Donor T Cell Activation Initiates Small Bowel Allograft Rejection Through an IFN-γ-Inducible Protein-10-Dependent Mechanism

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The poor success in controlling small bowel (SB) allograft rejection is partially attributed to the unique immune environment in the donor intestine. We hypothesized that Ag-induced activation of donor-derived T cells contributes to the initiation of SB allograft rejection. To address the role of donor T cell activation in SB transplantation, SB grafts from DO11.10 TCR transgenic mice (BALB/c, H-2Ld+/) were transplanted into BALB/c (isografts), or single class I MHC-mismatched (Ld-deficient) BALB/c H-2dm2 (dm2, H-2Ld−/) mutant mice (allografts). Graft survival was followed after injection of control or antigenic OVA323–339 peptide. Eighty percent of SB allografts developed severe rejection in mice treated with antigenic peptide, whereas <20% of allografts were rejected in mice treated with control peptide (p < 0.05). Isografts survived >30 days regardless of OVA323–339 administration. Activation of donor T cells increased intragraft expression of proinflammatory cytokine (IFN-γ) and CXC chemokine IFN-γ-inducible protein-10 mRNA and enhanced activation and accumulation of host NK and T cells in SB allografts. Treatment of mice with neutralizing anti-IFN-γ-inducible protein-10 mAb increased SB allograft survival in Ag-treated mice (67%; p < 0.05) and reduced accumulation of host T cells and NK cells in the lamina propria but not mesenteric lymph nodes. These results suggest that activation of donor T cells after SB allotransplantation induces production of a Th1-like profile of cytokines and CXC chemokines that enhance infiltration of host T cells and NK cells in SB allografts. Blocking this pathway may be of therapeutic value in controlling SB allograft rejection. The Journal of Immunology, 2002, 168: 3205–3212.

Small bowel (SB) transplantation is a potential solution for individuals with intestinal failure (1, 2). However, a poor survival rate limits the clinical usefulness of this technology. Rejection has been a significant impediment to extending SB allograft survival. SB allograft rejection is associated with the production of both Th1 and Th2 cytokines (IFN-γ, TNF, IL-4, and IL-5) (3). Both CD4+ and CD8+ T cell subsets are required for the development of SB allograft rejection (4). Compared with other solid organ allografts, a higher degree of immunosuppression is required to prevent SB allograft rejection (5, 6), and rejection episodes occur despite aggressive treatment. It has been suggested that difficulties encountered in controlling SB allograft rejection are due to the unique environment of the mucosal immune system (7, 8).

In the intestinal immune system, T cells respond to Ags derived from commensal and pathogenic enteric bacteria. Studies from our lab and others indicate that both APC as well as T cells localized to the intraepithelial and lamina propria (LP) compartments express surface and functional phenotypes consistent with their activated state, and are maintained as activated populations poised to deliver rapid effector immune responses (9, 10). Studies by Harper et al. (11) suggest that compared with splenic populations, LP APCs induce relatively high levels of IFN-γ and TGF-β from responding T cells in the absence of other cytokines (IL-2 and IL-5). The induction of IFN-γ by enteric Ag may increase mucosal permeability as well as enhance local responses by up-regulating expression of class I and II MHC and costimulatory molecules (12, 13). Based on these data, we postulated that the unique environment of the mucosal immune system contributes to the relatively poor success rate of SB allograft transplantation.

Acute SB allograft rejection is characterized by massive lymphocyte infiltration and extensive epithelial cell apoptosis, followed by mucosal destruction (8, 14). Recent data from several models of organ transplantation (skin, heart, kidney, and lung) (15–18) suggest that recruitment of host leukocytes into the allograft involves chemokine-mediated pathways. IFN-γ-inducible CXC chemokines such as IFN-γ-inducible protein-10 (IP-10) and monokine induced by IFN-γ (Mig) are important in Th1-like immune responses, including those associated with acute allograft rejection (19–21). Data from Hancock et al. (22) suggest that production of IP-10 by donor rather than infiltrating host cells is required for the initiation of cardiac allograft rejection. In complementary studies, CXCR3 expression by host cells was shown to be
required for rejection of cardiac allografts (23). While no studies evaluating the role of chemokines in SB allograft rejection have been performed, the SB LP is a potentially rich source of IP-10 and other IFN-γ-inducible chemokines (24). Therefore, we considered the possibility that activation of large numbers of graft-derived T cells may induce IFN-γ-inducible chemokines and contribute to the aggressive SB allograft rejection.

