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Frequency of Natural Regulatory CD4<sup>+</sup>CD25<sup>+</sup> T Lymphocytes Determines the Outcome of Tolerance across Fully Mismatched MHC Barrier through Linked Recognition of Self and Allogeneic Stimuli<sup>1</sup>

Rita Fucs,* Joszilene T. Jesus,* Paulo H. N. Souza Junior,* Larissa Franco,* Mauricio Verícimo,* Maria Bellio,† and Alberto Nobrega²†

We show in this study that long-term tolerance to allogeneic skin grafts can be established in the absence of immunosuppression by the combination of the following elements: 1) augmenting the frequency of regulatory CD4<sup>+</sup>CD25<sup>+</sup> T cells (Treg) and 2) presentation of the allogeneic stimuli through linked recognition of allo- and self-epitopes on semiallogeneic F<sub>1</sub> APCs. BALB/c spleen cells enriched for CD4<sup>+</sup>CD25<sup>+</sup> T lymphocytes were transferred either to BALB/c nu/nu mice or to BALB/c nu/nu previously injected with F<sub>1</sub>(BALB/c × B6.Ba) spleen cells, or else grafted with F<sub>1</sub>(BALB/c × B6.Ba) skin (chimeric BALB/c nu/nu-F<sub>1</sub>). Chimeric BALB/c nu/nu-F<sub>1</sub> reconstituted with syngeneic CD25<sup>+</sup>-enriched spleen cells were unable to reject the previously transferred F<sub>1</sub>(BALB/c × B6.Ba) spleen cells or F<sub>1</sub>(BALB/c × B6.Ba) skin grafts, and a specific tolerance to a secondary B6 graft was obtained, with rejection of third-party CBA grafts. BALB/c nu/nu mice reconstituted only with syngeneic CD25<sup>+</sup>-enriched spleen cells rejected both B6 and CBA skin grafts. In contrast, when chimeric BALB/c nu/nu-F<sub>1</sub> were reconstituted with spleen populations comprising normal frequencies of Treg cells, the linked recognition of allo and self resulted in breaking of self tolerance and rejection of syngeneic grafts, strongly suggesting that linked recognition works in both directions, either to establish tolerance to allo, or to break tolerance to self, the critical parameter being the relative number of Treg cells. *The Journal of Immunology, 2006, 176: 2324–2329.

In the last few years, the unequivocal description of regulatory T cells and their prominent role in the maintenance of peripheral tissue tolerance (reviewed in Refs. 1–5) led to a conceptual reformulation of the self/nonself discrimination paradigm, classically based on the assumptions of clonal deletion or anergy of the self-reactive lymphocytes. The role of CD4<sup>+</sup>CD25<sup>+</sup> regulatory T cells (Treg) in experimentally induced transplantation tolerance has been repeatedly demonstrated (5–9) and several protocols using Abs or drugs that temporarily inhibit the activation of alloreactive T lymphocytes result in the induction of regulatory lymphocytes, able to establish an indefinite dominant tolerance to the allogeneic graft (10). It is not yet clear whether these experimentally induced regulatory lymphocytes correspond to a distinct differentiation stage of the “conventional” Ag-specific T cell, or if they correspond to the same lineage of CD4<sup>+</sup>CD25<sup>+</sup>Foxp3<sup>+</sup> lymphocytes which emerge from the thymus already compromised with a regulatory function (“natural” Treg cells) (11). Both possibilities were already suggested by studies using different experimental protocols. The selection of human regulatory T cells specific for a defined allopeptide from peripheral precommitted CD4<sup>+</sup>CD25<sup>+</sup> has been reported (12), suggesting that polyclonal “natural” Treg involved in the maintenance of self tolerance may include clones cross-reactive with nonself epitopes. In contrast, alloantigen-specific CD4<sup>+</sup>CD25<sup>+</sup> regulatory cells can develop from CD4<sup>+</sup>CD25<sup>+</sup> precursors in response to pretreatment of mice with allogeneic donor cells and anti-CD4 therapy. In this case, the generation of the Treg cells was shown to be independent from an intact thymus as well as from the expansion of CD4<sup>+</sup>CD25<sup>+</sup> lymphocytes (13).

