Immunosuppressive Drugs and Tregs: A Critical Evaluation!

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To define therapeutic strategies that promote tolerance, it is of critical importance to determine the effects of immunosuppressive drugs on regulatory T cells (Tregs). This review discusses the current knowledge about the physiology of Tregs in humans, the role or Tregs in transplantation, and the impact of the different types of immunosuppressive agents on the frequency and functionality of Tregs in *in vitro* and *in vivo* systems.


The recent improvement in the therapeutic arsenal in transplantation has led to considerable success in short-term allograft outcomes (1); however, it has had a marginal impact on long-term graft survival and resulted in a disturbing adverse effect profile (2,3). In this context, the achievement of immunologic tolerance, whereby the recipient’s immune system accepts the graft in the absence of maintenance immunosuppression, is more than ever a valuable goal (4). A first step in this endeavor is to define the therapeutic strategies that promote donor-specific hyporesponsiveness and determine the mechanisms of tolerance in humans (5).

One of the mechanisms involved in tolerance/hyporesponsiveness is the suppression of alloreactive effector T cells (Teffs) by regulatory T cells (Tregs). It has been suggested that the balance between Teffs and Tregs plays a major role in the state of rejection versus tolerance of the graft (6). It is thus of utmost importance to ascertain the effect of the currently used and future immunosuppressive agents on the fate of Tregs, with regard not only to frequency but also to function of these cells. This review focuses on these last two aspects, with the ultimate goal of developing novel therapeutic strategies that promote regulation and protect the graft from alloreactivity, thereby paving the way for immunosuppression withdrawal and tolerance. Although several populations of Tregs have been described, including CD4+/CD8−, CD3+CD4−CD8−, γδ, and NK T cells (7), we concentrate on the population with the phenotype most widely studied in transplantation, namely CD4+/CD25+Foxp3+ T cells.

Tregs, a Subpopulation of T Cells

**Physiology of Tregs in Humans**

Although some controversy persisted until the early 1990s about the existence of Tregs, in 1995, Sakaguchi et al. (8) provided the first evidence that the α chain of IL-2 receptor CD25 identifies a subpopulation of CD4+ cells with regulatory properties. They showed that the transfer of CD25-depleted CD4+ cells into a lymphocyte-deficient mouse leads to autoimmune disease that could be prevented by the co-transfer of CD25+ cells. Further studies confirmed the presence *in vitro* and *in vivo* of CD4+ T cells with bright CD25 staining (CD4+CD25(high)) and suppressive capacity in both healthy volunteers and patients with autoimmune conditions (9–13).

Because only the most brightly staining CD25+ cells show regulatory properties, the use of this marker for Tregs study is arbitrary (9). Moreover, a proportion of Tregs lack CD25 expression (14,15). In 2003, three independent groups showed that Forkhead box protein 3 (Foxp3), a nuclear transcription factor defective in the multisystemic autoimmune disease IPEX (immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome) (16) was expressed in CD4+CD25+ Tregs (17–19). Foxp3 was shown to correlate with suppressive activity and, interestingly, to regulate the expression of several cell surface molecules previously used to identify Tregs, such as CTLA-4, GITR, and CD25 itself (18). Two limitations of Foxp3 marker for Tregs were (1) its intracellular localization, rendering it useless for Treg isolation, and (2) its transient expression in nonsuppressive, recently activated CD4+ T cells (20). More recently, it was demonstrated that the IL-7 receptor CD127 is a reliable surface marker for Treg identification and isolation (21,22). CD127 is downregulated on all T cells after activation, but, contrary to effector and memory T cells that reexpress it, Tregs remain CD127+/low and are suppressive *in vitro*.

