

Dendritic Cell Therapy for Tolerance Induction to Stem Cell Transplants

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Abstract: With rapid progress in identification of a variety of adult stem cells, there is an urgent need for basic studies on immunomodulatory protocols relevant to stem cell transplantation. There are new possibilities for immunomodulation invoking the function of dendritic cells (DC) in the induction of tolerance. This paper addresses the application of DC immunotherapy for establishing and maintaining peripheral tolerance to stem cell or tissue allografts. While recent approaches target immature DC and their role in peripheral tolerance, many questions can be raised about the tolerogenic properties of those cells and the clinical feasibility of their use. Procedures published to date for preparation of DC differ significantly in terms of the source of cells and methods for culture and expansion of immature, apparently tolerogenic DC. With evidence for tolerogenicity associated with all classes or lineages of DC, the hypothesis is advanced that the tolerogenicity of DC is determined during hematopoiesis and may be best established by immunotherapy using DC progenitors. It is expected that peripheral tolerance and central or thymic-based tolerance may complement each other as two essential mechanisms for transplantation tolerance since different clinical situations may invoke the need for different procedures for tolerance induction.

Keywords: Dendritic cells, immunogenic, tolerance, allograft, stem cells.

INTRODUCTION

Dendritic cells (DC) are generally recognised for their capacity to endocytose antigen, activate T cells and induce an immune response. DC present in peripheral tissues are resting, immature cells. Upon exposure to pathogens or inflammatory mediators these cells mature and become activated into an immunogenic state characterised by upregulated expression of major histocompatibility complex [MHC] molecules and costimulators like CD80 and CD86. This activates DC into a state ready for T cell activation. However, under steady-state conditions, in the absence of microbial stimulation, DC play another important role in tolerance [1, 2]. In the steady-state, peripheral DC, or DC precursors in blood are immature cells which continuously endocytose environmental antigens. They traffick antigen to secondary lymphoid organs for presentation to T cells [3]. This process maintains peripheral tolerance by the inactivation of autoreactive T cells.

Lymph nodes and spleen also contain endogenous immature DC which exist in close proximity to large numbers of naïve T cells and are well placed to induce rapid inactivation of autoreactive T cells for tolerance induction. The majority of DC in spleen are immature, endogenous DC which endocytose bloodborne antigens and participate in the establishment of tolerance by T cell inactivation [4]. Apoptotic cells represent a major source of self antigen [5]. Cells dying due to normal tissue turnover are taken up by resting DC and presentation of these antigens to T cells by DC in the steady state represents a critical process for maintenance of peripheral tolerance [6, 7]. DC which endocytose apoptotic cells do not become activated [8]. The outcome of T cell stimulation by steady-state DC appears to

depend on the state of maturation of DC and is manifest either as deletion or anergy of T cells, or by the development of regulatory T cells [9]. The important unanswered questions, however, are which DC are tolerogenic and which signals dictate DC differentiation into the tolerogenic versus immunogenic state.

PLASTICITY IN DC HEMATOPOIESIS

For those in the field, the study of DC development and the definition of lineage relationships between phenotypically distinct DC subsets has been more difficult than anticipated. The reason for this now appears to relate to plasticity in DC development, uncharacteristic of other hematopoietic lineages [10]. Theories on the myeloid or lymphoid lineage relationship between DC subsets have been disputed and corrected over time [11]. Both *in vivo* and *in vitro* studies now confirm that under steady-state or non-inflammatory conditions, there are two main classes of immature DC resident in peripheral lymphoid tissues of humans and mice: conventional and plasmacytoid DC. The 'conventional' DC in mice have also been defined as myeloid-like CD11c⁺CD11b⁺CD8⁻ DC and lymphoid-like CD11c⁺CD11b⁻CD8⁺ DC. Both mice and humans also contain the plasmacytoid [p] precursor DC which expresses CD8 upon activation in mice [12], along with lymphoid markers like pT and early D-J rearrangement at the IgH locus [13]. Monocyte-derived DC represent a very different type of DC, developing *in vivo* under inflammatory conditions. DC development is dependent on cytokines like GM-CSF and TNF- which drive cells from blood into lymph nodes for antigen presentation [14].

