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Studies on the participation of different T cell subsets in rat liver allograft rejection

Comparison of liver with heart graft

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Abstract. In this study, we investigated which subsets of rat T cells (CD8 + vs. CD4 +) are involved in the rejection of liver allografts by the in vivo administration of monoclonal antibody (OX-8 or OX-38, and W3/25 MAb) into thymectomized recipient Lewis (RTI^l) rats prior to DA (RTI^a) liver transplantation. We also compared the results of allograft survival of liver and heart transplants under the same experimental conditions. In order to deplete either CD8 + T cells or CD4 + T cells from recipient animals, 0.4 ml of OX-8 (ascitic form) or a 0.8 ml cocktail of MAb W3/25 and OX-38 (0.4 ml each) was injected into thymectomized recipient rats, respectively. Untreated Lewis rats consistently rejected donor DA liver grafts between 9 and 11 days ($n = 7$, 9.8 days ± 1.1 days). In contrast, anti-CD8 MAb pretreatment extended the survival times of DA liver grafts for up to 40 days ($n = 5$, 26.8 days ± 8.4 days). Furthermore, survival of DA liver grafts was significantly prolonged in Lewis rats that had been pretreated with anti-CD4 MAb ($n = 7$, 35.6 days ± 17.9 days). Two out of seven recipient animals survived for more than 60 days. For heart transplantation, untreated Lewis rats rejected DA heart grafts between 6 and 8 days after operation ($n = 6$, 6.5 days ± 1.2 days). Anti-CD4 MAb treatment prolonged heart graft survival for more than 60 days in all cases ($n = 3$, > 60 days). However, there was virtually no effect of anti-CD8 MAb treatment on heart graft survival ($n = 4$, 7.0 days ± 0.9 days). These results suggested that when whole MHC disparity prevailed between donor and recipient, both subsets of T cells were required for the rejection of liver allografts and that class II reactive T cells predominantly mediated liver graft rejection. Furthermore, CD8 + T cells played a differential role in the rejection of rat liver and heart allograft.

Key words: Allograft rejection – CD4 + /CD8 + T cell – Rat liver graft

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It has been well established that allograft rejection is primarily mediated by T cells [11]. The relative role of CD4 + T cells and CD8 + T cells in allograft rejection depends partly upon the MHC disparity between donor and recipient [14] and partly upon whether the CD8 + T cells need help from the CD4 + T cells [1] but it is not yet fully determined. Experiments using either the adoptive transfer system or in vivo administration of MAb specific for T cell subsets show that CD4 + T cells play a central and essential role in mediating the allograft rejection while CD8 + T cells do not. If CD8 + T cells do play a role in graft rejection, it is a specialized one.

Liver allografts, in particular strain combinations of inbred rats and pigs, are not rejected but induce a state of donor specific transplantation tolerance [7]. Liver also has a potent regenerative capacity, and it has been shown that liver secretes an immunosuppressive moiety, "soluble class I MHC antigen" into the blood circulation [12]. These observations differ from those of other organ grafts such as heart and kidney, and tissue grafts such as skin and islet. It is, therefore, in our interest to study the participation of each T cell subset in liver allograft rejection. We also compared the results of allograft survival in liver and heart transplants.

Materials and methods

Rats. Inbred strains of male Lewis (RTI^l) and DA (RTI^a) rats weighing 160–260 g were purchased from CLEA Ltd., Japan.

Organ transplantation. Orthotopic liver transplantation was performed as we have described in an earlier study [8]. Reconstruction of the hepatic artery was not performed. No blood transfusion was administered. Autopsy was done on all rats after death, and the livers were subjected to microscopic examination. Heterotopic heart transplantation was performed in the right neck of the recipient animal according to the modified methods originally described by Heron [4]. Grafts were inspected and palpated daily, and rejection was defined by cessation of beating of the graft and confirmed histologically in all cases.

Thymectomy. Adult thymectomy of the recipient Lewis rats was performed 2 weeks prior to heart and liver transplantation according to the standard procedures [5].

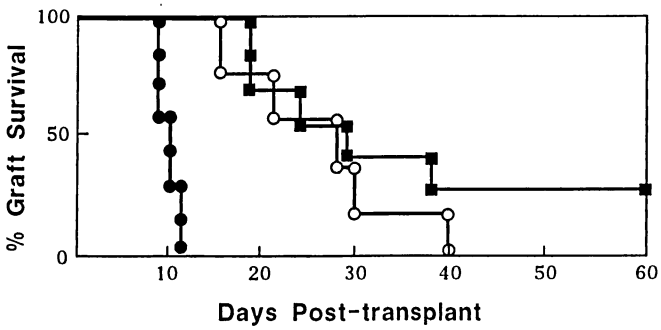

Fig. 1. Survival times of Lewis rats transplanted with DA liver grafts. Untreated Lewis rats (●), anti-CD4 MAb therapy and thymectomy (■), anti-CD8 MAb therapy and thymectomy (○)