The mechanisms by which Tregs suppress T cell activation are still incompletely understood. Tregs achieve self-tolerance by what is commonly referred to as dominant tolerance, meaning that they “silence” or suppress the function of autoreactive Teffs. This mechanism is in opposition to recessive tolerance, or negative selection, whereby T cells that react to self undergo clonal deletion or become unresponsive (anergic) (6). Other than their effect on Teffs, Tregs are known to suppress several populations of immune cells, including B cells, monocytes, and dendritic cells (7). A crucial feature of Tregs is that they express a T cell receptor with antigenic specificity (23). Upon encounter with their specific ligand, activated Tregs extravasate in the inflamed tissues (24). Experimental studies have shown that transfer of antigen-specific Tregs can reverse autoimmune pro-
cesses, whereas the injection of 10 times more non-antigen-specific Tregs has no effect (25,26). It is also known that activated Tregs are capable of “bystander suppression”—that is, suppression of non-antigen-specific effector cells in their neighborhood (27). It has been suggested that one form of specificity for activated Tregs is their suppressive effect on cells that they “meet” at the scene of inflammation (24).

Another important concept about Treg physiology is that, depending on their origin, Tregs can be distinguished as natural (n) or adaptive/induced (i). nTregs are generated intrathymically upon presentation of self-antigen by thymic epithelial cells. Whether co-stimulation molecules such as CD28 are essential to this process is still a matter of debate. iTregs are produced in the periphery, after encounter of either self or foreign antigens. Our group and others have confirmed that mature animal and human T cells can be converted from CD25+ to CD25+ or Foxp3+ to Foxp3+ in different experimental settings (28–32); these converted cells present with suppressive activity in vitro and in vivo, although for the latter results are mainly from adoptive transfer studies in T cell–deficient animals (29). Although both types of Tregs share similarities, nTregs seems to have more stable expression of Foxp3 (33). At present, it is not known whether these differences have clinical implications.

A new, increasingly recognized concept is that Tregs not only have various origins but also, similar to helper T cells, show plasticity in their development and lineage differentiation. Recent work showed that, depending on the cytokine milieu, CD4+CD25+ T cells expanded ex vivo in rats displayed functionally distinct phenotypes peculiar to Th1 and Th2 differentiation pathways (34). Even more striking is the recent evidence that CD4+CD25+ Foxp3+ not only can trigger the expansion of IL-17–producing (Th17) T cells but also can differentiate themselves into Th17 T cells in vitro in mouse and human upon stimulation with allogeneic antigen-presenting cells (35,36). It thus seems likely that, in vivo, the specific conditions at the site of recruitment of Tregs have a significant influence on their eventual role in the inflammatory process.

Tregs in Transplantation

For achievement of tolerance, the interest in tracking and manipulating Tregs seems obvious (4). In several murine models, Tregs were identified in tolerant allograft recipients and have been demonstrated to traffic to the allograft tissue (37,38). Other models have shown that Tregs that are induced in vitro or in vivo or expanded ex vivo after contact with alloantigen can adoptively transfer allograft tolerance (39–42).

Our group was the first to provide evidence of the existence of antigen-specific Tregs that can suppress alloreponses to donor HLA peptides in stable human renal transplant recipients (43). That article was followed by several articles that reported a high proportion of circulating and intragraft Tregs in tolerant/stable patients in renal as well as liver and lung transplantation (44–47). In contrast, recipients with chronic rejection had significantly fewer Tregs and lower levels of Foxp3 transcripts than clinically tolerant patients and healthy individuals (48). Mechanistic studies in renal transplant recipients confirmed that donor hyporesponsiveness was abrogated by depletion of CD4+CD25high T cells (45).

After this brief presentation of Treg physiology, we now discuss the available data regarding the effect of various therapeutic agents on Tregs. Available in vitro and in vivo data are summarized in Tables 1 and 2, respectively.

Maintenance Immunosuppressive Agents in Common Use
Calcineurin Inhibitors

Most of the available data about calcineurin inhibitors (CNIs) and Tregs compared their effects with those of rapamycin. In mice, recent studies demonstrated that CNIs completely inhibit de novo conversion of alloantigen-specific Tregs, whereas rapamycin promotes it (49). Upon transfer, these in vivo–converted Tregs potently suppress the rejection of donor but not third-party skin grafts (49). In humans, comparative in vitro studies showed that the CNIs and anti–IL-2R significantly inhibit Foxp3 mRNA expression upon third-party cell stimulation, whereas rapamycin merely delays it from 5 to 7 d (50). Available in vivo human data suggest that, on the one hand, CNIs lead to lower circulating levels of Tregs than does rapamycin (51,52) and to CNI tapering (53) but, on the other hand, higher levels than in healthy donors (46). All in all, these data suggest that CNIs have a negative impact on the expansion and homeostasis of Tregs.

