USE OF HETEROLOGOUS ANTIRECEPTOR ANTIBODIES FOR SPECIFIC REVERSAL OF ACUTE HOMOLOGOUS DISEASE IN MICE

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Much attention has recently been paid to the study of antireceptor (antiidiotypical) antibodies (ARA) as a possible means of specific suppression of immunologic reactions [2, 5, 8, 10, 12]. ARA have also been used to study certain properties of antigen-recognizing receptors of T and B cells [7, 9, 11].

This paper describes an attempt to obtain ARA in a heterologous system against antigen-recognizing receptors of T lymphocytes, reacting to allogeneic transplantation antigen. It was proposed to determine the specificity of action of ARA in the complement-dependent cytotoxic test on immune T target cells. If the result of this test was positive, it was proposed to use ARA for specific inhibition of acute homologous disease in irradiated recipients.

EXPERIMENTAL METHOD

Male and female mice of the following strains — CBA ($H\text{-}2^k$), C57BL/6 ($H\text{-}2^b$), BALB/c ($H\text{-}2^d$), (CBA × C57BL/6)F_1 ($H\text{-}2^{k/b}$), (CBA × BALB/c)F_1 ($H\text{-}2^{k/d}$), — weighing 18-20 g were used.

ARA were obtained as follows. CBA and C57BL/6 mice were immunized subcutaneously at 5 points with 10^8 spleen cells of C57BL/6 and BALB/c mice, respectively. Seven days after immunization cells were obtained from the regional lymph node (submandibular, axillary, inguinal), and injected intravenously, in three doses of $5 \cdot 10^8$ cells per injection, at intervals of 1 month, into rabbits. The rabbits were exsanguinated 7 days after the last immunization. Immune rabbit serum was absorbed with liver, red blood cells, serum, and also with cells of the thymus, lymph nodes, and spleen of intact mice. Absorption was continued until activity of the sera in the hemagglutination test with mouse red blood cells, in the gel precipitation test with the serum of intact mice, and in the complement-dependent cytotoxic test with intact lymphocytes had completely disappeared [3].

The resulting antisera were described as anti-(CBA-anti-C57BL/6) (ARA-1), and anti-(C57BL/6-anti-BALB/c) (ARA-2). Four series of ARA were obtained and all possessed the same type of activity.

T lymphocytes, activated by allogeneic transplantation antigens, were used as target cells for the cytotoxic test [13]: 10^8 allogeneic thymocytes were injected intravenously into recipients irradiated in a dose of 850 R, and three days after the injection spleen cells were obtained and the cytotoxic action of the experimental sera on them was tested. These suspensions contained 30-60% of transformed lymphocytes. When specific antilinear sera and sera against T lymphocytes (ATS) were used it was found that all living cells in these suspensions were T lymphocytes with the donor's phenotype. The effect of ARA on B lymphocytes was assessed by studying its action in vitro in the presence of complement on antibody-forming cells (AFC) producing antibodies against sheep's red blood cells [6]. The action of ARA on lymphocytes responsible for the graft versus host reaction (GVHR) was investigated as follows: spleen cells of CBA mice were treated *in vitro* with ARA-1 in the

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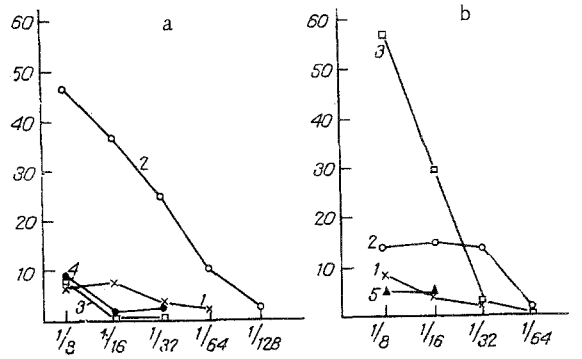

Fig. 1

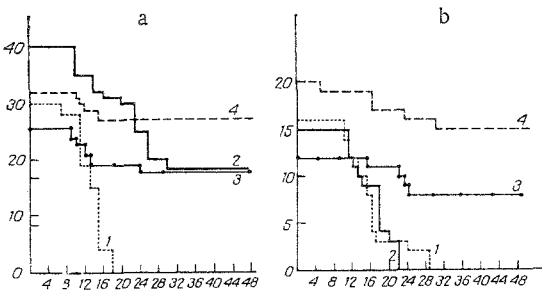

Fig. 2

Fig. 1. Effect of ARA-1 and ARA-2 on different target cells in the complement-dependent cytotoxic test. Abscissa, dilution of antiserum; ordinate, cytotoxic index (in percent). a) action of ARA-1; b) action of ARA-2. Target cells: 1) spleen, thymus, and lymph node cells of intact CBA, BALB/c, C57BL/6, and (CBA × C57BL/6)F_1 mice; 2) activated CBA-anti-(CBA × C57BL/6)F_1 T lymphocytes; 3) activated C57BL/6-anti-BALB/c T lymphocytes; 4) activated CBA-anti-BALB/c T lymphocytes; 5) activated BALB/c-anti-C57BL/6 T lymphocytes.

