Anti-CD45RB antibody deters xenograft rejection by modulating T cell priming and homing

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Abstract

Pancreatic islet xenotransplantation has been advocated as a way of overcoming the shortage of human donor tissue for the treatment of type 1 diabetes. However, the potent immune response against xenografts is a major barrier to their use. We show that a short course of the anti-CD45RB antibody, MB23G2, prolongs survival of fetal pig pancreas grafts in mice. To investigate this effect further we used an i.p. xenograft model in which both donor pig cells and host inflammatory cells can be expediently recovered and analyzed. Graft prolongation was associated with reduced T cell and macrophage infiltration, and reduced production of both Th1 and Th2 cytokines at the graft site. Graft survival was further increased and T cell infiltration further reduced by combining anti-CD45RB antibody with co-stimulation blockade. The primary effect of anti-CD45RB antibody may be on CD4 T cells, in keeping with the marked reduction in T cell cytokine production in both spleen and graft sites. This concurs with previous studies in allogeneic models that indicate that this antibody perturbs T cell responses by modifying signaling via the TCR. In addition, anti-CD45RB treatment led to reduced expression of LFA-1 and CD62 ligand (CD62L) on CD4 T cells, independent of antigenic challenge. LFA-1 may enhance co-stimulation, and both LFA-1 and CD62L are involved in T cell trafficking. Their reduced expression provides an explanation why the T cell pool is reduced in lymph nodes. We conclude that modulation of inflammation against xenografts by anti-CD45RB antibody is due to effects on both T cell priming and trafficking.

Introduction

Pancreas transplantation offers a cure for type 1 diabetes. Successful transplantation restores normoglycemia, eliminating the need for daily insulin injections, and reducing diabetes side effects such as nephropathy and neuropathy (1,2). Due to a shortage of human donor pancreas, there is considerable interest in alternative sources of pancreatic tissue including xenogeneic fetal pig pancreas (FPP). Hyperacute and acute vascular rejection pose a formidable barrier to xenotransplantation of organs that utilize blood vessels of donor origin (3). However, xenografts, such as those of FPP, which are neovascularized by the graft recipient are not subject to such rejection, but are rejected by T cell-dependent cellular mechanisms (3,4).

CD45 is a protein tyrosine phosphatase expressed on all nucleated hematopoietic cells, which plays an important role in regulating immune responses. By dephosphorylating Src-
family kinases, CD45 promotes antigen-specific B and T cell responses (5). Negative regulation of CD45 is facilitated by its homodimerization, and in the absence of this interaction mice are subject to lymphoproliferative and spontaneous autoimmune disease (6). CD45 plays an additional role in regulating selectin expression (7,8) and integrin function (9,10). Recently, CD45 has also been shown to negatively regulate cytokine receptor mediated signaling via JAK-family kinases, thus revealing a role for CD45 in multiple hematopoietic lineages (11). Multiple isoforms of CD45 are generated by alternative splicing of exons 4, 5 and 6 (also known as A, B and C) which encode part of the extracellular region of the molecule. However, their functional relevance is unclear. T cell activation results in a shift from high to low mol. wt isoforms, which can be detected by decreased intensity of staining with anti-CD45RB antibodies. The CD45RB\textsuperscript{low} phenotype has variously been detected by decreased intensity of staining with anti-CD45RB antibodies. The CD45RB\textsuperscript{low} phenotype has variously been associated with primed/memory T cells, increased secretion of T\textsubscript{h}2 cytokines and a population of T cells with regulatory function (12–15).

Several lines of investigation indicate that anti-CD45 antibody can prevent allogeneic graft rejection. Such antibodies are a major component of polyclonal anti-lymphocyte/anti-thymocyte globulin preparations (16) which are used clinically to reduce the incidence and severity of early rejection. Monoclonal anti-CD45 antibody has also shown promise in pre-clinical trials (17). In mice, a short course of the mAb, MB23G2, specific for the CD45RB isoform expressed on B and T cells, induces long-term donor specific tolerance in a proportion of recipients of allogeneic grafts (18–20). While the mechanism of allograft prolongation induced by anti-CD45RB antibody treatment has not been clearly defined, it is apparent that perturbations of T cell responses are involved. Increased tyrosine phosphorylation of phospholipase C\textsubscript{yt1}, increased production of T\textsubscript{h}2 cytokines, decreased expression of CD45RB and increased expression of the CTLA-4-regulatory molecule on CD4 T cells have been reported following treatment with the anti-CD45RB antibody, MB23G2 (18–21).