Rapamycin

Numerous studies have evaluated the effect of rapamycin on Tregs. Battaglia et al. (54) were the first to show that rapamycin selectively expands the murine naturally occurring Tregs in vitro. Such expanded Tregs prevent allograft rejection in an in vivo model of islet transplantation. Collectively, in vitro data in mice and humans showed either Treg expansion (54,55) or no expansion (56,57), the disparity being mainly attributable to different experimental settings: Expansion of isolated CD4+ versus CD4+CD25+ cells, different dosages of agent, various times of culture, and cytokine supplementation of the culture medium. In contrast, Valmori et al. (58) demonstrated that rapamycin promotes human CD4+ suppressive function by inducing suppressive functions in conventional CD4+ T cells rather than by selective expansion of naturally occurring Tregs. No studies specifically reported in vitro data on humans with rapamycin dosages within the whole blood therapeutic window of 5 to 15 ng/ml (59).

In vivo, renal recipients who underwent induction with alemtuzumab and received low-dosage rapamycin had consistent expansion of their Treg pool from as soon as 4 mo after transplantation, whereas the combination of alemtuzumab and low-dosage cyclosporin A had a marginal effect on Tregs (60). That study and others (45,61) suggested that rapamycin positively affects Treg function and frequency in the transplant setting; however, in a 30-mo prospective follow-up of the trial described, the Treg expansion did not translate into better clinical outcomes in terms of either GFR/proteinuria or C4d and chronic allograft damage score in protocol biopsies (62).
### Table 1. *In vitro* studies about the effect of agents on Treg population

