Published in final edited form as:
Clin Immunother. 1996 August ; 6(2): 89–96.

Prevention of Transplant Rejection:

Can Tolerance be Achieved with Immunosuppressive Treatment?

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Summary

Successful solid organ transplantation is generally attributed to the increasingly precise ability of drugs to control rejection. However, it was recently shown that a few donor haematolymphoid cells can survive for decades in recipients of successful organ allografts, a phenomenon called microchimaerism. The association for decades of haematolymphoid chimaerism with allograft tolerance in experimental transplantation suggests that immunosuppressive drugs merely create a milieu that enables an allograft and its complement of passenger leucocytes to prime the recipient for graft acceptance.

Exploitation of this concept requires a fundamental shift in the classical view of passenger leucocytes only as initiators of rejection. Microchimaerism has taught us that solid organ transplantation involves the transfer of two donor organ systems to the recipient: the allograft parenchyma and the donor haematolymphoid system in the form of donor stem cells contained within the passenger leucocyte compartment. Each has the potential to integrate with the corresponding recipient system and carry out normal physiological functions, such as immunological self definition. Resistance to initial integration by mature T cells requires some form of immunosuppression, but maintenance of donor immune system function will depend on renewable supply of cells, which can be provided by engrafted progenitors. Successful clinical application will depend on the development of low morbidity methods to enhance engraftment of donor haemopoietic stem cells.

The recent success of solid organ transplantation is generally attributed to the increasingly precise ability of drugs such as cyclosporin, and more recently tacrolimus, to control rejection by inhibiting signal transduction in activated T lymphocytes.[1,2] However, the observation that these or other drugs can control allograft rejection and prolong graft survival does not imply that, alone, they are capable of inducing donor-specific tolerance. In fact, most solid allograft recipients seem to uniformly require lifelong immunosuppressive therapy to maintain graft function. In this paper, we describe recent evidence suggesting that immunosuppressive drugs create a milieu which allows an allograft to prime the recipient for the induction of allogeneic tolerance. We suggest that every solid organ allograft transplanted under the cover of immunosuppression has this potential to induce tolerance.

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1. Donor-Specific Tolerance

Acquired donor-specific transplantation tolerance was first observed in cattle twins,[3] and was then intentionally induced over 40 years ago in experimental animal fetuses exposed to allogeneic haematolymphoid cells.[4] Tolerance in this setting was associated with mixed haematolymphoid chimaerism, a condition in which a single functionally integrated immune system is composed of cells from two different individuals.

Since the early experimental successes, many investigators have attempted to replicate this situation in adults in a clinically applicable setting.[5-12] Conditioning, or ablating, the recipient's immune system with cytoreductive drugs or irradiation, and then infusing donor haematolymphoid cells, results in engraftment of a significant number of the infused donor stem cells.[5-12] This results in the creation of a chimaeric immune system and, eventually, in the development of complete donor-specific tolerance, including unresponsiveness in *in vitro* assays of immunological reactivity, such as the mixed lymphocyte reaction.

In such animals, tolerance is associated with easily detectable chimerism (usually >1%) and is dependent on the persistence of donor haematolymphoid cells – remove the donor cells and the tolerance is lost.[12-17] Immunological mechanisms involved include 'central' pathways of clonal deletion,[15] presumably brought about by the presence of donor dendritic cells in the thymus during T cell development, a point elegantly illustrated in studies of intrathymic tissue transplantation.[18,19] Insertion of a complete islet tissue allograft into the thymus results in a state of systemic donor-specific tolerance.[18,19] However, if the intrathymic graft is first depleted of passenger leucocytes, rendering it 'nonimmunogenic', a subsequent extrathymic graft is rejected,[20,21] even though the intrathymic implant survives. Lastly, although central pathways have received the most attention, 'peripheral' regulatory control of donor reactive cells that escape thymic deletion has also been detected.[15]

Regardless of the mechanisms involved, complete and permanent tolerance to a variety of organs has been routinely produced in a variety of experimental settings using myeloablation and bone marrow transplantation.[5-12,22] However, the high morbidity and mortality associated with the conditioning regimes, in conjunction with the added risk of graft-versus-host disease, have prevented these strategies from being routinely applied in a clinical setting.

2. Persistence of Passenger Leucocytes

Solid organ transplantation gradually became a clinical success even without the treatment strategies that produced an ideal outcome in experimental models. Although the underpinnings of this success were thought to be the increasingly precise immunosuppressive drugs, a recent discovery has suggested that drugs only provide the milieu for a biological process that had been experimentally shown to be highly tolerogenic.

