CD8 T-cell recognition of acquired alloantigen promotes acute allograft rejection

Simon J. F. Harper¹, Jason M. Ali^{1,2}, Elizabeth Wlodek, Marg C. Negus, Ines G. Harper, Manu Chhabra, M. Saeed Qureshi, Mekhola Mallik, Eleanor Bolton, J. Andrew Bradley, and Gavin J. Pettigrew²

School of Clinical Medicine, University of Cambridge, Cambridge, CB2 0QQ, United Kingdom

Edited by Jonathan Sprent, Garvan Institute of Medical Research, Darlinghurst, NSW, and accepted by the Editorial Board September 3, 2015 (received for review July 9, 2015)

Adaptive CD8 T-cell immunity is the principal arm of the cellular alloimmune response, but its development requires help. This can be provided by CD4 T cells that recognize alloantigen “indirectly,” as self-restricted allopeptide, but this process remains unexplained, because the target epitopes for CD4 and CD8 T-cell recognition are “unlinked” on different cells (recipient and donor antigen presenting cells (APCs), respectively). Here, we test the hypothesis that the presentation of intact and processed MHC class I alloantigen by recipient dendritic cells (DCs) (the “semidirect” pathway) allows linked help to be delivered by indirect-pathway CD4 T cells for generating destructive cytotoxic CD8 T-cell alloresponses. We show that CD8 T-cell-mediated rejection of murine heart allografts that lack hematopoietic APCs requires host secondary lymphoid tissue (SLT). SLT is necessary because within it, recipient dendritic cells can acquire MHC from graft parenchymal cells and simultaneously present it as intact protein to alloreactive CD8 T cells and as processed peptide alloantigen for recognition by indirect-pathway CD4 T cells. This enables delivery of essential help for generating cytotoxic CD8 T-cell responses that cause rapid allograft rejection. In demonstrating the functional relevance of the semidirect pathway to transplant rejection, our findings provide a solution to a long-standing conundrum as to why SLT is required for CD8 T-cell allorecognition of graft parenchymal cells and suggest a mechanism by which indirect-pathway CD4 T cells provide help for generating effector cytotoxic CD8 T-cell alloresponses at late time points after transplantation.

semidirect pathway | semidirect allorecognition | CD8 cytotoxicity | allorecognition | alloimmunity

Cytotoxic CD8 T-cell responses directed against MHC class I alloantigens are one of the principal mediators of acute allograft rejection (1, 2). Exceptionally, at very high precursor frequency, cytotoxic CD8 T cells can effect graft rejection autonomously (3), but generally, differentiation of naïve CD8 T cells to fully functional cytotoxic effector cells capable of mediating acute allograft rejection requires help from activated CD4 T cells. How such help is delivered remains unclear. Studies of conventional CD8 T-cell responses against nontransplant antigens have demonstrated that CD4 T-cell help does not involve cognate cell-surface interaction between the helper CD4 T cell and cytotoxic CD8 T cell, but that instead, help is delivered to an intermediary antigen presenting cell (APC), which is then licensed to prime CD8 T cells (4–6). A critical requirement for such help is the expression of both the CD4 and CD8 T-cell epitopes on the same APC, and an analogous three-cell cluster model (Fig. 1A) in transplantation is only possible if help for CD8 T cells is provided by CD4 T cells that recognize intact MHC class II alloantigen on the surface of donor APCs, via the so-called “direct pathway” (7). Murine studies have confirmed that restricting CD4 T-cell help to the direct pathway generates strong cytotoxic CD8 T-cell alloresponses that effect rapid allograft rejection (8).

The indirect pathway of allorecognition, whereby CD4 T cells recognize processed alloantigen as self-restricted allopeptide (7, 9), is now viewed as being at least as important as the direct pathway for initiating and mediating allograft rejection. This importance

partly reflects the unique ability of indirect-pathway CD4 T cells to act as helper T cells for generating sophisticated alloantibody responses (10), but it is now also clear that effective cytotoxic CD8 T-cell responses can be generated when CD4 T-cell help is restricted exclusively to the indirect pathway (11). This observation is surprising, because the delivery of indirect-pathway T-cell help is only readily explained by postulating the unlikely formation of a cumbersome four-cell cluster, comprising CD4 and CD8 T lymphocytes and both recipient and donor APC (Fig. 1B), a cluster in which there are no apparent cell surface ligands to enable physical linkage between the donor APC/recipient CD8 T-cell couplet and the recipient APC/CD4 T-cell couplet. This scenario raises concerns of potentially uncontrolled CD8 T-cell alloimmunity, because such “unlinked” help could in principle be provided by concurrent exposure to any unrelated antigen.

