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Combining Autologous Dendritic Cell Therapy with CD3 Antibodies Promotes Regulatory T Cells and Permanent Islet Allograft Acceptance

Marije C. Baas,*†,1 Chantal Kuhn,*†,2 Fabrice Valette,*† Claire Mangez,*† Mercedes Segovia Duarte,‡,3 Marcelo Hill,‡,3 Alix Besançon,*† Lucienne Chatenoud,*† Maria-Cristina Cuturi,‡ and Sylvaine You*†

Cell therapy and the use of mAbs that interfere with T cell effector functions constitute promising approaches for the control of allograft rejection. In the current study, we investigated a novel approach combining administration of autologous tolerogenic dendritic cells with short-term treatment with CD3-specific Abs. Permanent acceptance of pancreatic islet allografts was achieved in mice treated with the combination therapy the day before transplantation but not in recipients treated with either therapy alone. The combination treatment induced a marked decrease in T cells infiltrating the allografts and a sustained reduction of antidonor responses. Importantly, CD4+Foxp3+ regulatory T cells appeared to play a crucial role in the long-term graft acceptance. Their frequency increased significantly in the spleen, draining lymph nodes, and transplanted islets and remained elevated over the long term; they exhibited increased donor-specific suppressive functions; and their removal at the time of transplantation abrogated the therapeutic effect of the combined therapy. These results support the therapeutic potential of protocols combining autologous dendritic cells and low-dose CD3 Abs, both currently in clinical development, and that act in synergy to control allogeneic immune responses and favor graft survival in a full-mismatch situation. The Journal of Immunology, 2014, 193: 000–000.

A major goal of transplantation research is the development of novel therapeutic approaches that allow permanent acceptance of the graft in the absence of continuous immunosuppressive therapy. Promising therapeutic tools include cell therapy and biological agents, such as mAbs directed against key molecules involved in effector lymphocyte activation. Major efforts were devoted to the production and characterization of dendritic cells (DCs) endowed with tolerogenic potential (1, 2). Several protocols have been proposed to generate in vitro tolerogenic DCs using specific culture conditions, pharmacologic treatment (exposure to immunosuppressive drugs or cytokines), or genetic manipulation (3–9). These DCs can be of donor or recipient origin, and their capacity to modulate alloimmune responses and induce tolerance was demonstrated in various experimental transplant models (5, 9–14). The mode of action of these tolerogenic DCs resides in their ability to induce T cell hyporesponsiveness to the alloantigens and/or to favor the expansion/generation of CD4+ Foxp3+ regulatory T cells (Tregs) (15–18).

Using a fully mismatched rat model of heart transplantation, we previously demonstrated that autologous tolerogenic DCs (ATDCs) injected the day before transplantation induced a significant prolongation of cardiac allograft survival that was associated with decreased antidonor humoral and cellular responses (9). These ATDCs were generated from rat bone marrow progenitors cultured with low doses of GM-CSF and IL-4 and corresponded to the adherent population exhibiting an immature phenotype. In contrast to the classical nonadherent bone marrow DCs, they were unable to stimulate allogeneic T cells in vitro and efficiently inhibited MLCs (9). We also showed that by combining these ATDCs with suboptimal treatment with LF 15-0195, a potent and less toxic derivative of the immunosuppressive drug 15-deoxyspergualine, whose main mode of action was reported to be the inhibition of DC maturation in vivo, we were able to promote transplantation tolerance (19–22). Recently, we showed in a minor mismatched skin allograft model in mice that the combined administration of ATDCs and a short course of a CD3-specific mAb prolonged allograft survival (23).

To implement these results, in this study we proposed to test the protocol based on administration of ATDCs combined with CD3 Abs in a fully mismatched pancreatic islet allograft model. CD3 Abs are potent immunosuppressants that were used in renal transplantation medicine in the 1980s to prevent and/or treat acute rejection (24, 25). More importantly, these Abs are also able to induce tolerance in the experimental autoimmune and the transplant settings (26–28). In this article, we show that CD3 Abs
synergize with ATDCs to promote CD4\(^+\)Foxp3\(^+\) Tregs and to switch the balance from rejection toward long-term acceptance of fully mismatched grafts.