This discovery was that passenger leucocytes, or donor non-parenchymal leucocytes contained within the stroma of all organs, were found to persist in small numbers (usually less than 1:1000) throughout recipient tissues for decades after successful solid organ transplantation.[23-25] The same observations were made in experimental animals,[26,27] even if they spontaneously accepted allografts without immunosuppression.[28]

Classically, passenger leucocytes had been viewed only as initiators of rejection, because of their proven role as stimulators of the alloreaction.[29] Their persistence in the tissues of long term organ allograft recipients had escaped notice because few had actually looked for them, and newer sophisticated techniques were required for their detection. However, their

presence after solid organ transplantation is analogous to the mixed haematolymphoid chimaerism which has been associated with allogeneic tolerance for many years.[4] it is thought that the major differences between the two forms of chimaerism appear to be the number of donor cells circulating in the recipient, and possibly the location of progenitor cells.

3. Clinical Observations on Withdrawal of Immunosuppression

The development of tacrolimus, with its powerful ability to reverse acute rejection,[30] made it possible to attempt withdrawal of immunosuppressive drugs from long-surviving liver allograft recipients,[31] in whom persistent donor passenger leucocytes had been found. The entry criteria used were:

- 5 years post-transplant
- 2 years without rejection
- good history of compliance
- immunosuppression-related complications
- baseline liver biopsy showing freedom from rejection and disease.

59 patients were gradually weaned from a variety of immunosuppressive regimens. Complete weaning was accomplished in 27.1% with 3 to 19 months of drug-free follow-up. Drug weaning was progressing in 47.4%, but had failed in 25.4% although there were no graft losses or irreversible damage. It was notable that the ease with which patients could be weaned off immunosuppression was independent of their starting regimen, suggesting the effect to be independent of drug type. However, successful weaning in this situation appeared to be dependent on a slow staged withdrawal of drugs (unpublished observation). Such studies show that solid organ allograft transplantation under the cover of immunosuppression can eventually result in allograft acceptance without the need for continued immunosuppressive treatment.

The exact mechanism of allogeneic tolerance induction in this setting has not been defined, but clonal deletion is usually not evident and donor-specific reactivity in a mixed lymphocyte reaction may be depressed, although usually detectable.[32-34] This may be related to the small number of persistent donor haematolymphoid cells, with few keeping residence in the thymus,[27] although maintenance of thymic function does not seem to be necessary in this circumstance.[35] These observations suggest that peripheral or immune regulatory pathways are likely to play a significant role in the maintenance of allograft function, although a component of central control or deletion cannot be entirely excluded.

In particular, we have been interested in peripheral regulation as an active phenomenon, based on the immune network theory initially proposed by Jerne[36] and furthered by Coutinho.[37,38] It is possible that the donor cells set up a self-referential system that successfully integrates into the recipient immune network in patients who are able to be successfully weaned from drugs. Such a system would include anti-idiotypic clones of recipient T and B cells (anti-self), produced as part of the response to donor alloantigen.[39] In this line of reasoning, rejection or an alloreaction is the signal for tolerance induction, and control of the immune response involves autoregulatory or ‘autoimmune’ reactions. Thus, without an alloresponse or rejection, there will be no tolerance.

To date, the majority of patients who have been successfully weaned from immunosuppression are liver allograft recipients who were on potent immunosuppression for many years. Similar weaning protocols have been less successful after kidney

transplantation, and suggest that the liver may be more tolerogenic (section 4).[40] Working retrospectively through the data to determine what factors, if any, are common to these patients other than haematolymphoid chimaerism is certainly a worthy goal. However, such a process would take many years and may even produce equivocal or confusing results. Since it is already known from experimental studies that chimaerism is necessary for the maintenance of tolerance, it may be more useful to examine the actions of immunosuppressive drugs and events that occur after organ transplantation to identify potential points of therapeutic intervention.