The appreciation that many types of cells, but particularly dendritic cells (DCs), can capture (“trogoctose”) membrane fragments from other cells (12, 13) has prompted the proposal that cytotoxic CD8 T-cell alloimmunity may be initiated by a “semi-direct” pathway, whereby intact donor MHC class I alloantigen is recognized after its acquisition onto the surface of recipient DCs. In support, murine studies have detailed in vivo capture of membrane alloantigen by host cells (14, 15). This phenomenon provides a possible mechanism by which the same recipient APC as represents intact MHC class I alloantigen could simultaneously present processed allopeptide for recognition by indirect-pathway CD4 T cells. This scenario would enable formation of a linked three-cell cluster (Fig. 1C) that obviates many of the concerns

Significance

Cytotoxic CD8 T-cell responses against mismatched MHC class I alloantigen are the principal arm of the cellular response against a transplanted organ. How CD4 T cells deliver essential help for development of these cytotoxic responses remains unclear. Here we show that recipient dendritic cells present acquired MHC alloantigen both as intact protein, for recognition by cytotoxic CD8 T cells, and as processed allopeptide, for recognition by helper CD4 T cells. Our findings suggest a mechanism by which help is provided for generating effector cytotoxic CD8 T-cell alloresponses at late time points after transplantation and solve a long-standing conundrum as to why host lymphoid tissue is required for CD8 T-cell allorecognition of graft parenchymal cells.

Author contributions: S.J.F.H., E.B., J.A.B., and G.J.P. designed research; S.J.F.H., J.M.A., E.W., M.C.N., I.G.H., M.C., M.S.Q., and M.M. performed research; E.W. and M.C.N. contributed new reagents/analytic tools; S.J.F.H., J.M.A., E.B., J.A.B., and G.J.P. analyzed data; and J.M.A., J.A.B., and G.J.P. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission. J.S. is a guest editor invited by the Editorial Board.

¹S.J.F.H. and J.M.A. contributed equally to this work.

²To whom correspondence may be addressed. Email: ja297@cam.ac.uk or gjp25@cam.ac.uk.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1513533112/-DCSupplemental.

Fig. 1. Possible mechanisms for provision of CD4 T-cell help to alloreactive CD8 T cells. Analogous to provision of CD4 T-cell help for cytotoxic CD8 T-cell responses against nominal protein antigen, simultaneous linked direct-pathway allorecognition of MHC class I and class II alloantigen on donor antigen presenting cells (APCs) by cytotoxic CD8 and helper CD4 T cells, respectively, is considered the dominant helper mechanism (A). Nevertheless, indirect-pathway CD4 T cells that recognize self-restricted alloantigen following internalization and processing by recipient APCs can also provide help; an unlinked four-cell cluster model has been proposed (B). Provision of linked help by indirect pathway CD4 T cells may be achieved by a three-cell cluster model in which the recipient APC simultaneously presents intact and processed MHC class I alloantigen to, respectively, the cytotoxic CD8 T-cell and helper CD4 T-cell (C).

associated with the above four-cell cluster model. However, although we have recently demonstrated simultaneous presentation of intact and processed alloantigen by recipient DCs following heart transplantation (16), the contribution of trogocytosis of alloantigen to graft rejection has yet to be clarified. To have functional relevance, presentation of intact donor alloantigen by recipient APCs must, at least in certain circumstances, be more effective for triggering cytotoxic alloimmunity than conventional encounter on donor APCs, and this has not been shown. Addressing this key concern is challenging, because it requires development of an experimental model in which CD8 T-cell allorecognition of target MHC class I alloantigen is limited to the surface of recipient, but not donor APCs. One way this scenario could perhaps be achieved is by resolving the paradox created by the seminal observations that CD8 T cells can still effect allograft rejection when target MHC class I alloantigen is expressed by graft parenchyma only (17), but that rejection of vascularized allografts does not occur in the absence of secondary lymphoid tissue (SLT) (18). Why SLT is necessary for CD8 T-cell allorecognition of graft parenchymal cells has not been explained, but one elegant and compelling solution is that alloreactive CD8 T-cell activation occurs in lymphoid tissue because shed, intact MHC class I alloantigen is only presented by recipient APCs at these sites.