In vivo reconstitution studies in mice have confirmed that both conventional DC and p-DC derive from the Flt3⁺ subset of both common lymphoid and common myeloid progenitors [15, 16]. They can all be derived by *in vitro* culture of Flt3⁺ bone marrow [BM] cells using cocktails of

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growth factors containing Flt3L [17, 18]. It appears that DC development mediated by Flt3L can occur *via* multiple pathways from Flt3⁺ BM precursors, although Flt3L is not specific for DC, and can stimulate expansion of other hematopoietic lineages [19]. Differences between mice and humans in the number and type of DC subsets could relate to differences in the developmental potential of lymphoid and myeloid progenitors in the two species. *In vivo* evidence in support of plasticity in DC development was obtained after LCMV infection of mice which resulted in transdifferentiation of p-DC into myeloid-like DC [20]. In these mice, plasticity was first detected as an increase in the number of myeloid DC over p-DC following LCMV infection. Subsequently p-DC derived from infected BM were shown to differentiate into myeloid-like DC upon *in vitro* culture with Flt3L [20]. In the least, these two DC subsets must share an immediate common precursor responsive to Flt3L. A model for DC development involving plasticity could incorporate the characteristic downregulation of gene expression in stem cells at lineage commitment. Such a model would account for multiple pathways for development triggered by environmental stimuli leading to a range of DC-like cell subsets with a range of either lymphoid or myeloid characteristics. This could also account for differences between subsets present in humans and mice.

No further committed murine DC progenitor has been identified beyond the Flt3⁺ subset in BM, although some evidence points to a lineage of DC distinct from 'Flt3L-induced' cells. McKenna *et al.* [21] showed that Flt3^{-/-} mice still maintain a population of functional DC in lymphoid organs. Others have predicted a population of Flt3⁻CD11c⁺MHC-II⁻ DC in blood and spleen suggesting a separate lineage of DC refractory to Flt3L stimulation [16, 22]. In this lab, spleen has been shown to be a source of DC progenitors which can be maintained in long-term cultures of splenic stromal cells which do not express Flt3L but which support production of immature myeloid-like DC [23-25]. This work raises the possibility that a more committed progenitor of myeloid DC is maintained in spleen. Consistent with this hypothesis is evidence that spleen contains a majority of endogenous, immature DC [4], thought to be involved in the maintenance of peripheral tolerance [2]. The characteristics of a DC progenitor population in spleen are yet to be established.

A RANGE OF DC TYPES CAN INDUCE TOLERANCE

The immature DC is generally described as an antigen capturing cell, highly endocytic, with low expression of MHC and costimulatory molecules, and weak ability to activate T cells. The commonly accepted model is that immature DC are tolerogenic and mature DC are immunogenic [26, 27]. However, this model does not consider the lineage or type of DC with these distinct functions. It now appears however that some level of maturation is needed for development of tolerogenic capacity [5, 28]. Several reports now demonstrate partial maturation of DC for tolerance mediated by induction of T regulatory cells [27, 29]. An alternative model is that some level of DC activation is required for T cell activation [30]. The function of DC as tolerogenic versus immunogenic could then depend

on the state of activation of cells. Activation of otherwise mature or maturing DC by infection or inflammation can change their function from tolerogenicity to immunogenicity.

While it now appears that all DC types can function in both tolerance and immunity, an unresolved issue is whether the different types of DC like myeloid, lymphoid-like or p-DC have greater capacity to become tolerogenic or immunogenic. It is not yet clear which signals are needed to induce tolerogenic versus immunogenic DC, and how these relate to the development and maturation of different lineages of DC. One model is that the body needs a separate lineage of tolerising DC, and that it would not be feasible for the same cells to be involved in immunogenicity following environmental activation [8, 31]. The argument against this model is that a tolerogenic lineage would be an easy target for pathogens to induce tolerance to their own antigens [31]. The question then is how the same immature or maturing DC can adopt different states of function. In the absence of infection or 'danger', immature DC could induce anergy or apoptosis of T cells, while DC in more mature states could induce regulatory T cells [2, 30]. Exposure to 'danger' would lead to alternative maturation and development of immunogenic DC which can activate T cells. Different maturation signals are known to target regulators of MHC expression. More immature DC express low surface levels of MHC-II antigens due to sequestration into late endosomal/lysosomal MHC-II-rich compartments and rapid recycling of cell surface antigen by endocytosis [24, 32, 33]. Immunogenic DC adopt high sustaining cell surface expression of MHC antigens consistent with high antigen presenting capacity. One expectation is that for tolerogenic DC, maturation is associated with short temporal expression of MHC on the cell surface to avoid presentation of both self and non-self antigens [29].