Initially, the role of T cell activation in SB tissues was examined. We found that T cell activation induced rapid production of both proinflammatory cytokines as well as CXC and CC chemokines. This result led us to hypothesize that donor T cell activation enhances allograft rejection by inducing local production of CXC chemokines. Using a mouse-vascularized SB transplant model, we show that activation of donor CD4⁺ T cells by foreign Ag triggered rejection of class I MHC-mismatched SB allografts. Furthermore, our data indicate that neutralization of IP-10 significantly prolonged SB allograft survival following donor T cell activation. Taken together, results from these studies suggest that donor T cell activation enhances SB allograft rejection by inducing local production of CXC chemokines that promote infiltration of host leukocytes.

Materials and Methods

Animals

Male BALB/c (H-2d, Ld) and BALB/c-H-2m2 mice (dn2, Ld) were purchased from The Jackson Laboratory (Bar Harbor, ME). The dn2 mice are BALB/c mutants that lack class I Ld Ag (25). DO11.10 mice (26), expressing a Vε31ββ.2 TCR specific for a class II MHC-restricted peptide of chicken OVA (OVAaa,aa) in >85% of the CD4⁺ T cells (a gift from D. Lobs, nippon Research Center, Kamakura, Japan), were backcrossed to BALB/c (H-2b, Lb) mice for over 14 generations, and the progeny were screened for expression of the TCR transgene by flow cytometry using the clonotypic mAb, KJ1-26.1 (Caltag Laboratories, South San Francisco, CA). All strains were housed in a barrier facility at Northwestern University Medical School (Chicago, IL) and were used at 8–12 wk of age.

Quantitation of RNA transcripts by real time RT-PCR

Real time RT-PCR assays were used to specifically quantitate mouse cytokine and chemokine transcripts. Total cellular RNA was extracted and samples were heated at 95°C for 10 min, chilled, and reverse transcribed with Superscript II reverse transcriptase (Life Technologies, Rockville, MD) with random hexamers and oligo(dT). Equivalent amounts of individual cDNAs from similarly treated mice were combined to create pooled samples for real time RT-PCR. Primers were either obtained from PerkinElmer/Cetus (Norwalk, CT) or generated with Primer Express software (PerkinElmer/Cetus) and synthesized in our core facility. Whenever possible, primer pairs were designed to span intron/exon borders. Samples were subjected to 40 cycles of amplification of 95°C for 15 s, followed by 60°C for 1 min using a GeneAmp 5700 sequence detection system (PE Applied Biosystems, Foster City, CA) and SYBR green buffer. PCR amplification of the housekeeping gene ubiquitin was performed for each sample to control for sample loading and to allow normalization between samples. Both water and genomic DNA controls were included to insure specificity. Each data point was examined for integrity by analysis of the amplification plot and dissociation curves. The ubiquitin-normalized data were expressed as the fold induction of gene expression in treated mice compared with that in control mice.

Marine SB transplantation

Intestinal transplantation was performed, as described previously (27), with minor modifications. Briefly, small intestines were isolated from donors and perfused in situ with cold heparinized lactated Ringer’s solution. End-to-side anastomoses were performed between donor and recipient aorta and donor portal vein and recipient inferior vena cava. The proximal end was exteriorized as a stoma, while the distal end was anastomosed, end to side, to the recipient intestine. Recipient mice were evaluated twice daily for clinical evidence of rejection, including increased mucous output, stomal necrosis, and palpable abdominal mass. Mice with clear clinical evidence of rejection were sacrificed, and healthy mice were followed for 30 days and sacrificed. Mice that died within 4 days after transplantation were considered technical failures (<15%) and excluded from analysis. In some cases, grafts were removed on postoperative days (POD) 5, 7, 8, and 14 for evaluation of tissue histology, cell apoptosis, and infragraft cytokine expression. Cells were isolated from LP, mesenteric lymph nodes (MLN), and spleen, and analyzed by flow cytometry.