It is generally believed that xenograft rejection is more potent than allograft rejection. Nevertheless, a common feature of the cellular responses to both allog- and xenografts is dependence on T cells, in particular CD4 T cells (4,22,23). Thus, we were interested in testing the potential of MB23G2 anti-CD45RB antibody to inhibit the cellular xenoreactivity to avascular pig tissue. The efficacy of MB23G2 was tested in two models: FPP transplantation under the kidney capsule and transplantation of a pig kidney cell line PK15 into the peritoneum. The former model is commonly used as a surrogate for FPP transplantation into humans. The latter model facilitates recovery by peritoneal lavage of both graft and graft-infiltrating cells, enabling simple quantitation and functional analysis (24).

Methods

Animals

Mice were bred and maintained at the Walter and Eliza Hall Institute for Medical Research and used at 6–10 weeks of age. Wild-type mice were either BALB/c or B6.C-H-2\textsuperscript{bm1} (termed bm1, C57BL/6 with the bm1 mutation of H-2K\textsuperscript{b}). The production of Th2 cytokines, decreased expression of antibody treatment has not been clearly defined, it is apparent that CD45RBlow phenotype has variously been detected by decreased intensity of staining with anti-CD45RB antibodies. The CD45RBlow phenotype has variously been associated with primed/memory T cells, increased secretion of T\textsubscript{h}2 cytokines and a population of T cells with regulatory function (12–15).

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generally was ~5 ml. Reported cell recoveries were adjusted to reflect the total in 5.5 ml.

Flow cytometry

Single-cell suspensions of spleen or lavage cells on ice in PBS containing 1% BSA and 0.05% sodium azide were blocked with 2.4G2 anti-Fcγ receptor antibody, and then stained. The mAb used for staining were directly conjugated to fluorochromes or biotinylated and detected using streptavidin–phycoerythrin (Caltag). Cells were analyzed on a FACScan (Becton Dickinson, Mountain View, CA) with CellQuest software.

Cytospins

Peritoneal cells were spun through an FCS cushion in a cytospin onto glass slides and stained with DIFF-Quik (Lab Aids, Narrabeen, NSW, Australia). Between 500 and 800 cells/slide were scored morphologically.

Assessment of cytokine production ex vivo

Splenic CD4 T cells positively selected by MACS (~90% CD4+ by flow cytometry) or unfractionated peritoneal cells were cultured at 3 x 10^6/well in 1.5 ml of RPMI supplemented with 10% FCS in 24-well plates at 37°C/5% CO₂ without antigen. Supernatants were recovered on day 3 and assayed for the presence of IL-4, IL-5, IL-10 or IFN-γ by ELISA. Recombinant cytokines used as standards, coating antibody and biotinylated antibody for detection were obtained from PharMingen, and used according to the manufacturer’s instructions.

Results

Anti-CD45RB delays inflammation and prolongs xenograft survival

Anti-CD45RB-treated BALB/c mice were grafted with FPP, and graft survival and inflammation assessed histologically. Two features were observed: infiltration of the graft was delayed and graft survival was prolonged by anti-CD45RB treatment (Table 1). Grafts were well defined with minimal infiltration in both groups at day 4 post-transplant. However, in mice treated with control antibody, all grafts (six of six) were completely destroyed by 7 days post-transplant, at which time a dense infiltrate was observed at the graft site (Table 1 and Fig. 1A). The infiltrate consisted of lymphocytes, macrophages and eosinophils. By contrast, graft tissue was identified in four of four anti-CD45RB-treated mice at day 7 post-transplant (Table 1 and Fig. 1B). Delayed rejection was also indicated by reduced infiltration of grafts from the anti-CD45RB-treated mice (Table 1 and Fig. 1B). Insulin-positive graft tissue persisted in five of six anti-CD45RB-treated mice at day 14 post-transplant (Table 1); organized clusters of cells indicative

