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CD8⁺ T Cells Resistant to Costimulatory Blockade Are Controlled by an Antagonist Interleukin-15/Fc Protein

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Abstract

Background—Although permanent engraftment is often achieved with new therapeutics, chronic rejection and graft failure still occur. As the importance of CD8⁺ cells in rejection processes has been underlined in various transplant models, and as interleukin (IL)-15 is involved in the activation of CD8⁺ T cells, we hypothesize that CD8⁺ T cell "escape" from costimulation blockade might be a IL-15/IL-15R dependent process.

Method—In a murine islet allograft model employing a fully major histocompatibility complex-mismatched strain combination of Balb/c donors to CD4^{−/−} C57BL/6 recipients, a monotherapy with the IL-15 antagonist, IL-15 mutant/ Fc 2a, or the costimulatory blockade molecule, CTLA4/ Fc, was used. In addition to monitoring graft survival, infiltration of alloreactive immune cells was analyzed by histology and immunohistochemistry, and alloimmune response of proliferative CD8⁺ T cells was measured in vivo.

Results—Sixty percent of the recipients treated with CTLA4/Fc acutely rejected their islet allograft, comparable to untreated control animals (50% survival). In contrast, the IL-15 antagonist proved to be highly effective, with 100% of recipients accepting their allograft. Immunohistology study demonstrated a remarkable decrease of CD8⁺ T-Cell intra-graft infiltration in IL-15 mutant/ Fc 2a treated animals with well-preserved islet architecture and a reduced frequency of proliferating alloreactive CD8⁺ T cells in comparison with that of untreated and CTLA4/Fc treated groups.

Conclusions—In this study, we determined the efficacy and potential therapeutic benefit of the IL-15 antagonist on CD4-independent CD8⁺ T-cell responses to alloantigens. Targeting the IL-15/IL-15R pathway represents a potent strategy to prevent rejection driven by CD8⁺ T cells resistant to costimulation blockade.

Keywords

Tolerance; Suppression; T-lymphocytes; Cytokines; Immunotherapy

The primary goal in designing new therapies for transplantation is the induction and maintenance of a state of donor antigen (Ag)-specific immunologic tolerance. In organ

transplantation, CD4⁺ T cells play a critical role in initiating the immune response leading ultimately to the effector mechanisms mediating allograft rejection. Interestingly, several reports have pointed out that CD8⁺ T cells could play an important role in graft rejection and particularly in chronic rejection, as CD8⁺ T cell-dependent processes appear to be responsible for costimulation blockade-resistant rejection (1-5). Despite costimulation blockade, engraftment and induction of tolerance in skin, kidney, or heart allografts requires adjunctive treatments depleting CD8⁺ T cells, such as anti-CD8 monoclonal antibody (mAb) (2, 4) or anti-GM1⁺CD8⁺ mAb (5), as these lymphocyte subsets are suspected to be the cells responsible for costimulation blockade-resistant rejection.

Therefore, targeting CD8⁺ T cells appears to be an essential step to control graft survival. Among the cytokines responsible for CD8⁺ T lymphocyte survival and proliferation, interleukin (IL)-15 appears to be a good candidate as it drives the proliferation and survival of CD8⁺ T cells and natural killer (NK) cells (6). The phenotype of IL-15^{-/-} (7) and IL-15R^{-/-} (8) mice demonstrates that IL-15 selectively provides support for the homeostasis and proliferation of memory CD8⁺ T cells. The description of IL-15 transgenic mice developing fatal leukemia with a CD8⁺ T cell phenotype (9) confirms that IL-15 plays an essential role in the homeostatic control of these cells. These findings raise the following question: Can expression of IL-15 be linked to CD8-dependent costimulation resistant rejection? As intragraft gene expression of IL-15 is consistently found in rejected tissues from mice and humans (10, 11) and costimulation blockade cannot act directly to decrease IL-15 expression or inhibit the activation of alloreactive CD8⁺ T cells (1, 3, 5), we hypothesized that targeting the IL-15/IL-15R system might provide a new tool for the induction of allograft tolerance.