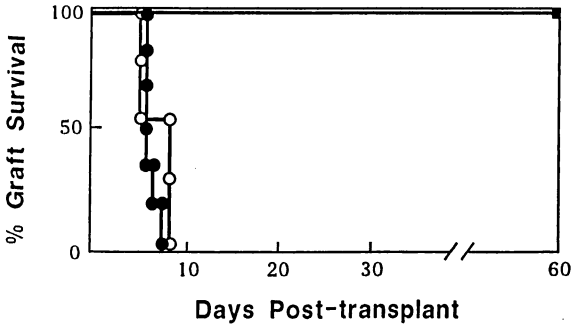

Fig. 2. Survival times of DA heart grafts in Lewis recipient rats. Untreated Lewis rats (●), anti-CD4 MAb therapy and thymectomy (■), anti-CD8 MAb therapy and thymectomy (○)

Flow cytometry (FACS) analysis. Peripheral blood obtained from the tail vein was diluted 1:3 in phosphate-buffered saline containing heparin, and the mononuclear cells were recovered from Ficoll/Hypaque gradient centrifugation. For a single color analysis, the cell suspensions were divided into two aliquots, one for staining with a saturating amount of monoclonal antibody and the other without primary antibody. After incubation on ice for 30 min, the cell suspension was washed and binding of the primary antibody was revealed by affinity-purified FITC-conjugated F(ab')2 rabbit anti-mouse Ig antibodies (heavy chain and light chain specific) purchased from Southern Biotechnology Association (Birmingham, Ala.). The latter exhibits some cross-reactivity with rat Ig, but this was minimized by passing the antibody through a Sepharose 4B (Pharmacia Fine Chemicals, Piscataway, N.J.) column cross-linked with rat IgG. For two-color analysis, phycoerythrin-tagged OX 19 (PF-OX19) and FITC-tagged OX 8 or OX-35 (FITC-OX8 or FITC-OX35) were used for direct staining. Using EPICS, 10^5 cells were analyzed.

Statistical analysis. The significance of differences in graft survival between control and treatment groups was assessed by the generalized Wilcoxon test.

Monoclonal antibodies. MAbs used in this study were W3/25, OX-35 and OX-38 for anti-CD4, and OX-8 for anti-CD8. These MAbs were generously provided to us by A. F. Williams and D. W. Mason (Oxford). PE-OX 19 (anti-CD5), FITC-OX 8 and FITC-OX 35 were obtained from Serotec (Kidlington, England).

Depletion of CD8 positive T cells and CD4 positive T cells in vivo. In order to deplete CD8 + T cells from recipient animals, 0.4 ml of OX-8 MAb (ascetic form) was dissolved in 2.0 ml saline and sterilized by passing through a membrane filter (0.45 μm pore size) and injected intravenously into thymectomized Lewis rats 3 days prior to organ transplantation. For the depletion of CD4 + T cells, 0.8 ml cocktail of MAbs W3/25 and OX-38 (0.4 ml each) was diluted 1:1 with saline and 1.6 ml of this was injected into recipient animals 3 days before organ transplantation.

Results

Survival times of DA liver and heart graft in Lewis recipient are shown in Figs. 1 and 2. We prepared donor liver grafts from DA rats and employed Lewis rats as recipients. Major rejection of the liver graft occurred in this combination, unlike the DA into PVG ($RT1^c$) combination where the liver grafts are often tolerated in the allogeneic recipients without immunosuppressive reagents. Indeed, untreated Lewis rats rejected donor DA liver grafts and died consistently between 9 and 11 days. Survival of DA liver grafts was significantly prolonged in anti-CD8 MAb treated rats with simultaneous thymectomy (26.8 days ± 8.4 days) ($P < 0.001$). The administration of anti-CD4 MAb to thymectomized Lewis recipients caused marked prolongation of DA liver allografts; two out of seven animals survived for more than 60 days. This effect was significantly better than that of anti-CD8 MAb treatment ($P < 0.05$).

Untreated Lewis rats rejected DA heart grafts between 6 and 8 days after operation ($n = 6$, 6.5 days ± 1.2 days). Anti-CD4 MAb treatment prolonged heart graft survival for up to 60 days in all cases ($n = 3$). However, there was virtually no effect of anti-CD8 MAb treatment on heart graft survival ($n = 4$, 7.0 days ± 0.9 days).