Experimental protocols using blocking anti-CD4/CD8 Abs were able to induce long-term tolerance to allogenic grafts (10). The allospecific tolerance state could be transferred to naive hosts injected with T lymphocytes from the tolerant animal, showing the dominant effect of regulatory T cells upon the naive T lymphocytes of the recipient animal. Furthermore, it has been demonstrated that the donor Treg recruits new Tregs from the hosts, that are able to establish allospecific tolerance if transferred to a naive recipient, a phenomenon described as “infectious tolerance” (14). It has also been shown that tolerance may be extended to other unrelated Ags when both are recognized in the same APC, linked recognition, as demonstrated for F<sub>1</sub> grafts (15). Induction of tolerance by linked recognition could be firmly established across minor histocompatibility barriers, but much less efficiently across fully mismatched MHC. It is probable that tolerance induction by linked recognition depends on the frequency of Tregs and anti-allo-T cells participating in the immune response. By manipulating the ratio of Tregs/anti-allo-T cells it could eventually be possible to establish allogeneic tolerance across MHC barriers as well.
In the present study, we evaluated tolerance induction by linked recognition of allo- and self-epitopes presented by the same APC, in conditions where the frequencies of “natural” Treg were modified. Recipient BALB/c nu/nu mice, which have been previously injected with semiallogeneic (BALB/c × B6)F1 spleenocytes or grafted with skin from (BALB/c × B6)F1, were injected with different spleen cell suspensions from BALB/c nu/nu donors: 1) CD25+ -depleted cells, 2) CD25+ -enriched cells, or 3) total spleen cell suspensions. The persistence of the semiallogeneic F1 spleen cells or skin graft, and the tolerance to secondary B6 skin grafts were analyzed in the three groups and we found that animals receiving CD25+ -enriched spleen cells developed tolerance to fully allogeneic B6 skin grafts while rejecting third-party allografts. The results strongly suggest that “natural” Treg cells are critically involved in tolerance to fully allogeneic grafts, through a mechanism of linked recognition. Furthermore, a significant percentage of BALB/c nu/nu mice, that have been previously injected with semiallogeneic (BALB/c × B6)F1 spleen cells and further reconstituted with total BALB/c spleenocytes (not enriched for Treg cells), rejected syngeneic skin grafts, suggesting that linked recognition of allo and self can also lead to the opposite consequence, and result in breaking of tolerance to self, depending on the relative frequency of Treg cells injected in the host.

Materials and Methods

Mice

C57BL/6.Thy1.1+ (B6.Thy1.1+) (H-2b), BALB/c nu/nu (BALB/c), (B6.Thy1.1+ × BALB/c nu/nu)F1 (F1), and CBA/J (H-2d) mice, bred in our conventional animal house (Nucleo de Animais de Laboratorio/Universidade Federal Fluminense (UFF)), were used as donors of spleen cell suspensions and tail skin for graft rejection tests. BALB/c nu/nu (H-2b) mice, used as hosts, were housed in microisolation cages and received sterilized food and water.

Cell transfers

Spleen cell suspensions were diluted in PBS after red blood cell lysis by ammonium chloride and counted in presence of trypan blue. Nylon wool-T lymphocyte-enriched spleen cells were submitted to magnetic separation: CD25+ -depleted and -enriched fractions were obtained by negative and positive selections, respectively, following MACS protocol using anti-CD25 biotin (BD Biosciences/BD Pharmingen) and streptavidin-magnetic microbeads (Miltenyi Biotec-MACS). The resulting percentage of CD25+ cells in each fraction was evaluated by flow cytometry. All cell suspensions were injected i.v. into 20- to 30-day-old BALB/c nu/nu recipient mice. Cell numbers injected in each experiment and time schedule are indicated in the figure legends. T cell-depleted spleen cells from C57BL/6 were prepared by two consecutive rounds of lysis by anti-Thy1 and complement; T cell depletion was ascertained by FACS analysis and percentages of CD3+ cells were below 1%.