We have previously reported on the therapeutic effects of an IL-15 mutant/Fc 2a protein possessing a prolonged circulating half-life, a high affinity IL-15R site specific antagonist function (12) and Fc-related cytotoxic potential against IL-15R⁺ cells upon allograft survival (13,14). To determine whether treatment with the IL-15 mutant/Fc 2a protein inhibits alloreactive CD8⁺ T cell mediated rejection, we compared the therapeutic activity of the IL-15 related protein to that of a costimulatory blockade agent, CTLA4/Fc, in a CD4-independent, CD8-mediated islet allograft model using CD4 knockout mice as recipients. We analyzed the role of CD8⁺ T cells in allograft rejection and determined the fate of CD8⁺ T in vivo by analyzing CD8⁺ T proliferation in a model mimicking a graft-versus-host (GVH) response in CD4 knockout (CD4KO) mice. Our data suggests that IL-15R⁺ cells are an important component of allograft rejection and that these cells play a pivotal role in CD8⁺ T-cell mediated CTLA4/Fc blockade-resistant rejection.

METHODS

Animals

BALB/c (H-2^d) and C57BL/6 (H-2^b) mice, 8 to 10 weeks old, were obtained from Taconic Farms (Germantown, NY). C57BL/6-^{cd4tm1knw} (CD4KO, H-2^b) were obtained from The Jackson Laboratory (Bar Harbor, ME).

Islet Transplantation

Allogeneic BALB/c islet cell grafts were transplanted into 8- to 10-week-old C57BL/6 recipient mice rendered diabetic by a single intraperitoneal (i.p.) injection of streptozotocin (270 mg/kg). Islet cell transplantation was performed as previously described (15). Briefly, islets were isolated from donor BALB/c pancreata through collagenase digestion and centrifugation on a discontinuous Ficoll gradient. The crude islet isolates containing islets, vascular tissue, ductal fragments, and lymph nodes were divided into aliquots of ca. 300

islets and were transplanted under the renal capsule into C57BL/6-^{cd4^{tm1knw}} (CD4KO, H-2^b) recipients. We used these crude islet preparations by intent, as they are not rejected and confer long-term graft survival and function in syngeneic recipients, but are more immunogenic than more highly purified islet preparations in allogeneic recipients (16, 17). Initial allograft function was verified by sequential blood glucose measurements with levels under 200 mg/dL on days three to five after transplantation, and graft rejection was defined as a rise in blood glucose levels exceeding 300 mg/dL following a period of primary graft function.

Treatment Protocol

Murine CTLA4/Fc (18) and human IL-15 mutant/ Fc 2a (12) proteins were constructed and expressed in our laboratory. CTLA4/Fc protein used in these studies bears active FcR binding and complement binding domains (18). Treatment of islet allograft recipients with CTLA4/Fc consisted of 0.1 mg/day i.p. for 10 consecutive days after transplantation which is the optimal treatment period in this model (14). Using a previously established treatment protocol (14,19), islet allograft recipients treated with IL-15 antagonist received 1.5 µg of IL-15 mutant/Fc 2a per day i.p. for 21 consecutive days after transplantation.

Histopathology and Immunohistology

The left kidney bearing the islet graft was removed from the recipients after 12 days and embedded in optimum cutting temperature (OCT) compound (Tissue TCK, Miles Scientific, Elkhart, IN). Cryostat sections of islets (n=3/group) were fixed in paraformaldehyde-lysine-periodate for analysis of leukocyte antigens, and stained by a four-layer peroxidaseantiperoxidase (PAP) method involving overnight incubation with mAb, followed by mouse immunoglobulin (Ig)-absorbed goat anti-rat Ig, rabbit anti-goat Ig, goat PAP complexes and diaminobenzidine substrate. Rat antimouse mAbs and isotype-matched control mAbs, were purchased from BD Biosciences PharMingen (San Diego, CA) and included mAbs to CD4⁺ (H129.19) and CD8⁺ (53–6.7) T cells. Sections were counterstained in hematoxylin and mounted. Isotype-matched mAbs and a control Ab were analyzed for endogenous peroxidase activity in each experiment. Samples were assigned a random number and processed and evaluated in a blinded fashion; each sample was evaluated at five different levels of sectioning. Immunohistochemistry sections were evaluated and the number of CD8-positive cells in five consecutive high-power fields were enumerated, with data expressed as labeled cells per high-power field (mean±SD).