IMPORTANCE OF PERIPHERAL TOLERANCE IN TRANSPLANTATION

The process of T cell development in the thymus incorporates effective deletion of self-reactive T cells, but the process leading to central tolerance is not always complete. Many T cells reactive to a self-peptide are known to escape clonal deletion in the thymus and to enter the periphery [34]. These T cells include low-affinity autoreactive T cells, T cells specific for self antigens not presented in the thymus, and T cells specific for environmental antigens found in the respiratory tract or intestines [35, 36]. Peripheral tolerance is now known to be critical for immune homeostasis and prevention of autoimmune disease, and there is increasing evidence for regulatory cells which maintain this. These include the well-defined regulatory CD4⁺CD25⁺ T cells [2] and more recently, subsets of regulatory DC [37, 38]. A role for regulatory T cells in transplant tolerance is well documented. It has also been proposed that regulatory T cells can modulate DC hematopoiesis to induce higher numbers of immature or regulatory DC to sustain the tolerant state [39]. There is also evidence that peripheral tolerance can be mediated by clonal deletion which could represent a more effective long-term solution for tissue transplantation [40].

With increased prospects for isolation and use of adult stem cells like hematopoietic stem cells [HSC] or mesenchymal stem cells in cell therapy, there is increased need to establish effective protocols for transplantation tolerance. Allogeneic mesenchymal stem cells or HSC are immunogenic and are subject to rejection by an immunocompetent host. Alloantigen presentation to T lymphocytes by both donor and recipient type DC can induce acute rejection after tissue transplantation. While immunosuppressive drugs like cyclosporin A are very effective in early graft survival, chronic rejection leading to late loss of grafts is a complication which could be avoided by induction of tolerance to donor tissues. Although autografts will always be the preferred regimen, the feasibility of using allografts has improved with development of hematopoietic cell therapies given prior to tissue transplantation [41, 42]. In this case, transplantation success depends on reprogramming the immune system during development of a hematopoietic chimera to ensure survival of the allograft.

Possible ways to induce tolerance include transplantation of allogeneic donor type HSC to produce chimerism ahead of allografting, the infusion of donor type DC to induce 'operational' or peripheral tolerance by either deletion or suppression of T cell function, and the induction of suppressor or regulatory T cells which is also dependent on DC. Host conditioning prior to grafting is also an important consideration in the success of transplantation. Variations on this can include sublethal and partial total body irradiation, thymic irradiation, removal of T cells with drugs or anti-lymphocyte antibodies, as well as costimulatory blockade. Each of these procedures target functional alloreactive T cells and create hematopoietic 'space' allowing stem cell migration into developmental niches. Myeloablative conditioning is a toxic, life-threatening procedure making it difficult to achieve allochimerism. The production of mixed chimeras by transplantation of both donor and host hematopoietic cells through various non-myeloablative treatments is an alternative approach which can be successful [41]. The opportunity also exists to infuse host type DC, or DC progenitors, which have either been pulsed with donor type cell lysates or transfected with donor type MHC antigens for induction of tolerance to allografts. Another more ambitious model is transplantation of allogeneic embryonic stem [ES] cells which could act as a source of HSC for reprogramming the immune system for donor tolerance as well as providing a source of stem cells for tissue replacement. While this is an interesting possibility, it is one which will require extensive investigation to establish feasibility [43, 44].