Here, we show that rejection of heart allografts in mice lacking hematopoietic APCs is mediated by alloreactive CD8 T cells, and that the participation of CD8 T-cells is dependent upon the acquisition and presentation of intact MHC class I alloantigen by recipient DCs within host SLT. We further demonstrate that representation of intact alloantigen enables the delivery of essential help from indirect-pathway CD4 T cells. These findings provide strong functional support for the provision of indirect-pathway CD4 T-cell help to alloreactive CD8 T cells via a “linked” three-cell cluster model.

Results

The Requirement for Secondary Lymphoid Tissue for Differentiation of Effector Cytotoxic CD8 T-Cell Alloimmune Responses. Key to the proposed semidirect pathway for generating CD8 T-cell alloimmunity is presentation of intact MHC class I alloantigen by recipient APCs. In the expectation that this presentation would occur chiefly within host SLT, we first sought to determine under what circumstances lymphoid tissue was required for effector CD8 T-cell activation and cardiac allograft rejection, by incorporating alymphoplastic (*aly/aly*) mice that lack lymph nodes and Peyer’s patches due to a point mutation in the NF- κ B-inducing kinase gene (19). We also incorporated T-cell receptor (TCR)-transgenic *Rag1*^{-/-} 2C TCR (2C) mice, whose CD8 T cells recognize H-2L^d alloantigen (20), in the anticipation that when

present at high frequency, 2C CD8 T-cell activation would not require T-cell help, and that this would, for the initial set of experiments, eliminate the confounding role of SLT in initiating helper CD4 T-cell responses.

As previously reported (18), splenectomized *aly/aly* H-2^b mice did not reject BALB/c cardiac allografts (Fig. 2A). However, increasing the precursor frequency of the alloreactive CD8 T-cell population in the recipients by adoptive transfer of 2C CD8 T cells into the splenectomized *aly/aly* recipients restored prompt rejection of BALB/c heart grafts [median survival time (MST) = 10 d, Fig. 2A]. We reasoned that the rapid graft rejection mediated by 2C CD8 T cells without a requirement for SLT reflected the ability of the transferred 2C CD8 T cells to interact with professional donor APCs bearing target MHC class I alloantigen, perhaps within the parenchyma of the heart graft. To test this possibility, heart donors were depleted of hematopoietic APCs before heart graft retrieval by administering lethal irradiation (Fig. S1); a strategy that resulted in depletion of all MHC class II-expressing APCs from the graft parenchyma, as demonstrated by the inability of heart grafts from irradiated mice to provoke direct-pathway CD4 T-cell alloresponses (Fig. 2B). Heart grafts that were depleted of hematopoietic cells were not rejected by recipients that lacked SLT, even after adoptive transfer of a 2C CD8 T-cell effector population (Fig. 2C). In contrast, when irradiated BALB/c grafts were transplanted into unmodified *Rag1*^{-/-} 2C TCR mice (that harbored SLT), rapid rejection ensued and strong IFN- γ CD8 T cells responses were elicited in the 2C CD8 T-cell population (Fig. 2C and D). The inability of the transferred 2C CD8 T cells to effect rejection in SLT-deficient recipients was not due to lack of target alloantigen within the irradiated hearts, because transfer of activated 2C CD8 T cells provoked rapid heart graft rejection (Fig. 2C). Similarly, irradiated BALB/c hearts were rejected in wild-type C57BL/6 (B6) recipients (albeit at a slightly slower tempo than when transplanted into *Rag1*^{-/-} 2C TCR mice) (Fig. 2C) and provoked an IFN- γ CD8 T-cell alloresponse within the endogenous CD8 T-cell population that, although initially weaker than the response provoked by an unmodified BALB/c heart graft, was, by day 12, of similar intensity (Fig. 2E). Thus, although heart allografts can be rejected in the absence of host lymphoid tissue, this rejection is dependent upon the presence of donor hematopoietic APCs within the graft, and requires an unusually high precursor frequency of alloreactive CD8 T cells; lymphoid tissue is normally required to facilitate productive effector CD8 T-cell alloresponses and for rejection of heart allografts devoid of hematopoietic APCs.