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Table 1. Anti-CD45RB treatment delays inflammation and prolongs FPP xenograft survival

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Morphological analysis of the PK15 xenograft site shows increased graft survival and decreased inflammation. Cytospins were prepared from peritoneal lavage cells of individual BALB/c mice 5 days post i.p. transplant with 10^7 PK15 cells and stained with Diff-Quik prior to scoring of 500–800 cells/mouse. (A) Total cells (leukocytes + PK15) and leukocyte subsets (mφ = macrophage/monocyte, Ly = lymphocyte, Poly = eosinophils, basophils and neutrophils) and (B) PK15 cells at the graft site (ND = none detected in isotype control-treated mice). Graphs show recovery/mouse as the mean ± SD for four individual host mice which were PK15 grafted and treated with either isotype control (hatched bars) or anti-CD45RB (filled bars) antibody. Dashed lines indicate resident cell number in ungrafted, untreated mice (mean of two mice). (C) Cytospin from a PK15-grafted, anti-CD45RB antibody-treated mouse showing representative cell morphologies.

Fig. 2. Morphological analysis of the PK15 xenograft site shows increased graft survival and decreased inflammation. Cytospins were prepared from peritoneal lavage cells of individual BALB/c mice 5 days post i.p. transplant with 10^7 PK15 cells and stained with Diff-Quik prior to scoring of 500–800 cells/mouse. (A) Total cells (leukocytes + PK15) and leukocyte subsets (mφ = macrophage/monocyte, Ly = lymphocyte, Poly = eosinophils, basophils and neutrophils) and (B) PK15 cells at the graft site (ND = none detected in isotype control-treated mice). Graphs show recovery/mouse as the mean ± SD for four individual host mice which were PK15 grafted and treated with either isotype control (hatched bars) or anti-CD45RB (filled bars) antibody. Dashed lines indicate resident cell number in ungrafted, untreated mice (mean of two mice). (C) Cytospin from a PK15-grafted, anti-CD45RB antibody-treated mouse showing representative cell morphologies.

To confirm that anti-CD45RB antibody reduces cell infiltration, we used an i.p. xenograft model (24). Assessment of histological sections in the FPP model, although useful, is at best semi-quantitative. The advantage of the i.p. model is that the graft and graft-infiltrating cells can be recovered by peritoneal lavage, enabling both quantitative and functional analysis. Cells of the pig kidney cell line PK15 were grafted i.p. into BALB/c mice treated with anti-CD45RB or control antibody. Lavages were performed on day 5 post-transplant, corresponding to the peak of T cell infiltration (24). The effect of anti-CD45RB treatment mirrored that observed for FPP grafts under the kidney capsule, i.e. graft survival was prolonged and the inflammatory response was reduced. Indeed, the number of lavage cells was reduced from 16.7 to 4.4 × 10^6 (e.g. Fig. 2A, 3.3 ± 0.7-fold reduction, n = 8 experiments). Notably, the number of lavage cells from anti-CD45RB-treated, PK15-grafted mice was no different from that of normal mice (Fig. 2A). PK15-grafted cells were identified morphologically in cytospins (Fig. 2C) and by their mouse class I, high SSC profile in FACSs (Fig. 3A, upper panels). Anti-CD45RB treatment protected PK15 xenograft cells as shown by the increased number of grafted cells recovered (Figs 2B and 3C).

Morphological assessment of cytospins showed that the inflammatory response in the i.p. PK15 challenge model also resembled that of FPP grafts, with macrophages, lymphocytes and polymorphs identified at the graft site (Fig. 2A and C). Anti-CD45RB treatment led to a dramatic reduction in the number of macrophages/monocytes in the lavage (7.5 ± 5.2-fold reduction, n = 3 experiments, e.g. Fig. 2A). Lymphocyte numbers were also reduced, although less dramatically than for macrophages/monocytes (2.2 ± 1.2-fold reduction, n = 3 experiments, e.g. Fig. 2A). Both eosinophils and neutrophils were present in lavages (Fig. 2C), but their numbers were not reproducibly reduced by anti-CD45RB treatment.