FACS analysis

Immunofluorescent staining and analysis of peripheral blood leukocytes and spleen cell suspensions were performed by flow cytometry (FACS-Calibur; BD Biosciences), using the mAbs anti-Thy-1.1 FITC (MRC OX-7; Serotec), anti-Thy-1.2 PE (30-H12; Immunotech), anti-CD4 FITC (H129.19; BD Pharmingen), anti-TCRβ1 biotin (H57-597; Invitrogen Life Technologies), anti-CD25 FITC (PC61; BD Pharmingen). Streptavidin-PE (Sigma 125H8876) and Avidin-FITC (Sigma 083H4825) were used in conjunction with biotin-labeled mAbs.

Skin grafting

Full-thickness tail skins from female BALB/c, B6, F1, and CBA/J mice were grafted on the dorsum of each experimental nude mouse. Grafts were inspected two to three times per week for hair growth and integrity of the grafted skin (acceptance) or were considered to be rejected when total absence of hair growth together with numerous disruption in tissue integrity, leaving <20% of the original graft, were noted.

Results

Chimeric BALB/c nu/nu-F1 recipients of CD25+ -enriched BALB/c lymphocytes do not eliminate F1 (BALB/c × B6.Ba) spleen cells

Three groups of BALB/c nu/nu hosts, previously reconstituted with F1 (BALB/c × B6.Ba) spleenocytes (chimeric BALB/c nu/nu-F1), were injected with different preparations of syngeneic BALB/c nu/nu spleen cells: the first group received nonseparated, total spleen cell suspensions and the other two received the CD25+ -depleted or CD25+ -enriched fractions. Typically, the CD25+ -depleted fraction contained <0.5% CD25+ cells and the fraction used as CD25+ -enriched comprised 30–60% of CD25+ lymphocytes, corresponding to a 6- to 10-fold enrichment of the original cell suspension. The transfer of syngeneic cells obtained from the nonseparated spleen or from the CD25+ -depleted fraction resulted in the complete elimination of the circulating F1 T cells from the nude hosts within the first month after injection (Fig. 1). In contrast, nude hosts receiving comparable numbers of the CD25+ -enriched BALB/c spleen cells were unable to reject the semiallogeneic F1 cells. Mice of this group were studied for 11 mo after the BALB/c transfer and a high frequency of F1 T cells was still found (Fig. 1). Similar results in the three groups were obtained with different inoculums of BALB/c cells, ranging from 3 × 10^6 to 15 × 10^6 cells. The CD25+ -enriched transferred cells were not rejected by CD25+ -enriched BALB/c spleen cells. BALB/c nu/nu hosts were injected with F1 spleen cells (Thy-1.1+/Thy-1.2−) at day 0 and further reconstituted either with CD25+ -depleted (n = 5) or CD25+ -enriched (n = 6) BALB/c spleen cells (Thy-1.2+), or total spleenocytes (n = 20). The persistence of F1 cells in blood samples was investigated 30 days after reconstitution. Typical Thy-1.1+/Thy-1.2− staining of peripheral blood, representative of one animal of each group, are shown. Similar results were obtained injecting cell numbers varying from 1 to 15 × 10^6 BALB/c cells in each group. F1 T cells were rapidly eliminated in BALB/c nu/nu hosts injected with total spleen or CD25+ -depleted splenocytes (Fig. 1, upper left and lower left). Short-term and long-term persistence of F1 cells in BALB/c nu/nu hosts injected with CD25+ -enriched spleen cells were demonstrated after 1 and 11 mo after reconstitution (Fig. 1, lower right and upper right).

