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An Antagonist IL-15/Fc Protein Prevents Costimulation Blockade-Resistant Rejection

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IL-15 is a powerful T cell growth factor (TCGF) with particular importance for the maintenance of CD8+ T cells. Because costimulation blockade does not result in universal tolerance, we hypothesized that “escape” from costimulation blockade might represent a CD8+ and IL-15/IL-15Rα-dependent process. For this analysis, we have used an IL-15 mutant/Fcγ2a protein, a potentially cytolytic protein that is also a high-affinity receptor site specific antagonist for the IL-15Rα receptor protein, as a therapeutic agent. The IL-15-related fusion protein was used as monotherapy or in combination with CTLA4/Fc in murine islet allograft models. As monotherapies, CTLA4/Fc and an IL-15 mutant/Fcγ2a were comparatively effective in a semiallogeneic model system, and combined treatment with IL-15 mutant/Fcγ2a plus CTLA4/Fc produced universal permanent engraftment. In a fully MHC-mismatched strain combination known to be refractory to costimulation blockade treatment, combined treatment with both fusion proteins proved to be highly effective; >70% of recipients were tolerated. The analysis revealed that the IL-15 mutant/Fc treatment confers partial protection from both CD4+ and CD8+ T cell graft infiltration. In rejections occurring despite CTLA4/Fc treatment, concomitant treatment with the IL-15 mutant/Fcγ2a protein blocked a CD8+ T cell-dominated rejection processes. This protection was linked to a blunted proliferative response of alloreactive T cells as well silencing of CTL-related gene expression events. Hence, we have demonstrated that targeting the IL-15/IL-15R pathway represents a new and potent strategy to prevent costimulation blockade-resistant CD8+ T cell-driven rejection. The Journal of Immunology, 2001, 167: 3478–3485.
may be mediated by CD8 T cells and IL-15 is essential for the homeostasis and proliferation of CD8 T cells, we have now tested the hypothesis that targeting the IL-15/IL-15R system in concert with costimulation blockade might provide a new tool for the induction of allograft tolerance.

The trimolecular IL-15R expressed upon T cells includes the constitutively expressed IL-2Rβ and IL-2R common γ-chains shared with the IL-2R. A unique IL-15Rα chain (25, 26) is expressed upon activated, but not resting, mononuclear leukocytes (27). The IL-15Rs is expressed in lesser abundance upon activated epithelial cells, macrophages, and fibroblasts (24) We have previously reported on the specificity of a unique IL-15 mutant/Fcγ2a protein possessing a prolonged circulating half-life, high-affinity IL-15Rα site-specific antagonist function and both complement- and Fc-related cytoidal potential against IL-15Rα+ cells (28). Kim et al. (28) have demonstrated that this IL-15 mutant/Fc fusion protein specifically binds to the IL-15Rα; IL-15 mutant/Fcγ2a binding to activated T cells was blocked by IL-15, but not by IL-2 or anti-IL-2/IL-15Rγ chain Abs. In this report, we also demonstrated that this IL-15 mutant/Fc protein efficiently blocks delayed-type hypersensitivity in mice (28). In the present report, the therapeutic activity of the IL-15-related protein was compared with that of a CTLA4/Fc. CTLA4/Fc treatment is known to promote engraftment and often leads to tolerance (5, 29). Combined treatment with CTLA4/Fc and IL-15 mutant/Fc proteins was also studied. Our data suggest that IL-15Rα cells are an important component of allograft rejection and these cells play a pivotal role in CD8 T cell-mediated CTLA4/Fc blockade-resistant rejection.

Materials and Methods

Animals

BALB/c (H-2d), DBA/2 (H-2b), B6AF1 (H-2b/d), and C57BL/6 (H-2b) mice, 8–10 wk old, were obtained from Taconic Farms (Germantown, NY). C57BL6/129sc2m1(CDK4KO, H-2b) and C57BL6/129sc2m1(CDK8KO, H-2d) were obtained from The Jackson Laboratory (Bar Harbor, ME).

