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Passenger donor lymphocytes: ~~t~~To affinity and beyond~~.~~

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Graft-versus-host recognition by passenger donor lymphocytes within organ transplants can trigger host alloantibody production (Charmetant *et al*.).

Immune responses against transplanted tissues drive graft rejection. In particular, the development of antibody responses against major histocompatibility complex (MHC) alloantigens derived from donor organs is now recognized as a contributor to early failure of organ transplants. Analogous to the requirement of $CD4^+$ T cell help for class-switched antibody production against conventional protein antigens, help for alloantibody production has long been considered to be provided exclusively by "indirect-pathway" $CD4^+$ T cells. These $CD4^+$ T cells are thought to recognize processed target alloantigen presented as peptide fragments complexed to the MHC II complex of a B cell. However, in this issue of *Science Translational Medicine*, Charmetant *et al.* (*1*) have outlined an unexpected pathway for donor-specific alloantibody production, which the authors term an "inverted" direct response. The authors found, using a murine cardiac allograft model, that recipient B cells were instead activated as a consequence of help provided by donor $CD4^+$ T cells that were passengers within the allograft. These donor-derived $CD4^+$ T cells recognized intact MHC II on the surface of the host B cell through the direct allorecognition pathway. The authors further supported these observations by comparing the frequency of $CD4^+$ T cells in human kidney, lung, and intestinal grafts with the incidence of early alloantibody generation, finding that grafts with a higher frequency of passenger $CD4^+$ T cells developed more donor-specific alloantibodies.

DIRECT-PATHWAY ALLORECOGNITION BY PASSENGER DONOR $CD4^+$ T CELLS

T cell activation through the direct pathway is unique to transplantation. It is characterized by a large precursor frequency (up to 10% of recipient $CD4^+$ T cell clones), because not only is every surface MHC II alloantigen on the donor antigen-presenting cell recognized as non-self~~,~~ but **also** multiple epitopes are generated by the spectrum of peptides bound in the groove of MHC II (*2*). At such a high ligand density, even T cell clones with lower affinity receptors can become activated. Donor-derived $CD4^+$ T cells could thus provide help to every host B cell, irrespective of its specificity, in a "peptide-degenerate" fashion (*3*). ~~Yet~~**However**, not all B cells undergo differentiation into antibody-producing plasma cells. Rather, concurrent signaling through the B cell receptor (BCR) is essential for antibody production. Charmetant and colleagues now demonstrate that increased B cell proliferation and up-regulation of MHC II occurred if B cells were co-cultured with third-party T cells, but only if the BCR was simultaneously cross-linked (*1*). Similarly, we previously reported that adoptively- transferred third-party $CD4^+$ T cells could provide help in T cell-deficient mice for antibody responses against a model antigen, but only if simultaneously challenged with that antigen (*3*).

Consequently, and somewhat counterintuitively, donor T cells can provide help for an alloantibody response directed against all MHC alloantigens on their own surface, a response that ultimately leads to their destruction. Such responses would be expected to contribute to graft rejection. To that end, Charmetant and colleagues report intragraft microvascular lesions associated with donor T cell-mediated alloantibody production (*1*). Similarly, in our murine model of chronic antibody-mediated rejection, allograft vasculopathy was diminished, and heart graft survival prolonged, when donor hearts were first depleted of T cells (*3*). In contrast, heart grafts were rejected more rapidly when retrieved from donors that were previously sensitized against their recipient through skin grafting, because an expanded memory $CD4^+$ T cell population with specificity for host MHC II was also transferred. These $CD4^+$ T cells presumably interact more effectively with MHC II complexes on B cells, leading to enhanced alloantibody responses.

WHAT IS THE CLINICAL RELEVANCE OF INVERTED DIRECT ALLORECOGNITION?

The parsing of alloimmunity through highly refined murine models is elegant, but as models become more elaborate, the relevance of their findings to clinical transplantation becomes less immediately apparent. After standard retrieval techniques, human organ transplants still contain substantial numbers of T cells (*1*, *3*), including resident memory T cells that exhibit heterologous, cross-reactive immunity against alloantigens (*4*), but demonstrating functional relevance is more challenging. Charmetant and colleagues reason that, because transferred donor T cells will mediate early donor-specific alloantibody responses, alloantibody development in a large proportion of lung and intestinal transplant recipients likely reflects the abundance of $CD4^+$ T cells contained within, and transplanted with, these organs. Although an intriguing observation, other explanations are possible. For example, the large numbers of professional antigen-presenting cells transferred with lung and intestinal allografts would be expected to provoke stronger recipient helper T cell responses. Charmentant and colleagues also suggest that the relatively short duration of the early alloantibody responses observed in lung and intestinal transplant recipients, with ~~the majority~~**most** decaying by 100 ~~-~~days after transplant, reflects the transient survival of donor T cells in the recipient~~,~~ and**,** consequently, an inability to provide help for long-term humoral immunity. However, this observation raises additional questions. If particularly short-lived, then donor $CD4^+$ T cells may only trigger extrafollicular alloantibody responses, which typically decay within a few weeks after transplant. ~~But~~ **However,** if donor T cells can differentiate to T follicular helper cells and promote development of germinal center responses, **then** this would generate long-lived plasma cells that endure long after involution of the lymph node germinal center. In any event, once activated, B cells can solicit their own help from additional $CD4^+$ T cells (*5*), which would include the recipient's own $CD4^+$ T cell population. The findings of Charmetant *et al.*, along with our investigations (*6*)**,** have suggested that complex interactions between alloreactive B cells and both donor and recipient $CD4^+$ T cell fractions underpin development of germinal center responses and durable humoral memory against alloantigens (Fig. 1).