FIGURE 1. (BALB/c × B6)F1 cells are not rejected by CD25+ -enriched BALB/c spleen cells. BALB/c nu/nu hosts were injected with F1 spleen cells (Thy-1.1+/Thy-1.2−) at day 0 and further reconstituted either with CD25+ -depleted (n = 5) or CD25+ -enriched (n = 6) BALB/c spleen cells (Thy-1.2+), or total spleenocytes (n = 20). The persistence of F1 cells in blood samples was investigated 30 days after reconstitution. Typical Thy-1.1+/Thy-1.2− staining of peripheral blood, representative of one animal of each group, are shown. Similar results were obtained injecting cell numbers varying from 1 to 15 × 10^6 BALB/c cells in each group. F1 T cells were rapidly eliminated in BALB/c nu/nu hosts injected with total spleen or CD25+ -depleted splenocytes (Fig. 1, upper left and lower left). Short-term and long-term persistence of F1 cells in BALB/c nu/nu hosts injected with CD25+ -enriched spleen cells were demonstrated after 1 and 11 mo after reconstitution (Fig. 1, lower right and upper right).

Statistical analysis

Statistical comparisons of the survival time of grafts were done using the nonparametric Mann-Whitney U test.

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T cells occasionally had a diminished initial expansion when compared with mice receiving the CD25^+ -depleted fraction (data not shown). However, as can be seen in Fig. 1, after 1 mo, donor-derived lymphocytes correspond to half of the T cell population in the chimeric hosts.

Chimeric BALB/c nu/nu-F1 recipients of CD25^+ -enriched BALB/c lymphocytes are specifically tolerant to B6 skin grafts

The three groups of chimeric mice described above were tested for their ability to reject B6 skin grafts. Sixty days after syngeneic BALB/c spleen cell transfers, the mice were grafted with tail skin obtained from three different donors: BALB/c nu^+^, C57BL/6 and CBA/J (third party alloimmune haplotype). In line with their tolerance to H-2^b on (B6 × BALB/c)F1, T cells, the BALB/c nude hosts transferred with the CD25^+ -enriched population were also mostly unable to reject the B6 skin (Fig. 2A, F1nu/nu/CD25^+^), being statistically equivalent to animals grafted with syngeneic skin (p = 0.46, Mann-Whitney U test). Healthy grafts, with no signs of rejection crises and abundant hair growth, were observed for at least 11 mo in 80% of the animals in this group. The specificity of this tolerance was established by the complete rejection of CBA skin grafts in 100% of these hosts.

BALB/c nu/nu mice receiving only CD25^+ -enriched syngeneic cells, in the absence of a previously transferred F1 population, were fully able to reject allogeneic B6 skin grafts, showing that previous contact with semiallogeneic cells was necessary to induce tolerance to B6. As shown in Fig. 2B, these mice were fully able to reject CBA and B6 skin grafts (p < 0.04, Mann-Whitney U test when compared with syngeneic BALB/c graft), showing that the transferred population of CD25^+ -enriched T cells is functional and immunocompetent. It is important to observe that contact with allogeneic Ags on a fully mismatched combination does not lead to skin graft tolerance, as demonstrated by BALB/c nu/nu mice previously injected with T cell-depleted splenocytes from B6 donors, and reconstituted with CD25^+ -enriched syngeneic cells (Fig. 2A, B6 → nu/nu/CD25^+^). Tolerance could only be established by linked presentation of self and nonself on F1 spleenocytes.