**Materials and Methods**

**Mice**

C57BL/6, C57BL/6 Foxp3\(^{GFP}\), congenic CD45.1 C57BL/6, and BALB/c mice were bred in our animal facility under specific pathogen–free conditions. Blood glucose was measured using an ACCU-CHEK Performa glucometer (Roche Diagnostics). Experiments were conducted according to European Directives (86/609/EEC) and were approved by the Ethical Committee of Paris Descartes University (registered number: P2.SY.162.10).

**Pancreatic islet isolation and transplantation**

Pancreatic islets were separated by density gradient centrifugation (Histopaque; Sigma-Aldrich), after in situ digestion with collagenase P (Roche Applied Science), and transplanted (300 islets) under the kidney capsule of diabetic recipients. Diabetes was induced by a single injection of streptozotocin (Sigma-Aldrich) at 225 mg/kg. Diagnosis of graft rejection was made after two glucose measurements > 250 mg/dl.

**DC preparation**

ATDCs were generated from C57BL/6 mouse bone marrow, as previously described (29). Briefly, bone marrow cells were cultured in the presence of rGM-CSF for 8 d. Nonadherent cells were discarded, and adherent DCs were used for in vivo experiments: 1 × 10\(^4\) ATDCs was injected i.v. into C57BL/6 recipients that were transplanted the next day with BALB/c islets.

**Abs**

The cell line producing the engineered CD3 Ab F(ab\(^\prime\))\(_2\) fragments (30) was kindly provided by J.A. Bluestone (University of California, San Francisco, San Francisco, CA). The Abs were produced and purified in-house. PC61 Abs were obtained from Bio X Cell (West Lebanon, NH) and were used at the dose of 300 μg/injection. All Abs used for flow cytometry were from BD Biosciences or eBioscience.

**Confocal microscopy**

Frozen sections of selected organs were fixed in acetone and incubated for 1 h with primary Abs. The slides were washed, incubated for 30 min with secondary Abs, and mounted with Fluoromount-G (Southern Biotech). Confocal images were acquired on a Leica SP5 AOBS microscope and analyzed using ImageJ software.

**IFN-γ ELISPOT**

Polyvinylidene difluoride plates were coated with anti–IFN-γ Ab (U-CyTech). Responder cells (10\(^4\)/well) were incubated with 10\(^4\) irradiated splenocytes from C57BL/6, BALB/c, or C3H mice. After a 20-h culture, IFN-γ was detected using biotinylated anti–IFN-γ Ab, streptavidin-HRP, and SIGMAFAST BCIP/NBT (Sigma-Aldrich). IFN-γ spot readouts were expressed as spot-forming cells/10\(^4\) cells.

**In vitro inhibition assays**

CD4\(^+\)CD25\(^-\) T cells (5 × 10\(^5\) cells/well) from naive C57BL/6 mice were coincubated with CD4\(^+\)CD25\(^-\) T cells (1 × 10\(^5\) cells or 5 × 10\(^5\) cells/well) isolated from transplanted recipients (treated or not with CD3 Abs and ATDCs) and stimulated with irradiated splenocytes from BALB/c or C3H mice (2 × 10\(^6\) cells/well). After 5 d at 37°C, cells were pulsed with \([\text{H}]\)thymidine (Amersham). Data are expressed as cpm.

**Statistical analysis**

Cumulative graft survival was calculated using the Kaplan–Meier method. The statistical comparison was performed using the log-rank (Mantel–Cox) test. When appropriate, the Student t test was used. A p value < 0.05 was considered significant.

**Results**

**Combination of ATDCs and CD3 Abs promotes long-term islet graft survival**

ATDCs were produced from the bone marrow of C57BL/6 mice, as previously described (29). After an 8-d culture in the presence of rGM-CSF, nonadherent and adherent DCs were recovered and tested for their capacity to induce allogeneic T cell responses in vitro. In contrast to nonadherent DCs, adherent DCs from C57BL/6 mice were unable to efficiently prime T cells from BALB/c donors (Fig. 1A). In addition, adherent DCs inhibited the proliferation of C57BL/6 T cells in response to BALB/c DC stimulation in an MLR assay (Fig. 1B). The adherent DCs were named ATDCs.

