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Cellular mechanisms of alloimmune non-responsiveness in tolerant mixed lymphocyte chimeras induced by vascularized bone marrow transplants

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Abstract It has been demonstrated that the development of stable mixed lymphocyte chimerism is associated with alloimmune tolerance induction in vascularized bone marrow transplant (VBMT) recipients. The underlying mechanisms of immune non-responsiveness in tolerant VBMT chimeras remains unclear. Our VBMT model involves the transplantation of a parental donor limb (Lewis rats) onto a hybrid (Lewis × Brown Norway) F_1 recipient. Tolerogenic mechanisms and cellular immune regulation to self and host allodeterminants were investigated during the early post-transplant phase of tolerance induction. Flow cytometric analysis of sIg^+-depleted experimental peripheral blood lymphocytes from tolerant VBMT recipients demonstrated low level stable mixed immune chimerism. Chimeric cells tested for responsiveness against self-LEW determinants showed activated proliferation and immune dysregulation 30 days post-transplantation. However, direct immunocytolytic activity against LEW determinants was not found. Tolerant chimeras also demonstrated elevated cellular proliferation and cytolytic responses against host-specific BN allodeterminants at 30 days. Consistent with these in vitro findings, limited clinical signs compatible with GVH reactivity were evident in vivo at this time. Following this initial period, the tolerant VBMT animals returned to normal clinical condition and remained otherwise healthy throughout the study. Consistent with these results, VBMT chimeras then showed declining proliferative responses from the elevated values seen at 30 days against self-LEW determinants. Proliferative and immunocytolytic responses also decreased against host-specific BN allodeterminants from peak levels at 30 days. In conclusion, these results provide evidence that the initial phases of tolerance induction in VBMT chimeras consist of self- and alloimmune regulation that follow an early period of immune dysregulation. Sequential phases of immune dysregulation and re-regulation elucidated in VBMT stable mixed chimeras within the first 100-day period may represent important mechanisms of tolerance induction.

Key words Tolerance · Chimerism
Composite tissue allografts
Limb transplantation

Introduction

It has been demonstrated that the development of stable mixed lymphocyte chimerism is associated with alloimmune tolerance induction in vascularized bone marrow transplant (VBMT) recipients and other models [1–7]. The underlying mechanisms of immune non-responsiveness in the tolerant VBMT chimeras remain unclear. The VBMT model is provided by transplantation of composite tissue allografts [1–6]. The VBMT/CTA model that we have used involves hind limb transplantation from parenteral LEW onto hybrid LBN F_1 rat recipients with reattachment of neurovascular pedicle. Long-term follow-up studies of indefinitely surviving tolerant VBMT animals has been previously reported [5, 6]. The development of T-cell chimerism and antigen-specific tolerance to host allodeterminants were found at more than 100 days. In this preliminary report tolerogenic mechanisms and cellular immune regulation to self- and host allodeterminants were investigated during the early post-transplant phase of tolerance induction. T-cell chimerism and in vitro cellular immune responsiveness were analysed in long-term surviving VBMT chimeras known to be freee of graft versus host disease (GVHD).

Materials and methods

The VBMT model utilized in these studies involved the transplantation of a parental donor limb (LEW rat) onto a hybrid (Lewis & Brown Norway, LBN) F_1 recipient [1–6]. Inbred LBN rats were obtained from Harlan Sprague Dawley (Indianapolis, Ind.), and LEW and BN rats from Charles River (Wilmington, Mass.) All animals were assessed clinically, histopathologically and immunologically for GVHD and tolerance. Long-term surviving tolerant VBMT recipients ($n = 15$) were the subjects of the present investigation. Cellular immune regulation to self and host determinants during the early phase of tolerance induction was studied.