Islet transplantation

Allogeneic DBA/2 islet cell grafts were transplanted into 8- to 10-wk-old B6AF1 recipient mice rendered diabetic by a single i.p. injection of streptozotocin (225 mg/kg; Sigma, St Louis, MO). Allogeneic BALB/c islet cell grafts were transplanted into 8- to 10-wk-old C57BL/6 recipient mice rendered diabetic by a single i.p. injection of streptozotocin (270 mg/kg). Islet cell transplantation was performed as previously described (30). Briefly, islets were isolated from donor DBA/2 or 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 ~300 islets and were transplanted under the renal capsule into B6AF1 or C57BL/6 recipients. By intent, we use these crude islet preparations which are more immunogenic than more pure islet preparations (our unpublished observations). Initial allograft function was verified by sequential blood glucose measurements with levels under 200 mg/dl on days 3–5 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 (31) and human IL-15 mutant/Fcγ2a (28) proteins were constructed and expressed in our laboratory. CTLA4/Fc protein used in these studies bears active FcR binding and complement-binding domains (29). 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 (our unpublished data). Islet allograft recipients received 1.5 μg of IL-15 mutant/Fcγ2a/day i.p. for 21 consecutive days after transplantation. An IgG2a protein, bearing the same Fc sequence as the CTLA4/Fc and IL-15 mutant/Fcγ2a proteins, was used as a control treatment at 1.5 μg/day for 10 days.

Histopathology and immunohistology

The left kidney bearing the islet graft was removed from the recipients after 8 days and embedded in 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 Ags and stained by a four-layer peroxidase-antiperoxidase method involving overnight incubation with mAb, followed by mouse Ig-adsorbed goat-anti-rat Ig, rabbit anti-goat Ig, goat peroxidase-antiperoxidase complexes, and diaminobenzidine substrate. Rat anti-mouse mAbs and isotype-matched control mAbs were purchased from BD PharMingen (San Francisco, 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 two to three different levels of sectioning.

PCR analysis

Intragraft mRNA analysis was performed via template RT-PCR as previously described (16). The specific primers used for hybridization to murin IL-2, TCR Cγ, perforin, Fas ligand (FasL), B, and GAPDH cDNA, the latter as an internal control, have been previously described (16). The PCR amplification was performed in a thermocycler (Gene Amp, PCR system 2400; PerkinElmerCetus, Norwalk, CT); denaturing at 94°C for 30 s, annealing at 57°C for IL-2 and FasL, at 60°C for perforin and FasL, at 60°C for TCR Cγ, and at 57°C for GAPDH for 30 s, and extension at 72°C for 30 s for each cycle, for a total of 40 cycles. A negative control was included for each PCR amplification and consisted of the omission of cDNA in the PCR mixture. To further confirm data, a semiquantitative competitor template RT-PCR analysis was also performed with IL-2 using the gene-specific relative RT-PCR kit from Ambion (Austin, TX). To quantitate the relative amounts of TCR Cγ gene transcripts between samples, each PCR was performed by coamplifying the cDNA of interest with an internal PCR control. A gene-specific competitive template cDNA was designed and the semiquantitative PCR was performed as described by Steiger et al. (16). After amplification, the samples (10 μl) were separated on ethidium bromide-stained 1.5% agarose gel and the DNA was visualized and photographed using UV transilluminator (Gel Doc 1000; Bio-Rad, Hercules, CA).

CFSE labeling and analysis of T cell proliferation in vivo

Spleen and lymph node cells from wild-type C57BL6, CD8−/−, or CD4−/− mice (C57BL6, H-2b) were harvested, processed, and labeled with CFSE (Molecular Probes, Portland, OR) as described previously (32). Cells were incubated in DSMO and added into the cell suspension at a final concentration of 5 μl for 3 min at room temperature. The reaction was stopped by the addition of HBSS/1% FCS. The cells were washed in HBSS/1% FCS and resuspended in the same solution before injection. Recipient BALB/c mice were sublethally irradiated (1000 rad with a GammaCell irradiator, Kanata, Ontario, Canada) before injection of the CFSE-labeled cells via the lateral tail vein. From that day, untreated recipient mice or mice receiving an i.p. injection of CTLA4-4Fc (0.1 mg/mouse) or IL-15 mutant/Fcγ2a protein (1.5 μg/mouse) for 3 days were studied. Adoptive transfer experiments using syngeneic CFSE-labeled lymphocytes was also performed as a control. On day 3, recipient spleen and lymph node cells were removed and cell suspensions were processed as before. Cells were stained with anti-CD4-PE conjugate (L3T4, 2 μg/ml) and anti-CD8a (53-6.7, 2 μg/ml; BD PharMingen) for 30 min at 4°C. After staining, cells were washed once and resuspended in 0.5 ml of HBSS for analysis by flow cytometry using a BD Biosciences FACSort equipped with CellQuest software (Mountain View, CA). Live cells were collected and analyzed by gating on CD4+ or CD8+ CFSE− cells.

