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

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Introduction

We previously reported that unmanipulated donor-derived CD4+CD25+ regulatory T cells suppress lethal acute graft-versus-host disease (GVHD) 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 after aGVHD induced by an inoculum of 500,000 CD4+CD25+ donor spleen 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-1 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.

The CD4+CD25+ regulatory T cells are heterogeneous with respect to expression of the lymph node homing receptor CD62L. The CD62L+/− subpopulations of CD4+CD25+ T cells differ in their chemokine receptor expression pattern and chemokine-receptor responsiveness suggesting that differential trafficking is the reason why only the CD4+CD25+CD62L+ regulatory T cells protect from aGVHD induced by CD4+CD25+ T cells (Stano, Ermann et al. J Immunol 2002). We analyzed the 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− T cells to CD4+CD25+ T cells in vitro. This indicated that both subsets were regulatory T cells. However CD4+CD25+CD62L− T cells but not the CD4+CD25+CD62L− subset prevented early death from aGVHD in vivo. CD4+CD25− 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 spleens 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 organs 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.

Conclusions

1. CD4+CD25+CD62L− T cell subsets of CD4+CD25+ Tregs do not protect from lethal acute GVHD

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

- Acute GVHD in mice can be effectively controlled by donor-derived CD4+CD25+ Treg cells (1-3).
- Although the allo-reactive response of CD4+CD25+ CD4+ and CD8+ T cells is alleviated, a beneficial graft-versus-leukemia effect can still be observed (4) making donor-derived CD4+CD25+ Tregs a very attractive target for clinical applications in humans.

Experimental Setup

- 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) This is in agreement with the described data that CD4+CD25+ regulatory T cells are heterogeneous with respect to expression of the lymph node homing receptor CD62L.

Experiments and Results

- Both the CD62L+ and CD62L− CD4+CD25+ Tregs express comparable levels of FoxP3.
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- 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+CD44lo are induced to become CD62L−CD44hi T cells after chronic stimulation in vitro. The CD4+CD25+ Treg cells which are CD62L+CD44lo were also shown not to suppress T cell proliferation in vitro when stimulated with PMA/Ionomycin and exogenous IL-2. They were anergic to stimulation with PMA/Ionomycin.

Conclusions

- Only the CD62L+ subpopulation of CD4+CD25+ T cells protected from lethal acute GVHD.
- CD4+CD25− T cells transferred alone did not result in lethal aGVHD. In vitro both the CD62L+ and CD62L− subpopulations of CD4+CD25+ T cells were suppressive in vitro.
- The absence of CD4+CD25− donor T cells lead to reduced accumulation of donor CD4+ T cells in the large intestine and prolonged host survival.