<table>
<thead>
<tr>
<th>Study</th>
<th>Subjects</th>
<th>Responder</th>
<th>Stimulus</th>
<th>Result</th>
</tr>
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<tbody>
<tr>
<td><strong>Maintenance agents</strong>&lt;br&gt;CNIs&lt;br&gt;Baan et al. (50), 2005</td>
<td>Healthy donors PBMC</td>
<td>Third-party PBMCs with CNI versus Rapa versus anti–IL-2R for 7 d</td>
<td>Significant inhibition of Foxp3 mRNA proliferation with CNI and anti–IL-2R; delayed expression with Rapa</td>
<td></td>
</tr>
<tr>
<td><strong>Rapamycin</strong>&lt;br&gt;Battaglia et al. (54), 2005</td>
<td>Mouse CD4+</td>
<td>APCs/OVA332-339 (3 wk) plus IL-2, then resting in IL-2 (1 wk)</td>
<td>Significant enrichment in CD4+CD25high in Rapa versus medium alone with preserved suppressive function</td>
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</tr>
<tr>
<td><strong>Battaglia et al.</strong>&lt;br&gt;(55), 2006</td>
<td>Healthy donors CD4+</td>
<td>Anti-CD3/28 (3 wk), then resting in IL-2 (1 wk)</td>
<td>Significant expansion of CD4+Foxp3+; expanded Tregs are suppressive</td>
<td></td>
</tr>
<tr>
<td><strong>Coenen et al.</strong>&lt;br&gt;(57), 2006</td>
<td>Healthy donors CD4+CD25+</td>
<td>Fully mismatched PBMCs plus IL-2/IL-15 (1 wk)</td>
<td>Expanded CD4+CD25+ 16 times more potent at suppression after incubation with Rapa versus CsA</td>
<td></td>
</tr>
<tr>
<td><strong>Valmori et al.</strong>&lt;br&gt;(58), 2006</td>
<td>Healthy donors CD4+</td>
<td>Anti-CD3/CD8 plus IL-2 and CD4+CD8−APCs (weekly intervals)</td>
<td>Increased suppressive function of CD4+ not due to selective expansion of CD4+CD25high but rather to the induction of suppressive function in the total CD4+ population</td>
<td></td>
</tr>
<tr>
<td><strong>Lim et al.</strong>&lt;br&gt;(56), 2007</td>
<td>Mouse CD4+CD25+</td>
<td>Allogeneic splenocytes (1 wk)</td>
<td>Decrease in the expansion of Tregs with Rapa, CsA, and methylprednisolone versus medium, MMF, anti-CD40L, and CTLA4Ig; suppressive capacity preserved</td>
<td></td>
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<tr>
<td><strong>MMF</strong>&lt;br&gt;Lim et al. (56), 2007</td>
<td>Mouse CD4+CD25+</td>
<td>Allogeneic splenocytes (1 wk)</td>
<td>No effect on Treg expansion with MMF versus medium; suppressive capacity preserved</td>
<td></td>
</tr>
<tr>
<td><strong>Induction agents</strong>&lt;br&gt;ATG&lt;br&gt;Lopez et al. (32), 2002</td>
<td>Healthy donors PBMCs</td>
<td>Medium alone (up to 96 h)</td>
<td>Significant expansion of CD4+CD25+ with maintained suppressive activity</td>
<td></td>
</tr>
<tr>
<td>Feng et al. (65), 2008</td>
<td>Healthy donors PBMCs</td>
<td>Medium alone (up to 72 h)</td>
<td>Significant expansion of CD4+CD25+ with rabbit ATG but not horse ATG</td>
<td></td>
</tr>
<tr>
<td><strong>Biologics</strong>&lt;br&gt;Natalizumab&lt;br&gt;Stenner et al. (79), 2008</td>
<td>Healthy donors CD4+CD25high−CD127low</td>
<td>Anti-CD3/28 or autologous dendritic cells</td>
<td>No effect on CD4+Foxp3+ frequency or suppression of autologous CD4+CD25−proliferation</td>
<td></td>
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</tbody>
</table>

APC, antigen-presenting cell; CsA, cyclosporin A; MMF, mycophenolate mofetil; OVA332-339, stimulation peptide; PBMC, peripheral blood mononuclear cells; Rapa, rapamycin.
### Table 2. In vivo studies about the effect of agents on Treg population