We evaluated the in vivo therapeutic effect of ATDCs in a fully mismatched pancreatic islet allograft mouse model. C57BL/6 mice, rendered diabetic following one injection of streptozotocin, were grafted under the kidney capsule with pancreatic islets isolated from BALB/c mice. Rejection occurred within 3 wk in untreated recipients (Fig. 2). Administration of ATDCs to C57BL/6 hosts 1 d prior to islet transplantation (day −1) did not significantly delay graft rejection. In contrast, combining ATDCs with short-term T cell–depleting CD3 Abs (one injection each day for five consecutive days starting on the day before transplant) induced long-term survival of fully allogeneic islets in half of the treated mice. This result was observed using either the intact mitogenic form of CD3 Ab (clone 145 2C11) or its nonmitogenic F(ab\(^\prime\))\(_2\) fragments (Fig. 2). As already shown (27), the Ab by itself significantly prolonged graft survival as a result of its potent immunosuppressive properties, but all grafts were eventually rejected when the T cell compartment was reconstituted.

In vivo tracking experiments, using ATDCs from CD45.2 C57BL/6 mice infused into congenic CD45.1 recipients that were treated with CD3 Abs and grafted with BALB/c pancreatic islets,
Combination therapy using autologous DCs and CD3 Abs induced long-term islet graft survival. Islets isolated from BALB/c mice were grafted under the kidney capsule of diabetic C57BL/6 mice. (A) Recipients were left untreated (n = 10), injected i.v. with 10^6 ATDCs on day –1 (n = 6), treated with intact CD3-specific Ab 145-2C11 (5 μg/injection, five injections, one every other day, n = 5), or treated with a combination of ATDCs and CD3 Abs (n = 8). (B) Recipients were untreated (n = 14) or received five injections of 145-2C11 F(ab')_2 fragments (50 μg/injection) alone (n = 10) or in combination with ATDCs (n = 14). In all cases, treatment started the day before transplantation.

We next investigated the effect of the combination therapy on T cell compartments in the spleen, the draining (renal) LNs, and the islet allografts, as reflected by an intense insulin staining (Fig. 3A). This was in clear contrast to what was observed in untreated hosts, where the size, shape, and insulin content of islet allografts were reduced. This was associated with a prominent infiltrate of immune cells, notably CD8^+ T cells that invaded and destroyed the islet allografts (Fig. 3). In contrast, in recipients treated with ATDCs plus CD3 Abs, T cell infiltration was moderate and confined to the periphery of the islets. This was also observed in long-term surviving grafts (118 d). CD8^+ T cells from recipients treated with CD3 Abs alone had recovered to levels similar to those observed in untreated recipients (Fig. 4D). In contrast, those in mice treated with ATDCs plus CD3 Abs and evaluated on day 90–100 posttransplant remained significantly low, revealing donor-specific hyporesponsiveness. We performed similar experiments using Treg-depleted Foxp3^- T cells sorted from the spleen of Foxp3^GFP reporter C57BL/6-transplanted mice treated with CD3 Abs alone or combined with ATDCs (Fig. 4E).

Defective migration and donor-specific hyporesponsiveness of CD8^+ T cells from recipients treated with CD3 Abs and ATDCs

We also focused our attention on CD4^+Foxp3^+ Tregs and how they might be affected by the treatments. We (27, 33) and other investigators (34) reported that Tregs were more resistant to CD3-induced depletion than were conventional T cells. As a consequence, the proportion of Tregs increased at the periphery during the early posttransplant period, but they returned to normal levels as the T cell compartment was reconstituted. We could reproduce these findings in the current study; interestingly, however, on day within the graft, in contrast to what was observed after treatment with CD3 Abs alone (Fig. 4B, lower panel). This impaired migration was restricted to the CD8 compartment, as CD4^+ T cell infiltrates were detected within the target tissue, independently of the treatment (Fig. 4B, upper panel).