Fig. 2. Effect of ARA-1 on development of a lethal GVHR. Abscissa, days after transplantation; ordinate, number of surviving mice. a) Dynamics of death of (CBA × C57BL/6)F_1 recipients; b) dynamics of death of (CBA × BALB/c)F_1 recipients. 1) serum of intact rabbits; 2) ARA-1; 3) ATS; 4) irradiation control.

presence of rabbit complement [4], incubated for 45 min at 37°C, washed, and injected in a dose of $3 \cdot 10^7$ cells into (CBA × C57BL/6)F_1 and (CBA × BALB/c)F_1 mice irradiated in a dose of 650 R. The intensity of the GVHR was estimated from the survival rate of the recipients.

EXPERIMENTAL RESULTS

The results showing the action of ARA-1 and ARA-2 on different target cells in the cytotoxic test are illustrated in Fig. 1. They show that neither serum was toxic for intact thymus, spleen, and lymph node cells of CBA, C57BL/6, BALB/c, and (CBA × C57BL/6)F_1 mice. The experimental sera likewise did not inhibit AFC: incubation of AFC with ARA and complement did not affect the number of AFC (the results are not given). Meanwhile ARA-1 and ARA-2 had a marked specific action against corresponding target cells: ARA-1—anti-(CBA-anti-C57BL/6) was cytotoxic only for activated CBA-anti-(CBA × C57BL/6)F_1 T cells (maximal cytotoxic index 46%) and virtually did not interact with activated CBA-anti-BALB/c and C57BL/6-anti-BALB/c T cells. ARA-2—anti-C57BL/6-anti-BALB/c) killed 56% of activated C57BL/6-anti-BALB/c T cells, did not affect BALB/c-anti-C7 BL/6 T lymphocytes, and reacted with only 15% of the CBA-anti-C57BL/6 cells (Fig. 1).

The results of the cytotoxic test served as a basis for experiments using ARA-1 and the GVHR (Fig. 2). Treatment of CBA spleen cells with inactivated normal rabbit serum did not lead to abolition of the GVHR: 80-100% of the (CBA × C57BL/6)F_1 and (CBA × BALB/c)F_1 recipients died by the end of the 3rd week after injection of the donor's cells. In experiments in which the irradiated hybrids did not receive parental cells, a high survival rate was observed among the animals. ATS gave a marked protective effect: After three weeks 73-91% of the recipients, and after eight weeks 66-69%, were still alive. ATS abolished the ability of CBA lymphocytes to induce a GVHR when injected into both (CBA × C57BL/6)F_1 and (CBA × BALB/c)F_1 recipients. These results show that, in accordance with the generally accepted view [1, 7, 8], T lymphocytes take part in the mechanism of the GVHR, and ATS acts equally on CBA-anti-C57BL/6 and CBA-anti-BALB/c T cells. The effect of ARA-2 on the ability of CBA lymphocytes to induce the GVHR was twofold (Fig. 2). ARA-1 virtually did not prevent the GVHR in (CBA × BALB/c)F_1 mice. After three weeks the mortality among the experimental animals was 100%. Meanwhile, it was shown with the aid of ARA-1 that the GVHR could be prevented in (CBA × C57BL/6)F_1 hybrids: 3 and 8 weeks after the beginning of the experiment, 65 and 40% of recipients, respectively, were still alive.

It can be concluded from these facts that ARA-1 evidently eliminates lymphocytes with original CBA-anti-C57BL/6 specificity selectively from a population of intact CBA spleen

cells, with consequent inhibition of the intensity of the GVHR effected by this "defective" population. Data relating to the role of T lymphocytes in the mechanism of the GVHR (Fig. 2) and to the specific action of ARA-1 on activated CBA-anti-C57BL/6 T cells (Fig. 1) suggest that the targets for the action of ARA-1 during abolition of the GVHR is intact CBA-anti-C57BL/6 T lymphocytes. These results are in agreement with data in the literature showing that the GVHR can be abolished by means of ARA [2, 8, 12]. However, most workers have used ARA obtained in first generation hybrids and have used it to immunize these hybrids with parental cells or with alloantiserum. Clearly, this method of obtaining ARA has certain limitations. Kraskina [2] showed that lethal homologous diseases in (CBA × C57BL/6)F_1 mice can be prevented by heterologous ARA. However, that investigation lacked a sufficiently adequate control of specificity of action of the ARA: the effect of ARA on CBA-anti-C57BL/6 lymphocytes was compared with the action of ARA on C57BL/6-anti-CBA lymphocytes, and not on CBA lymphocytes reacting against targets with a different haplotype.

In a heterologous system we evidently succeeded in obtaining ARA with the power: a) to produce specific lysis in the cytotoxic test of T cells of definite idiotypical specificity, activated by transplantation antigen (in the absence of lysis of normal T and B lymphocytes, of activated T cells of other specificity, and of AFC producing antibodies against sheep's red blood cells); b) to specifically abolish a lethal GVHR.

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