CFSE Labeling and Analysis of T-cell Proliferation In Vivo

Spleen and lymph node cells from wild-type C57BL/6 or CD4^{-/-} mice (C57BF/6, H-2^b) were harvested, processed, and labeled with fluorochrome 5-carboxyfluorescein diacetate succinimidyl ester (CFSE, Molecular Probe Inc., Portland, OR), as described previously (20). CFSE was dissolved in dimethylsulfoxide and added into the cell suspension at a final concentration of 5 nM for three min at room temperature. The reaction was stopped by the addition of Hank's balance salt solution (HBSS)/1% fetal calf serum (FCS). The cells were washed in HBSS/1% FCS and resuspended in the same solution prior to injection. Recipient Balb/c mice were sublethally irradiated (1000 rad with a GammaCell irradiator, Ontario, Canada) prior to injection of 30×10^6 CFSE-labeled cells via the lateral tail vein. Mice were separated in three groups, untreated recipient or recipients receiving an i.p. injection of CTLA4/Fc (0.1 mg/mouse) or IL-15 mutant/Fc- 2a protein (1.5 µg/mouse) for three days. Adoptive transfer experiments using syngeneic CFSE-labeled lymphocytes were also performed as a control. On day three, recipient spleen and lymph node cells were removed and processed as before. Spleen and lymph node cells were analyzed for CD8⁺ T-cell proliferative responses by using a biotinylated mAb against mouse CD8a (53–6.7) and phycoerythrin-conjugated Annexin-V (BD Biosciences PharMingen, San Diego, CA). After

staining, cells were washed once and resuspended in 0.5 mL of HBSS for analysis by flow cytometry using a Becton-Dickinson FACS Sort equipped with CellQuest Software. Live events were collected and analyzed by gating on CD8⁺CFSE⁺ cells.

Calculation of the Frequency of Proliferating T Cells

Analysis of CD8⁺ T cell proliferation in response to alloantigen stimulation was performed according to Noorchashm et al. (21). With each round of cell division, the CFSE dye partitions equally between the daughter cells. By using the FACS acquisition software (CellQuest), the total number of cells in each generation of proliferation can be calculated and the number of precursors that generated the daughter cells was determined by using the following formula: $y/2^n$ (y = absolute number of cells in each peak, n = number of cell division). The calculation of the frequency of T-cell proliferation was then analyzed by dividing the total number of precursors by the total CFSE-labeled cells.

Statistics

Evaluation on the immunohistochemistry sections, and numbers of CD8-positive cells in five consecutive highpower fields were enumerated, with data expressed as labeled cells per high-power field (mean±SD; Fig. 3, and were analyzed using a two-factor analysis of variance (ANOVA) test. $P<0.002$ was defined as significant (Statview 5.1, SAS Institute Inc., Cary, NC and GraphPad Prism 3.02).

RESULTS

Survival of Islet Allografts in CD4^{-/-} C57BL/6 Recipients

We used CD4 T-cell deficient C57BL/6 (H-2^d) recipients in a fully major histocompatibility complex-mismatched Balb/c (H-2^b) islet allograft model to investigate the effect of an IL-15 antagonist on CD8⁺ T-cell mediated alloimmune response. In untreated control group, 50% CD4^{-/-} recipients rejected BALB/c islet allografts at a mean survival time (MST) of 35 days (Fig. 1A). CTLA4/Fc treatment did not prolong islet allograft survival with 60% CD4^{-/-} recipients acutely rejecting the allograft with a MST of 38 days (Fig. 1A). In contrast, a dramatic improvement of allograft survival was noted among CD4^{-/-} recipients treated with the IL-15 mutant/Fc protein as 100% recipients accepted islet allograft indefinitely (MST >120 days; Fig. 1A). Surgical removal of the islet allograft in each of five recipients 150 days after transplantation led to hyperglycemia, demonstrating that the euglycemic state was maintained by the islet allografts. Subsequent to removal of the primary allograft, two IL-15 mutant/Fc-treated recipient mice were successfully engrafted with a second BALB/c islet allograft in the absence of further immunosuppressive treatment, indicating that treatment with the IL-15 antagonist induces peripheral tolerance (Fig. 1B). One untreated and one CTLA4/Fc-treated recipient received a second BALB/c islet allograft in the absence of further immunosuppressive treatment, and allografts were rejected at day 17 and day 27 posttransplantation, respectively (Fig. 1B), which suggests that these recipient mice were not in a tolerant state. As reported earlier and shown in the present study, CD8⁺ driven rejection may be sustained by CTLA4/Fc treatment, as costimulatory blockade may preserve or even increase alloreactive CD8⁺ T-cell responses. In contrast, our results indicate that CD8⁺ T cells, resistant to CTLA4/Fc blockade, may be controlled by an IL-15 antagonist.

