Dominant Transplantation Tolerance

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The ideal therapy for the prevention of graft rejection would be one given short-term to achieve life-long tolerance without incurring side effects nor diminishing immunocompetence to infectious agents. Recent advances in the understanding of peripheral transplantation tolerance suggest that this may eventually be possible. The demonstration of regulatory cells with the ability to tame alloreactive clones may provide the framework for this advance. This review focuses on the challenging prospects of dominant tolerance and some of its characteristics, namely linked suppression and infectious tolerance.

ABBREVIATIONS:

AICD	Activation-induced cell death
CsA	Cyclosporin-A
MHC	Major histocompatibility complex

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Transplantation tolerance (Fig. 1) can be achieved therapeutically through two distinct approaches: inactivation of alloreactive clones and the induction of regulatory circuits. Although the approaches might seem incompatible, we here argue that most tolerance induction strategies involve, to a certain degree, both inactivation of alloreactive cells and the amplification of regulatory cells.

Current immunosuppressive regimens target the whole immune system. However, an elective ablation of only the alloreactive clones, if feasible, offers a way of preventing graft rejection while sparing host immunocompetence. One possible approach to achieving this involves the transfer of a high dose of bone marrow cells from the donor to establish mixed hemopoietic chimerism or macrochimerism.[1-3] This permits in vitro monitoring of the tolerant state by sampling lymphocytes from the host and testing their reactivity against donor-type cells. Such "functional" assays may be impracticable, inconvenient and not always reliable. Furthermore, it might prove difficult to deplete all alloreactive T-cell clones, and any expansion of residual cells might give rise to delayed transplant rejection.

The complementary strategy aims to control alloreactive cells in a different way. It is based on the induction of a dominant tolerance state (Fig. 2) and its hallmark is the emergence of regulatory $CD4^+$ T cells.[4] Unlike tolerance by deletion, here cells with the ability to react in vitro with donor type cells may still be demonstrated, but grafts are still accepted indefinitely. Furthermore, tolerance is very robust and resists the adoptive transfer of cells with the potential to mediate graft rejection—the reason why it is termed dominant.[5,6] The regulatory cells can even do more than just "suppress": if they are allowed to coexist with the naïve cells, they have the capacity to recruit new regulatory $CD4^+$ T cells from that naïve pool. After this recruitment, the initial regulatory T cells can be removed experimentally and one observes that the new regulators can maintain tolerance themselves.[7] This process can be repeated experimentally for several cell transfers, and has therefore been named infectious tolerance (Fig. 3).[8]

Achieving Dominant Transplantation Tolerance

Short courses of therapeutic antibodies have been shown to lead to long-term acceptance of foreign grafts in several experimental systems (reviewed in ref. 4). The first examples of peripheral tolerance induced with monoclonal antibodies were reported in 1986.[9,10] In these experiments tolerance to foreign immunoglobulins was achieved after a short-term treatment with depleting anti-CD4 antibodies. It was soon demonstrated that depletion of $CD4^+$ cells was not required for tolerance induction, as similar results were found using F(ab')2 fragments,[11-13] non-depleting isotypes[5] or non-depleting doses of synergistic pairs of anti-CD4 antibodies.[14] Treatment with anti-CD4 antibodies was also shown to lead to long-term acceptance of skin grafts differing by "multiple-minor" antigens[5] even