More clinical evidence is thus required to support the relevance of the donor T cell fraction in promoting host alloantibody production in humans. One strategy would be to examine donor-specific alloantibody production following maternal-to-child living kidney donation, in anticipation that grafts from mothers who are sensitized to their child (and who therefore have detectable donor-specific alloantibodies at the time of donation) will contain memory T cells with direct allospecificity for the recipient~~,~~ and that their transfer will promote stronger donor-specific alloantibody responses in the recipient. If this comprises a substantial component of the alloimmune response, or perpetuates additional effector mechanisms, **then** this may compromise long-term graft outcomes and be detectable in large transplant registry analysis. This phenomenon would be further restricted to those offspring who are both mismatched at the MHC II loci (enabling donor-to-recipient direct allorecognition) and matched at the killer cell immunoglobulin receptor loci, as previous studies suggest that avoidance of immediate killing by host natural killer cells is crucial for interactions between donor T cells and recipient B cells (*3*).

Transfer of donor $CD4^+$ T cells may trigger antibody specificities other than against donor alloantigen, and the promiscuous help they provide could theoretically activate self-reactive B cells. Charmetant and colleagues did not detect autoantibody generation in their model, but classical murine models of humoral autoimmunity rely on transfer of third-party $CD4^+$ T cells as the trigger; we have previously reported that $CD4^+$ T cells generate potent germinal center autoimmunity against nuclear

antigen (*6*). Although the contribution of autoimmunity to organ transplant rejection continues to be debated, Zorn and colleagues have recently reported that increases in "natural" **immunoglobulin G (**IgG**)** concentrations after transplantation are associated with poorer long-term graft survival (*7*). The understanding of natural antibodies continues to evolve because, although traditionally considered to be present in the body ~~prior to~~**before** encountering cognate ligand, natural antibodies can be class-switched and even somatically mutated, suggesting a role for T cell help in their generation. Assuming **that** this help can be provided by donor $CD4^+$ T cells, greater increases in concentrations of natural antibodies may be observed in recipients of lung and intestinal transplants than in kidney transplant recipients.

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Although essentially a form of graft-versus-host recognition, there is a peculiarity with inverted direct allorecognition as described by Charmetant and colleagues. Graft-versus-host recognition is considered the immunological antithesis to host-versus-graft recognition, and ~~it's~~ **it is** not expected that the two would occur simultaneously. ~~But~~ **However,** here, graft-versus-host recognition by the donor T cell fraction triggers a host-versus-graft response from recipient B cells. This may have relevance beyond solid organ transplantation, most immediately in mixed chimeric states that typically develop ~~following~~ **after** reduced intensity conditioning and hematopoietic stem cell transplantation for hematological malignancies. The co~~-~~existence of donor and recipient lymphocyte fractions may promote spontaneous humoral immunity; indeed, isolated reports describe the generation of anti~~-~~tumor antibodies associated with hematopoietic mixed chimerism (*8*).

In summary, Charmetant *et al.* provide compelling experimental support for a paradigm in which graft-versus-host activity by donor T cells can provide help to host B cells for production of alloantibodies. Human organs for transplantation can contain substantial numbers of donor lymphocytes, and the data presented by Charmetant *et al.* suggest that their transfer may contribute to alloantibody production immediately after transplantation. Donor T cell transfer may also explain the development of early autoantibody responses in human transplant recipients. The most immediate relevance of these findings is likely to be in the rapidly expanding field of ex vivo perfusion, wherein organs are perfused after retrieval, typically with warm oxygenated blood, to ameliorate ischemia-reperfusion injury on subsequent implantation. These technologies offer the scope to alter the organ microenvironment. It would be possible, for example, to administer high doses of depleting anti-$CD4^+$ monoclonal antibodies during ex vivo perfusion to prevent donor T cell~~-~~–mediated promotion of the recipient's alloimmune response. ~~C~~**However, c**aution would need to be exercised~~, however,~~ because donor-derived natural regulatory T cells that are transferred with the graft would be expected to broadly inhibit host humoral alloimmunity. Perhaps more excitingly, this ability of third~~-~~party $CD4^+$ effector and regulatory T cells to promote or inhibit B cell responses irrespective of BCR specificity may hold potential beyond transplantation for use as a cellular therapy in cancer and autoimmunity.

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Fig. 1. Donor CD4⁺ T cells mediate inverted direct allorecognition and development of donor-~~-~~specific alloantibodies. ~~Following~~ **After** transplantation, donor-derived CD4⁺ T cells migrate from the transplanted graft into host secondary lymphoid tissue, such as lymph nodes, where they can (1) interact with recipient B cells (irrespective of their specificity**)**, as a consequence of peptide-degenerate or inverted ~~-~~direct allorecognition of MHC II complexes on the recipient B cell surface. Despite the promiscuous help provided by donor T cells, differentiation to an antibody-secreting cell remains antigen~~-~~ specific, because binding of target antigen, in this case**,** alloantigen, to the BCR is also required. (2) Although interactions with the donor T cells are expected to be short-lived and lead predominantly to transient alloantibody production by extrafollicular plasmablasts, once activated, the recipient B cells can potentially mediate long-lived humoral immunity by (3) secondary interactions with recipient CD4⁺ T cells~~,~~ and (4) follicular localization within the germinal center. (5) Somatic hypermutation of B cells within the germinal center will generate (6) both memory B cells and bone~~-~~ marrow~~-~~–resident long-lived plasma cells with the potential to secrete high-affinity alloantibodies for many years that (7) contribute to **the** progression of allograft vasculopathy. **TCR, T cell receptor.** CREDIT: ASHLEY MASTIN/*SCIENCE TRANSLATIONAL MEDICINE*