Calculation of the frequency of proliferating T cell

Analysis of CD4+ and CD8+ T cell proliferation in response to alloantigen stimulation was performed according to Noorshash et al. (33). 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 = n (n = 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.
performed on transplantation. Nephrectomy of the left kidney was
third part islet allograft (B10. BR, H-2k; F) ing a state of tolerance (F).
One mouse received a second transplant for more than 50 days, demonstrat-
ing a state of tolerance (F). All of them accepted the capsule (following the same procedure) without fur-
second BALB/c islet allograft under the right renal
tation. Then four nephrectomized mice received a
grafts were transplanted into B6AF 1 (H-2 b/d.k ) mice. Untreated
whereas in recipients receiving the combined treat-
ment, the MST was of 120 days (F).
Results
Survival of islet allografts in B6AF 1 and C57BL/6 recipients
To probe for the role of the IL-15/IL-15R network in the allograft
response, partially MHC-mismatched DBA/2 (H-2 b) islet allo-
grafts were transplanted into B6AF 1 (H-2 b/d.k) mice. Untreated
recipients rejected DBA/2 islet allografts with a mean survival
time (MST) of 14 days (Table I) while B6AF 1 recipient mice
with treated with CTLA4/Fc or IL-15 mutant/Fc had a MST of 70
and 77 days, respectively. All B6AF 1 recipients of DBA/2 islet
allografts treated with a combination of IL-15 mutant/Fcγ2a and
CTLA4/Fc were permanently engrafted. Surgical removal of the
left kidney bearing the islet allograft was performed on two recipients
150 days after transplantation. Six days later, a second
DBA/2 islet allograft was successfully engrafted without rejection
in the absence of further immunosuppressive therapy.
To test the hypothesis that targeting the IL-15/IL-15R system
would block CD8 + -driven CTLA4/Fc-resistant rejection, fully
MHC-mismatched BALB/c (H-2 d) donor islet allografts
were transplanted into C57BL/6 (H-2 b) recipient mice. In this model,
the recipients are refractory to the tolerizing effects of optimal
doses of CTLA4/Fc. Untreated C57BL/6 recipients rejected
BALB/c islet allografts (MST, 20 days; Fig. 1 a). As additional
controls, C57BL/6 recipients were also treated with a control
IgG2a protein (1.5 μg/ml). In other experiments, C57BL/6 received
CTLA4/Fc (0.1 mg/ml) plus IgG2a (1.5 μg/ml). Recipients
treated with IgG2a rejected rapidly their allografts (MST, 14 days;
Fig. 1 a), and the recipients treated with CTLA4/Fc and IgG2a
rejected allografts at the same tempo as recipients treated with
CTLA4/Fc alone (Fig. 1 a). These data confirm that the prolonged
engraftment observed with IL-15 mutant/Fcγ2a fusion protein
treatment is not produced solely by Fc sequences shared with
IgG2a. Engraftment was prolonged in C57BL/6 recipients treated
with CTLA4/Fc or with IL-15 mutant/Fcγ2a protein (MST, 30
days; Fig. 1 a), some grafts functioned permanently following
monotherapy with either agent. A dramatic improvement was
noted among C57BL/6 recipients treated with a combination of
IL-15 mutant/Fcγ2a and CTLA4/Fc (MST 120 days; Fig. 1 a). Sur-
gical removal of the islet allograft in each of five recipients 150

FIGURE 1. Survival of islet allografts with a combined treatment of CTLA4/Fc and IL-15 mutant/
Fcγ2a. a. Islets from BALB/c (H-2 d) donors were transplanted under the renal capsule of C57BL/6 (H-2 b)
recipients. Recipients were treated with CTLA4/Fc (0.1 mg), IL-15 mutant/Fcγ2a (1.5 μg), or a combination of both proteins as indicated in Materials and Methods. Untreated recipients (□) or recipients treated with a control IgG2a protein (1.5 μg) rejected allografts with a MST of 15 days and 14
days, respectively (□). Graft rejection was delayed by 30 days in recipients treated with CTLA4/Fc (○),
CTLA4/Fc plus IgG2a (●), or IL-15 mutant/Fcγ2a (○), whereas in recipients receiving the combined treatment,
the MST was of 120 days (●). b. Second islet transplantation. Nephrectomy of the left kidney was
performed on five recipients 150 days after transplantation. Then four nephrectomized mice received a
second BALB/c islet allograft under the right renal capsule (following the same procedure) without fur-
ther immunosuppression. All of them accepted the second transplant for more than 50 days, demonstrat-
ing a state of tolerance (●). One mouse received a third part islet allograft (B10. BR, H-2 b; ○) which
was rejected at day 12, confirming that the immune response is intact.