Activation of DO11.10 transgenic donor cells

Control (myelin basic protein) and OVAaa,aa,aa peptides were synthesized in the Northwestern molecular biology core facility using a PE Applied Biosystems Synergy Peptide Synthesizer with purity assessed by HPLC and mass spectroscopy. To activate T cells, OVAaa,aa,aa (200 μg/mouse) was administered to BALB/c or dn2 mice with DO11.10 SB transplants on POD 5, 6, and 7, via i.p. injection.

In vivo blocking of IP-10 following SB transplant

Hamster anti-mouse IP-10 mAb (1F11) (21) and control hamster IgG (UCB-189; American Type Culture Collection, Manassas, VA) were grown in serum-free medium and then purified over protein G columns using standard techniques. For in vivo neutralization of IP-10 in SB transplants, 100 μg IP-10 mAb was administered on POD 5 and every other day thereafter until sacrifice. For all mAbs and peptides, endpoint levels were tested using the Limulus amebocyte assay (BioWhittaker, Walkersville, MD) and were <0.1 endotoxin U/ml.

Pathologic evaluation of the intestinal graft

At necropsy, a section of each graft was fixed in 10% buffered formaldehyde, embedded in paraffin, and then stained with H&E for routine pathology. Microscopic sections were examined blindly by a pathologist using previously established criteria (8, 28). Briefly, slides were assessed for mononuclear cell infiltration, villous atrophy and sloughing, epithelial erosion, and crypt apoptosis. Scores of 0 (no change), 1 (focal), or 2 (diffuse) were assigned for each feature of acute rejection. After assignment of scores, the overall severity of rejection was based on the total score and designated as follows: 0, no rejection; 1–5, mild rejection; 6–10, moderate rejection; 11–14, severe rejection.

Isolation of cells

Lamina propria. LP lymphocytes were isolated as described (29). Briefly, small intestines were extracted and rinsed with cold PBS. After Peyer’s patches were removed, tissues were minced and epithelial cells were liberated by incubation with 5 mM EDTA and 10% newborn calf serum (Life Technologies). Mucosal pieces were washed, returned to digestion buffer, and incubated six times for 30 min with collagenase (100 U/ml; Sigma-Aldrich, St. Louis, MO). Cells released into the supernatant were washed and kept overnight before separation on a Nycodren 1.077 gradient (Accurate Chemical, Westbury, NY).

Mesenteric lymph nodes. Nodes were mechanically dissociated and fat eliminated by passage of the cell suspensions through nylon mesh. Cell suspensions were washed and resuspended in 5% DMEM and stored on ice.

Splenocytes. Splenocytes were isolated as previously described. Briefly, spleens were mechanically dissociated, and RBCs were lysed in ammonium phosphate/chloride lysis buffer. Cell suspensions were washed and stored in DMEM (Life Technologies) with 5% FCS (5% DMEM) on ice until used.

Abs for flow cytometry

The following primary mAbs used in flow cytometry, either unconjugated or FITC, PE, or allophycocyanin conjugated, were purchased from BD PharMingen (San Diego, CA), unless otherwise defined. These include: anti-Thy-1.2 (53-2.1), anti-CD4 (L3T4a, GK1.5), anti-CD8 (Ly-2, 2.43, and HO2.2), anti-mouse pan-NK (DX5), anti-mouse CD3 (145-2C11 and 50A3), anti-mouse CD69 (H12F6), anti-mouse H-2Ld (H-2Dd) (28-14-8), and isotype-matched control mAbs. The clonotypic anti-TCR mAb KJ1-26.1 conjugated to PE (Caltag Laboratories) was used to identify the OVAaa,aa,aa-specific transgenic TCR.

Flow cytometric analysis

Three-color flow cytometric analysis was conducted on freshly isolated cells from recipient splenocytes and MLN, and graft-derived MLN or LP, using standard techniques. Briefly, cell suspensions were prepared as described and washed twice in FACS buffer (PBS with 1% FCS, 0.1% sodium azide) before blocking of FcR (2.4G2) and staining with FITC-, PE-, or allophycocyanin-conjugated mAbs. Nonviable cells were excluded from analysis on the basis of propidium iodide staining, and data were analyzed...
on a FACSCalibur using CellQuest software (BD Biosciences, San Jose, CA).