Flow cytometric analysis of T-cell chimerism was done as follows [5, 6]. Ig^+ cells were depleted by anti-Ig-coated magnetic beads. Cells were incubated with a polyclonal LEW anti-BN alloantibody. Negative controls were incubated with normal LEW serum. FITC-conjugated affinity-purified rabbit anti-rat IgG was used for staining. Experimental labelling was determined by regression analysis of standard curves employing varying donor/host cell populations and inverse prediction [5, 6]. Cellular alloimmune responses were studied by mixed lymphocyte culture and direct immunocytolysis. Responder cells used included normal LBN or VBMT chimeric populations. Target and stimulatory cells included parental LEW and BN strains. Stimulator cells were irradiated with 1200 rad prior to coculture. Target cells in direct immunocytolysis assays were prepared with 450 μCi of ^{51}Cr per 10^7 cells for 4 h. Effector to target ratios of 100:1 were used in 4-h assays. Self-immune regulation in tolerant VBMT recipients, as determined by immunocytolytic and mixed lymphocyte responses, were expressed as percent of normal LBN anti-LEW and LBN anti-BN reactions following subtraction of appropriate background controls. VBMT responses at T_0 were represented by normal LBN anti-LEW and LBN anti-BN reactions and were set at 100% response.

Results

As reported previously, the majority (60–70%) of VBMT recipients consistently fail to express GVHD and develop tolerance [5, 6]. During the early post-transplant period (100 days) tolerant VBMT recipients demonstrated stable low-level mixed donor T-cell chimerism in the peripheral immune compartment (Fig. 1). Although tolerant VBMT recipients did not demonstrate GVHD during the early post-transplant period, they did show limited subclinical and reversible symptoms compatible with a GVH reaction and immune dysregulation. This was evident at approximately 30 days. However, the tolerant chimeras returned to normal and remained otherwise healthy throughout the study.

During this period, tolerant VBMT recipients were studied for self-immune regulation as determined by immunocytolytic and mixed lymphocyte responses to parental self-LEW and host-BN allodeterminants. Chimeric cells tested for responsiveness against self-LEW determinants, showed activated proliferation and immune dysregulation 30 days post-transplantation (T_{30}) (Fig. 2). However, direct immunocytolytic activity against self-determinants (LEW) was not found at T_{30} (Fig. 2). Similarly, tolerant chimeras demonstrated elevated cellular proliferation against host BN determinants at T_{30} (Fig. 3). However, in contrast to previous results, VBMT chimeras also showed elevated cytotoxic responses against host-specific BN determinants. The levels of BN-specific immune proliferation and immunocytolysis were not significantly different from those

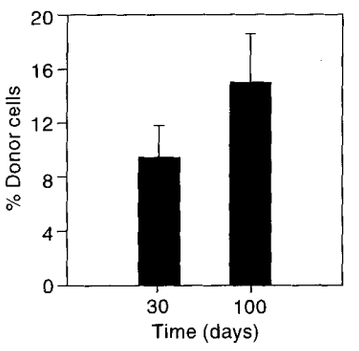

Fig. 1 Stable mixed allogeneic T-cell chimerism in tolerant VBMT chimeras. Chimerism was determined by flow cytometry, regression analysis and inverse prediction

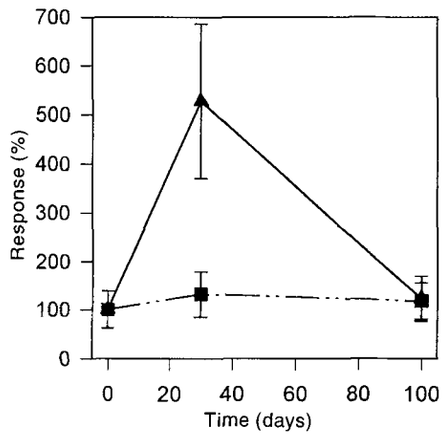

Fig. 2 Cellular immune regulation to self-LEW determinants in tolerant VBMT chimeras. Self-LEW (stimulator) antigen activated mixed lymphocyte responses were determined over time with immunocytes from tolerant VBMT recipients (responder). Self-LEW antigen (target)-activated immunocytolytic responses, were determined over time with effector/target immunocytes from tolerant VBMT recipients (effector) at an effector/target ratio of 100:1 (▲ Immune proliferation, ■ direct immunocytolysis)

