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FOCUS ON: TRANSPLANTATION

Update on transplantation tolerance

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SUMMARY

The induction of transplantation tolerance has become a major goal, because modern immunosuppressive therapy has not improved chronic rejection rates, and is associated with significant side effects. This article aims to explain the principles of immunological tolerance. Mechanisms of central tolerance involve deletion of self-reactive T cells. Mechanisms of peripheral tolerance are reviewed and also the identification of a subset of regulatory T cells which are characterised by the expression of the transcription factor FoxP3.

Interesting recent insights on the role of the 'anti-inflammatory' cytokine transforming growth factor β which can ultimately lead to the generation of inhibitory Tregs or inflammatory Th17 cells (CD4 helper T cells which secrete the pro-inflammatory cytokine IL17) are discussed.

There are many ways to induce experimental tolerance in animals, however these are difficult to translate tolerance into the clinical context. In addition, standard immunosuppressive agents are calcineurin inhibitors which block T cell activation and IL-2 production. These drugs not only inhibit the activation of effector T cells, but also Tregs, therefore inhibiting Treg driven tolerance induction. Other classes of immunosuppressive drugs should be introduced into the clinic to allow for the possibility of tolerance induction. Strategies to modulate immune responses following transplantation and their potential risks are discussed.

1. Introduction

Transplantation is an effective treatment for end stage organ failure. However, it requires that patients take immunosuppressive drugs for life. These have side effects (eg nephrotoxicity), increase the risk of infection and cancer, but most importantly fail to prevent chronic graft rejection. Chronic rejection is arguably the biggest problem following transplantation, and its development is linked to the incidence and severity of acute rejection episodes. The goal of transplant immunologists has been to harness the immune system to specifically ignore the graft, but respond fully to pathogens/tumour cells, without long term immunosuppression.

The idea of self tolerance was first put forward by Paul Elrich in 1901 when he failed to immunise goats with autologous red cells. He reasoned there must be mechanisms to prevent immune responses to self tissue under normal circumstances, and coined the term 'horror autotoxicus' as a prediction of what would happen if this were not the case.[1] However understanding the mechanisms by which we are self tolerant and exploiting them to prevent disease has proved extremely difficult.

Sir Peter Medawar was awarded a Nobel Prize in 1960 for his discovery of 'acquired immunological tolerance'. He demonstrated that transplant rejection was an immunological response and that tolerance to skin allografts could be induced experimentally in fetal mice and chick embryos.[2] So where are we fifty years later – are immunologists any nearer their goal of turning theory into reality and inducing a donor specific tolerance following transplantation?.

In order to explain where the field of transplantation tolerance is now, a brief overview of tolerance and how this is linked to clinical and experimental tolerance induction will be made.

2. Central tolerance

Immature thymocytes are produced in the bone marrow and travel to the thymus where they undergo a random process of receptor rearrangement followed by thymic selection. The newly formed T cell antigen receptors (TCR) are first positively selected by their ability to bind with low affinity to self MHC/peptide complexes on thymic epithelial cells *ie* if the newly formed TCR are not 'useful', they die by neglect. However, since the process is random, it is essential to delete those TCR with high affinity for self MHC/peptide and reduce the risk of autoimmunity. In recent years, it has been appreciated how much effort is made to express tissue specific proteins within the thymus under the

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control of the 'Auto-immune Regulator Element' AIRE.[3,4] Therefore, tissue specific proteins, like insulin, are expressed in the pancreas and the thymus. Thymic expression is driven by the AIRE promoter, so that newly rearranged TCR will be exposed to MHC/insulin peptide complexes on thymic epithelial cells. This will enable the deletion of potentially auto-reactive insulin-specific T cells and therefore reduce the risk of autoimmunity. Genetic manipulation of insulin gene expression in the thymus has been shown to affect whether insulin-specific T cells survive or not. Elimination of insulin from the thymus results in the escape of insulin-specific T cells into the periphery and the development of auto-immune diabetes in a murine model.[5]

The very fact that TCR are selected for their ability to bind to self MHC/peptide complexes with a low affinity most likely explains why the T cells from a patient can directly recognise the MHC molecules expressed on donor tissue.[6] This high frequency of 'alloreactive' T cells is responsible for the intensity of the rejection response, and is several orders of magnitude higher than the immune response to a pathogen.[7]

The original experiments by Medawar and colleagues were essentially inducing a central tolerance to subsequent skin grafts. However manipulating the immune system of a newborn is not a feasible strategy for use in clinical transplantation.