T-cell Infiltration in the Allografts

T cell infiltration in the BALB/c donor allografts placed into C57BL/6 CD4^{-/-} recipients was assessed by histological analysis of the allografts. The histological data demonstrates a massive lymphocytic infiltrate in untreated recipients (Fig. 2A), a moderate lymphocytic infiltration in CTLA/Fc-treated recipients (Fig. 2B) and a remarkable decrease of infiltrating

cells in IL-15 mutant/Fc-treated recipients (Fig. 2C) at day 12 posttransplantation. Islet architecture and insulin production were well preserved in recipient mice treated with IL-15 mutant/Fc 2a (Fig. 2C–F) as compared to a less preserved islet architecture but stable insulin production in CTLA/Fc-treated recipients (Fig. 2B–E). Untreated recipients presented a complete destruction of the injected islets and undetectable insulin staining (Fig. 2A–D). In untreated recipients, dense graft infiltration by mononuclear leukocytes, composed predominantly of CD8⁺ T cells, was noted (Fig. 3A)). In recipients treated with CTLA4/Fc (Fig. 3C), a decreased graft infiltration by CD8⁺ T cells was observed, but CD8⁺ staining remained in loci of increased cell infiltrates (CD8-positive cells CTLA4/Fc: 85±9 vs. untreated: 181 ± 10; $P<0.001$). By comparison, the cellular infiltration was dramatically reduced in recipients treated with IL-15 mutant/Fc 2a (Fig. 3B; CD8-positive cells IL-15 mutant/Fc 2a: 50 ± 6 vs. CTLA4/Fc: 85± 9; $P<0.002$). As expected CD4⁺ staining cells were absent in the allograft of CD4 KO recipients (Fig. 3, D and E).

IL-15 Mutant/Fc Treatment Inhibits Alloreactive CD8⁺ T-cell Proliferation In Vivo

To evaluate the proliferation of alloreactive CD8⁺ T cells in vivo, splenic and lymph node leukocytes from C57BL/6 CD4^{-/-} mice were labeled with CFSE. The dye-labeled leukocytes were injected i.v. into irradiated BALB/c mice. The recipient mice were then either left untreated or treated with CTLA4/Fc or IL-15 mutant/Fc 2a for three days. On the third day following adoptive cell transfer, mice were sacrificed and their spleen and lymph nodes recovered and stained with a biotinylated anti-CD8a mAb for further analysis of the proliferative responses by flow cytometry. In untreated hosts, ~30% and 29% of the CFSE-labeled allogeneic CD8⁺ T cells proliferated in the host spleen and lymph nodes, respectively (Fig. 4A and B). CTLA4/Fc treatment did not decrease proliferation of CFSE-labeled alloreactive CD8⁺ T cells in vivo (31% and 29%, respectively). In contrast, treatment with IL-15 mutant/Fc markedly inhibited the proliferation of alloreactive CD8⁺ T cells with only 17 and 13% of the CFSE-labeled CD8⁺ T cells proliferating in vivo (Fig. 4). Using syngeneic controls (CFSE-labeled C57BL/6 CD4^{-/-} lymphocytes injected into C57BL/6 mice) proliferation of CD8⁺ T cells was not detected (data not shown). Control of T cell survival and apoptosis is one of the key events regulating peripheral immune homeostasis. As IL-15 plays an important role in the homeostasis and proliferation of CD8⁺ T cells, we therefore assessed whether IL-15 mutant/Fc would control the proliferation of alloreactive CD8⁺ T cells by promoting their apoptosis. In the above adoptive transfer experiment, we determined apoptotic events through annexin V labeling of alloreactive CD8⁺ T cells. Surprisingly, the decrease in proliferation of alloreactive CD8⁺ T cells observed with IL-15 mutant/Fc treatment (Fig. 4) is not correlated with a relative increase of death events in these cells (Fig. 5A and B). Overall, the data suggests that IL-15 mutant/Fc 2a represents an important therapeutic agent capable of controlling the proliferation of alloreactive CD8⁺ T cells resistant to costimulation blockade action.

