Transplant International ISSN 0934-0874

INVITED COMMENTARY

Harnessing negative T-cell costimulatory pathways to promote engraftment

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Received: 31 August 2007
Accepted: 3 September 2007

doi:10.1111/j.1432-2277.2007.00565.x

Our understanding of lymphocyte activation in the context of allo- and auto-immunity has come a long way since the propounding of the 'historical' theories of self-nonself discrimination [1]. Indeed, on account of the increasingly sophisticated molecular and immunological tools, our knowledge of the complexities of cell–cell interaction continues to evolve. The concept of a single second signal to promote and sustain T-cell response in addition to the signal 1 delivered by the T-cell receptor (TCR)-CD3 complex as a direct consequence of antigen recognition no longer applies. Examination of the molecular interaction that occurs when a lymphocyte encounters an antigen-presenting cell (APC) has revealed that T cells require multiple signals to become fully activated. The additional signals that are necessary for activation are provided when accessory and co-stimulatory molecules engage their ligands. Moreover, we now know that the 'second signal' takes place through several different co-stimulatory pathways [2]. Perhaps even more fascinating is the discovery that co-stimulatory signals maybe positive or negative, and that it is the interplay between these pathways that determines the ultimate outcome of the immune response *in-vivo* [3,4].

The reason for the seemingly abundance of pathways providing signals for T-cell activation remains unclear. On the other hand, this could be due to each one playing a distinct role and exhibiting unique or possibly accumulative functions within the immune system. Another possible theory would be that several pathways perform the same function indicating redundancy within the immune system. A further elucidation could be one of hierarchy among the pathways with defining roles for each pathway at distinct stages of the immune response.

The programmed death-1 (PD-1) molecule (the subject of the *Chen et al.* paper [5]) and its ligands PD-L1 (B7-H1) and PD-L2 (B7-DC) are members of the CD28:B7 family which have been shown to negatively regulate the immune response [6]. The PD-1:PD-L1/PD-L2 pathway has been shown to play a critical role in modulating T-cell activation and tolerance. Negative signaling may result in limitation, attenuation or even complete termination of T-cell responses, their ultimate outcome appears to be as a result of a variety of factors such as the immune setting T-cell tolerance or autoimmunity. In the experimental setting adopted models, strain combinations and addition of other immunomodulatory agents need to be taken into account.

The PD-1:PD-L1/PD-L2 pathway has generated considerable interest in recent years although it has not been shown to be as potent as cytotoxic T lymphocyte associ-

ated antigen-4 (CTLA-4), the high affinity receptor for both B7-1 and B7-2. It is clear that the expression of the earlier discovered ligands B7-1 and B7-2 is largely restricted to professional antigen presenting cells (APCs); by contrast, the novel ligands B7H-3, B7H-4, ICOS, PD-L1, and to a lesser extent, PD-L2 have the unique characteristic of being widely expressed by nonlymphoid (endothelial tissues, tumors) cells, as well as on the antigen-presenting and other bone marrow-derived hemopoietic cells. Indeed, the ubiquitous expression of the PD-1 ligands has contributed to providing us with further insight into the complexities of the role that co-stimulatory pathways play. The expression of PD-L1 has been shown to contribute to a wide variety of allogeneic, autoimmune and infectious responses; for instance, in the islets [7], heart [8] and skin [9] transplants, in the brain in a model of experimental autoimmune allergic encephalomyelitis [10], the placenta in allogeneic foetomaternal tolerance [11] and in viral immunity via the liver [12].

Harnessing physiological mechanisms that regulate allo-immunity should lead to development of novel strategies, which can be utilized to induce durable and reproducible transplantation tolerance. In this issue of Transplantation International, *Chen et el* [5] seek to address the role of up-regulation of constitutive expression of PD-1 as a means of inhibiting allograft rejection. Perhaps most important is the direct approach it takes in attempting to address the mechanics of over-expression of PD-1 by utilizing PD-1 transgenic mice. The authors showed that in addition to reducing CD28-mediated PI3K activation, PD-1 ligation also inhibits TCR-dependent PI3K activation. This paper adds to previous literature, which has shown that PD-1 engagement reduces TCR-mediated activation of PKC-θ [13].

The authors demonstrated that PD-1 cross linking augmented PTEN phosphorylation leading to initiation of PI3K activation and its downstream targets Akt and PKC-θ by enhancing PTEN activation *in-vitro*. Despite this, they found that only T-cell responses to minor but not major mismatches were reduced *in-vitro*. The PD-1-Tg mice only exhibited prolonged cardiac survival in mice transplanted with heart allografts expressing multiple minor mismatches and treated with low dose cyclosporine-A. Though the authors conclude that genetic engineering of T cells to constitutively express PD-1 only has a mild impact on allograft survival, we believe it would be important to study these recipients in other models and strain combinations, such as the bm12 into B6 model of chronic allograft rejection. Using this model, it has been shown that blockade of the PD-L1 pathway with PD-L1 antibody results in accelerated graft arterial disease in cardiac allografts [14]. Indeed the PD pathway may potentially exert its maximal effect in smoldering rejection such as is seen in chronic rejection. Additionally, it maybe more beneficial to target ligands and over-express these in donors as experience with transgenic models has shown considerable variability in regard to their robustness [15]. The authors clearly demonstrated upregulation of PD-1 expression on T cells particularly in transgenic Line-24; however, there was no illustration of achievement of increased expression on macrophages and B cells. Confirmation of this would be pertinent as this is one of the key factors differentiating the PD-1 negative regulatory CD28 receptor from CTLA-4 [6]. Furthermore, perhaps, in order to achieve true tolerance through targeting T-cell co-stimulatory pathways it may be necessary to block the positive pathways, as well as enhance negative T-cell costimulatory signals. In that regard, it may be useful to treat the PD-1 transgenic recipients of fully allogeneic grafts with agents to block positive costimulatory signals such as CTLA4Ig, MR1 or anti-ICOS/B7H antibodies. Indeed two of our recent manuscripts [16,17] suggest interesting interactions between the positive costimulatory pathways and the PD-1 pathway in regulating alloimmune responses.

Acknowledgements

JP is a recipient of The Transplantation Society basic science research Fellowship.

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