4. Potential Points of Therapeutic Intervention

In the absence of immunosuppression, multilineage passenger leucocytes contained within the allograft disseminate by haematogenous migratory routes,[27,41] and can be found throughout the primary lymphoid organs of the recipient within 24 hours.[42,43] The number and type of donor cells that leave the allograft is dependent on the organ transplanted.[40] Tolerogenic bone marrow and liver allografts are rich in haemopoietic stem cells.[44] In fact, an adult liver allograft flushed free of blood by preservation fluid can completely reconstitute the haematolymphoid system of a lethally irradiated syngeneic recipient.[44] Other allografts that are less tolerogenic, such as the heart, are stem cell poor[44] and organs like the intestines, which regularly cause graft-versus-host disease when transplanted, are T cell rich.[40]

Recipient haematolymphoid cells also enter the allograft, where T cell infiltration can be documented within 48 hours of transplantation.[43,45] Mixing of donor and recipient immunocompetent cells at these ‘central’ and ‘peripheral’ sites results in an *in vivo* equivalent of a mixed lymphocyte reaction that is initiated by dendritic cells and allogeneic T cells.[43,45] This causes T cell activation within 24 to 48 hours, secretion of cytokines and initiation of effector cascades capable of destroying the organ, such as cytotoxic T lymphocytes and allo-antibodies. For these reasons, passenger leucocytes have been considered deleterious to allograft survival. However, this fate can be dramatically altered by administering an immunosuppressive agent.

With the exception of transplants between a limited number of experimental animal strains,[27,28,46] some form of at least transient immunosuppressive control is essential both for survival of the allograft and subsequent induction of tolerance. However, the exact site of drug action appears to be irrelevant – whether at the level of antigen presentation [gusperimus (deoxyspergualin)], gene transcription (tacrolimus, cyclosporin), cytokine action [sirolimus (rapamycin)] or clonal expansion and inhibition of DNA synthesis (azathioprine, mizoribine, cyclophosphamide and mycophenolate mofetil). Control of the alloreaction can also be achieved with monoclonal antibodies to a variety of antigens, including CD4, adhesion molecules and the interleukin-2 receptor.[47,48] In fact, as reviewed recently,[47,48] every potent inimunosuppressant studied has been claimed to induce tolerance in some experimental system. Thus, it is likely that the common end result seen with all of these drugs is related to a permissive effect, rather than a variety of separate causative effects, that facilitates the migration and survival of donor passenger leucocytes and modulates the context of allorecognition.[25,27,28,39,49]

In order to exploit this knowledge, however, a fundamental shift in our thinking must occur.[39] It is tacitly accepted that an orthotopic solid organ allograft integrates into the appropriate physiological system of the recipient. For example, a newly transplanted liver accepts nutrients absorbed by allogeneic intestines and then secretes serum proteins utilised by the remainder of the body. The allograft also secretes bile, manufactures cholesterol and, altogether, it is really no surprise that the organ is carrying out its normal physiological

functions in an allogeneic environment. Microchimaerism has taught us that solid organ transplantation involves the transfer of two donor organ systems to the recipient – the organ itself and the seeds or essence of the donor haematolymphoid system.[44] Like the allograft parenchyma, the donor haematolymphoid system has the potential to integrate with the recipient and carry out some of its normal physiological functions, although this integration is resisted by the mature elements (especially T cells) in both populations. Thus, the initial encounter requires immunosuppression or deletion of the T cells. Thereafter, successful integration will depend on continual supply of donor haematolymphoid cells, which can be provided by engrafted progenitors. Successful introduction of a few new donor peptides on major histocompatibility complex (MHC) molecules into the existing recipient immune network can have profound immunological consequences. Hypothetical outcomes of this integration would include: (a) establishing a new profile of resistance to infection and autoimmune disorders; and (b) an ability to react to allogeneic but not syngeneic tissues.

There has been recent discussion, and even sanctioned debate, on whether the parenchyma of the allograft or ‘chimaerism’ in the periphery is the key to maintenance of allograft acceptance. To us, this is a moot point and illustrates the need for a shift in perspective. In the broadest terms, any organ allograft recipient is chimaeric, although the cells accounting for the chimaerism may differ (parenchyma versus haematolymphoid cells). In fact, in both situations, donor antigen is present and can stimulate regulatory pathways of (or anergise) the recipient. However, it seems reasonable to conclude that the parenchyma is designed by nature for purposes other than tolerance induction, so it will not perform as well as haematolymphoid cells, an important function of which is the establishment of immunological boundaries.