Flow cytometry of the lavage confirmed the findings of the cytospin histology (Fig. 3). Anti-CD45RB treatment reduced infiltration by CD4 (2.6 ± 0.2-fold reduction, n = 4 experiments e.g. Fig. 3A and B) and CD8 (4.9 ± 1.6-fold reduction, n = 2 experiments, e.g. Fig. 3A and B) lymphocytes. However, the most dramatic effect of the anti-CD45RB treatment was the marked reduction in macrophages/monocytes (7.8 ± 3.9-fold reduction, n = 3 experiments, e.g. Fig. 3B), as determined by F4/80 staining (Fig. 3A).

Anti-CD45RB reduces cytokine production at the graft site

A preferential increase in IL-4 and IL-10 (as determined by RT-PCR) occurs in allografts from anti-CD45RB-treated mice (18, 20). By contrast, we have not found any evidence of cytokine skewing in xenografted mice treated with anti-CD45RB antibody. Perhaps this is because the xenograft response is already dominated by T<sub>0</sub>,2 cytokines (28). Peritoneal lavage cells were recovered 5 days after PK15 transplantation and cultured in vitro without adding PK15 cells. Cytokine secretion into culture medium was quantified by ELISA. Secretion of IL-4, IL-5, IL-10 and IFN-γ was
Anti-CD45RB reduces CD4 T cell responses, but not numbers in the spleen

The rapid rejection of neovascular xenografts is dependent on CD4 T cells (22,23). For instance, we have shown that a proportion of FPP grafts survive beyond 8 weeks in mice rendered permanently and completely CD4 deficient by the transgenic expression of anti-CD4 antibody (23), compared to rejection within 7 days in wild-type mice (e.g. Fig. 1B). Thus, we wished to examine the effect of anti-CD45RB treatment on priming of xenoreactive CD4 T cells in the spleen. CD4 T cell recovery from PK15 cell-challenged mice was not reduced by anti-CD45RB treatment (Fig. 5A). Over five experiments, the ratio of CD4 T cells in the spleen of anti-CD45RB + PK15:control antibody + PK15-treated mice was 1.3 ± 0.3. Thus, anti-CD45RB antibody did not simply deplete splenic CD4 T cells.

Assessment of cytokine secretion showed that anti-CD45RB treatment clearly had a negative effect on CD4 T cell priming. Splenic CD4 T cells were enriched to ~90% by positive selection on MACS and cultured without re-stimulation, enabling assessment of ex vivo cytokine production in ELISA. A comparison of CD4 T cells from mice given PK15 grafts plus control antibody treatment with those from normal mice showed that graft-activated T cells secreted IL-4, IL-5, IL-10 and IFN-γ (Fig. 5B). Anti-CD45RB treatment impaired production of each of these cytokines (Fig. 5B). IL-2 levels after 20 h of culture were <16 pg/ml in all test groups. These trends were observed in two experiments, as well as for ex vivo cultures of unfractionated spleen cells (data not shown).

