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Role of B7-H1 in Immune Privilege of the Eye

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Background

The normal eye possesses immunologic privilege. When grafted orthotopically to the eyes of experimental animals, allogeneic corneas enjoy a relatively high level of acceptance, compared with orthotopic grafts of other types of solid tissue. Even corneas grafted into eyes of preimmunized recipients are often not subject to immune rejection. To understand the molecular mechanisms of immune privilege of the eye, we studied the role of new B7 family co-stimulatory molecules in the maintenance of the immunosuppressive microenvironment of the eye. Programmed death 1 (PD-1) is a new member of the CD28/CTLA-4 family which has been implicated in the maintenance of peripheral tolerance. B7-H1 (PD-L1) and B7-DC (PD-L2), new members of the B7 family, have been identified as ligands for PD-1, but their expression and function in the eye remain largely unknown. The purpose of the present study was to determine whether the PD-1/PD-Ls pathway plays a role in the immune privilege of corneal allografts and, if so, to investigate its mechanism.

Methods

Normal corneas of C57BL/6 mice were transplanted orthotopically into the normal eyes of BALB/c mice. Recipients were given intraperitoneal injections of 0.2 mg of anti-PD-1, anti-B7-H1, anti-B7-DC monoclonal antibodies (mAbs) or control rat IgG 3 times a week for 8 weeks after grafting. Graft survival was assessed clinically and was compared. Expression of PD-1, B7-H1, and B7-DC in normal corneas and in allografts was assessed immunohistochemically with confocal microscopy. Terminal deoxyribonucleotidyl transferase-mediated deoxyuridine triphosphate nick-end labeling (TUNEL) staining was performed to detect cells undergoing apoptosis in corneal allografts. To further substantiate the B7-H1-mediated protection of corneal allografts from effector T cells, we evaluated the destruction of corneal endothelial cells (CECs) by alloreactive T cells in vitro. Fresh normal corneas from C57BL/6 eyes were incubated with anti-B7-H1 mAbs or control rat IgG for 2 hours. Magnetic cell sorting and separation was used to purify T cells from the spleen of BALB/c mice that had been presensitized by subcutaneous immunization with C57BL/6 spleen cells or with third-party (C3H/He) spleen cells, or from the spleen of naive BALB/c, C57BL/6, or C3H/He mice. The corneas pretreated with anti-B7-H1 mAbs or control rat IgG were incubated with 2.5×10^5 T cells. The unfixed corneal samples were incubated with 50 μg/mL of propidium iodide to stain nuclei of dead CECs. Cells stained with propidium iodide were counted at 3 randomly selected areas in the corneal endothelium of each corneal sample by means of confocal microscopy.

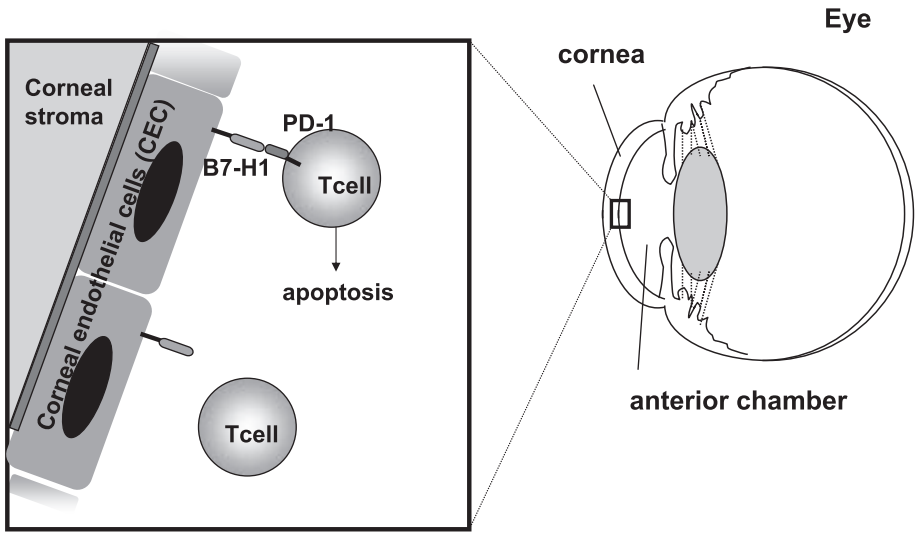

Fig. 1 Constitutive expression of B7-H1 on corneal endothelial cells (CECs) induces apoptosis of effector T cells via PD-1, and protects CEC from destruction by allo-reactive effector T cells.

Results

Allograft survival in the anti-PD-1 group (0%) and that in the anti-B7-H1 group (0%) were significantly less than that in the control group (50%) at 8 weeks. Differences in the allograft survival rate between the anti-B7-DC group and the control group were not statistically significant. B7-H1 was expressed on the endothelium of both normal corneas and allografts. PD-1 expression was found on inflammatory cells in the posterior surface of allografts. Inflammatory cell infiltration was found at 2 weeks in both control allografts and allografts treated with anti-B7-H1mAbs. TUNEL assay revealed that many of the infiltrating cells were undergoing apoptosis in B7-H1-positive control allografts at 2 weeks. These allografts showed little inflammation at 4 weeks and survived more than 8 weeks. On the other hand, allografts treated with anti-B7-H1mAbs were occupied by TUNEL-negative inflammatory cells, including PD-1-positive CD4 T cells, at 2 weeks. At 4 weeks, the majority of corneal cells, i.e., epithelial, stromal, and endothelial cells, and infiltrating cells in these allografts were undergoing apoptosis, and the tissue was destroyed. In an in vitro co-culture of cornea and T cells, the killing of CECs by alloreactive T cells was significantly enhanced in the corneas pretreated with anti-B7-H1mAbs as compared with those pretreated with control IgG (p=0.0016). Interestingly, B7-H1-mediated protection was also observed after incubation with third-party-reactive T cells (p=0.0248). Moreover, the B7-H1-mediated protection was still observed even after incubation with naive allogeneic T cells (p=0.0051). In contrast, no significant difference was observed between corneas treated with anti-B7-H1 mAbs or the control rat IgG when syngeneic C57BL/6 T cells were used as the effector.

Conclusions

Constitutive expression of B7-H1 on CECs induces apoptosis of effector T cells via PD-1 and plays a substantial role in the protection of CECs from destruction by alloreactive effector T cells (**Fig. 1**). B7-H1 protects the cornea from bystander injury by activated T cells. Moreover, constitutive expression of B7-H1 on CECs may inhibit peripheral sensitization, since naive T cells were sensitized by B7-H1-blocked allogeneic CECs to promote their injury. These results indicate that the PD-1/B7-H1 pathway plays a role in the peripheral deletion of alloreactive effector T cells within the cornea to maintain the immune privilege of corneal allografts.