<table>
<thead>
<tr>
<th>Study</th>
<th>Subjects</th>
<th>Follow-up</th>
<th>Therapy</th>
<th>Results</th>
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<tbody>
<tr>
<td><strong>Maintenance agents</strong></td>
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<tr>
<td>CNI</td>
<td>Meloni et al. (46), 2006</td>
<td>Lung recipients</td>
<td>1661-1669, 2009</td>
<td>CNI + steroids ± AZA ± MMF</td>
</tr>
<tr>
<td>CNI</td>
<td>Segundo et al. (51), 2006</td>
<td>Renal recipients</td>
<td>4-12 mo</td>
<td>CNI versus Rapa</td>
</tr>
<tr>
<td>CNI</td>
<td>Gao et al. (49), 2007</td>
<td>Mouse</td>
<td>4 d</td>
<td>CNI versus Rapa ± anti-CD154</td>
</tr>
<tr>
<td>CNI</td>
<td>Pascual et al. (53), 2008</td>
<td>Renal recipients</td>
<td>6 mo</td>
<td>Alectuzumab induction; CNI maintenance versus taper</td>
</tr>
<tr>
<td>CNI</td>
<td>Levitski et al. (52), 2009</td>
<td>Liver recipients</td>
<td>6 mo</td>
<td>Monotherapy TAC versus Rapa versus MMF</td>
</tr>
<tr>
<td><strong>Rapamycin</strong></td>
<td>Norris et al. (60) and Ruggenti et al. (62), 2007</td>
<td>Renal recipients</td>
<td>30 mo</td>
<td>Alectuzumab induction; maintenance with MMF + low-dose CsA versus low-dose Rapa</td>
</tr>
<tr>
<td>MMF</td>
<td>Monti et al. (61), 2008</td>
<td>Islet recipient</td>
<td>1 to 5 mo</td>
<td>Rapa monotherapy</td>
</tr>
<tr>
<td>MMF</td>
<td>Gregory et al. (63), 2001</td>
<td>Mouse islet transplants</td>
<td>1 mo</td>
<td>MMF ± vitamin D&lt;sub&gt;3&lt;/sub&gt;</td>
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<tr>
<td>MMF</td>
<td>Velhuis et al. (64), 2006</td>
<td>Renal recipients</td>
<td>84 mo</td>
<td>MMF + prednisone</td>
</tr>
<tr>
<td>MMF</td>
<td>Levitski et al. (52), 2009</td>
<td>Liver recipients</td>
<td>6 mo</td>
<td>Monotherapy TAC versus Rapa versus MMF</td>
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<tr>
<td><strong>Induction agents</strong></td>
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<tr>
<td>ATG/Alectuzumab/anti-IL-2R</td>
<td>Ciancio et al. (67), 2005</td>
<td>Renal recipients</td>
<td>15 mo</td>
<td>ATG versus Alectuzumab versus daclizumab; maintenance with TAC/MMF ± prednisone</td>
</tr>
<tr>
<td></td>
<td>Bloom et al. (66), 2008</td>
<td>Renal recipients</td>
<td>36 mo</td>
<td>Alectuzumab versus basiliximab; maintenance mostly with Rapa</td>
</tr>
<tr>
<td></td>
<td>Toso et al. (68), 2009</td>
<td>Islet recipients</td>
<td>18 mo</td>
<td>ATG versus alectuzumab versus daclizumab; maintenance with TAC ± MMF ± Rapa</td>
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<tr>
<td><strong>Biologics</strong></td>
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<tr>
<td>Belatacept</td>
<td>Tang et al. (71), 2003</td>
<td>Mouse</td>
<td>10 d</td>
<td>B7 blockade</td>
</tr>
<tr>
<td></td>
<td>Chavez et al. (73), 2007</td>
<td>Renal recipients</td>
<td>6 mo</td>
<td>Belatacept versus CNI</td>
</tr>
<tr>
<td></td>
<td>Bluestone et al. (72), 2008</td>
<td>Renal recipients</td>
<td>3 mo and 3 to 5 yr</td>
<td>Belatacept versus CNI; all patients received basiliximab/MMF and corticosteroid taper</td>
</tr>
<tr>
<td>Efalizumab</td>
<td>Mortensen et al. (74), 2005</td>
<td>Patients with psoriasis</td>
<td>12 wk</td>
<td>Efalizumab monotherapy</td>
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<tr>
<td></td>
<td>Alefacept</td>
<td>Vaishnav et al. (77), 2002</td>
<td>Healthy volunteers</td>
<td>Single dose</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Larsen et al. (78), 2007</td>
<td>Patients with psoriasis</td>
<td>12 wk</td>
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<tr>
<td></td>
<td>Natalizumab</td>
<td>Stenner et al. (79), 2008</td>
<td>Patients with MS</td>
<td>1 to 6 mo</td>
</tr>
<tr>
<td>Small molecules</td>
<td>CP690 550</td>
<td>CP690 550 + MMF + prednisolone</td>
<td>2 mo</td>
<td>Initial reduction in CD4&lt;sup&gt;+&lt;/sup&gt;CD25&lt;sup&gt;high&lt;/sup&gt; with partial recovery at 2 mo; no effect on suppressive activity at 1 mo versus pre-Tx</td>
</tr>
</tbody>
</table>

AZA, azathioprine; CsA, cyclosporin A; MMF, mycophenolate mofetil; MS, multiple sclerosis; pre-Tx, pretransplantation; post-Tx, posttransplantation; Rapa, rapamycin; TAC, tacrolimus.
Mycophenolate Mofetil