TOLEROGENIC DC IN PRECLINICAL MODELS OF ALLOGRAFT TRANSPLANTATION

The usefulness of inbred mouse strains as preclinical models for allografting has been demonstrated extensively. It is essential to prevent graft-versus-host disease [GVHD] and this depends on an absence of T cells in the graft. In contrast to non-hematopoietic grafts, engraftment with HSC can lead to development of a new immune system tolerant of both donor and host cells [45-47]. Purified stem cell populations also contain no T cells which can induce GVHD upon

adoptive transfer. HSC chimeric mice can demonstrate donor-specific tolerance for life [46]. Purified HSC can reconstitute lethally irradiated allogeneic mice without producing GVHD although high numbers of cells are needed and success rates are not 100% [47]. Effective protocols for transplantation can therefore involve prior infusions of HSC to facilitate tolerance to a subsequent tissue graft from the same donor given 4-6 weeks later [48]. Successful engraftment of HSC is dependent on other BM cells, and CD8⁺ DC have been identified as candidates in mice [49]. More recently, plasmacytoid precursor DC were also identified as facilitating cells for HSC engraftment [50]. The need for DC to facilitate HSC engraftment emphasises the important role for peripheral tolerance mechanisms in successful allografting. However, it is possible that a range of DC types which are tolerogenic can facilitate grafting.

Tolerogenic DC are now a recognised target for prevention of tissue or cell rejection. Apparently 'immature' DC derived from BM cultured with low doses of GM-CSF have been shown to prolong allograft survival [51]. Another common approach has been to induce an apparent tolerogenic state amongst blood monocyte-derived DC using cytokines and blockers of signalling pathways to inhibit DC differentiation and to induce non-stimulatory DC which cannot activate T cells [52, 53]. While this may have some effectiveness as a therapeutic approach, the procedure is fraught with many unknowns and appears to be more reflective of downregulation of immunity than development of 'tolerance' *per se*. For example, these procedures can involve use of pharmacological agents like IL-10, TGF- β , VEGF, aspirin, vitamin D3 and immunosuppressive drugs like dexamethasone, cyclosporin A and rapamycin to inhibit DC maturation [2, 54]. Another more specific approach is to induce formation of immature recipient type DC deficient in costimulatory molecules. In one case, this involved *in vitro* propagation of DC using cytokines followed by treatment with oligodeoxyribonucleotides specific for NF-kappaB to inhibit DC differentiation and induce unresponsiveness in T cells. After donor antigen pulsing and reinfusion, these DC preparations can delay the onset of allograft rejection [53].

With rapid advancement in stem cell identification with a view to transplantation therapy, there is an urgent need for development of immunomodulatory protocols to facilitate allogeneic cell engraftment. With respect to immunotherapy involving tolerogenic DC, there remain many issues over the best approach and clinical feasibility. There is a question over which DC subset represents an appropriate tolerogenic DC, and whether its function is maintained following adoptive transfer. Procedures published to date for isolation of cell subsets and culture of immature DC *in vitro* using cytokines, can in fact, activate DC leading to loss of immature characteristics and tolerogenic potential. It may not be possible to isolate immature DC *ex vivo* such that they can maintain their tolerogenic capacity following adoptive transfer. Since tolerogenicity appears to be associated with all classes and lineages of DC, it appears to reflect a DC property determined during hematopoiesis. It may therefore be necessary to consider the need for DC progenitors and to develop ways to characterise and isolate these cells. They could then be used in immunomodulation therapy prior to stem cell transplantation.

DEFINING A CLINICALLY RELEVANT SOURCE OF DC PROGENITORS

To date there has been no report of long-term donor-specific tolerance induced by transplantation of only donor-derived DC. This reflects an inability to define a committed progenitor of the DC lineage which can colonise the host and produce progeny DC. A test of this approach would involve transplantation of candidate cells into an allogeneic host with a view to induction of 'DC chimerism' for development of long-term tolerance to alloantigen. The tolerance induced is expected to be peripheral but could also involve central tolerance depending on the ability of DC progenitors to seed the thymus along with other lymphoid compartments. Long-term chimerism may depend on deletional tolerance both in the periphery and in the thymus [41]. Cell therapy for tolerance induction should precede transplantation of allograft tissue. However, this is only possible when there is a live donor. Another approach could also involve cell therapy using host DC progenitors engineered to express MHC antigens of the donor cell type.

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