The effect of anti-CD45RB is enhanced by co-stimulation blockade

The aforementioned experiments indicated that anti-CD45RB treatment prolonged xenograft survival, but did not prevent eventual rejection. Co-stimulation blockade has also received considerable attention as a means of prolonging allograft and xenograft survival. In particular, blockade of the co-stimulatory interaction of CD28 on T cells with CD80/86 on antigen-presenting cells (APC) and that of CD154 on T cells with CD40 on APC has been effective in many model systems (29–31). Hence, we also examined the relative efficacy of co-stimulation blockade alone or combined with anti-CD45RB treatment. CD28–CD80/86 blockade was achieved by the use of recipient mice transgenic for CTLA-4–Ig. CD154–CD40 blockade was achieved by the use of CD154KO recipient mice. These experiments demonstrated that the combination of anti-CD45RB with co-stimulation blockade was advantageous. Co-stimulation blockade, as was the case for anti-CD45RB antibody, reduced CD4 T cell infiltration, but the combination was more effective (Fig. 6A). Indeed, anti-CD45RB treatment of CTLA-4–Ig/CD154KO mice essentially prevented CD4 T cell infiltration (Fig. 6A). Co-stimulation blockade alone was ineffective at enhancing PK15 graft survival, few PK15 cells being recovered from CTLA-4–Ig, CD154KO or CTLA-4–Ig/CD154KO mice (Fig. 6B). Anti-CD45RB antibody alone was as effective as co-stimulation blockade (Fig. 6B). Notably, anti-CD45RB treatment and co-stimulation blockade synergized to enhance PK15 graft survival. The most pronounced effect was reduced by anti-CD45RB treatment of the mice (Fig. 4). Given that cytokine secretion by lavage cells obtained from normal mice or anti-CD45RB + PK15-treated mice was frequently below the limit of detection, we are unable to report the relative fold reduction. However, a reduction in production of each of these cytokines was observed in three independent experiments.
observed when anti-CD45RB antibody was combined with blockade of both CD28 and CD154 co-stimulatory pathways (Fig. 6B). Thus, the number of grafted cells surviving after antibody treatment was 50,000 cells and CD28/CD40 ligand co-stimulation blockade was 25,000 cells, whereas the two together was 1,700,000 cells.

CD62L and LFA-1 expression is reduced after anti-CD45RB antibody treatment

Various T cell activation markers were examined in order to determine if anti-CD45RB treatment blocked the activation of xenoreactive T cells. In the spleen of PK15-grafted mice treated with anti-CD45RB versus control antibody there was no consistent reduction in the numbers of CD4 cells positive for CD25 or CD44 (data not shown). By contrast, CD62L expression was reduced, the CD62Lhigh population observed in control antibody-treated PK15-grafted mice being replaced by a CD62Llow population (Fig. 7). Notably, the reduction in CD62L expression was not restricted to xenoreactive T cells, but rather appeared to be a direct affect of the anti-CD45RB antibody alone. Thus, the shift affected the majority of cells (Fig. 7A) and a similar reduction was obtained in the absence of PK15 challenge (data not shown). Anti-CD45RB treatment of PK15-grafted mice similarly resulted in a reduced intensity of staining for LFA-1 (detected using antibody specific for the anti-CD11a chain of LFA-1, Fig. 7). Again, this shift was not
restricted to xenoreactive CD4 cells, but rather affected the majority of cells (Fig. 7), and was also observed for CD4 cells from untransplanted mice (data not shown).

**Anti-CD45RB reduces CD4 T cell numbers in lymph nodes, but not in spleen**

CD62LKO and LFA-1KO mice show reduced homing of lymphocytes to lymph nodes, but not the spleen (32,33). Hence, we wished to determine if reduced expression of CD62L and LFA-1 after anti-CD45RB treatment similarly affected the homing of CD4 T cells. Ungrafted mice were treated with anti-CD45RB or control antibody and analyzed 3 days later. CD4 T cell recovery from inguinal and iliac lymph nodes was reduced 2- to 3-fold (Fig. 8), whereas the recovery from the spleen was not reduced (Fig. 8). This result was confirmed in two additional experiments in which anti-CD45RB treatment selectively reduced CD4 T cells in the inguinal nodes (but not spleen) by 2- to 3-fold (data not shown). CD8 T cell recovery in the inguinal nodes was similarly reduced (data not shown).

**Discussion**

Antibodies against different CD45 isoforms or different epitopes of the same isoform can elicit different biological effects and only some have been shown to prolong allograft survival (16-20,34). One such antibody, MB23G2, has been shown to promote establishment of allografts (renal and islets) and result in a state of donor-specific tolerance in a proportion of recipients (18,19). However, this antibody alone had little effect on the survival of vascular xenografts (cardiac and renal) (35). Humoral and cellular responses both contribute to rejection of such xenografts that utilize blood vessels of donor origin, and it was proposed that the failure of MB23G2 alone to prolong graft survival was due to a failure to prevent production of xenoreactive antibodies (35). By contrast, we have
shown for the first time that this anti-CD45RB antibody prolongs survival of neovascularized FPP and avascular PK15 xenografts. The rejection of such xenografts which do not depend on donor vasculature is predominantly mediated by cellular mechanisms, leading to the conclusion that the anti-CD45RB antibody-induced xenograft prolongation is a result of perturbation of these cellular responses.