Evaluation of the antidonor reactivity by IFN-γ ELISPOT showed an impaired response on day 14 posttransplant in mice treated with CD3 Abs, combined or not with ATDCs (Fig. 4C). Responses against third-party C3H Ags also were reduced, illustrating the global immunosuppressive effect of the Ab. On day 50–60 posttransplant, anti-BALB/c responses mediated by spleen cells from mice treated with CD3 Abs alone had recovered to levels similar to those observed in untreated recipients (Fig. 4D). In contrast, those in mice treated with ATDCs plus CD3 Abs and evaluated on day 90–100 posttransplant remained significantly low, revealing donor-specific hyporesponsiveness. We performed similar experiments using Treg-depleted Foxp3^- T cells sorted from the spleen of Foxp3^GFP reporter C57BL/6-transplanted mice treated with CD3 Abs alone or combined with ATDCs (Fig. 4E). Responses to donor Ag were higher compared with the ones obtained with total T cells. However, alloreactivity of Foxp3^- T cells toward BALB/c Ags was still strongly reduced in mice treated with ATDCs plus CD3 Abs compared with mice treated with CD3 Abs alone, further demonstrating the inhibition of donor-reactive responses.

The therapeutic effect of the combination therapy is dependent on Treg expansion and acquisition of Ag-specific suppressive functions

We also focused our attention on CD4^+Foxp3^+ Tregs and how they might be affected by the treatments. We (27, 33) and other investigators (34) reported that Tregs were more resistant to CD3-induced depletion than were conventional T cells. As a consequence, the proportion of Tregs increased at the periphery during the early posttransplant period, but they returned to normal levels as the T cell compartment was reconstituted. We could reproduce these findings in the current study; interestingly, however, on day 90–100 posttransplant, the T cell compartment was completely reconstituted, and T cells had returned to their pretreatment levels in the spleen and renal LNs (Fig. 4B). However, in recipients treated with ATDCs plus CD3 Abs (analyzed on day 90–100 posttransplant), CD8^+ T cells did not accumulate with ATDCs (Fig. 2). Recipients were untreated (n = 10), injected i.v. with 10^6 ATDCs on day –1 (n = 6), treated with intact CD3-specific Ab 145-2C11 (5 μg/injection, five injections, one every other day, n = 5), or treated with a combination of ATDCs and CD3 Abs (n = 8). (B) Recipients were untreated (n = 14) or received five injections of 145-2C11 F(ab')_2 fragments (50 μg/injection) alone (n = 10) or in combination with ATDCs (n = 14). In all cases, treatment started the day before transplantation.

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>19.6 ± 0.9</td>
</tr>
<tr>
<td>ATDCs</td>
<td>24.9 ± 4.5</td>
</tr>
<tr>
<td>CD3 Ab</td>
<td>38.9 ± 6.1</td>
</tr>
<tr>
<td>CD3 Ab + ATDCs</td>
<td>77.4 ± 10.7</td>
</tr>
</tbody>
</table>

The therapeutic effect of the combination therapy is dependent on Treg expansion and acquisition of Ag-specific suppressive functions

We also focused our attention on CD4^+Foxp3^+ Tregs and how they might be affected by the treatments. We (27, 33) and other investigators (34) reported that Tregs were more resistant to CD3-induced depletion than were conventional T cells. As a consequence, the proportion of Tregs increased at the periphery during the early posttransplant period, but they returned to normal levels as the T cell compartment was reconstituted. We could reproduce these findings in the current study; interestingly, however, on day...
14 posttransplant, the increase in Treg frequency was significantly more pronounced after the combination therapy compared with CD3 Abs alone in the spleen, the renal LNs, and the islet allografts (37.9, 32.5, and 48.5% of total CD4+ T cells versus 23.5, 21.4, and 28.3%, respectively) (Fig. 5A, left panel). In terms of numbers, Tregs also were increased in the spleen and draining LNs of mice treated with ATDCs plus CD3 Abs compared with those treated with CD3 Abs alone (although an overall reduction was observed compared with untreated recipient mice) (Supplemental Fig. 2A). In contrast, Treg numbers detected within the islet allografts were similar between the two groups, suggesting that the increased Treg frequency was due to a more profound depletion of effector T cells induced by ATDCs plus CD3 Abs compared with CD3 Abs alone (Fig. 4A). Accordingly, the intragraft Treg/CD4+ T cell and Treg/CD8+ T cell ratios were significantly enhanced after administration of the combination therapy (Supplemental Fig. 2B). In all organs, Tregs proliferated more vigorously than did Tregs from mice that were not treated or from those that were treated with CD3 Abs alone, as illustrated by the expression of the proliferation marker Ki67 (Fig. 5A, right panel).