FACS analysis of peripheral blood from the recipient Lewis rats treated with anti-CD8 MAb revealed profound reduction of OX 19 + and OX-8 + T cells which are believed to contain class I restricted killer T cells (15.2% ± 2.3% before treatment and 0.2% ± 0.03% at 24 h after treatment). In contrast, elimination of CD4 + T cells by anti-CD4 MAb was incomplete (58.7% ± 4.42% before treatment and 24.4% ± 5.18% at 24 h after treatment).

Discussion

In vitro studies demonstrate that the CD4 + helper T cells are class II MHC reactive T cells, the CD8 + cytotoxic T cells are class I MHC reactive T cells, and naive class I reactive CD8 + T cells cannot be activated without help from activated CD4 + T cells in most strains. These studies indicate that CD4 + T cells may play a dominant role in allograft rejection, and suppression of CD4 + cell function by anti-CD4 MAb and/or matching for class II MHC may result in effective inhibition of rejection. On the other hand, in particular strains of mice such as B6 [10] and high responder rats such as Lewis and W/F [9], CD8 + T cells can be activated and can effect rejection independent of help from CD4 + cells. In these situations, anti-CD4 MAb therapy and/or matching for class II have limited use. Thus, the relative roles of CD4 + and CD8 + T cells in mediating allograft rejection are dependent upon whether CD8 + T cells are provided help by CD4 + T cells.

In our study, heart graft rejection was completely suppressed by anti-CD4 MAb therapy. All heart grafts sur-

vived over 60 days with no evidence of rejection. This result was consistent with the results of Herbert [3] and Ilano [6], who have demonstrated that CD4 + T cells play an essential role in cardiac rejection by using in vivo administration of OX-35 and/or OX-38 to the recipient of neonatal or vascularized heart grafts. Also in agreement with the results of Herbert and Ilano, the rejection of liver allografts in our study was markedly delayed by anti-CD4 MAb therapy. Thus, anti-CD4 MAb treatment was an effective regimen for the prolongation of heart and liver allograft survival, suggesting that CD4 + T cells participate predominantly in allograft (both heart and liver) rejection. In contrast, anti-CD8 MAb therapy had no effect on heart graft survival. All heart grafts were rejected in the same time period as the control group despite complete depletion of CD8 + T cells. This was, however, in sharp contrast to the results of liver allografts. The depletion of CD8 + T cells from the recipient caused marked prolongation of the liver graft survival.

In our study, the strain combination studied used DA rats as donors and high responder Lewis rats as recipients differing at class I and class II MHC and non-MHC loci. Thus, the differences in efficacy of anti-CD8 MAb could be attributed to graft factors alone. Why CD8 + T cells played a differential role in the rejection of liver and heart allograft was not clear. One explanation could be the difference in the susceptibility of the allografts to the immune response. That is, an RTI^a heart graft in a high responder RTI^l recipient may be principally rejected through an antibody-mediated pathway rather than a cell-mediated pathway. It has been shown that the CD4 + T cell alone is sufficient to reject a graft either independently or in collaboration with other cells. It can provide help for B cells to generate alloantibody against graft antigen. This is seen especially in high responder RTI^u recipients of $RTIA^a$ class I disparate kidney grafts. RTI^u recipients rejected class I disparate kidney grafts not by CD8 + T cytotoxic T cell but by alloantibody, and this alloantibody can be transferred to cyclosporin-treated RTI^u recipients to restore their ability to reject an $RTIA^a$ graft in an antigen specific manner [2].

The other difference could be in the immunogenicity of the organ graft. It has been shown that liver is richer in class I MHC antigen than other organs, and that liver secretes a soluble class I antigen into the blood circulation. The serum of Lewis (RTI^l) rats that had received DA (RTI^a) livers shows a high titer of $RTIA^a$ class I activity which includes not only soluble form (Mw: 38–40 Kd) class I antigen but an aggregated form or cell membrane fraction (Mw: > 200 Kd). The latter are believed to be the products of destruction of liver tissue by immune attack [13]. This class I activity, however, was not detected in the serum of Lewis rats of a DA heart (unpublished data). Therefore, it may well offer the hypothesis that these materials may stimulate CD8 + T cells and, subsequently, recruit them to participate in liver graft rejection.

In conclusion, survival of heart and liver allografts was significantly prolonged by the in vivo administration of anti-CD4 MAb to thymectomized recipients prior to organ transplantation. Profound and sustained elimination of CD8 + T cells by the combined therapy of MAb administration with thymectomy led to a marked prolongation of liver graft survival but did not affect the survival of heart allograft. These results suggest that CD4 + T cells played a central and essential role in liver and heart allograft rejection, and that CD8 + T cells also played an essential role in liver graft rejection, but not in heart allograft rejection. For the clinical application of monoclonal antibody, we should take into account organ specificity in selecting an effective MAb.

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