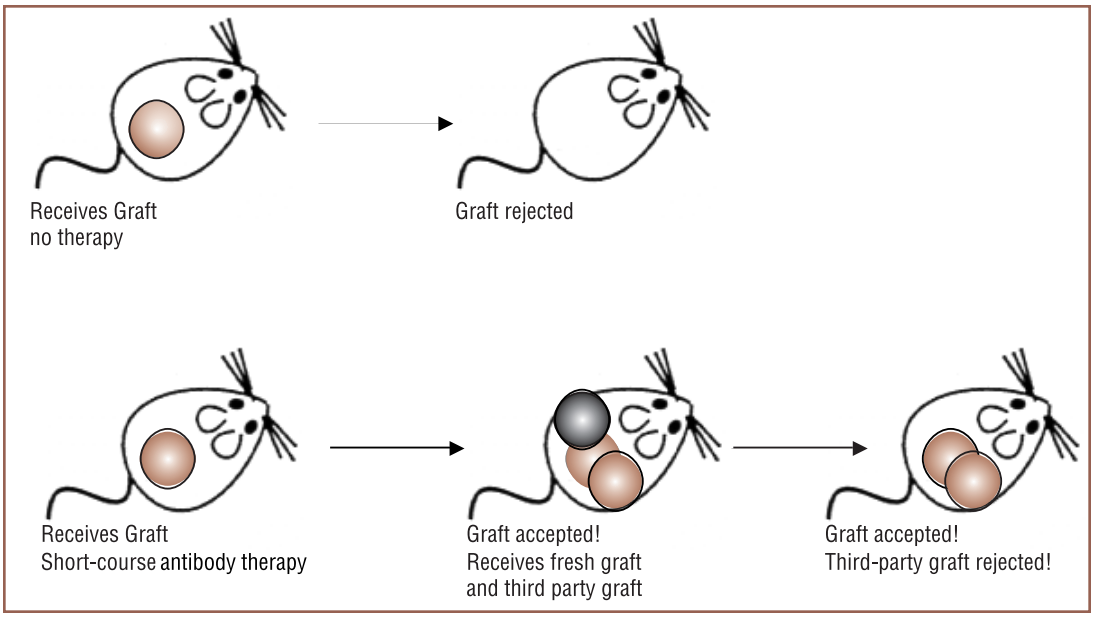


Figure 1. Demonstration of tolerance in antibody treated animals. Mice accept a second challenge with a graft of the same type, but readily reject third party grafts. Alloreactive cells, as demonstrated by proliferation assays, are present at any time point.

TRANSPLANTATION TOLERANCE:

Long-term acceptance of an allogeneic transplant without the need for continuous immunosuppression. The recipient remains fully competent to reject unrelated (third-party) grafts.

in pre-sensitized recipients.[15] The same results were also demonstrated for heart grafts across MHC barriers[16,17] or concordant xenografts.[16]

Further demonstrations of transplantation tolerance were later reported with anti-LFA-1 antibodies, alone[13] or in combination with anti-ICAM-1[18] and also with anti-CD2 and anti-CD3 antibodies.[19]

More recently, co-stimulation blockade of CD28,[20] CD40L (CD154)[21] or both in combination[22] has been shown effective. These findings have recently been extended to non-human primates. In one study, long-term survival of renal allografts was achieved following blockade of CD40L alone.[23] Another group achieved prolonged islet allograft acceptance after a similar treatment.[24] Interestingly, the association of tacrolimus or steroids to the therapeutic regime abrogated tolerance.[23]

Infectious Tolerance

Models of transplantation tolerance induced with anti-CD4 or anti-CD40L antibodies showed that tolerant mice did not reject the grafts even after the adoptive transfer of lymphocytes from a non-tolerant animal.[5,6,25] It was also demonstrated that spleen cells from animals made tolerant to skin and heart grafts using anti-CD4 or anti-CD40L antibodies could regulate naïve T cells, and in so doing, render them regulatory in their own right.[7,26,25] Using transgenic mouse strains carrying specific cell surface markers in their lymphocytes, it was possible to selectively eliminate the host-type T cells from the tolerant animal.[7,25] If this cell-depletion was performed immediately after cell transfer, the tolerance state was broken and indicator grafts were readily rejected by the transferred non-tolerant lymphocytes.[7,25] If, however, the host cells were allowed to coexist with those adoptively transferred for 4-6 weeks, then tolerance was maintained even after the depletion of the host cells.[7,25] The remaining cells were nevertheless fully competent to reject an unrelated graft. Not only were they unable to reject a graft from a similar donor, but they could now regulate another population of spleen cells from a non-tolerant animal in a similar transfer experiment.[7] This effect, named "infectious tolerance" (Fig. 3), provides compelling evidence for the existence of regulatory T cells: the regulatory cells from a tolerant animal can suppress the aggressive action of graft-reactive T-cells and induce members of that population to become regulatory as well.

A further important finding underlining the significance of infectious tolerance comes from the demonstration of a phenomenon named "linked suppression" (Fig. 4). In the original experiment[27] mice of type A made tolerant to type B skin grafts with non-depleting anti-CD4 and anti-CD8 treatment, readily rejected a third party graft of type C. However, if they were grafted with (BxC)F1 skin instead, rejection was delayed or absent while (AxC)F1 grafts were readily rejected. Furthermore, mice that accepted the (BxC)F1 skin grafts later accepted C type skin. The same phenomenon was recently demonstrated for anti-CD40L antibody induced tolerance.[28]