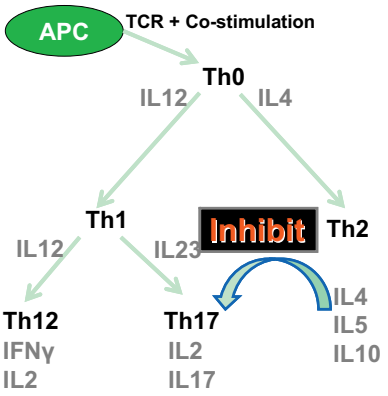

Fig. 1. Simplified schematic of T cell activation and the development of polarised T cell responses. A naïve uncommitted T cell is referred to as a Th0 cell. Depending on the cytokine signal received during T cell activation, the T cell can be polarised and will differentiate into a T helper 1 subset (Th1) which is typically inflammatory and associated with graft rejection. Recently, two subpopulations of Th1 cells have been identified: those Th1 cells that produce interleukin 12 (are referred to as 'Th12' cells) or those that produce interleukin 17 (and are referred to as 'Th17' cells). Alternative cytokine signals (eg interleukin 4) will drive a Th0 cell down a different pathway and the cell will develop into a T helper 2 subset (Th2). These are typically associated with allergy/parasitic infections and the production of antibodies.

3. Peripheral tolerance

Multiple mechanisms have been proposed to explain tolerance outside the thymus. Interfering with antigen presentation has been postulated to induce tolerance. A 'naive' T cell requires three signals for activation and differentiation into an effector T cell:

Signal 1: TCR binding to a cognate MHC/peptide complex (on antigen presenting cells).

Signal 2: Costimulation (CD28 binding to CD80/86 on antigen presenting cells and/or CD154 binding to CD40 on antigen presenting cells).

Signal 3: Cytokine signalling (eg Interleukin-12 will drive the generation of the Th1 subset of helper T cells; Interleukin-4 the polarisation of Th2 helper T cell subsets *etc*).

Activation of a naïve T cell requires all three signals and takes places in lymph nodes. Dendritic cells are the most effective antigen presenting cells to deliver these signals, and the original tissue micro-environment where the dendritic cell was 'primed' is associated with a particular cytokine 'message' which will instruct the T cell response required (Fig. 1). In the case of transplantation, Interleukin 23 (IL-23) produced by dendritic cells will drive the differentiation of pro-inflammatory Th17 T cells which is particularly associated with acute graft rejection.

In contrast, signal 1 in the absence of signal 2 leads to a specific hypo-responsiveness, or anergy.[8,9] Anergic cells are functionally inactive, and may inhibit other T cells by competition for space and growth factors. Significantly, this state of unresponsiveness is not overcome if an anergic T cell subsequently receives signal 1 and signal 2 (unless high levels of the T cell growth factor, interleukin-2 are also provided).[10] The costimulatory molecules on a T cell include CD28 which binds to CD80/CD86 on antigen presenting cells and CD154 (CD40L) which binds to CD40 on antigen presenting cells. T cells also possess a negative regulator of costimulation, CTLA4 (CD152). Normally intracellular, CTLA4 is expressed on the T cell surface at the end of an immune response where it has a higher affinity for CD80/CD86 than CD28. CLTA4 ligation delivers an inhibitory signal to T cells.[11]

Investigations of the normal phenomena of oral tolerance have demonstrated the role of 'anti-inflammatory' cytokines, particularly transforming growth factor β (TGFβ) and interleukin 10. TGFβ inhibits the proliferation of Th1 and Th2 lymphocyte subsets. Weiner[12] introduced a 3rd subset of T helper cells associated with mucosal surfaces, Th3, characterised by their production of TGFβ.[13]

Most significantly, a subset of 'regulatory' T cells has been identified that can suppress the responses of activated T cells and turn an 'aggressive' or 'pathogenic' immune response off. They were first identified by several groups in animal models of auto-immune diseases. Adoptive transfer models demonstrated the role of 'pathogenic' T cells in transferring disease (such as colitis, thyroiditis), but also indicated a population of regulatory T cells which could inhibit them.[14,15] Originally described in the CD4 + CD25 + memory population, these are now characterised by the expression of a transcription factor, FoxP3. FoxP3 is the 'master switch' which controls Treg development, and is predominantly (but not exclusively) expressed by CD4 + CD25 + T cells in both the thymus and periphery.[16]

It has been demonstrated that these Treg normally constitute ~10% of peripheral CD4 + T cells, and they are also found in the thymus ('natural' Tregs), where it is proposed those T cells bearing TCR with the highest affinity for self MHC/peptide are pre-programmed by FoxP3 to be inhibitory. They proliferate poorly following TCR stimulation, don't produce IL-2 and constitutively express high levels of the glucocorticoid-induced TNF-related receptor (GITR) and a high proportion (~50%) express CTLA-4. Expression of the IL-2 receptor (CD25) and IL7 receptor (CD127) discriminates between Tregs and activated T cells.[17] Consequently many studies have shown that CD4 + CD25hi CD127low/neg T cells effectively identifies Tregs (Fig. 2), and correlates with FoxP3 expression/regulatory function.

The discovery of Tregs has been a major milestone in our understanding of tolerance, and consequently there has been much speculation about their induction to treat inflammatory diseases, including transplantation rejection. The goal of inducing a donor specific tolerance could be closer if Tregs that control the pathogenic effector T cells responsible for acute graft rejection could be induced.

Interestingly TGFβ has been shown to play a role in the differentiation of both Tregs, but also surprisingly, inflammatory Th17 cells. At low concentrations, TGFβ synergises with interleukin 6 and interleukin 21 to promote the IL-23 receptor and the differentiation