**Evaluation of cytokine and chemokine mRNA expression in SB transplants using RT-PCR**

Total cellular RNA was extracted using TRIzol ( Molecular Research Center, Cincinnati, OH) in nuclease-free water. Isolated RNA was incubated with 10 U DNase I (Boehringer Mannheim, Indianapolis, IN) in the presence of RNasin (Promega, Madison, WI) for 30 min at 37°C. Samples were heated at 95°C for 10 min, chilled, and reverse transcribed with SuperScript II reverse transcriptase (Life Technologies) with random hexamers and oligo(dT). Primers specific for IFN-γ, Mig, and IP-10 were used to amplify relevant sequences. The amount of mRNA for β-actin was assessed to normalize RNA from each specimen. Target cDNA was amplified for a range of 22–40 cycles, and subsequently electrophoresed in 2% agarose gels containing ethidium bromide.

**Statistical analyses**

Statistical comparisons of survival curves between groups were performed by the log-rank test using GraphPad Prism 3.0 (GraphPad, San Diego, CA) software. Statistical significance of cytokine levels and apoptosis between treated groups was assessed using a one-tailed ANOVA with group means compared using the Scheffe’s multiple comparison test (30). Values of $p < 0.05$ were considered significant.

**Results**

**Donor SB T cell activation induces cytokine/chemokine production in SB tissue**

To examine the effect of T cell activation on SB cytokine and chemokine induction, mRNA expression for a panel of cytokines and chemokines was evaluated by real time PCR in SB tissues following OVA323–339 (200 μg/mouse, i.p.) administration to DO11.10 mice. We had previously shown that systemic administration of Ag (i.p. OVA323–339) activates cells in peripheral lymphoid tissue as well as in intestinal LP and Peyer’s patches (29). Results in Fig. 1A reveal that a single injection of OVA323–339 increased mRNA expression of several effector cytokines, including IFN-γ (13-fold), TNF (6-fold), IL-4 (7-fold), and IL-10 (9-fold) by 3 h postinjection. More notably, OVA323–339 administration induced a dramatic increase in SB mRNA for chemokines including IP-10 (90-fold) and Mig (75-fold), macrophage-inflammatory protein-1α (8-fold), macrophage-inflammatory protein-1β (25-fold), and monocyte chemoattractant protein-1 (30-fold) by 3 h postinjection. Increased mRNA expression persisted for 24 h after OVA323–339 administration (Fig. 1B).

**Donor-derived SB T cell activation accelerates SB allograft rejection**

Based on the findings of increased cytokine and chemokine expression in SB tissues, we hypothesized that donor T cell activation would initiate SB allograft rejection. To address the role of donor-derived intestinal T cell activation on SB allograft rejection, SB from DO11.10 mice were transplanted into syngeneic BALB/c mice (isograft) or Ld-deficient dm2 mice (allograft). These donor/recipient combinations were used for several reasons. First, evaluation of a single Ag mismatch facilitated a more focused examination of the influence of donor CD4+ T cell activation on the host alloresponse. Second, while SB allografts with a complete class I and II MHC mismatch develop acute rejection by POD 8 (31), it was anticipated that a single class I MHC Ag disparity would be associated with a more prolonged graft survival. The less aggressive rejection response was utilized to allow for examination of more subtle effects of donor T cell activation on allograft rejection. Finally, the choice of strains eliminated the potential for graft-vs-host responses, as both DO11.10 and dm2 mice strains used are on the BALB/c background.

**FIGURE 1.** Cytokine (A) and chemokine (B) mRNA induction after T cell activation in the SB. Normal DO11.10 mice were given OVA323–339 peptide (200 μg i.p.). RNA was isolated from three pooled whole SB at each of the selected time points following OVA323–339 administration. Real-time PCR analysis was performed as described in Materials and Methods. Shown is the fold induction of ubiquitin-normalized mRNA expression compared with levels detected in unstimulated mice.

Following transplantation, isografts and allografts were treated with i.p. injection of either control or OVA323–339 peptide on POD 5, 6, and 7 to activate DO11.10 T cells. Peptide injections were delayed until POD 5 to allow for adequate recovery time from surgery.