Differences in vascularity (neovascularized versus avascular) and graft site (kidney versus peritoneum) may result in varied effectors of FPP and PK15 xenograft destruction being evoked. Nevertheless, a number of studies have indicated that the potency of the cellular xenograft response is controlled by T cells, in particular CD4 T cells (22,23,36). Hence, we exploited the versatile and quantitative nature of the PK15 graft model to study the effect of anti-CD45RB antibody on T cells. The action of anti-CD45RB antibody appears to be multifaceted. Firstly, a modest (2-fold) reduction of blood lymphocytes (19) and lymph node CD8 T cells (20) results from anti-CD45RB treatment. We also observed a 2- to 3-fold reduction in CD4 and CD8 T cell numbers in lymph nodes. By contrast, there is no reduction in T cell (both CD4 and CD8) numbers in the spleen. This paradox is not readily reconciled by a depletion mechanism. Instead, the restriction of this reduction in T cell numbers to lymph nodes and blood can be explained by altered trafficking, especially given the changes in the CD62L and LFA-1 homing/adhesion molecules. Secondly, the reduction in cytokine production by splenic CD4 T cells (without a reduction in numbers) supports the view that anti-CD45RB directly affects T cell priming. Thirdly, anti-CD45RB may affect T cell trafficking to the inflammatory site. The expression of CD62L and LFA-1 is reduced, and the numbers of cells in the graft site is reduced in the i.p. xenograft model. These issues are discussed further below.

Although not expressly shown by our study, the existing literature supports the view that the effects of anti-CD45RB antibody treatment on T cell priming are direct. CD45 has an important role in positive regulation of T cell responses (5). Previous reports have indicated that some anti-CD45RB antibodies directly affect TCR-mediated signaling. For example, anti-CD45RB antibody increases phosphorylation of phospholipase Cγ1 in an anti-CD3 activated T cell hybridoma suggesting direct modification of signaling via the TCR (19). Furthermore, anti-CD45RB treatment increases CD4 T cell expression of the negative regulator of co-stimulation, CTLA-4 (21). Our finding that anti-CD45RB treatment reduces splenic CD4 T cell cytokine production, but not number, or expression of activation markers such as CD25 and CD44, suggests that the frequency of responding CD4 T cells is not reduced in the spleen, but rather that outcome of antigen recognition is qualitatively altered. The production of both Th1 (IFN-γ) and Th2 cytokines (IL-4, IL-5 and IL-10) was reduced by anti-CD45RB treatment, indicative of a dampened rather than a deviated immune response. This was true for both afferent sites (e.g. unfractionated spleen cells and purified splenic CD4 T cells) and effector sites (e.g. lavage cells in the peritoneal graft site). This contrasts with what has been described for allografts (18,20), where the levels of Th2 cytokines IL-4 and IL-10 was increased while that of IFN-γ was unaltered. In addition to reduced T cell cytokine production and infiltration, there was a dramatic reduction in macrophage numbers at the graft site of anti-CD45RB-treated mice. This is of interest as macrophages are abundant in rejecting FPP and PK15 xenografts, and activated macrophages can directly destroy xenografts (24). T cells produce proinflammatory cytokines (37,38) that promote macrophage infiltration and/or expansion (38–40) at the graft site. Thus, the reduction in macrophage numbers may be attributed to reduced T cell infiltration and function. Nevertheless, we have found that CD45RB is expressed on a subset of macrophages (data not shown) such that it is possible that anti-CD45RB antibody treatment may also directly affect macrophage infiltration or expansion.