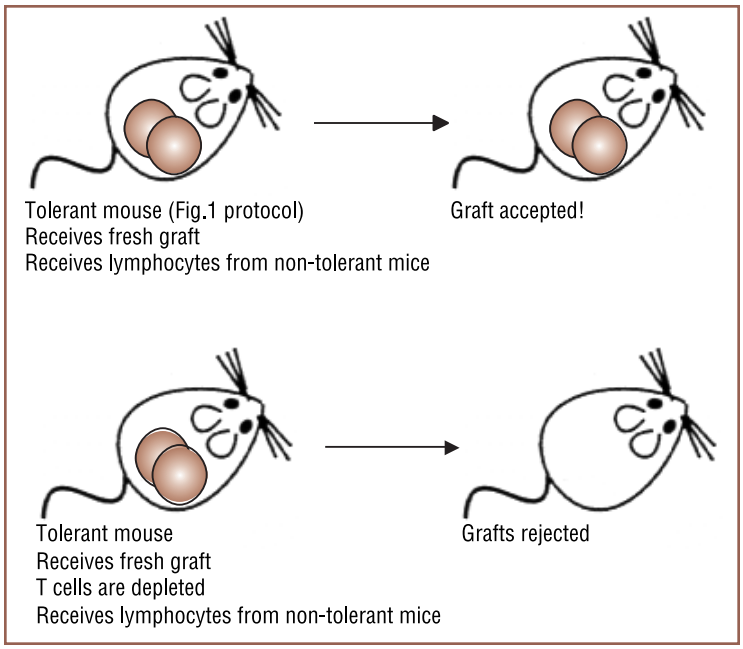


Figure 2. Demonstration of dominant tolerance. This requires the demonstration of tolerance being imposed on cells with the ability to reject a graft in the absence of regulatory cells.

DOMINANT TOLERANCE:

A state where the immune system of the tolerant recipient actively prevents rejection of the transplant even following the administration of immunocompetent T cells. In this situation, the tolerance is self-maintained despite continuous regeneration of naïve lymphocytes from the thymus.

T-Cell Regulation

Evidence for the existence of regulatory T cells does not come exclusively from studies of transplantation tolerance. Regulatory T cells have been found in several autoimmunity models (reviewed in 29). Even among the T cell population of normal individuals, T cells with the capacity of causing autoimmune disease have been identified, as well as regulatory cells that prevent this pathological autoaggression.[30,31] It is therefore likely that, in addition to thymic tolerance, peripheral tolerance mechanisms operate to safeguard tolerance to extrathymic antigens.

The phenotype of these regulatory cells and their proposed mechanisms of action are not yet totally clear. Although it is possible to induce transplantation tolerance with mAbs in thymectomized mice,[14,28] there is evidence suggesting that regulatory cells in some autoimmunity models are a defined lineage originating in the thymus (reviewed in ref 32). This lineage was shown to have some distinctive surface markers: they are included in the $CD45RC_{lo}$ population of $CD4^+$ cells in the rat,[30] or in the $CD45RB_{lo}$ in the mice.[33] It also seems that expression of the IL-2 receptor α-chain (CD25) reflects the presence of a putative regulatory $CD4^+$ cell that further subdivides the $CD45RB_{lo}$ population.[34,35] Given that CD25 seems to be a marker of suppressor cells it may seem paradoxical that an antibody targeting CD25 is licensed for use as immunosuppressive agent in clinical transplantation (reviewed in ref. 36). A theoretical risk for a therapy that targets CD25 expressing cells might be the loss of potential to induce tolerance to the graft, as well as a possible disruption of normal regulatory mechanisms that prevent autoimmunity.

Other markers, such as L-selectin[37] or CD38,[38] have also been suggested as possible surface markers of regulatory cells. It is hoped that purification and cloning of these elusive regulatory cells will allow a better understanding of their biology.

Tolerance and Cell Death

There are now many examples where evidence is found for alloreactive T-cell death in response to transplanted tissue without the need for purposeful chimerism. For example, two interesting recent papers demonstrate that tolerance induction with therapeutic anti-CD40L mAbs requires cell death.[39,40] In fact, blockade of activation induced cell death (AICD) either by using transgenic mice resistant to apoptosis[40] or by using cyclosporin A (CsA)[39] resulted in graft rejection in animals subjected to antibody blockade of CD28 and CD40L.

In spite of the importance of AICD in anti-CD40L transplantation tolerance, regulatory cells also play a role in its maintenance. In fact, tolerance induction with therapeutic anti-CD40L results in linked suppression[28] and in infectious tolerance.[25] Thus, regulatory $CD4^+$ T cells emerge, following tolerance induction and actively enforce a dominant tolerance state.