There are few data regarding Tregs and mycophenolate mofetil (MMF). In mice in vitro, Lim et al. (56) observed no more expansion of CD4⁺CD25⁺ cells than with medium alone. An in vitro islet transplant model showed increased Tregs in lymphoid tissues, but this was in the setting of a combined MMF and vitamin D₃ protocol (63). One in vivo/ex vivo study compared Tregs in 26 living-donor renal recipients who were receiving MMF and prednisone (64). After a mean follow-up of 7.2 yr, 69% (18 of 26) of recipients were donor nonresponsive as assessed by mixed lymphocyte reaction assay. There was no difference in CD4⁺CD25⁺ frequency in donor responsiveness versus nonresponsiveness. Notably, 19% (five of 26) of recipients showed donor-specific Tregs, as demonstrated by abrogation of nonresponsiveness after CD25⁺ depletion.

Induction Agents: Anti-thymocyte globulin, Alemtuzumab, and Anti-IL-2R

Our group was the first to demonstrate relative expansion of functional CD4⁺CD25⁺Foxp3⁺ Tregs in vitro with rabbit anti-thymocyte globulin (ATG) at a concentration 10 times lower than clinical dosage (32). This effect supervened with incubation in medium alone; was apparent already after 18 h; and was not observed with alemtuzumab, basiliximab, or daclizumab. Moreover, the increase in the Treg population was attributable to conversion of CD4⁺CD25⁻ T cells rather than merely expansion of a preexisting CD4⁺CD25⁺ pool. In contrast to rabbit ATG, horse ATG was unable to promote expansion of human Tregs (65).

In in vivo Treg studies involving depleting agents, one has to be careful in considering relative versus absolute levels of cell subpopulations. In renal and islet transplant recipients, studies with up to 36 mo of follow-up showed that alemtuzumab increased by up to three-fold the relative frequency of Tregs, ATG has either a smaller increase or no effect, and daclizumab had decreased Tregs (45,66–68). It is interesting that Bloom et al. (66) observed that the ratio of Tregs/Teffs in renal recipients who underwent induction with alemtuzumab rose from 0.04 before transplantation to 0.16 at 6 mo after transplantation, whereas the ratio decreased from 0.05 to 0.02 with basiliximab. There was no difference between the two agents in suppressive activity of Tregs in vitro. Another group that studied renal recipients found that, although in proportion of the CD4⁺ population, the highest levels of CD4⁺CD25⁺ T cells were observed with alemtuzumab, looking at absolute numbers at 240 d after transplantation, the highest levels were produced in decreasing order by daclizumab, ATG, and alemtuzumab (67).

Biologics

Belatacept (LEA29Y)

Belatacept is a high-affinity variant of CTLA4-Ig that blocks the CD28-CD86/80 co-stimulatory pathway and seems to regulate both naive and memory T cell responses (69,70). There have been concerns about the detrimental effect of CD28-CD86/80 blockade on Tregs after studies showed that this co-stimulatory pathway was essential for thymic production of nTregs and peripheral homeostasis of iTregs in an in vivo mouse model (71); however, in a cohort of renal recipients who were randomly assigned to Belatacept versus CNI maintenance therapy, Bluestone et al. (72) noted a transient (3 mo) reduction in the CD25⁺ population of CD4⁺Foxp3⁺ Tregs. With the caveat that all patients in that study underwent induction with Basiliximab, it was argued that the decrease was most probably due to the induction therapy; however, they and others observed no change in either the frequency or the potency of Tregs in 2 to 5 yr of follow-up (72,73).

Efalizumab (Raptiva)

Efalizumab is a humanized anti-CD11a (LFA-1) mab that is now used for the treatment of psoriasis. It inhibits LFA-1 binding to intracellular adhesion molecule 1, thereby preventing adhesion and activation of lymphocytes (74). In a protocol based on Thymoglobulin induction followed by efalizumab and rapamycin, patients who had diabetes and received islet transplantation had a marked increase in circulating Tregs (75). In patients with psoriasis, administration for 12 wk led to more than doubling of circulating T and B cells that was apparent within 7 d of the first dose and lasted up to 5 to 8 wk after treatment was stopped (74). Efalizumab was withdrawn from the market in early 2009 because of potential risks for developing progressive multifocal leukoencephalopathy (PML).

