Transplantation tolerance: a journey from ignorance to memory

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Transplantation tolerance can be defined as long-term allograft survival in the absence of continuous immunosuppressive therapy. Implicit to this definition is that tolerant recipients of organ transplants are unresponsive to donor antigens but maintain reactivity to other (third-party) antigens. In other words, a tolerant patient is capable of mounting an effective immune response against microbial pathogens but is incapable of rejecting the transplanted organ.

Despite a wealth of information on how tolerance to self-antigens is maintained (Figure 1), the induction of tolerance to a transplanted organ remains elusive because of several biological barriers. These barriers include the relatively large magnitude of the alloimmune response, the limitations of peripheral tolerance mechanisms, and the unavoidable fact that immune responses to foreign antigens, by virtue of evolutionary design, are destined to generate immunologic memory [1]. Here, I would like to relay the trials and tribulations of my research group, as well as those of others, to understand how tolerance to a transplanted organ can be achieved.
Introduction

When faced with a daunting scientific quest, it is perhaps best to start by laying out a simple road map of the journey that lies ahead. A useful road map in the pursuit of transplantation tolerance is that of the primary immune response (Figure 2). Upon exposure to a foreign antigen, whether a potentially lethal virus or a life-saving organ transplant, antigen-specific T cells proliferate extensively (the expansion phase) and acquire effector functions that allow them, with the help of B lymphocytes and other mononuclear cells, to eliminate the foreign intruder. T cell expansion, however, does not continue indefinitely but comes to a quick halt as autoregulatory mechanisms ensure that most effector T cells generated during the immune response are eliminated by apoptosis (the death phase). The few lucky T cells that survive the death phase become long-lived memory T cells that confer life-long protection against the foreign antigen (the memory phase). Therefore, the central question in the quest for transplantation tolerance is how to coerce an immune response determined to generate T cell memory into a state of blissful unresponsiveness.

Ignorance is bliss

To tackle this central question, we first asked whether preventing the primary immune response from occur-
ring within the correct anatomical context leads to tolerance. In other words, does interfering with the primary step required for launching the adaptive immune response, the interaction of antigen presenting cells (APC) with T cells (Figure 2, point A), cause immunologic unresponsiveness? To answer this question, we transplanted fully vascularized cardiac allografts to mice that lack secondary lymphoid organs [2]. Secondary lymphoid organs (the spleen, lymph nodes and mucosal lymphoid tissues) are the seat of primary immune activation as they provide the optimal environment for APC–T cell interaction. As expected, we found that such mice are unable to reject their allografts because they cannot mount primary immune responses. Alloreactive T cells in these recipients, however, remained immunocompetent as they quickly mediated allograft rejection when transferred to an immunodeficient host that had a full complement of secondary lymphoid organs. Therefore, chance encounter between T cells and alloantigens outside the context of secondary lymphoid organs does not lead to T cell tolerance but, instead, the transplanted organ is ignored and the alloreactive T cells remain functionally intact. This finding implies that clinical strategies based on global immunosuppression, which prevents primary T cell activation, are unlikely to achieve tolerance.

Undesirable memories

A cardinal feature of the adaptive immune response is its ability to generate long-lived populations of memory T lymphocytes (Figure 2) [9]. Memory T cells are specific to the antigen encountered during the primary immune response and react rapidly and vigorously upon re-encounter with the same antigen. Memory T cells that recognize microbial antigens provide the organism with long-lasting protection against potentially fatal infections. On the other hand, memory T cells that recognize donor alloantigens jeopardize the survival of life-saving organ transplants [10]. Memory T cells constitute a formidable hurdle to tolerance induction because they have several functional advantages over their naïve counterparts: they live much longer, have access to both lymphoid and non-lymphoid tissues and have much less stringent activation requirements. Using a murine model of heart transplantation, we recently addressed the anatomic requirements for the activation of allospecific memory T cells. We found that, unlike naïve T cells, antigen-experienced memory T cells mount a productive immune response that leads to allograft rejection and beget more memory T cells independent of secondary lymphoid organs [11]. As little as a few thousand allospecific CD8 memory T cells were able to migrate directly to the allograft and mediate its rejection in an immunodeficient host that lacks secondary lymphoid tissues. The memory T cell response in these experiments could not be suppressed by agents that effectively block naïve T cell costimulation, hinting at the formidable challenge that lies ahead of us if we were to successfully prevent allospecific memory responses. Equally surprising was our finding that the long-term maintenance of CD8 memory T cell populations is inhibited by IL-2 [12], again indicating that IL-2 blockade may hinder the induction of stable tolerance.

Cytokines: friends or foes?

It is generally assumed that blocking mitogenic T cell cytokines, particularly interleukin-2 (IL-2), not only prevents the proliferation of activated T cells but also coerces these cells to die or become anergic. Therefore, we and others asked whether targeting the expansion phase of the alloimmune response (Figure 2) leads to transplantation tolerance by studying allograft survival in mice that lack IL-2. The results of these experiments were completely unexpected. First, allograft rejection occurred unhindered in the absence of IL-2 [3,4]. Secondly, long-term allograft survival or tolerance could not be induced in IL-2-deficient mice, even when effective immunosuppressive or immunomodulatory agents were administered [4,5]. Unknown to transplant immunologists at the time was the fact that IL-2 plays a dual in vivo role. On one hand, it is an important but dispensable T cell mitogen. On the other hand, it is indispensable for preparing activated T cells for apoptosis, a phenomenon often referred to as activation-induced cell death (AICD) [4]. The role of IL-2 in mediating AICD cannot be substituted for by other T cell mitogens [6]. In addition to IL-2, interferon-γ and perforin, which were initially believed to be essential mediators of rejection, turned out to be critical for down-regulating alloimmune responses [7,8]. Therefore, conventional immunosuppressive strategies, which block cytokine production or function, specifically that of IL-2, are unlikely to facilitate tolerance induction but, instead, may hinder it.
Concluding remarks

The journey towards transplantation tolerance has been full of surprises. We have come to realize that T cell activation in the presence of IL-2 is a pre-requisite for tolerance induction, and that memory T cells remain a major hurdle to achieving donor-specific immunologic unresponsiveness in transplant recipients. Perhaps tolerance will be achieved only after we carefully assess our current understanding of the alloimmune response. We can only hope that through further research our ignorance will one day turn into memorable scientific reality.

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References


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Stopping a medical research project for financial reasons

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Recently, a multicentre clinical trial set up by nephrology groups in several countries in the European Union, under the instigation and with the active logistical support of the Zambon group in Italy, has been stopped at its very start, for undeclared reasons. In fact, the week before the first patients were to start their treatment protocol, the investigators were informed that the study was cancelled.

The purpose of the study was to examine possible beneficial effects of the antioxidant drug N-acetylcysteine (NAC), marketed by Zambon, on the anaemia of chronic haemodialysis patients. In particular, the intention was to test the hypothesis that patients receiving NAC might require less recombinant human erythropoietin to maintain blood haemoglobin at a constant level than patients receiving no NAC supplementation. It is not uncommon that uraemic patients exhibit a less than normal response to erythropoietin. These patients must therefore be considered as partially resistant to the hormone’s action. Their management requires upward adjustments of the weekly erythropoietin dose. One of the factors involved in the anaemia of patients with chronic kidney disease and erythropoietin hyporesponsiveness is oxidative stress, which is caused by an increased...