DISCUSSION

Analysis of the phenotype of IL-15^{-/-} mice (7) and IL-15R^{-/-} mice (8) demonstrates that IL-15 is a critical factor for innate and adaptive immune responses. Not only does IL-15 chemoattract and promote proliferation of T cells and NK cells (7), but it also selectively induces the homeostasis and proliferation of memory CD8⁺ T cells (6). The role of CD8⁺ T lymphocytes in transplant rejection has been the subject of controversy, but cumulative data is now clearly demonstrating that CD8 lymphocytes, in the absence of CD4 lymphocyte help, are sufficient to promote allograft rejection (22,23). The allogeneic immune response of human CD8⁺ T cells may be induced by different signals including cytokines such as IL-15 (6,24), immune cells such as NK cells (24), and costimulatory molecules such as ICOS, OX40, and PD-L1 (25). CD8⁺ T cells may be responsible for costimulation blockade-

resistant rejection (1, 2, 26), and this resistance could be explained by the poor effect of CTLA4Ig (4, 5) and D 154 Abs (3) in regulating activation and proliferation of CD8⁺ T cells as compared to CD4⁺ T cells (27). Clearly, a rapid deletion of CD8⁺ T cells would be required for allograft survival (4). We have previously reported on the combination of CTLA4/Fc and IL-15 mutant/Fc in a model of allogeneic islet transplantation. As monotherapies, CTLA4/Fc and IL-15 mutant/Fc were comparably effective in a semiallogeneic model system, and combined treatment with these two fusion proteins produced universal permanent engraftment (14).

In this report, we examined the effect of an antagonist for the IL-15 receptor, IL-15 mutant/Fc 2a protein, on the regulation of CD8⁺ T cells alloimmune response on islet allograft survival in animals lacking CD4⁺ alloresponsive T cells. As IL-15 is essential for the homeostasis and proliferation of CD8⁺ T cells and as CD8⁺ T cells are responsible for costimulation blockade-resistant rejection, we hypothesized that targeting the IL-15/IL-15R system might provide a potential therapeutic agent for inducing allograft tolerance (19). In this study, the administration of IL-15 mutant/Fc 2a was compared to that of costimulation blockade with CTLA4/Fc. CTLA4/Fc treatment alone failed to prevent islet allograft rejection. In contrast, prolonged graft survival was evident in IL-15 mutant/Fc 2a treated recipients, as allograft rejection was prevented in 100% of the recipient mice. The beneficial effect of IL-15 mutant/Fc 2a treatment was accompanied by a net decrease in CD8⁺ T cell infiltration in graft tissues. CTLA4/Fc treatment had a weak effect on CD8⁺ T cell infiltration, and more dramatic effects upon graft infiltration of CD4⁺ T cells were reported previously (28). The successful use of soluble IL-15 receptor α -chain proteins as an adjunct to anti-CD4 treatment (29) attests to our hypothesis that blocking both CD4⁺ and IL-15/IL-15R dependent CD8⁺ T cell activation is required to gain long-term graft acceptance.