Anti-CD45RB antibody targeting of signaling via the TCR has recently been shown to synergize with co-stimulation blockade (with anti-CD154 antibody) to promote allograft survival (20). To determine whether this synergism also applied to the xenograft response, we investigated the effect of anti-CD45RB treatment combined with blockade of CD28 and CD154. The combination of anti-CD45RB antibody with blockade of either CD154 or CD28 co-stimulation was advantageous in both reducing CD4 T cell infiltration of the graft and promoting xenograft survival. The effect was even more marked when anti-CD45RB antibody was combined with blockade of both CD28 and CD154. Not only was there an additional enhancement of xenograft survival, but CD4 T cell infiltration was essentially ablated. We suggest that the synergy of these agents reflects not only compatibility of targeting both TCR signaling and co-stimulation, but also the advantage of blocking multiple co-stimulatory interactions. Many studies including our own have demonstrated the advantage of combined co-stimulation blockade, including combined blockade of CD28 and CD154, and CD28 and LFA-1 [e.g. (29,41,42)]. Reduced LFA-1 expression in anti-CD45RB-treated mice might also have contributed to the reduction in co-stimulation, given the importance of LFA-1 in interactions between T cells and APC (38,43,44). Of relevance to our findings above, one study showed that activation of transgenic CD4 T cells without ICAM-1/LFA-1

**Fig. 8.** Anti-CD45RB treatment reduced CD4 cell numbers in the lymph nodes, but not spleen of ungrafted mice. Mice were treated with either isotype control (hatched bars) or anti-CD45RB (filled bars) antibody as indicated on the x-axis. Three days later, spleen and lymph nodes were recovered and CD4 cells were quantified by FACS analysis. Graphs show the mean ± SD recovery/mouse for three mice/group.
interaction resulted in normal proliferation, but impaired production of CCL3 and CCL4 chemokines (38). Upon adoptive transfer, such T cells had a reduced capacity to recruit macrophages.

Our finding of decreased CD4 T cells in peripheral lymph nodes, but not spleen of anti-CD45RB-treated mice is suggestive of altered trafficking of these cells rather than direct elimination. The anti-CD45RB-induced reduction in expression of CD62L and LFA-1 is consistent with this possibility. CD62L (the ligand for peripheral node addressin) and LFA-1 (an adhesion molecule with many functions including binding to vascular endothelium) promote lymphocyte homing to peripheral lymph nodes such that CD62LKO and LFA-1KO mice show a similar phenotype to anti-CD45RB-treated mice, i.e. a reduction in lymphocyte numbers in peripheral lymph nodes, but not the spleen (32,33). A role for CD45 in regulating lymphoid cell traffic is also supported by the finding that restoration of CD45 expression in CD45KO mice promotes lymphocyte accumulation in the lymph nodes, but not spleen (45). Both LFA-1 and CD62L have also been implicated in directing leukocytes to sites of inflammation (32,46–48). It is probable that the anti-CD45RB antibody exerts its effects on lymphocyte number and expression of CD62L and LFA-1 directly. Thus, we have shown that these effects are produced by anti-CD45RB treatment in the absence of an antigenic challenge. Furthermore, several studies have indicated a role for CD45 in regulating CD62L expression and the function of various integrins including LFA-1. Anti-CD45RB treatment in vitro resulted in down-regulation of surface CD62L (7,8). Comparison of CD45- and CD45+ cells has shown that CD45 can regulate adhesive interactions mediated by β1 and β2 integrins (9,10). Furthermore, certain anti-CD45 antibodies (but not all) can inhibit LFA-1-dependent homotypic aggregation of lymphocytes (49,50), possibly by inhibiting tyrosine phosphorylation of molecules following LFA-1 engagement (51). Our finding of decreased CD4 T cells in peripheral lymph node populations and on the homing/adhesion molecules, it is likely that altered trafficking (as well as the altered T cell activation described above) contributes to the reduced level of infiltration in the i.p. xenograft model.

Impressive progress has been made in the area of immunosuppression for clinical allotransplantation. Indeed, recent improvements in the immunosuppressive protocol for recipients of allogeneic pancreatic islet transplants have enabled the majority of recipients to become insulin independent (51). Nevertheless, the supply of human donor pancreas is inadequate to meet demand. The use of xenogeneic (e.g. pig) pancreas would overcome the problem of supply, but not of rejection. Thus, the demonstration that anti-CD45RB antibody treatment enhances the survival of avascular pig xenografts is notable, particularly given its multifaceted action and synergism with co-stimulation blockade.

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Abbreviations

APC antigen-presenting cell
CD62L CD62 ligand
FPP fetal pig pancreas
KO knockout

References


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