In recipients treated with ATDCs plus CD3 Abs, the proportion and numbers of Tregs remained elevated in the long-term (day 90–100 posttransplant) (Fig. 5B, Supplemental Fig. 2A). In contrast, in mice that were treated with CD3 Abs alone and analyzed at the time of rejection (day 50–60 posttransplant), Treg frequency was similar to control animals. Tregs accounted for 17.5, 17.9, and 35.9% of total CD4+ T cells versus 11.6, 11.5, and 23.9% in the spleen, draining LNs, and islet allografts of recipients treated with the combination therapy versus CD3 Abs alone, respectively. In long-term surviving grafts, Treg/CD4+ T cell and Treg/CD8+ T cell ratios were increased 2- and 5-fold in recipients of the combination therapy versus CD3 Abs alone, respectively (Supplemental Fig. 2B).

We next investigated the suppressive capacities of Tregs recovered from mice showing permanent graft acceptance after treatment with ATDCs plus CD3 Abs (day 90–100 posttransplant). Using the classical coculture assay, we showed that these Tregs were more efficient at inhibiting the proliferation of effector CD4+ CD25+ T cells from C57BL/6 mice stimulated by irradiated BALB/c splenocytes than Tregs isolated from untreated recipients (57.2% inhibition versus 0% at a 5:1 effector T cell/Treg ratio) (Fig. 5C). This was not observed when using the C3H third-party stimulator. Similar results were observed with regard to IFN-γ production by allogeneic T cells (Fig. 5D).

Lastly, to further investigate whether the synergistic tolerogenic effect of treatment with ATDCs plus CD3 Abs is dependent on Foxp3+ Tregs, we performed pancreatic islet allografts in Treg-depleted recipients. C57BL/6 mice received two injections of anti-CD25 Abs (PC61) before transplantation and administration of ATDCs and CD3 Abs (days 23 and 21). Under these conditions, none of the recipients that received the combined therapy showed long-term graft survival (mean survival: 34.5 ± 8 d) (Fig. 5E).

**Discussion**

In this study, using a mouse model of pancreatic islet allograft, we demonstrated that combination therapy with CD3 Abs and ATDCs,
two products presently in clinical development, is applicable to transplantation for the long-term survival of fully mismatched allografts. The main findings were that both treatments acted in synergy to inhibit antidonor T cell responses and migration to the grafted tissue and to promote a sustained expansion of CD4+ Foxp3+ Tregs, at the periphery and in the transplant; these Tregs were alloantigen-specific and mandatory for induction of long-term graft survival.

The central role of DCs in controlling immunity provides the possibility of using them as cellular mediators of Ag-specific tolerance. Therapeutic strategies have been developed using recipient or donor DCs generated and manipulated ex vivo for the acquisition of suppressive functions. They have the capacity to inhibit the alloimmune response and to induce T cell hyporesponsiveness (5, 6, 9, 13, 14, 35). This feature of tolerogenic DCs is associated with an inadequate cell surface expression of MHC and costimulatory molecules and the absence of danger or proinflammatory signals that otherwise promote DC maturation (1, 2, 17). We already demonstrated that ATDCs (adherent bone marrow DCs) displayed an immature phenotype; in this study, we confirmed that they were unable to stimulate allogeneic T cells and they efficiently inhibited MLR (9, 29). However, in contrast to results obtained in the rat cardiac transplantation model, ATDCs by themselves were unable to prolong islet allograft survival in fully mismatched mouse recipients. Immunosuppressive drugs can promote the suppressive functions of DCs, and we reported that a suboptimal dose of LF 15-0195, but not rapamycin, synergized with ATDCs to achieve long-term acceptance of rat heart allografts (19). Because production of LF 15-0195 was stopped, we moved to CD3-specific Abs, which have proven efficacy in