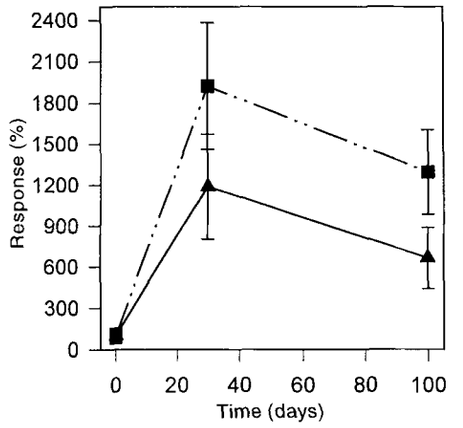

Fig. 3 Cellular immune regulation to host-BN allodeterminants in tolerant VBMT chimeras. Allo-BN (stimulator) antigen-activated mixed lymphocyte responses were determined over time with immunocytes from tolerant VBMT recipients (responder). Allo-BN antigen (target)-activated immunocytolytic respons were determined over time with immunocytes from tolerant VBMT recipients (effector) at an effector/target ratio of 100:1 (▲ Alloimmune proliferation, ■ direct immunocytolysis)

obtained with normal responder LEW cells (data not shown). VBMT chimeras then showed declining proliferative responses and apparent self-immune re-regulation from the elevated values seen at T_{30} against LEW determinants (Fig. 2). Recipients also demonstrated declining proliferative and immunocytolytic responses from the increased levels seen at T_{30} against host-specific BN allodeterminants (Fig. 3). These results provide evidence that the chimeras were undergoing alloimmune regulation and tolerance induction during this early period following an early phase of immune dysregulation. Both immunocytolytic and in vitro alloimmune responses eventually demonstrated antigen-specific long-term immune non-responsiveness [5, 6]. However, at T_{100} the process had not yet been completed and responses still remained elevated compared with controls.

Discussion

Vascularized bone marrow transplantation presents several unique and distinguishing features in comparison with conventional approaches. Through surgical intervention VBMT provides immediate marrow engraftment along with complete transfer of the donor syngeneic microenvironment of stromal cells and extracellular matrix. Thus, VBMT may facilitate tolerizing mechanisms and engraftment. The haemopoietic potential of the VBMT model has been compared favourably with conventional marrow transplantation [8].

In the present investigation, tolerogenic mechanisms and cellular immune regulation to self and host determinants were investigated during the early post-transplant phase of tolerance induction. Self- and alloimmune dysregulation was observed in tolerant VBMT recipients during this period of tolerance induction. Results from the VBMT anti-LEW experiments showed that immune dysregulation was confined to proliferative responses as opposed to cytolytic effector function. This suggested two different mechanisms that may be operative. The first possibility is that self-immune activation in VBMT chimeras may be restricted to $CD4^+$ Th cell populations since these cells are primarily responsible for proliferation in mixed culture. The second possible mechanism involves a phasic maturation process of self- and alloimmune tolerance induction. In this model both host LBN and donor LEW cells may undergo new self-tolerance induction with inactivation of certain forbidden clones in the new chimeric environment. The cells responsible for antigen-specific non-responsiveness could be either host or donor suppressor cells, but most probably represent donor and host cytolytic veto cells reactive against forbidden self- and alloreactive idiotypes. Upon introduction of a new set of naive LEW cells into the culture, including the eliminated clones, $CD4^+$ Th cells would be activated. However, $CD8^+$ and other cytolytic effector cells would remain undetectable in direct cytotox-

icity assays because they would have already undergone clonal expansion and contraction in vivo and would be unavailable for immediate reactivity in vitro. Similar mechanisms would be operative with respect to alloimmune tolerance induction with LEW anti-LBN clones, but many more clones would be involved in this immunoregulatory process. The duration of this process would be much longer so proliferating $CD4^+$, activated $CD8^+$ and other cytolytic effector cells would be immediately identifiable on assay. This model is consistent with the results. Both proliferating and effector populations were significantly elevated against BN determinants at T_{30} with a down-regulation thereafter to T_{100}. These phasic mechanisms of alloimmune suppression in tolerant VBMT recipients developed in synchrony with a stable but growing chimeric environment.

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