Tolerance to B6 grafts in recipients of CD25^+ -enriched splenocytes could also be induced by the previous grafting of the nude host with F1(BALB/c × B6.Ba) skin instead of the transfer of F1(BALB/c × B6.Ba) spleen cells (Fig. 3A, F1nu/nu/CD25^+^). Again, tolerance to B6 is strictly dependent on the presence of the alloantigen in the F1 skin graft. As shown in Fig. 3A, BALB/c nu/nu mice previously grafted with B6 and BALB/c skin and reconstituted with CD25^+ -enriched splenocytes rejected B6 (Fig. 3A, B6 → nu/nu/CD25^+^). Tolerance was dependent on the enrichment for CD25^+ cells, as BALB/c nu/nu mice grafted with F1 skin and injected with total BALB/c spleen cells rejected B6 skin grafts within 40 days after grafting (Fig. 3B, F1nu/nu/spleen). The minimum relative enrichment for CD25^+ cells needed to confer tolerance was not systematically investigated. However, in experiments described in Figs. 2 and 3, CD25^+ cells varied from 30 to 60% of injected cells (Table I), representing a 6- to 10-fold enrichment, when compared with total splenocytes.

**FIGURE 2.** CD25^+ -enriched BALB/c spleen cells are tolerant also to B6 skin grafts. A, BALB/c nu/nu previously injected with F1 spleen cells and further injected with CD25^+ -enriched BALB/c spleen cells (F1nu/nu/CD25^+^) were simultaneously grafted 1 mo later with skins from CBA (Δ), (F1nu/nu/CD25^+^ (CBA)), BALB/c (○) (F1nu/nu/CD25^+^ (BALB/c)), and B6 (□) (F1nu/nu/CD25^+^ (B6)) (n = 5), and analyzed for the kinetics of graft rejection. In the legend, the origin of the skin graft is indicated within brackets. Statistical comparisons between graft rejections gave BALB/c × CBA, p < 0.009; BALB/c × B6, p = 0.46; B6 × CBA, p < 0.05, Mann-Whitney U test. All BALB/c nu/nu previously injected with T cell-depleted B6 splenocytes and further reconstituted with CD25^+ -enriched BALB/c spleen cells rejected B6 skin (■) (B6 → nu/nu/CD25^+^ (B6)). B, Kinetics of graft rejection by normal BALB/c nu/nu reconstituted with CD25^+ -enriched BALB/c spleen cells (nu/CD25^+^) (n = 6) and further grafted with skin from CBA (Δ) (nu/CD25^+^ (CBA)), BALB/c (○) (nu/CD25^+^ (BALB/c)), and B6 (□) (nu/CD25^+^ (B6)). Statistical comparisons between graft rejections gave: BALB/c × CBA, p < 0.04, and BALB/c × B6, p < 0.04, Mann-Whitney U test.

**Breaking tolerance to syngeneic grafts**

Animals in our colony of BALB/c nu/nu hosts reconstituted with syngeneic total spleen cells behave as expected, with acute rejection (within 30 days) of allogeneic skin grafts (B6 and CBA), and tolerance to the syngeneic BALB/c skin. However, when these mice received F1(BALB/c × B6.Ba) spleen cells before the transfer of syngeneic BALB/c spleenocytes, a disturbance on the tolerance to syngeneic BALB/c skin was clearly observed. Besides rejection of B6 and CBA grafts, >60% of the animals also showed a complete rejection of the syngeneic skin until 70 days after grafting (F1nu/nu/spleen (BALB/c)–Fig. 4A). Most of the BALB/c grafts in the remaining hosts had macroscopic signs of partial rejection, with no hair left and a marked reduction in the donor tissue present in the graft bed. The rejection of syngeneic grafts was only observed when BALB/c nu/nu were previously injected with F1 spleenocytes, but not when BALB/c nu/nu were previously grafted with F1 skin (Fig. 3B). Breaking of tolerance to self could only be achieved in mice exposed to alloantigen on semiallogeneic F1 spleen cells (Fig. 4B).