Table I. Survival of DBA/2 islet allografts in B6AF 1 recipients treated with CTLA4/Fc and IL-15 mutant/Fc

<table>
<thead>
<tr>
<th>Donor</th>
<th>Recipient</th>
<th>Treatment</th>
<th>Islet Graft Survival (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DBA/2</td>
<td>B6AF 1</td>
<td>Untreated</td>
<td>11, 11, 11, 12, 13, 13, 15, 17, 17, 20</td>
</tr>
<tr>
<td>DBA/2</td>
<td>B6AF 1</td>
<td>CTLA4/Fc</td>
<td>20, 31, 34, 51, &gt;120, &gt;120, &gt;120, &gt;120</td>
</tr>
<tr>
<td>DBA/2</td>
<td>B6AF 1</td>
<td>IL-15m/Fc</td>
<td>15, 22, 29, 32, &gt;120, &gt;120, &gt;120, &gt;120</td>
</tr>
<tr>
<td>DBA/2</td>
<td>B6AF 1</td>
<td>CTLA4/Fc + IL-15m/Fc</td>
<td>&gt;120, &gt;120, &gt;120, &gt;120</td>
</tr>
</tbody>
</table>

* Treatments: CTLA4/Fc, 0.1 mg/day, i.p. from days 0 to 11 posttransplantation; IL-15m/Fc, 1.5 μg/day, i.p. from days 0 to 21 posttransplantation.
days after transplantation led to hyperglycemia, demonstrating that the euglycemic state was maintained by the islet allografts. Subsequent to removal of the primary allograft, four recipient mice were successfully engrafted with a second BALB/c islet allograft in the absence of further immunosuppressive treatment (Fig. 1b). One recipient received a third-party islet allograft (B10.BR, H-2k) that was rejected at day 12 posttransplantation (Fig. 1c). This result is in keeping with the hypothesis that costimulation-resistant rejection is a CD8+ T cell process (Fig. 1d). By comparison (and as anticipated), a decrease in CD8+ T cells was not detected in untreated mice (Fig. 1d). This result is in keeping with the hypothesis that costimulation-resistant rejection is a CD8+ T cell process. In contrast, in recipients treated with IL-15 mutant/Fcγ2a (Fig. 3a, e, and f), a decrease in CD8+ T cell infiltration without a major change in CD4+ T cell infiltration was evident. Note that recipient mice treated with both CTLA4/Fc and IL-15 mutant/Fcγ2a (Fig. 3g and h) show a near absence of CD4+ and CD8+ graft-infiltrating T cells. The effect of treatment upon intragraft IL-2 and TCR Cβ gene expression was analyzed by RT-PCR at day 8 posttransplantation. As the constant chain of TCR Cβ is constitutively expressed by T cells, we observed that CTLA4/Fc treatment inhibits alloreactive proliferating CD8+ T cells in vivo. To determine whether treatment with the IL-15 mutant/Fcγ2a protein blunts the proliferation of alloreactive CD8+ T cells in vivo, we labeled splenic lymphocytes from C57BL/6 CD4-/- mice with CFSE. The dye-labeled C57BL/6 CD4-/- lymphocytes were injected i.v. into irradiated untreated or treated (for 3 days) BALB/c mice. On the third day following adoptive cell transfer, CFSE-stained leukocytes from hosts were recovered and stained with a biotinylated anti-CDF mAb. Since CFSE partitions equally between daughter cells following cell division, the pattern and frequency of proliferation of alloreactive CD8+ T cells in vivo can be analyzed via the CFSE staining pattern (31). In untreated hosts, ~23% of CFSE-labeled allogeneic CD8+ T cells proliferated in the host spleen (Fig. 5b). In CTLA4/Fc-treated hosts, CD8+ T cells (~21%) divide for multiple generations (Fig. 5b). In contrast, treatment with IL-15 mutant/Fcγ alone or in combination with CTLA4/Fc markedly inhibited proliferation of alloreactive CD8+ T cells with only 15 and 14%, respectively, of CFSE-labeled CD8+ T cells proliferating in vivo (Fig. 5b). 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). By contrast, when similar experiments were performed with splenic lymphocytes from C57BL/6 CD8-/- mice to analyze the fate of CD4+ T cells, we observed that CTLA4/Fc treatment markedly decreased the frequency of proliferating
CFSE-labeled alloreactive CD4⁺ T cells in vivo (Fig. 5a). Treatment with IL-15 mutant/Fcγ2a reduces the frequency of proliferating CD4⁺ and CD8⁺ T cells, although a lesser effect on CD4⁺ T cells as compared with CD8⁺ T cells was noted. Similar results were observed when wild-type C57BL/6 mice were used as donor (data not shown). IL-15 mutant/Fcγ2a may represent an important therapeutic agent capable of controlling the proliferation of alloreactive CD8⁺ (costimulation-resistant) T cells. Proliferation of CD4⁺, but not CD8⁺, T cells is controlled by B7 blockade agents.

