Only the CD62L+ subpopulation of CD4+CD25+ regulatory T cells protects against lethal acute GVHD

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Abstract

We previously reported that unmanipulated donor-derived CD4+CD25+ regulatory T cells suppress lethal acute graft-versus-host disease (aGVHD) after allogeneic bone marrow transplantation. In the model used for these studies, irradiated BALB/c (H-2d) recipients of C57BL/6 (H-2b) T cell-depleted bone marrow die within 28 days from aGVHD induced by an inoculum of 500,000 CD4+CD25- donor splenic T cells. The co-transfer of an equal number of CD4+CD25+ T cells prevents death and results in long-term survival (Hoffmann et al. J Exp Med 2002).

To address where the CD4+CD25+ T cells exert their protective function, CD4+CD25+/- T cells were sorted from Thy-congenic mice and their tissue distribution was analyzed by FACS and immunohistochemistry. Five days after transfer the progeny of CD4+CD25- T cells were highly activated as judged by FACS analysis of forward scatter profile and CD25 expression. They could be detected in liver and gut, as well as in secondary lymphoid tissues, irrespective of whether regulatory T cells had been co-transferred or not. Importantly, the progeny of CD4+CD25+ T cells could be found in the same locations, including the target organs liver and gut. CD4+CD25+ regulatory T cells are heterogeneous with regard to expression of the lymph node homing receptor L-selectin (CD62L). The CD62L+/- subpopulations of CD4+CD25+ T cells differ in their chemokine receptor expression pattern and chemokine-responsiveness suggesting that differential trafficking is the reason why only the CD4+CD25+CD62L+ regulatory T cells protect in an adoptive transfer model of diabetes (Szanya, Ermann et al. J Immunol 2002). We analyzed the CD62L+ and CD62L- subsets of CD4+CD25+ T cells in the aGVHD model. Both subpopulations expressed GITR and Foxp3 and both suppressed the allo-response of CD4+CD25- C57BL/6 T cells against BALB/c stimulators in vitro. This indicated that both subsets were T reg cells. However CD4+CD25+CD62L+ T cells but not the CD4+CD25+CD62L- subset prevented early death from aGVHD in vivo. CD4+CD25+CD62L- T cells transferred alone did not result in lethal aGVHD. We found that on day 5 after transfer of CD4+CD25+CD62L- T cells (together with CD4+CD25- T cells) significantly fewer regulatory T cells could be detected in the mesenteric lymph nodes and spleen than after co-transfer of the CD4+CD25+CD62L+ subset. The expansion of CD4+CD25- T cells correlated inversely with the presence of regulatory T cells at these sites. In summary, CD4+CD25+ regulatory T cells can be found in inflamed target tissues and protection from lethal acute GVHD appears to require their accumulation in secondary lymphoid organs where they inhibit the expansion of conventional alloreactive T cells.

Introduction

- Acute GVHD in mice can be effectively controlled by donor-derived CD4+CD25+ Treg cells. (1-3).
- While the allo-reactive response of CD25-CD4+ and CD8+ T cells is alleviated, a beneficial graft-versus-leukemia effect can still be observed (4) making donor-derived CD4+CD25+ Treg cells a very attractive target for clinical applications in humans.
- When tested for suppressor activity in vitro no significant differences in the suppressive capacity of CD4+CD25+ subpopulations defined by CD69, CD62L, CD38, CD103 were found. (5-7)

Studying CD4+CD25+CD62L+/- subpopulations in the NOD mouse (8) we recently reported that in vitro CD4+CD25+CD62L+ and CD4+CD25+CD62L- cells suppressed equally well, while in vivo only the CD62L+ subpopulation caused a significant delay of disease onset in an adoptive transfer model of diabetes into NOD.scid mice.

- Recent publications reported that naïve CD4+ T cells which are CD62L+CD45RAhi induced acute GVHD while CD62L-CD44hi memory T cells did not. (9-10)
- In this study we looked at the effect of CD62L+/- subpopulations of CD4+CD25+ T cells in GvHD.
- We also studied the role of the CD62L molecule in the induction of GVHD by CD4+ T cells in a C57BL/6 to BALB/C model of bone marrow transplantation.