Interestingly, rejection of syngeneic BALB/c skin was also a statistically significant result occurring in >50% of mice reconstituted only with syngeneic CD25^+ -depleted spleen cells, even in the absence of the previous injection of F1 spleenocytes (Fig. 5), corroborating the notion that pathological autoreactivity often arises from this T cell population in the absence of Treg cells (16).
We noted that the absence of linked recognition resulted in more vigorous rejection in animals previously injected with B6 splenocytes when compared with nudes previously grafted with B6 skin (Figs. 2A and 3A). In the latter, complete rejection of allogeneic grafts in some animals was observed after only 200 days. We speculate that the reduced number of fully allogeneic APCs in animals grafted only with skin may favor the indirect pathway of recognition of self and nonself. Differently, in BALB/c nu/nu injected with T cell-depleted B6 splenocytes, T cells are directly exposed to fully allogeneic stimuli in higher numbers of donor APCs, in the absence of linked recognition.

Long-term chimera of donor lymphocytes is a hallmark of the tolerant state, as originally described by Medawar and colleagues (17). The continuous presence of semiallogeneic F1 splenic cells, as shown in Fig 1, could allow the recruitment of anti-allogregulatory T cells or promote anergy of alloreactive lymphocytes, such that chimera could be the essential element determining tolerance in our experimental system. It is thus important to consider why semiallogeneic F1 cells are not eliminated by the CD25+ enriched spleen cells. It could be argued that the CD25+ enriched fraction would have limited expansion in the nude hosts, which might compromise their potential to reject the F1 cells. However, these animals are fully competent to reject CBA grafts.

Table 1. Phenotype and numbers of BALB/c transferred cells

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<th>Total Number of Transferred Cells</th>
<th>% CD25+</th>
<th>% F1 T Cells</th>
<th>% BALB/c T Cells</th>
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* For each chimeric F1 → nu/CD25+ animal used in experiments described in Figs. 2, 3A, or 3D, the total number of transferred cells and the relative enrichment for CD25+ T cells in the transferred population are shown. For animals used in the experiments described in Fig. 2A, the percentages of F1 T cells and donor BALB/c T cells in peripheral blood, 1 mo after the injection of BALB/c cells, are also shown. The identifications of F1 and BALB/c T lymphocytes was based on the expression of Thy1.1 and Thy1.2 markers.
elimination of F1 cells because they are inhibited by the presence, in high frequency, of CD25+ regulatory T lymphocytes. This inhibition of CD25− T cells would probably require close cellular interactions of T CD25− and T CD25+ cells on F1 APCs, through linked recognition of self and nonself epitopes (18, 19). CD4+CD25+ Tregs may then influence the allospecific lymphocytes. This in - teractions of T CD25+ T lymphocytes (20). In transplantation tolerance, Treg could be obtained by protocols which blocked acute rejection, and it has been proposed that a sustained but incomplete signaling by donor Ags, in the absence of “danger” signals, would anergize the alloreactive cells (6). Alternatively, the prevention of an initial activation of anti-alloeffector T cells may favor the indirect phase of recognition of allogeneic epitopes in host APCs, such that anti- allopeptide Treg are probably generated under the influence of natural Treg cells through linked recognition of self and allopeptide presented by the host APC. The beneficial effect of pretransplant blood transfusion on allogeneic graft acceptance was recently ascribed to the induction of CD4+CD25+ Treg cells. Although allospecificity is not always required in these protocols (21), the obligatory sharing of at least one MHC class II allele between donor and recipient in humans favors the need for linked recognition and the participation of self-reactive Treg cells for the tolerance induction (22). Also, in a model of tolerance induced by modified dendritic cells, it was shown that the coexpression of allogeneic and self epitopes and the presence of CD25+ regulatory T cells are essential to cause unresponsiveness in the indirect pathway of allore cognition (23).