As shown in Fig. 2, all BALB/c isografts, regardless of whether they received OVA323–339 ($n = 5$) or not ($n = 4$), survived until sacrifice on POD 30 with weight gain, healthy stoma, and normal

**FIGURE 2.** SB transplant graft survival. SB from DO11.10 mice were transplanted into syngeneic BALB/c mice (isograft) (A and B) or Ld-deficient dm2 mice (allograft) (C and D) with MBP (control) (A and C) and with OVA323–339 (B and D) peptide administration (200 μg i.p.) on POD 5, 6, and 7. Graft survival was monitored as described in Materials and Methods. *, Values of $p > 0.05$ vs A and B; **, $p < 0.05$ vs A–C.
Donor T cell activation had no effect on isograft survival. Allografts (n = 6) that were not given OVA323-339 exhibited a transient increase in mucus output at about POD 9–10, but five of six had no further evidence of rejection throughout the study period (30 days). One allograft (17%) rejected on POD 20 (mean survival time (MST) = 28 days). Serial histological examination of other surviving allografts revealed transient focal cellular infiltrates of grafts on POD 8. However, these infiltrates resolved spontaneously and were not detected on POD 10, 14, or 30 (total histological scores <3 at these time points).

In contrast, when donor T cells were activated by OVA323-339 administration, 83% of allografts (n = 6) developed rejection by POD 20 (MST = 16 days, p < 0.05 for OVA323-339 vs control peptide-treated group). Rejection was manifested by increased mucus output (POD 8), followed by the development of a palpable abdominal mass. Histological findings were consistent with severe rejection (scores >12), including lymphocytic infiltration, distortion of villous architecture, and mucosal sloughing (Fig. 3, A and B). TUNEL staining for apoptotic cells revealed that donor T cell activation induced apoptosis of crypt enterocytes on POD 7 in SB allografts (Fig. 3, C and D). Quantification of apoptosis indices (apoptotic index: apoptotic epithelial cells per 100 total epithelial cells) indicated that donor T cell activation increased levels of epithelial apoptosis by >4-fold from 3.3 ± 2.6 in control peptide-treated SB allografts to 13.1 ± 4.3 in OVA323-339-treated SB allografts (p < 0.01). Overall, these results suggest that donor T cell activation induced vigorous rejection of murine SB allografts mismatched at a single class I MHC locus.

Donor SB T cell activation enhances host T cell activation

To determine whether donor T cell activation affected the activation state of host T cell, host splenocytes from DO11.10 (SB donor)→dm2 (host) SB transplants on POD 8 were analyzed for expression of surface activation markers, including CD69 (very early activation Ag) and CD25 (IL-2R α-chain). Results in Fig. 4 suggest that transplantation of SB allografts alone increased the proportion of activated dm2-recipient T cells (Thy-1.2*, Ld−). Compared with naive dm2 mice, increased numbers of activated host T cells were detected in the spleen of dm2 recipients of DO11.10 SB allografts (POD 5). Host T cell activation was further enhanced on POD 8 when donor T cells were activated by administration of OVA323-339 peptide on POD 5, 6, and 7 following DO11.10→dm2 SB allotransplantation, but was not detected in recipient spleens of either control or OVA323-339-treated SB
isografts (DO11.10→BALB/c) on POD 8 (data not shown). Taken together, these data suggest that donor T cell activation increased the proportion of activated recipient T cells localized to peripheral lymphoid tissues.