Discussion

T cell costimulation blockade treatment with CTLA4/Fc or Abs against CD154 have often produced permanent engraftment and donor-specific tolerance (5–8). Unfortunately, recent reports have underlined the importance of activated CD8⁺ T cells in a costimulation-resistant rejection process observed in various transplant models such as skin, intestine, and heart (11–15). It is notable that costimulatory blockade prolongs allograft survival in CD8 KO mice (11, 14) because in those models in which tolerance is not produced by costimulation blockade, tolerance can be achieved with provision of anti-CD8 mAb or other treatments that clear alloreactive CD8⁺ T cells (12–15). Apparently costimulatory blockade-resistant activation of alloreactive CD8⁺ T cells can cause allograft rejection.

Since the molecular targets of costimulation blockade are primarily expressed upon CD4⁺, and not upon CD8⁺ T cells, the reason that CD8⁺ T cells play a prominent role in costimulation blockade escape rejection process is enigmatic. Studies using IL-15⁻/⁻ mice and IL-15Rα⁻/⁻ mice (19, 20) have emphasized the role of IL-15 in support of the activation and maintenance of CD8⁺ T cells (22). Because costimulation blockade results in diminished expression of IL-2 by CD4⁺ T cells (7, 9), we hypothesized that escape from costimulation blockade might represent an IL-15/IL-15R-dependent process. Moreover, costimulation blockade does not target epithelial cells, the principle source of IL-15 (23). Thus, we wanted to study the effect of IL-15 mutant/Fcγ2a on an islet allograft model and its potential to enhance the effectiveness of costimulatory blockade.

For our experiments, we first chose a partial MHC-mismatch combination (H-2ᵈ/H-2ᵇ/d.k) to examine the effect of the IL-15 mutant/Fcγ2a. In this islet allograft model, the administration of IL-15 mutant/Fcγ2a produces permanent engraftment and tolerance in 50% of the recipient mice (Table I). Identical results were obtained with CTLA4/Fc administration. It is notable that combined treatment with CTLA4/Fc and IL-15 mutant/Fcγ2a produced permanent engraftment and tolerance in all recipients. Next, we chose to study a fully MHC-mismatched strain combination and used a recipient strain (C57BL/6) that is refractory to costimulation blockade therapy (15). A modest beneficial effect of CTLA4/Fc treatment was observed (MST, 30 days vs 13 days in the controls; Fig. 1a) and
performed and the results are as follows: untreated mice: 0.733 ± 0.25, 0.362 ± 0.15, and 2.72 ± 0.57, respectively; CTLA4/Fc-treated mice: 0.98 ± 0.71, 0.56 ± 0.27, and 1.09 ± 0.24, respectively; IL-15 mutant/Fc-treated mice: 0.094 ± 0.09, 0.03 ± 0.03, and 0.13 ± 0.15, respectively; CTLA4/Fc plus IL-15 mutant/Fc-treated mice: 0.123 ± 0.15, 0.017 ± 0.02, and 0, respectively. Molecular weight markers are in the left part of the gel.