As shown by Nishimura et al. (24), tolerance to the fully mismatched allograft could be obtained if the animals were reconstituted with highly purified CD4+CD25+ T cells. These results suggest that “natural” self-reactive Treg cells may have some cross-reactivity with allo-MHC+ peptides, but the frequency of cross-reactive clones seems to be low, as the presence of a small number of CD25− does not allow tolerance to be established. Here, the data we obtained show that tolerance can be established in the presence of a significant percentage of CD25− T cells, provided that the allogeneic stimulators are linked to self. It is interesting to observe that the need of linked recognition of allo and self may be less stringent in different genetic combinations of host and donor mouse strains (25). The impact of linked recognition together with CD25+ cells frequency on allograft rejection may also explain the very recent results reported by Benghiat et al. (26), published while this manuscript was in preparation. They observed that F1(B6×bm12) skin is often spontaneously accepted by nonmanipulated B6 mice (50% of the recipients), in contrast to bm12 skin, which expresses only the donor MHC class II, and is rejected by all the B6 hosts studied. The tolerance to the F1 skin is apparently dependent on CD4+CD25+ Treg cells, because it is abolished by CD25− in vivo depletion. Because in this case donor and

Evidence for the inhibition of Ag-specific T lymphocytes by Tregs has been obtained in different experimental models of peripheral tolerance and it has recently been demonstrated, in vivo, that Treg cells are able to inhibit both proliferation and differentiation of CD25− T lymphocytes (20). In transplantation tolerance, Treg could be obtained by protocols which blocked acute rejection,
recipient MHC difference is restricted to three amino acids, the linked presentation of their molecules in F1 grafts may be sufficient to tolerize T cell populations harboring the normal frequency of CD25+ cells.

When CD25+-depleted cells were injected in normal BALB/c nu/nu hosts, not only the allogeneic B6 graft was rejected, but also we often verified the rejection of syngeneic skin grafts (Fig. 5). Statistical analysis comparing BALB/c nu/nu reconstituted with CD25+-depleted BALB/c spleen cells with the control group of BALB/c nu/nu reconstituted with syngeneic nonseparated splenocytes gave p < 0.03, supporting the conclusion that rejection of syngeneic grafts is indeed determined by the absence of CD25+ T reg cells. This rejection adds to the general picture of systemic autoimmunity observed in immunodeficient hosts injected with CD4+CD25+ T cells, characterized by multiple organ infiltration of effector autoreactive T cells (16). The inflammatory signals present in the grafted tissue may focus the autoimmune response to the syngeneic graft, leading to its rejection. These results indicate that lymphocytes specific for self-epitopes in the skin are not deleted by thymic selection and may be peripherally controlled by Treg cells. Studies analyzing the genes expressed by T cells infiltrating tolerated allografts, or syngeneic skin grafts, showed comparable gene expression profiles (27). Similar mechanisms may, thus, function to maintain an induced tolerance to allogeneic tissues and the natural tolerance to self-skin, both apparently depending on regulatory T cells.

More interestingly, the injection of total spleen cells in chimeric BALB/c nu/nu-F1 hosts often resulted in the rejection of syngeneic skin grafts as well (Fig. 4A). Rejection of syngeneic grafts do not occur in chimeric BALB/c nu/nu-F1, reconstituted with CD25+-enriched splenocytes (Fig. 2A). These results suggest that the frequency of Tregs is also the critical parameter controlling the outcome of autoimmunity triggered by a potent allogeneic response, through linked recognition. We speculate that a high frequency of allogeneic-specific effector lymphocytes interacting with autoreactive T cells in the same APCs, through linked interaction, as occurred in the response to F1, may revert the tolerizing influence of “natural” self-reactive regulatory cells, resulting in the activation of the anti-self lymphocytes with an effector profile. That would imply that linked recognition works in both directions, either to establish tolerance to allo, or to break tolerance to self, the critical parameter being the relative number of self-reactive Treg cells vs self-reactive effector T cells.

In conclusion, our results point to a great potential of linked recognition to control both anti-self and anti-nonself responses, that could be the basis of new strategies to control graft rejection or autoimmunity. The linked recognition of allogeneic epitopes by a high frequency of natural CD4+CD25+ cells may allow long-term or indefinite acceptance of allogeneic grafts, circumventing the need for immunosuppression.

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References