The mechanisms by which immunosuppressive drugs might directly or indirectly affect the recipient response to donor passenger leucocytes have been described previously in some detail,[47] and are outlined in table I. Briefly, adequate immunosuppression prevents the early destruction of passenger leucocytes that have migrated out of the allograft, and allows their redistribution from the primary lymphoid organs throughout all tissues of the recipient.[27] Agents such as cyclosporin and tacrolimus can injure the thymic medulla and thus create a need for an emigration of dendritic cell progenitors, some of which may be supplied by donor cells.[27,53,54] Exposing immature recipient T cells to potent donor MHC antigens during thymic selection increases the likelihood of clonal deletion. These agents also increase the egress of immature thymocytes into the periphery. In addition, the inhibition of antigen-presenting cell function caused by many immunosuppressants may block delivery of the second signal[55] and induce T cell anergy,[56] further contributed to by the continuous low grade antigenic stimulation caused by persisting donor passenger leucocytes.[56,57] All of these hypothetical scenarios are dependent on a continual supply of immunogenic donor haematolymphoid cells, best satisfied by the transfer and engraftment of donor stem cells. Therefore, novel methods with low morbidity of enhancing engraftment of simultaneously transferred bone marrow cells should be the key to successful clinical application of this concept.

5. Experimental Links between Allo- and Auto-Immunity

As mentioned in section 4, we are particularly interested in active regulatory control of allo-reactivity as an important component in the tolerance observed after solid organ transplantation. Other evidence supporting the importance of low grade immune activity in the maintenance of tolerance has been provided by a recent report that showed that the interleukin-2 receptor β chain is required to keep the activation programmes of T cells under control.[58] Deletion of the gene for this receptor allowed loss of immune control, with consequent death from autoimmunity.[58]

To test whether transferred donor haematolymphoid cells change other properties of the recipient immune system, a series of experiments were carried out in the autoimmunity-prone Brown-Norway (BN) rat, which develops autoreactive T cells after exposure to mercuric chloride. After a series of injections, BN rats develop systemic vasculitis, lymphocytic infiltration and damage to multiple visceral organs and the skin, polyclonal B cell activation and a host of autoantibodies. However, if the animal survives the acute syndrome and recovers, by immune regulation it becomes resistant to further mercuric chloride injections.

Lewis (LEW) rats do not develop clinical autoimmune disease after injections of mercuric chloride. In BN rats made chimaeric (and tolerant to LEW grafts) by injection of allogeneic (LEW) bone marrow and transient immunosuppression, the continued presence of allogeneic cells was associated with low grade activation of the host immune system.[59] More importantly, these animals were also resistant to the mercuric chloride injections, showing that the presence of allogeneic cells triggered some of the same autoregulatory mechanisms involved in alloimmunity. Reciprocal experiments carried out to determine the effect of autoimmunity on allogeneic tolerance induction have shown that cells capable of passively transferring resistance to mercuric chloride autoimmunity can also prolong allograft survival (CP Delaney et al., unpublished observations).

6. Conclusions

In summary, the simple answer to the question ‘can tolerance be achieved with immunosuppressive treatment?’ is ‘yes’, with the following caveats. First, too much immunosuppression is likely to be harmful to the process for the reasons outlined in sections 4 and 5. Second, despite the possibility of inducing tolerance after solid organ transplantation with immunosuppression, currently only a minority of all allograft recipients can be weaned from therapy. The key to success is the development of low morbidity treatment modalities to enhance engraftment of haemopoietic stem cells transplanted as a part of the organ, or an additional inoculum of donor bone marrow. Thus, it is not an argument about whether the glass is half empty or half full, because nature has shown us that it is possible to fill the entire container.

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Table I

Potential mechanisms by which immunosuppressive drugs might promote the induction of allograft tolerance

Mechanism	Agents
Attenuate rejection, thereby preventing destruction of allogeneic donor passenger leucocytes in the recipient lymphoid and nonlymphoid tissues	Not drug-specific
Allow B cell activation (in response to surviving donor cell surface antigens) which may contribute to network control of the immune system[a]	Not drug-specific
Increase thymic accrual of donor dendritic cells, allowing exposure of the immature host immune system to donor alloantigens	Tacrolimus, cyclosporin[50,51]
Allow movement of immature thymocytes to the periphery, resulting in further exposure of immature T cells to donor alloantigens	Tacrolimus, cyclosporin[52]
Permit replication and widespread dispersal of the donor passenger leucocyte population, with consequent continuous presentation of donor alloantigen to the recipient immune system[a]	Not drug-specific
Inhibit the second signal required for activation of host lymphocytes presented with foreign alloantigen, thus promoting an anergic response	Not drug-specific

[a] Although not drug-specific, these mechanisms would appear to be dependent on the avoidance of overimmunosuppression.