Prolonged graft survival was evident in IL-15 mutant/Fc2a-treated recipients (Fig. 1a). Treatment with a control IgG2a protein did not prolong engraftment (MST, 14 days). Furthermore, combined treatment with CTLA4/Fc and IL-15 mutant/Fc2a therapy. Permanent engraftment was obtained in most treated recipients (Fig. 1b). Further analysis demonstrated that these permanently engrafted mice were rendered specifically tolerant to donor strain grafts (Fig. 1b). CTLA4/Fc treatment had a far more dramatic effect upon CD4+ T cell infiltration into the graft than upon CD8+ T cell infiltration. While the principal effect of IL-15 mutant/Fc2a treatment in the combined CTLA4/Fc plus IL-15 mutant treatment group was exerted upon infiltration of CD8+ T cells into the graft (Fig. 3). The prolongation of islet allograft survival in recipient mice receiving both CTLA4/Fc and IL-15 mutant/Fc2a was accompanied by a decrease in T cell infiltration in graft tissues (Fig. 2). Immunohistologic analysis suggests that the potential benefit of a combined treatment is related to the drastic decrease of tissue infiltration by both allorreactive CD4+ and CD8+ T cells (Fig. 3). Molecular markers for activated CD8+ T cells, such as CTL genes, have been identified within the infiltrate of rejecting allografts (28), and expression of these CTL genes (granzyme B, FasL, perforin) has been associated with acute renal allograft rejection (18). It is thus interesting to observe that the expression of CTL genes are markedly decreased in IL-15 mutant/Fc2a-treated mice (Fig. 4), further attesting to the effect of IL-15 mutant/Fc2a treatment upon alloactivated CD8+ T cells.
To further analyze the effect of costimulation blockade and IL-15 mutant/Fcy2a, we studied alloantigen-driven proliferative responses in vivo using the CFSE dye system. CTLA4/Fc treatment has no effect on the CD8+ T cell proliferative response to alloantigen, whereas IL-15 mutant/Fcy2a has a potent inhibitory effect on the proliferation of alloreactive CD8+ T cells (Fig. 5). Because IL-15 is of essential importance for the homeostasis and proliferation of CD8+ T cells (22, 23), we cannot exclude the possibility that IL-15 mutant/Fcy2a treatment decreases the frequency of proliferating CD8+ T cell by promoting apoptosis of these responder cells. In contrast, the alloantigen-driven response of CD4+ T cells was decreased by either CTLA4/Fc or IL-15 mutant/Fcy2a monotherapy (Fig. 5b). The effect of CTLA4/Fc upon CD4+ T cells is particularly potent (Fig. 5a). The IL-15 mutant/Fcy2a, but not CTLA4/Fc, treatment targets CD8+ T cells (Fig. 5). Consequently combined IL-15 mutant/Fcy2a plus CTLA4/Fc treatment exert additive immunosuppressive effects in controlling allograft rejection (Fig. 5 and Table I).

We now confirm and extend previous observations that costimulation blockade treatment reduces the frequency of proliferating alloreactive CD4+ T cells in an allograft model (34), but is unable to inhibit the proliferation of alloreactive CD8+ T cells in vivo (13). The resistance of CD8+ T cells to costimulation blockade is related to the inability of CTLA4/Fc (35, 36) and anti-CD154 mAb (36) treatments to control alloactivated CD8+ T cells and is probably related to the restricted expression of CD28 and CD154 to CD8+ T cells (37). To obtain prolonged engraftment of heart allografts, treatment with a nondepleting anti-CD4 mAb alone is not sufficient. The successful use of soluble IL-15 receptor α-chain proteins as an adjunct to anti-CD4 treatment (19) is consistent with our hypothesis that blocking both CD4+ and IL-15/IL-15R-dependent CD8+ T cell activation is required to gain long-term graft acceptance.

IL-15 mutant/Fcy2a treatment mediates important effects on allograft survival that are exerted, at least in part, through the inhibition of the activation and proliferation of alloreactive CD8+ T cells (Figs. 3–5 and Table I). This finding emphasizes the role of the IL-15/IL-15R pathway as an important element in the rejection process. Moreover, we have recently noted that the initial five to six waves of the T cell proliferation to alloantigens in vivo are IL-15, not IL-2, dependent (38). The induction of permanent tolerance is still an elusive goal in clinical organ transplantation. By targeting IL-15/IL-15R+ cells to prevent costimulation blockade-resistant rejection, IL-15 mutant/Fcy2a appears to provide a promising new agent capable of interrupting allograft rejection mediated by CD8+ T cells. The combined effects of costimulation blockade plus targeting the IL-15/IL-15R pathways to target both CD4+ and CD8+ T cells may represent an effective approach to achieve permanent tolerance.

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