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Prevention of lethal graft-versus-host disease by monoclonal antibody treatment in vivo*

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Graft-versus-host disease (GVHD) is a major complication of allogeneic bone marrow transplantation (BMT). The disease is caused by mature T cells in the graft that recognize foreign antigens of the host and subsequently elicit an immune response to host tissues [1]. Although T-cell depletion of the graft strongly reduced the incidence and severity of GVHD, the overall survival of allogeneic BMT did not increase because of the increased rate of graft rejection and leukemic relapses [2]. New prophylactic and therapeutic approaches have to be developed to improve the outcome of allogeneic BMT. T-cell-specific monoclonal antibodies (mAb) administered in vivo to the allograft recipients seem to be promising in the prevention and treatment of lethal GVHD [3–5]. In this study we especially addressed the effect of in vivo treatment of recipients with anti-T-cell subset mAb in a murine model for acute GVHD. We also determined the long-term effects.

Key words: Bone marrow transplantation – Graft-versus-host disease – Monoclonal antibodies

Materials and methods

Mice. (C57BL/Ka × CBA/Rij)F1 (H-$2^{b/q}$) and BALB/c (H-2^{d}) mice were bred at the Department of Immunology of the Erasmus University. The mice were 12–18 weeks old at the start of the experiments. Mice were kept 2 per cage with access to acidified water and pelleted food ad libitum.

Induction of GVHD. Lethally irradiated (10 Gy) (C57BL × C-BA)F1 recipients were intravenously (i.v.) injected with 10^7 BALB/c spleen cells, 24 h after irradiation. Mice were examined daily for the development of signs of GVHD. Control mice that were injected with 10^7 syngeneic spleen cells survived > 250 days.

Antibodies. Purified rat anti-mouse mAb, anti-Thy-1 (YTS 154.7), anti-CD4 (YTS 191.5), and anti-CD8 (YTS 169.4), all of the IgG2b subclass, were purchased from Sera-lab, Sussex, U.K. Treatment with mAb was given within 4 h after irradiation.

Chimerism. To determine the degree of chimerism, peripheral blood cell samples were stained with fluorescein isothiocyanate (FITC) conjugated mouse anti-mouse H-$2K^d$ mAb (clone SF1-1.1) and mouse anti-mouse H-$2K^b$ mAb (clone AF6-88.5), which were purchased from Pharmingen, San Diego, Calif. Subsequently, the samples were analyzed using a flow cytofluorometer (FACScan, Becton Dickinson, Mountain View, Calif.).

Data analysis. Differences between groups were analyzed using the Wilcoxon-Mann Whitney statistic. Values of $P < 0.05$ were considered significant.

Results and discussion

We compared the effectiveness of mAb treatment given either by intravenous (i.v.), intraperitoneal (i.p.), or subcutaneous (s.c.) injection. To be able to detect minimal differences between these three routes of administration, we employed a dose of either 25 μg or 50 μg anti-Thy-1, which is suboptimal according to previous experiments [5]. In all three groups the higher dose resulted in a better survival rate than the lower dose. The survival of s.c.-injected mice was slightly but not significantly decreased in comparison with the i.v.- and i.p.-treated groups. This indicates that treatment via all above mentioned routes of administration is equally effective.

We further investigated the effect of anti-CD4 and anti-CD8 treatment as compared with the effect of anti-Thy-1. Earlier experiments showed that both anti-Thy-1 and anti-CD4 treatment decreased the morbidity and mortality of GVHD. A dose of 100 μg anti-Thy-1 resulted in 100% survival, whereas a similar dose of anti-CD4 was less effective. Anti-CD8 treatment did not decrease the

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Table 1. Effect of anti-Thy-1, anti-CD4, and anti-CD8 monoclonal antibody treatment on the survival of mice after allogeneic spleen cell transplantation

Recipient strain	*n*	Donor	Treatment	Survival at day 30 (%)	Survival at day 60 (%)
Experiment 1					
(C57BL × CBA) F1	10	BALB/c	100 μg anti-Thy-1	100	100
(C57BL × CBA) F1	10	BALB/c	100 μg anti-CD4	60	30
(C57BL × CBA) F1	10	BALB/c	100 μg anti-CD8	0	0
(C57BL × CBA) F1	10	BALB/c	none	0	0
Experiment 2					
(C57BL × CBA) F1	8	BALB/c	100 μg anti-Thy-1	100	100
(C57BL × CBA) F1	8	BALB/c	200 μg anti-CD4	100	100
(C57BL × CBA) F1	8	BALB/c	200 μg anti-CD8	0	0
(C57BL × CBA) F1	6	BALB/c	none	16	16

(C57BL × CBA) F1 mice were lethally irradiated, treated with the indicated amount of mAb, and reconstituted with 10^7 BALB/c spleen cells. The percentage survival is given at days 30 and 60 after reconstitution

morbidity but appeared to postpone mortality [5]. This indicated a major role for $CD4^+$ T-cells, the role of $CD8^+$ T cells being less clear. To clarify the involvement of the $CD4^+$ and $CD8^+$ T-cell subset, we repeated the experiment with a dose of either 100 μg or 200 μg anti-CD4 and anti-CD8 mAb. The results are summarized in Table 1. Treatment with a dose of 100 μg of anti-Thy-1 or anti-CD4 improved the percentage survival, whereas treatment with a similar dose of anti-CD8 did not (experiment 1). Treatment with a double dose of anti-CD4 appeared to be as effective as treatment with 100 μg anti-Thy-1 and resulted in 100% survival on day 60. However, even a dose of 200 μg anti-CD8 did not increase the survival at all (experiment 2). This is in harmony with the effect of mAb treatment on the development of clinical symptoms of GVHD. Symptoms of severe acute GVHD developed simultaneously in both the anti-CD8 (200 μg)-treated and the untreated group but were absent in the anti-Thy-1- and anti-CD4 (200 μg)-treated group. These data are consistent with our earlier observation that purified $CD4^+$ T cells were able to induce a lethal GVH reaction, whereas purified $CD8^+$ T cells could not. The discrepancy in the effect of anti-CD8 mAb as compared with previously reported data [5] might be explained by the fact that in the previous experiments the GVH reaction developed more slowly. $CD8^+$ T cells might play a role in chronic GVHD in this strain combination.

To exclude the possibility that the observed differences were due to a variable capacity of the mAb to eliminate the respective T-cell population in vivo, we analyzed the spleens from two mice of each group (experiment 2) for the presence of T-cell subsets 7 days after allogeneic reconstitution. It appeared that anti-CD8 mAb were even more effective than anti-CD4 mAb in eliminating their target cell population in vivo. This means that the differences in effectiveness cannot be explained by a difference in the capacity to eliminate the respective target cells. Together, these data indicate a major role for the $CD4^+$ T-cell subset in the induction of acute lethal GVHD in this model.

We further determined the state of chimerism and tolerance of the mice that had become long-term stable chimeras. The total number of spleen cells appeared to be ±50% of normal. The percentages of B and T lymphocytes were within the normal range. Since >99% of the spleen cells as well as of the peripheral white blood cells reacted with the anti-H-$2K^d$ mAb and <1% with the anti-H-$2K^b$ mAb, the chimeric mice can be considered as complete chimeras. This was confirmed by the observation that spleen cells from these stable chimeras were able to induce a lethal GVH reaction in recipients syngeneic to the original host, but not in recipients syngeneic to the original donor. This also indicates that the state of tolerance in these long-term chimeras is not due to clonal deletion. Preliminary data suggest that the tolerance is maintained by a suppressive mechanism.

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