We can safely speculate that amplification of regulatory cells and induction of AICD are probably general mechanisms exploited in the different

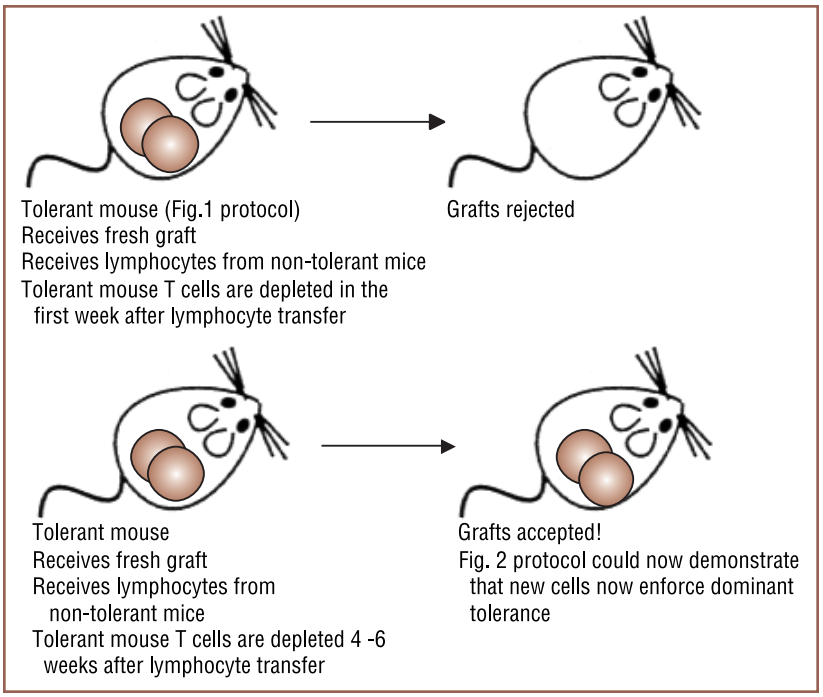


Figure 3. Demonstration of infectious tolerance. This requires the demonstration that cells with the ability to reject are converted into the regulatory type after coexistence with cells from a tolerant animal.

INFECTIOUS TOLERANCE:

A state of dominant tolerance characterized by the capacity of tolerant cells to impose similar regulatory behavior onto naïve T cells while both populations coexist together with the tolerated transplant.

tolerance inducing strategies. It is likely, although not yet demonstrated, that anti-CD4 therapeutic mAbs also require some cell death for the induction of transplantation tolerance. Recently it has been shown that anti-CD4 tolerance is independent of the Fas (CD95) pathway.[41] Probably in all tolerance inducing strategies some cell clones will remain fully committed towards an aggressive phenotype and their physical (AICD) or functional (anergy) deletion is required if tolerance induction is to be successful.

Information is lacking on whether therapeutic protocols that aim at the deletion of alloreactive clones, such as the ones based on macrochimerism,[2] also support the emergence of regulatory cells. Such studies need to be performed.

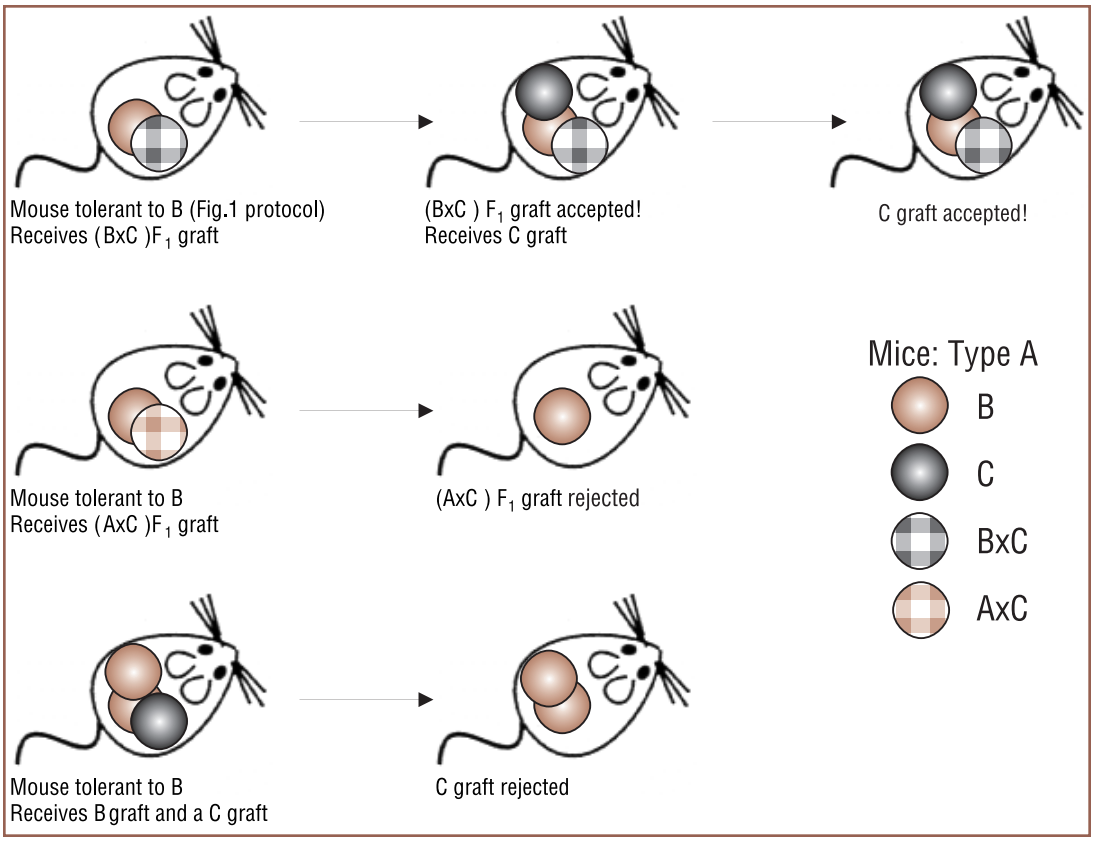
How Can This Knowledge Translate to the Clinic?

