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TLR agonists abrogate costimulation blockade-induced mixed chimerism and transplantation tolerance

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Abstract

We investigated the mechanisms by which toll-like receptor (TLR) agonists affect the induction of mixed chimerism and skin allograft survival in mice treated with costimulation blockade (CB).

We report that TLR agonists prevent the generation of mixed chimerism by breaking tolerance in the alloreactive CD4⁺ and CD8⁺ T cell compartments, and that type I interferon (IFN) is important in this process. Understanding how environmental perturbations affect CB-induced transplantation tolerance may lead to more effective regimens that can be used as an approach for the treatment of type 1 diabetes, for which the transplantation of pancreatic islets is a promising therapy.

Keywords

costimulation blockade; Toll-like receptors; hematopoietic chimerism; tolerance

Introduction

Allograft transplantation has proven to be a successful therapeutic modality for patients with a variety of disease states including liver failure, renal failure, and diabetes mellitus. Unfortunately, current transplantation protocols require the chronic administration of immunosuppressive drugs, which carry the additional risks of infection and neoplasia. Therefore, transplantation protocols that permit the survival of a functioning allograft in the absence of chronic immunosuppression are needed. The use of bone marrow (BM) transplantation to achieve hematopoietic chimerism has emerged as one of the most promising strategies to induce transplantation tolerance to a variety of allografts. To establish mixed hematopoietic chimerism and achieve allograft tolerance, our group has used a conditioning regimen comprised of a donor specific transfusion (DST) and anti-CD154 mAb. This protocol leads to stable mixed chimerism and the prolonged survival of donor-specific organ transplants [1]. However, our lab has shown that tolerance induction to solid-organ allografts using protocols based on CB can be prevented if the recipient is exposed to pathogenic perturbations, such as viral infection or exposure to TLR agonists [2]. In this study, we investigated the effects of TLR agonists on the induction of mixed chimerism and the development of transplantation tolerance.

Materials and Methods

Mice were given BM and skin graft transplants as previously described [3]. TLR agonists were administered on the first day of CB [2]. Anti-CD4 mAb (clone GK1.5, 0.5mg) or anti-CD8 mAb (clone 2.43, 0.5mg) was injected i.p. on day -10, -9, and -8, and anti-NK1.1mAb (clone PK136, 0.025mg) was injected on day -10, relative to transplantation on day 0.

Results and Discussion

We began our investigation by testing the hypothesis that TLR agonists given during tolerance induction would preclude the engraftment of allogeneic BM. To examine this, we treated mice with our conditioning regimen and gave them allogeneic BM and skin grafts with or without the TLR4 agonist LPS, or the TLR3 agonist poly I:C. Unlike mice treated with CB only, mice treated with CB plus LPS or poly I:C universally failed to exhibit hematopoietic mixed chimerism 8 weeks after transplantation, and displayed greatly shortened skin graft survival.

We next investigated the cellular mechanisms that control LPS-mediated abrogation of mixed chimerism by examining the role of host natural killer (NK), CD8⁺ and CD4⁺ T cells. We independently depleted each of these populations using monoclonal antibodies prior to the commencement of CB. We observed that allogeneic BM and skin grafts were readily accepted by CB-treated animals depleted of NK and/or CD8⁺ cells. However, mixed chimerism and skin graft tolerance were not achieved in CB-treated mice exposed to LPS and depleted of NK cells, CD8⁺ cells, or both. Mice depleted of CD4⁺ cells and treated with CB did not become chimeric, consistent with previously published data [3]. However, we did observe mixed chimerism and permanent skin graft tolerance in animals depleted of both CD4⁺ and CD8⁺ cells, even when exposed to LPS. These data indicate that administration of LPS breaks tolerance in both the CD4 and CD8 compartments.

We next investigated the molecular mechanisms that control LPS-mediated rejection of allogeneic BM by testing the hypothesis that TLR4 is required on host cells in order for LPS to mediate its effects. To test this, we transplanted TLR4 knockout mice with allogeneic BM and skin and treated them with CB with or without LPS. We observed that mice treated with CB and LPS became chimeric with allogeneic BM at a frequency similar to mice not treated with LPS. Both groups exhibited similar survival of allogeneic skin grafts.

Previously, we have shown that type I IFN is important for the TLR-mediated abrogation of tolerance to allogeneic skin grafts [4]. Therefore, we hypothesized that type I IFN would also be critical for LPS to break tolerance to allogeneic BM. To test this hypothesis, we treated mice that were deficient in the type I IFN receptor (IFNAR1) with CB with or without LPS, and transplanted them with allogeneic BM and skin grafts. We observed that in contrast to wild-type mice, IFNAR1 knockout mice became chimeric when treated with CB and given allogeneic BM in the presence of LPS.

We hypothesize that LPS breaks CB-induced tolerance to allogeneic BM by signaling through host TLR4 and upregulating type I IFN. This, we predict, results in the maturation of alloantigen-presenting APCs despite the presence of anti-CD154 mAb. Consequently, we postulate that these matured APCs present alloantigen to alloreactive T cells leading to activation rather than tolerance. Understanding how pathogens affect CB-induced transplantation may yield improved transplantation regimens that can be used as a treatment for type 1 diabetes.

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