**Donor SB T cell activation promotes host T cell infiltration into the SB allografts**

To address the effect of donor T cell activation on the composition of graft-associated MLN and LP T cell populations after SB transplantation, we isolated lymphocytes from LP and MLN of isografts as well as allografts on POD 8, and examined whether there were changes in numbers, morphological features (including size as measured by forward scatter and the nuclear/cytoplasmic complexity by side scatter) (32), and surface phenotypes. Expression of the donor Ld alloantigen was used to distinguish host from donor cells. Data in Fig. 5, A and B, indicate that little change in numbers and morphology was detected in the MLN and LP T cells from isografts regardless of donor T cell activation by OVA323–339 administration. An increase in lymphoblast-like T cells (increased size and nuclear/cytoplasmic complexity) was found in both MLN and LP following allotransplantation. By comparison, donor T cell activation resulted in further increase in the proportion of T lymphoblasts in LP of SB allografts (14 vs 43% in control vs Ag-treated allografts, respectively). Interestingly, injection of the Ag did not increase accumulation of T lymphoblasts in MLN of allografted mice. Phenotypic analysis (Fig. 5, C and D) revealed that donor T cells in the MLN of allografts were largely replaced by host cells by POD 8 regardless of whether donor T cells were activated with systemic Ag. In contrast, donor T cell activation led to a marked increase of host T cells, primarily CD8+ T cells (from 24 to 73%) in LP of SB allografts. These data suggest that donor T cell activation contributes to the initiation of allograft rejection by enhancing infiltration and expansion of host CD8+ T cells in the LP, and the LP is the primary focus of the allogeneic immune response.

**SB rejection is associated with IFN-γ-inducible CXC chemokine (IP-10, Mig) expression**

We hypothesized that the current model of SB allograft rejection was associated with increased IFN-γ-inducible CXC chemokine (IP-10, Mig) expression following donor T cell activation. To address whether donor T cell activation up-regulated cytokine/chemokine production in the transplanted SB tissues, mRNA expression levels were assessed by RT-PCR in SB isografts and allografts after treatment with control or antigenic peptide. In both isografts and allografts, administration of OVA323–339 resulted in an up-regulation in IFN-γ mRNA expression and up-regulated expression of the IFN-γ-inducible chemokines IP-10 and Mig (Fig. 6). This finding was consistent with those in nontransplanted SB tissues.

**IP-10 is involved in the initiation of SB allograft rejection by donor T cells**

To examine the role of IP-10 in SB allograft rejection, the Ag-treated, allografted mice were treated with a neutralizing mAb to mouse IP-10 (anti-IP-10 mAb) every other day, beginning on POD 5. Data in Fig. 7 indicate that neutralization of IP-10 prolonged graft survival in nearly all mice. Four of six (67%) grafts survived...
to the study end point (POD 30) with no clinical or histological evidence of rejection (MST = 30). In control IgG-treated mice, Ag treatment induced graft rejection in 86% of mice with a median survival of 15 days (p < 0.05). Three of four anti-IP-10 mAb-treated mice that were not sacrificed at POD 30, but were discontinued with anti-IP-10 mAb treatment, survived to POD 100 without clinical evidence of rejection.

**Anti-IP-10 treatment decreases infiltration of host T cell into LP of the SB allograft**

To determine whether anti-IP-10 prevents SB allograft rejection by inhibiting host cell infiltration of the allograft, cellular composition and cell numbers of LP infiltrates were examined. Compared with controls, LP infiltrates from mice treated with anti-IP-10-neutralizing mAb had significantly fewer host CD4\(^+\) T cells, CD8\(^+\) T cells, and NK cells (Fig. 8A). In addition to decreasing the overall numbers of infiltrating host cells in the LP, anti-IP-10 also influenced the donor-recipient T cell mix in the LP (Fig. 8B). In fact, the LP T cell infiltrates in the anti-IP-10 mAb-treated group closely resembled those of the group that did not receive OVA\(_{323-339}\). Taken together, these data suggest that IP-10 was involved in the recruitment of host T cells and NK cells following donor T cell activation.

**Anti-IP-10 treatment inhibits donor cell accumulation in the host spleen**

To determine whether anti-IP-10 treatment also affected cellular accumulation in host peripheral lymphoid tissues, we analyzed host spleens from POD 8 allografts treated with control mAb or anti-IP-10 mAb (see Materials and Methods) and analyzed on POD 8 by flow cytometry. A, Total numbers of host cells (L\(^+\)). The numbers were calculated based on assessments of total yield and flow cytometric analysis for percentages of CD4\(^+\) and CD8\(^+\) T cells and NK (DX5) cells of host origin (L\(^+\)) from two separate experiments. B, Composition of CD4\(^+\) and CD8\(^+\) T cells in LP of Ag-stimulated allografts treated with control or anti-IP-10 mAb. The cells were gated on Thy-1.2 \(^+\) populations. Quadrant percentages are reported in each plot. Data shown are representative of more than three experiments with similar results.
is expressed on populations of CD4+ T cells. Thus, it is possible that IL-2 are seen in the gut-associated lymphoid tissue before the histological appearance of host T cells (3, 14). Therefore, in the SB, effector T cells that release IFN-γ when challenged with Ag are found adjacent to large numbers of resident cells that produce IFN-γ-inducible chemokines such as IP-10.