Alefacept (Amevive)

Alefacept is an LFA-3/IgG1 recombinant protein that binds CD2 on T cells and FcγRIII on NK cells and macrophages. It inhibits T cell activation/proliferation and induces selective apoptosis (76). Initial studies on human volunteers showed selective reduction in circulating memory effector CD4⁺ as well as CD8⁺ T cells after a single dose (77). Twelve wk of maintenance therapy in patients with psoriasis induced a significant decrease in T cells but not of the CD4⁺CD25high subpopulation (28 and 11%, respectively) (78).

Natalizumab (Tysabri)

Natalizumab is an α4 (VLA4) integrin antagonist that selectively blocks trafficking of leukocytes to inflamed tissues and that may also play a role in co-stimulatory signals for T cells (79). Major concerns were raised about this molecule in 2005 after cases of PML were reported (80–82). A review of more than 3000 cases later concluded that the risk for PML was roughly 1/1000 patients at 18 mo (83). In patients with multiple sclerosis, natalizumab had no effect in CD4⁺Foxp3⁺ frequency or suppressive activity either in vitro or in vivo (79). Moreover, there was no preferential blockade or permissiveness for the transmigration of Tregs into tissues compared with conventional T cells.

Small Molecules

In this group, we include the new molecules ISA247 (Voclosporin), AEB-071, and CP 690 550. The only compound for which data are available on Tregs is CP 690 550. In combination with prednisolone and MMF for renal recipients, van Gurp et al. (84) noted an initial reduction of CD4⁺CD25⁺ T cells with partial recovery at 2 mo; there was no change in suppressive capacity at 1 mo compared with before transplantation. Another molecule, FK778, was able to induce Tregs with suppressive activity...
(85), but the development of this molecule for transplantation has been stopped.

Conclusions

The appraisal of the impact of therapeutic agents on Tregs is far from complete (Figure 1). For maintenance agents, aggregate data suggest that rapamycin has a more favorable effect than CNI for both frequency and suppressive activity. In the case of induction agents, looking at the proportion of T cells and especially at the ratio of Teffs/Tregs, it seems that alemtuzumab and ATG do better than anti–IL-2R with maintained potency, suggesting that these former agents might re-educate the immune system. For biologics and small molecules, there is actually a paucity of data, but initial in vivo results indicate no serious adverse effect on Treg populations.

For both in vitro and in vivo systems, there is still a gap of knowledge to fill. In vitro, we need to set more precisely under which conditions and which stimuli Tregs are produced. For in vivo studies, we need to establish what therapeutic combination predicts the most favorable Treg profile. It is tempting to speculate that the balance between Teffs and Tregs might well prove to be more relevant than the Tregs alone in the achievement of tolerance. In support of this concept, recent data from our group showed that the administration of low-dosage ATG and CTLA4-Ig was a very effective strategy to promote engraftment in a stringent skin transplant model, precisely by tipping the balance of Tregs/Teffs toward regulation (86).

The study of Tregs in transplantation brings some additional questions. Among them, the issue of antigen specificity in particular needs to be answered. This is of most relevance in the elaboration of successful tolerizing protocols, because reports cited herein suggested that increasing solely the frequency of Tregs does not per se translate into better clinical outcomes. Moreover, the clinical relevance of Tregs in peripheral blood is still unknown. Notably, it is not yet established whether the expansion of these cells in the periphery translates into more Tregs within the graft. We hope that mechanistic studies as well as ongoing multicenter clinical trials led by our center and others will provide us with enough data in the near future to address some of these pressing issues.

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Disclosures

M.H.S. is consultant to Roche.

References


Figure 1. Summary of the effect of agents on Treg biology. The effect on Treg frequency is represented on the horizontal axis and the effect on Treg potency on the vertical axis, each axis delineating a negative versus positive effect. §Agents for which data are available for frequency but not potency. rATG, rabbit ATG; hATG, human ATG.
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