FIGURE 4. The combination therapy decreased intragraft T cells and impaired antidonor responses. Spleen, renal-draining LNs, and pancreatic islet grafts were retrieved from recipients that were left untreated or treated with CD3 Ab F(ab')2 fragments alone or in combination with ATDCs. Frequency of CD4+ and CD8+ T cells in the different organs was analyzed on day 14 in the early posttransplant period (A) or on day ≥ 50 in the late posttransplant period [day 50–60 for the group treated with CD3 F(ab')2 alone and day 90–100 for the group treated with ATDCs plus CD3 F(ab')2] (B). *p < 0.02, **p < 0.005, ***p < 0.0007. Anti-donor responses also were evaluated at day 14 (C) or day ≥ 50 (D and E) by IFN-γ ELISPOT after a 20-h incubation of total spleen cells with irradiated T cell–depleted splenocytes (APCs) from C57BL/6, BALB/c, or C3H donors (3–10 mice/group). (E) IFN-γ ELISPOT using total T cells or Treg-depleted Foxp3+ T cells sorted from the spleen of Foxp3GFP reporter C57BL/6 mice on day ≥ 50 posttransplant and stimulated with donor BALB/c Ags. *p < 0.04, **p < 0.002, ***p < 0.0007.
inducing/restoring immune tolerance (26–28). Indeed, we showed in experimental models of islet or heart allografts that CD3 Abs, applied at the time of allogeneic effector T cell priming, could induce Ag-specific tolerance (27, 28). Recently, we showed synergy when combining a low-dose CD3 Ab treatment with ATDCs in a minor mismatched skin allograft model at the time of transplantation (23). In this study, we implemented these results in a fully mismatched pancreatic islet transplantation model, demonstrating a similar synergistic effect that led to permanent graft acceptance. Importantly, as discussed below, the immune mediators responsible for this sustained islet allograft survival were distinct from those reported previously (23).

Antidonor CD8+ T responses, measured by IFN-γ ELISpot, were considerably reduced after administration of the combination therapy, and this was maintained in the long-term. Removal of Foxp3+ Treg cells from the culture led to the absence of potent alloreactive effectors in mice treated with ATDCs plus CD3 Abs. This sustained T cell hyporesponsiveness was associated with a markedly reduced infiltration of CD8+ T cells in the transplanted islets, showing a preserved shape and insulin-productive capacities up to 100 d posttransplant. In contrast, recipients treated with CD3 Abs alone exhibited an increase in anti-BALB/c CD4+ T cell reactivity that was accompanied by increasing numbers of CD4+ and CD8+ T cells infiltrating the graft, corresponding to the kinetics of rejection of the islet allografts after reconstitution of the T cell compartment (27, 33). Thus, our results suggest that the ATDCs, displaying an impaired stimulatory ability, contributed to the inhibition of the T cell responses against donor alloantigens. Further investigations assessing the migration and expression of chemokines and adhesion molecules will provide important clues about how administration of the combined treatment impacts on the trafficking and activation of Ag-specific T cells.

Previous findings in the rat cardiac allograft model revealed that permanent graft acceptance, induced by administration of ATDCs and LF 15-0195, was associated with the accumulation of double-negative CD4+CD8+ TCRαβCD3+ T cells in the spleen of tolerant rats (36). ATDCs, characterized by the expression of the cytokine chain EBV-induced gene 3 (EBI3), promoted the production of IFN-γ by double-negative T cells, and long-term graft survival was abrogated upon blockade of EBI3 or IFN-γ (36). We did not detect such CD4+CD8− T cells in our model, but EBI3 expression by mouse ATDCs was confirmed (M. Segovia Duarte, M. Hill, and M.C. Cuturi, unpublished observations). Its role remains to be addressed. In the minor Ag skin graft mouse model, allograft survival that was induced by the combination of CD3 Abs and ATDCs was due to the capacity of ATDCs to cross-present alloantigens to CD8+CD11c+ Tregs, as revealed by the use of Tmem176b-deficient ATDCs that failed to synergize with CD3 Abs (23). Tmem176b has been involved in a phagosomal cation transport and is required for the control of phagosomal pH, a crucial parameter in the cross-presentation pathway (37). These data contrast with our present results in a fully mismatched islet allograft model in which CD8+CD11c+ T cells were not detected. In contrast, we found a predominant role for CD4+Foxp3+ Tregs in preventing graft rejection. These differences could be explained by the fact that the two models are different in terms of the graft (skin versus islets) and, more importantly, of the MHC barriers (minor versus major mismatch), thus involving distinct rejection mechanisms. From a conceptual point of view, such findings highlight the diversity of the immunoregulatory mechanisms that are operational in different allogeneic transplant contexts. This is
important in terms of clinical translation (DC therapy and CD3-specific Abs are in clinical development), because such combination treatment may efficiently target distinct rejection processes by setting up the appropriate regulatory programs to favor graft survival.