The current data suggest that bystander effects of donor T cell activation enhance the activation state of host T cells. In our model, OVA323 administration on POD 5 activated DO11.10 donor T cells and enhanced activation of host T cells in allografted mice (Fig. 4). In nontransplanted BALB/c control mice, no activation of endogenous T cells was detected within 72 h of i.v. OVA323 injection (data not shown). Thus, activation of host T cells after OVA323-induced activation of DO11.10 transgenic CD4+ T cells most likely represents a bystander activation. As these effects were not detected in Ag-treated recipients of SB isografts, we suspect that host cell activation by alloantigen was a prerequisite for bystander effects. This phenomenon has been previously demonstrated both in mice (38, 39) and humans (40, 41). Cytokines released from activated T cells induce nonspecific proliferation, prolonged expression of activation markers (42), and a break in T cell tolerance (43). Potentially, bystander activation of host T cells can occur in any location in which recipient and donor T cells are in close proximity, including the graft LP. In our model, LP T cells of the allograft were gradually replaced by host T cells so that by POD 5, a 3:1 mix of donor and recipient T cells existed (data not shown). By POD 8, the majority of CD8+ LP T cells were of host origin, while the majority of CD4+ T cells were of donor origin (Fig. 5D). We suspect that the mixture of host and donor T cells was favorable for the induction of bystander effects on activated host cells. Furthermore, donor T cell activation enhanced accumulation of T cells as well as NK cells through an IP-10-dependent mechanism (Fig. 8). It was possible that following donor T cell activation, the process was perpetuated by recruited host CD4+ and CD8+ T cells via activation by enteric Ags and alloantigens in the graft. Thus, the initial increase in cytokine levels in the graft induced by foreign Ag may have promoted migration and sustained activation of host T cells that induced allograft rejection.

In this study, an interesting effect of IP-10 neutralization was detected in host peripheral lymphoid tissue. We found that IP-10 blockade abrogated accumulation of Ag-specific donor T cells in host peripheral lymphoid tissue (Fig. 9). These findings suggest that IP-10 neutralization inhibited Ag-induced expansion of DO11.10 transgenic T cells that emigrated out of SB donor tissues into host spleen. These data are consistent with an earlier report by Khan et al. (21) that anti-IP-10 mAb reduced tissue inflammation as well as mononuclear cell expansion in a mouse model of parasitic infection. More recently, Pertl et al. (44) demonstrated that depletion of IP-10 impaired antitumor CTL and inhibited generation of Th1-type CD8+ T cell responses. Taken together, these results suggest the IP-10 may be involved in T cell priming. We suspect that in the current model of SB allograft rejection, IP-10 functioned not only as a potent chemoattractant, but also enhanced the activation of T cells. There are several (not mutually exclusive) potential explanations for these observations. First, CXCR3 signaling may enhance T cell responses to Ag. Second, IP-10 may deliver a survival signal to activated T cells that enhances accumulation of expanded clones. Third, CXCR3 may be involved in migration of APC that carry Ag to T cell areas within draining LNs. Investigation of these pathways is outside the scope of the current manuscript, but may explain the surprisingly effective results of IP-10 blockade in several models of allograft transplantation and inflammation (22, 44, 45).
The mechanism proposed in these studies for enhancing allograft rejection may not require donor T cell activation. In a successful allograft, host T cells ultimately replace the donor T cells of the allograft’s gut-associated lymphoid tissue (46), and therefore assume immunosurveillance functions. Foreign Ags continue to activate LP T cells, now of host origin, which release cytokines and chemokines and activate nearby allosensitized T cells. Therefore, the risk of acute rejection may persist longer after transplantation of a SB allograft than after other allografts. Taken together, these data suggest that responses to enteric Ag contribute to SB allograft rejection by enhancing the accumulation of alloreactive cells as well as promoting Th1 functional differentiation of T cells upon arrival in grafts.

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