Current immunosuppressive agents, although the best option available, are far from ideal drugs. However, their known efficacy in preventing acute allograft rejection makes it ethically difficult to displace them in clinical trials of potential tolerogenic drugs. CsA is known to hinder tolerance induction with therapeutic mAbs.[39] Is it wise though to give transplanted patients an experimental therapeutic regime in the absence of CsA?

One reason why CsA exerts a tolerance-blocking effect is due to its capacity to interfere with AICD.[39] In fact, both CsA and tacrolimus (FK506) are calcineurin inhibitors that block transcriptional activation of the IL-2 gene in response to antigen stimulation. As lymphocytes are prevented from being activated, AICD does not occur. In that respect the new immunosuppressive drug rapamycin might be a good alternative. It does not interfere with activation and AICD. It rather functions by arresting the cell cycle, rendering lymphocytes insensitive to proliferative signals. Therefore, although CsA prevents tolerance induction with anti-CD40L antibodies, rapamycin does not affect tolerance in this system.[39] One can predict that anti-CD4 tolerance induction might be achieved in spite of concomitant administration of rapamycin.

Another issue to bear in mind is the practical feasibility of any therapeutic strategy. Some of the experimental protocols might be too complex or involve potential side-effects too risky for widespread clinical use. The ideal agent should be easily administered and with low impact on the immune system as a whole.

A potentially simple approach that has not yet been exploited, though offering therapeutic potential, is linked suppression (Fig. 4). Although very little is known of its mechanisms, it can mediate powerful immunoregulatory effects: for example, after tolerance induction with non-depleting CD4 and CD8 mAbs to a minor mismatched skin, tolerant animals subsequently accept skin from donors that in addition to



LINKED SUPPRESSION:

T cells suppressing the response to one set of antigens are able to prevent rejection directed towards further antigens co-expressed in the transplanted tissue.

Figure 4. Demonstration of linked suppression. This requires the demonstration that tolerant animals accept grafts where a third party antigen is present in cells that also have the tolerated antigens (BxC), but reject third party grafts (C) if the tolerated antigen absent from the graft cells (even if a concomitant tolerated-type graft (B) is given). The animals that accept the grafts with the "linked" third party antigen (BxC) should accept later grafts of the third party (C).

the tolerized minors have a major histocompatibility mismatch.[27] In practice one might be able to "tolerize" to a series of polymorphic alloantigens in advance of a transplant to pre-expand regulatory cells. Following organ transplantation this first cohort of regulatory cells may facilitate spread of tolerance to clones reactive to the "linked" antigens. Thus inducing tolerance to the whole organ.

The administration of epitopes by oral,[42,43] nasal[44] and even intraperitoneal[45] routes can lead to tolerance. It is also possible to modify the characteristics of the peptide, such as the affinity for the MHC, to modulate this effect.[46]

As we get to know some of the most important or dominant epitopes involved in graft rejection, we may be able to use them to induce transplantation tolerance. Furthermore, tolerance induced with a few dominant epitopes might then "spread" by linked suppression to other epitopes that are also present in the graft. It should therefore be possible to "build tolerance in stages": it is probably not necessary to tolerize to the whole set of major and minor antigens of the allograft, since tolerance to a few dominant ones will subsequently spread to the rest. Ultimately it might be possible to identify a group of dominant epitopes that could be used as a universal therapy to induce transplantation tolerance in any host-donor combination.

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