In the early posttreatment period, on day 14 posttransplant, a marked increase in CD4+Foxp3+ Treg frequency was observed in the spleen, renal LNs, and transplanted islets of recipients of the combination therapy compared with untreated recipients or those who were treated with CD3 Abs alone. These Tregs displayed enhanced proliferative abilities (revealed by the proliferation marker Ki67), which may explain their significant expansion at the periphery and in the target tissue. We (27, 28) and other investigators (34) demonstrated that CD3 Abs induced a significant T cell depletion that primarily targets pathogenic T cells while sparing Tregs, resulting in an increased Treg frequency. Our data suggest that combination with ATDCs amplified this mechanism and favored Treg expansion. An elegant study by Nussenzweig’s group (38) showed that Treg expansion in vivo positively correlates with the number of DCs and that MHC class II expression is required to sustain Treg proliferation. Based on this knowledge and the fact that ATDCs accumulated in the spleen and draining LNs of treated animals and that absolute Treg numbers increased in these organs, we may hypothesize the existence of a key role for the interaction between the two subsets, which may promote Treg expansion in the periphery and migration to inflammatory sites.

At the time of complete T cell reconstitution, the majority of mice treated with CD3 Ab alone had rejected their grafts (day 50–60 posttransplant), and Treg proportion was back to the levels found in control animals. In mice that received combination therapy, increased percentages and numbers of CD4+Foxp3+ Tregs were still evident up to day 100 posttransplant in the spleen, renal LNs, and islet allograft. Intragraft Treg/CD4+ T cell and Treg/CD8+ T cell ratios were enhanced throughout the follow-up period in the group treated with ATDCs plus CD3 Abs, arguing for the establishment of immunoregulatory mechanisms overcoming effector responses. Our data further demonstrated that this sustained high frequency of Tregs contributed to the long-term survival of islet allografts, as in vivo depletion of Tregs abrogated the therapeutic effect of the combined therapy. Similarly, in vitro antigen responses displayed by Treg-depleted Foxp3+ T cells were increased compared with those measured for total T cells. These findings are reminiscent of publications highlighting the crucial role of CD4+Foxp3+ Tregs in transplant tolerance induced by mAbs interfering with T cell activation (31, 39–41). Our data further support the fact that resident intragraft Tregs may control effector T cells present in the same microenvironment and, thus, prevent invasive islet infiltration. Lastly, the combination treatment promoted the expansion of Tregs endowed with Ag-specific suppressive capacities as they inhibited proliferation and IFN-γ secretion of effector cells stimulated with donor Ags, but not third-party Ags, more efficiently than Tregs recovered from untreated mice.

In view of translating this therapeutic strategy to the clinical arena, the combination of ATDCs and CD3 Abs offers several advantages. First, tolerogenic DCs are derived from the patient awaiting a transplant and not from the donor. Therefore, application of our protocol is not restricted to living donation, in contrast to the use of donor DCs. Second, autologous DCs are not pulsed in vitro with allogeneic peptides before infusion into the recipient. Thus, risks of sensitization to donor Ags are minimal. Third, tolerogenic DCs can now be produced under good manufacturing practice conditions, and comparative studies have been performed assessing their survival, phenotype, and functions (42, 43). ATDCs are being tested in a European Commission – Seventh Framework Programme granted project, “the ONE study,” to evaluate their safety, tolerogenic properties, and therapeutic efficacy in living donor renal transplant recipients. In addition, our results show that CD3 Abs can be used concomitantly with ATDCs in vivo without altering their suppressive functions. This is important, because the in vivo stability of tolerogenic DCs, notably in the presence of high-dose immunosuppression and inflammatory signals, is a concern. Finally, humanized CD3 Abs have been used in autoimmunity, in type 1 diabetic patients. Promising results have been obtained in phase II and III trials in terms of preservation of the insulin-secreting capacity of endogenous pancreatic β cells (44–47). Therefore, our data argue for considering this combination strategy for a proof-of-concept trial in organ transplantation to achieve efficient control of the alloimmune effector responses while avoiding the hazards of long-term nonspecific immunosuppression.

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Disclosures

The authors have no financial conflicts of interest.

References