To assess alloantigen-driven proliferation of CD8⁺ T cells in response to costimulation blockade and IL-15 mutant/Fc 2a, the fate of CD4⁺ and CD8⁺ T cells was studied in vivo using a CFSE-labeled GVH model. The IL-15 mutant/Fc 2a, but not CTLA4/Fc, treatment targets CD8⁺ T cells, as it profoundly inhibits the proliferation of alloreactive CD8⁺ T cells, whereas CTLA4/Fc treatment has no effect on the CD8⁺ T cell proliferative response to alloantigen. Part of the decreased frequency of proliferating CD8⁺ T cells may be ascribed to the proapoptotic effects of treatment with IL-15 mutant/Fc 2a. As IL-15 may promote CD8⁺ T cells and other immune cell reconstitution after allogeneic bone marrow transplantation through a decrease in apoptotic CD8⁺ T cells and an increase in antiapoptotic (Bcl-2) and proliferative (Ki67) proteins (30), IL-15 mutant/Fc 2a administration may have the opposite effects. Zheng et al. (13) have demonstrated that the occurrence of T-cell apoptotic depletion after administration of rapamycin plus a murine antagonist IL-15-and agonist IL-2-related proteins contributes to the inhibition of allogeneic T lymphocyte proliferation. We now confirm previous observations that IL-15 mutant/Fc 2a treatment reduces the frequency of proliferating alloreactive CD8⁺ T cells in an islet allograft model (14), whereas CTLA4/Fc is unable to inhibit the proliferation of alloreactive CD8⁺ T cells in vivo (31). However, in our experiments we did not observe an increase in apoptotic events in alloreactive CD8⁺ T cells, suggesting that a simple lack of IL-15 signaling may not be sufficient to increase T cell apoptosis in this model, and emphasizing the role of IL-2 in stimulating activation-induced cell death (32). The resistance of CD⁺ T cells to costimulation blockade is related to the inability of CTLA4/Fc (31) treatments to control alloactivated CD8⁺ T cells, and is probably related to the restricted expression of CD28 on these cells (33).

Among the immune cells actively involved in the balance between graft rejection or tolerance, NK lymphocytes play a crucial role as they are involved in the crosstalk between innate and adaptive immune system. As immunoregulatory cells, NK lymphocytes are responsible for mediating graft-versus-leukemia (GvL) reactions but do not participate in,

and are even able to prevent the development of GVH disease. NK cells have also been described as key player in the development of islet allograft tolerance (34). IL-15 is capable of inducing proliferation and activation of NK cells, and plays a crucial part in NK-cell development (7, 35), as demonstrated by the quasi-absence of NK cells in IL-15R^{-/-} mice (IL-15R^{-/-}) (8). IL-15 also induces the expression of a number of natural killer receptors on NK cells (Ly-49, CD94, NKG2) and improves adherence and proliferation of human NK cells. However, NK cells from IL-15R^{-/-} mice can proliferate and mature in the presence of IL-7, flt3, and IL-15 suggesting that other factors can counteract IL-15 or that the α and β chain of the IL-15R are sufficient (36). These reports may explain why, in mice treated with the IL-15 antagonist we did not observe any decrease of NK cell proliferation in an adoptive transfer model (data not shown). From our observation, we can also suspect that blocking the IL-15/IL-15R pathway has no influence on the mature NK cells.

So far, classical immunosuppressive regimens do not lead to alloimmune tolerance and great hopes are placed in new therapeutic agents, such as costimulation blockade agents, as they provide a powerful inhibition of alloimmune responses in both rodents and primates (37, 38). Although many therapeutic strategies focused on inhibiting CD4⁺ allorecognition pathways, they may neglect the involvement of CD4 independent CD8⁺ T cell activity in organ transplant rejection. The findings reported here re-emphasize the role of CD8⁺ T cells and IL-15/IL-15R pathway as important components of the rejection process. By targeting IL-15/IL-15R⁺ cells to prevent allograft rejection mediated by CD8⁺ T cells, IL-15 mutant/Fc γ 2a may be a promising tool capable of blocking transplant rejection, particularly when CD8⁺ T cells contribute to the allograft rejection process. Therefore, the addition of a new immunosuppressive strategy to well-established immunosuppressive agents in order to control CD8⁺ T cell alloimmune response and resistance to costimulation blockade may play an important role in our goal to induce permanent tolerance.

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FIGURE 1.

Islet allograft survival in CD4^{-/-} C57BL/6 recipient mice. Islets from BALB/c (H-2^d) donors were transplanted under the renal capsule of CD4^{-/-} C57BL/6 recipient mice. Recipients were treated with CTLA4/Fc (0.1 mg) or IL-15 mutant/Fc 2a (1.5 μ g) as indicated in Materials and Methods. Untreated recipients () and CTLA4/Fc treated recipients () rejected allografts with an MST of 35 and 38 days, respectively. Graft rejection was significantly delayed in recipients treated IL-15 mutant/Fc 2a, with an MST of 120 days ().

FIGURE 2.

