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Direct versus Indirect Allorecognition Pathways: On the Right Track

G. Benichou[a,*] and **A. W. Thomson**[b]

[a]Transplant Research Center, Department of Surgery, Massachusetts General Hospital and Harvard Medical School, Boston, MA

[b]Starzl Transplantation Institute and Departments of Surgery and Immunology, University of Pittsburgh School of Medicine, Pittsburgh, PA

Allorecognition is initiated by $CD4^+$ and $CD8^+$ T cells recognizing either intact allo-MHC molecules (MHC class II and I, respectively) on donor antigen-presenting cells (APCs) (direct pathway) or allopeptides bound to self-MHC molecules on recipient APCs (indirect pathway) (1). The high precursor frequency of T cells capable of interacting directly with an allo-MHC molecule presumably accounts for the strength and polyclonality of the direct alloresponse, as observed during *in vitro* mixed leukocyte reactions (MLR). In contrast, T cells recognizing allopeptides on recipient MHC molecules (indirect pathway) are directed to a few dominant determinants and they display a limited T-cell receptor (TCR) Vβ gene usage (2–4). Likewise, it is traditionally accepted that direct alloreactivity represents the driving force behind early acute graft rejection. Alternatively, it is believed that the direct alloresponse rapidly subsides as donor passenger leukocytes vanish, while the indirect alloresponse persists and causes chronic rejection, presumably by promoting delayed-type hypersensitivity and the production of alloantibodies (5,6). However, these scenarios are based largely on circumstantial evidence and the actual contribution of direct versus indirect allorecognition to the alloresponse and transplant rejection remains open to question.

In this issue of the *American Journal of Transplantation*, Brennan and colleagues have revisited these questions using a mouse model in which TCR transgenic (tg) $CD4^+$ T cells recognizing allo-MHC antigens either directly (4C) or indirectly (TCR 75) were adoptively transferred to recipients of vascularized heart transplants. It was observed that T cells activated indirectly displayed much earlier and stronger proliferation rates and IFNγ production than T cells activated directly. Next, they assessed polyclonal $CD4^+$ T-cell responses using an ELISPOT assay in B6 mice transplanted with BALB/c skin. They observed that the $CD4^+$ T-cell indirect alloresponse represented 10% of the overall alloresponse 10 days posttransplantation but rose to 20% after 60 days (Table 1). Most strikingly, in recipients of allogeneic hearts, the indirect response represented up to 33% of the $CD4^+$ T-cell-mediated alloresponse when measured using T cells recovered from the transplant itself. In another set of experiments, B6 Rag 1 KO mice, that had accepted BALB/c grafts for 100 days, were adoptively transferred with TCR tg $CD4^+$ T cells. Strikingly, only those T cells interacting with alloantigens in an indirect fashion rejected the allografts. Co-transfer of T cells with allogeneic (donor) dendritic cells could restore allograft rejection via the direct pathway. This result suggests that the direct alloresponse becomes inoperative once the donor passenger leukocytes are no longer present, although this could also reflect tolerogenic effects of alloantigen presentation by graft parenchymal cells.

*Corresponding author: Gilles Benichou, gbenichou@partners.org.

Upon adoptive transfer, TCR tg monoclonal T cells activated indirectly exhibited an earlier and higher proliferation rate than T cells responding through the direct pathway. If we concede that both T-cell clones share similar avidities for their corresponding antigens, this difference can be attributed only to the level of antigen presentation in the secondary lymphoid organ considered. Indeed, it may take several days for donor passenger leukocytes to reach the recipient's spleen and trigger a direct alloresponse. In turn, it is possible that recipient APCs bearing donor antigens may traffic faster and in higher numbers to the host's lymphoid organs. It is also possible that shed alloMHC class I proteins may represent an immediate source of antigen for processing by recipient APCs and the initiation of an early, indirect response. Since the same phenomenon is observed with vascularized heart transplants, it is unlikely that the initial lack of vascularization of skin grafts accounts for the somewhat delayed nature of the direct alloresponse. It is also noteworthy that the investigators adoptively transferred equal numbers of directly and indirectly activated T cells to graft recipients. Since the number of T cells activated indirectly is normally much lower than that with direct reactivity, this may bias the alloresponse toward the indirect allorecognition pathway.