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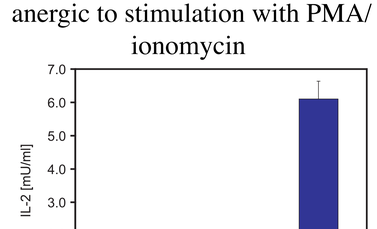
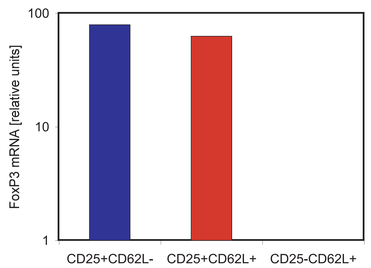
Experiments and Results

Both the CD62L+ and CD62L- subsets of CD4+CD25+ Tregs are suppressive in vitro

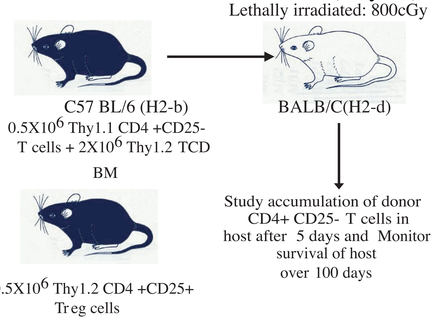


CD4+CD25+CD62L+ and CD4+CD25+CD62L- Tregs express comparable levels of FoxP3

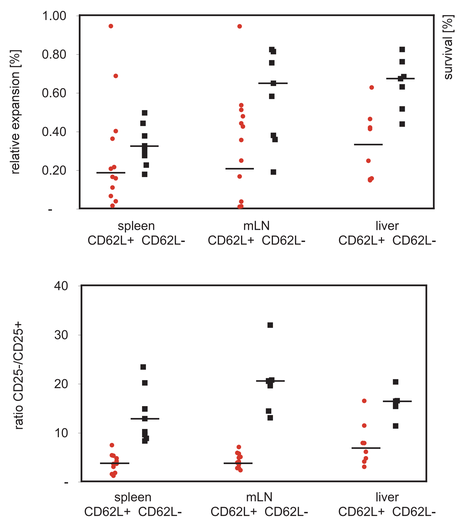


Both CD4+CD25+CD62L+ and CD4+CD25+ CD62L- cells are anergic to stimulation with PMA/ ionomycin

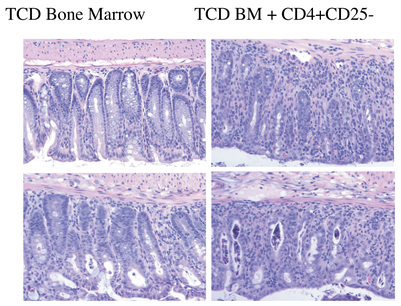
Experimental Setup



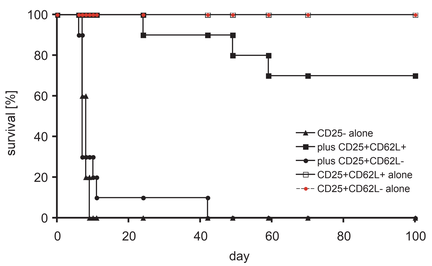
Expansion of CD4+CD25- T cells is reduced to a greater extent by CD4+CD25+CD62L+ than CD4+CD25+CD62L- Treg cells



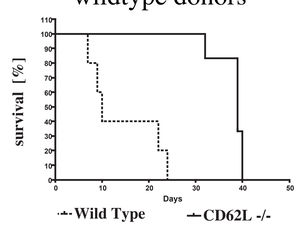
Tissue sections of large intestine of BALB/C hosts



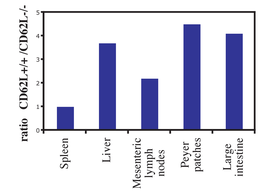
CD4+CD25+CD62L- Tregs do not protect from lethal acute GVHD



CD4+ T cells from CD62L-/- donors cause delayed death from GVHD compared to CD4+ T cells from wildtype donors



Reduced accumulation of CD4+ CD62L-/- donor T cells in lymph nodes and large intestine of hosts 6 days after transplantation



Conclusions

- Only the CD62L+ subpopulation of CD4+CD25+ T cells protected from lethal acute GVHD.
- CD4+CD25+CD62L+ T cells inhibited he expansion of donor CD4+CD25- T cells to a greater extend than CD4+CD25+CD62L- T cells. At the height of the expansion phase we consistently found a higher ratio of CD4+CD25+ to CD4+CD25- T cell progeny in lymphatic organs as well as GVHD target organs when CD4+CD25+CD62L+ T cells were transferred.
- In vitro both the CD62L+ and CD62L- subsets showed the characteristic features of CD4+CD25+ Treg cells. They suppressed the response of CD4+CD25- T cells in an allo-MLR to a similar extent. They were anergic to stimulation with PMA/Ionomycin and expressed comparable levels of GITR and Fox-P3.
- The absence of CD62L on donor cells lead to reduced accumulation of donor CD4+ T cells in the large intestine and prolonged host survival.