained good in the absence of both T cells and thymus.

ICO-10 McAb obtained by immunization of BALB/c mice with human embryonic thymus cells reveal Thy-1 antigen, which was present at all stages of differentiation of T cells with the Thy-1,1 and Thy-1,2 phenotype in the mice. In man Thy-1 antigen is expressed only on early lymphocytes and early B cells. Expression of Thy-1 antigen, detectable by ICO-10 McAb, moreover, was found in most cases on tumor cells of a neuroblastoma. Consequently, ICO-10 McAb are suitable for preliminary treatment of the bone marrow of neuroblastoma patients.

Under the conditions of this investigation it was thus shown that the use of McAb alone can give protection against GVHR. Attempts have been made clinically to use a mixture of three McAb to antigens SD-2, SD-5, and SD-7, but these do not completely prevent the development of GVHR.

The experiments with ICO-10 demonstrate the advisability of looking for new MCA to human T-cell antigens, which will be capable of completely eliminating all T cells and of producing a "universal" allogeneic bone marrow.

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