Histological assessment of islet allografts. Serial kidney sections were prepared 12 days after islet allograft transplantation. H&E staining showed high-grade cellular rejection with dense tissue infiltration by mononuclear cells in untreated recipients (A). Recipients receiving CTLA4/Fc evidenced a decreased tissue infiltration with some islets preserved (B). Recipients treated with IL-15mutant/Fc 2a present a rather normal histology with few mononuclear cell infiltrates and intact islets (C). Insulin staining is absent in untreated recipients (D), a result of complete destruction of the islets. In contrast, both CTLA4/Fc and IL-15 mutant/Fc 2a treated recipients maintained a high level of insulin production (E and F, respectively) (Cryostat sections, hematoxylin counterstain, $\times 100$ magnifications). These are representative sections from two allografts from each treatment group.

FIGURE 3.

Immunohistology evaluation of islet allografts. Serial kidney sections were isolated 12 days after islet allograft transplantation. Sections were evaluated and the number of CD8-positive cells in five consecutive high-power fields were counted and expressed as cells per high-power field (mean $\pm$ SD). Untreated recipients show dense tissue infiltration surrounding and invading the islets with a predominance of CD8⁺ T cells (A). Recipients treated with CTLA4/Fc show a decreased interstitial lymphocytic infiltrate with preservation of the tissue structure (B). Recipient mice receiving IL-15 mutant/Fc 2a present moderate CD8⁺ T cell infiltrates (C). Treatment with IL-15 mutant/Fc 2a prevents CD8 T cell infiltrates (CD8-positive cells IL-15 mutant/Fc 2a: 50 $\pm$ 6 vs. untreated: 181 $\pm$ 10; P <0,0001; CD8-positive cells IL-15mutant/ Fc 2a: 50 $\pm$ 6 vs. CTLA4/Fc: 85 $\pm$ 9; P <0.002). As expected, CD4⁺ T cells were undetectable in the grafts (D–F) (Cryostat sections, hematoxylin counterstain, $\times$ 100 magnification). These are representative sections from three recipients from each treatment group.

Figure 4.

In vivo proliferation of alloreactive CD8⁺ T cells. CFSE-labeled CD4^{-/-} C57BL/6 lymphocytes were injected into the lateral vein of irradiated BALB/c recipient mice. Representative histograms of proliferative responses of allogeneic CD8⁺ T cells from the host spleen (A) and the host lymph nodes (B) are shown. Mice were left untreated or treated with CTLA4/Fc (0.2 mg) or IL-15 mutant/Fc 2a (1.5 μ g) for three days. The mean frequency of proliferative alloreactive CD8⁺ T cells (R_F) from the host spleen (A) are 24.5 ± 6 for untreated mice, 25.75 ± 4.5 for CTLA4/Fc treated mice, and 14.5 ± 3 for IL-15 mutant/Fc 2a treated mice. The mean frequency of proliferative alloreactive CD8⁺ T cells (R_F) the host lymph nodes (B) are 25.5 ± 4 for untreated mice, 20 ± 5 for CTLA4/Fc treated mice and 10 ± 2.5 for IL-15 mutant/Fc 2a treated mice. Data is represented as the frequency of alloreactive CD8⁺ T cells (R_F) responding to antigens by proliferation. Data is representative of three identical and independent experiments.

Figure 5.

In vivo expression of annexin V on alloreactive CD8⁺ T cells, CFSE-labeled CD4^{-/-} C57BL/6 lymphocytes were injected into the lateral vein of irradiated BALB/c recipient mice. Representative histograms of annexin V expression by proliferating allogeneic CD8⁺ T cells (annexin V⁺ CD8 T cells) from the host spleen (A) and the host lymph nodes (B) are shown. The histograms shown are profiles for annexin V⁺ expression on gated CFSE⁺ CD8⁺ T cells (bold red lines) and profiles for proliferating alloreactive CD8⁺ T cells (gated on CFSE⁺ cells, black dotted lines). Mice were left untreated or treated with CTLA4/Fc (0.2 mg) or IL-15 mutant/ Fc 2a (1.5 μg) for three days. Data is represented as the frequency of proliferative alloreactive CD8 T cells (R_F) expressing annexin V on their cell surface. Data is representative of two additional identical experiments.