In naïve mice, the authors confirm the presence of direct but not indirect alloresponses by $CD4^+$ T cells producing IL-2 but no IFNγ. Such primary MLR responses reflect either the high precursor frequency of naïve T cells or the presence of preexisting memory T cells capable of recognizing allo-MHC proteins. The absence of IFNγ production rather supports a response mediated by naïve $CD4^+$ T cells. The observed frequencies of T cells activated via direct and indirect pathways in early polyclonal alloresponses following skin grafting are similar to those reported previously using the same ELISPOT assay (7). Apparently, over time, the memory T cells recognizing allopeptides tend to represent a higher percentage of the overall alloreactive population. This may reflect a higher level of cell death among T cells activated directly or the rapid elimination of donor professional APCs. Also, a higher rate of proliferation of indirectly activated T cells may account for this phenomenon. This finding supports the view that while T cells activated directly dominate the initial alloresponse, indirect alloreactivity becomes progressively more prominent. It also suggests that, upon re-exposure of a recipient to the same alloantigens, indirect alloimmunity may play a critical role in second set rejection. Interestingly, in the case of vascularized cardiac allotransplants, T cells activated indirectly account for a third of primed T cells recovered from the graft, while they represent only 6% of activated T cells in the spleen. This could reflect the massive and early infiltration of the allograft by recipient APCs. It also suggests that some T cells may be primed in the graft itself. Previous studies by Lakkis' group (8), using *aly/aly* mice devoid of lymph nodes and splenectomized, suggest that such 'peripheral sensitization' may take place in tertiary lymphoid structures formed in tissues after inflammation. The alternative interpretation of this observation is that T cells activated indirectly undergo faster activation and proliferation upon alloantigen presentation and infiltrate the graft at a higher pace than their directly activated T-cell counterparts.

Finally, the results shown in Figure 6 in the paper by Brennan et al. further suggest that T cells activated directly cannot reject an allograft on their own once donor passenger leukocytes have disappeared. In contrast, despite the lack of inflammation and 'danger' signals, T cells activated in an indirect fashion could reject a healed skin allograft. Chronic inflammation present in RAG KO mice as well as homeostatic proliferation of adoptively transferred T cells may favor the activation of transferred T cells in these mice. Indeed, it would be useful to determine whether the same phenomenon can be demonstrated in normal mice. In any case, this result further supports the view that indirect alloreactivity becomes the main route of rejection by $CD4^+$ T cells following the elimination of donor MHC class II^+ professional APCs. It is also important to keep in mind that this conclusion may not

pertain to directly activated $CD8^+$ T cells, whose activation can occur in the absence of $CD4^+$ T-cell help in certain mouse strains.

In conclusion, this interesting study indicates that the direct alloresponse is short-lived, while the indirect alloresponse eventually becomes the driving force behind the rejection process. This suggests that the achievement of stable tolerance may rely primarily on therapeutic control of the indirect allorecognition pathway.

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Table 1

Approximate numbers and percentages of donor-reactive activated $CD4^+$ T cells secreting IFNγ in direct and indirect fashion after allogeneic skin or heart transplantation. Modified after Brennan et al. (this issue)

	Skin allograft				Heart allotransplant			
	LN day 10		LN day 60		Spleen day 7		Heart transplant day 60	
Pathway	IFNγ spots	% response	IFNγspots	% response	IFNγ spots	% response	IFNγ spots	% response
Direct	>3000	90%	100	80%	2200	95%	50 000	67%
Indirect	300	10%	25	20%	100	5%	25 000	33%

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