Fig. 3. The generation of CD4 T-cell independent CD8 T-cell alloimmunity requires host dendritic cells. Heart allografts from lethally irradiated BALB/c donors were transplanted into CD4 T-cell-depleted CD11c-DTR-BM recipients. Control mice received no additional treatment, whereas experimental mice were adoptively transferred with *Rag1*^{-/-} 2C CD8 T cells alone (2C CD8) or in combination with high-dose diphtheria toxin (2C CD8 +DT) to ablate host DCs. (A) Kaplan–Meier survival analysis. (B) Minimal CD8 T-cell alloresponses, as assayed by IFN- γ ELISPOT, were observed in CD4 T-cell-depleted (but otherwise unmodified) CD11c-DTR-BM recipients of irradiated BALB/c heart allografts (control). These were significantly augmented by adoptive transfer of *Rag1*^{-/-} 2C CD8 T cells (2C CD8). This augmentation was dependent upon the host DC population (2C CD8 +DT; **P* < 0.05). Included for reference are the typical cytotoxic CD8 T-cell responses generated in WT C57BL/6 (B6) recipients of irradiated BALB/c grafts (WT B6). (C) Four days after transplantation with heart allografts from WT (WT donor) or irradiated BALB/c (irrad donor) donors, CD11c DCs were purified from B6 recipient spleens and adoptively transferred into naïve, secondary B6 hosts. Cytotoxic CD8 T-cell alloimmunity in the secondary hosts was assayed 10 d after transfer as above; included as control are the responses observed in naïve B6 mice. Individual data points represent responses observed in individual mice. (D) The response of purified B6 CD8 T cells to in vitro challenge with BALB/c splenocytes (SPCs) or cultured BALB/c endothelial cells (ECs) was quantified by standard mixed lymphocyte thymidine-incorporation assay. Responses to endothelial cell challenge were significantly increased by addition of B6 DC to culture. **P* < 0.05, ***P* < 0.01, ****P* < 0.001. (Kaplan–Meier in A; Mann–Whitney test in B–D). Data are representative of two independent experiments resulting in at least *n* = 6 in each group.

host B cells (Fig. 4A and Fig. S2). B-cell depletion did not compromise rejection of irradiated BALB/c heart allografts by B6 mice (Fig. 4B). All grafts were rejected rapidly, with rejection dependent upon host CD8 T cells (Fig. 4B) and associated with development of strong IFN- γ CD8 T-cell alloimmunity (Fig. 4C). As above (Fig. 3A), rejection was delayed by ablation of recipient DCs (Fig. 4B). Rapid graft loss was, however, also dependent upon host CD4 T cells (Fig. 4B and C), which critically, as a consequence of irradiation-induced death of donor hematopoietic cells, can only recognize alloantigen via the indirect pathway.

The graft rejection kinetics, coupled with the CD8 T-cell ELISPOT data, strongly suggest that the cytotoxic CD8 T-cell response is mediating graft rejection, but to confirm this possibility, B-cell-depleted “GzmBCreRosa26YFP” H-2^b mice (24) were challenged with an irradiated BALB/c heart allograft and tamoxifen administered concurrently. CD8 T cells in these recipients that differentiate into granzyme B-expressing effector cells are indelibly marked with enhanced yellow fluorescence protein (YFP). Ten days after transplantation, populations of YFP⁺ effector CD8 T cells were readily detectable within the spleen and within the rejecting

heart allograft (Fig. 4D), with a higher proportion of recovered CD8 T cells from the heart allograft expressing YFP (Fig. 4E). Similarly, because irradiated heart allografts do not provoke a direct-pathway CD4 T-cell response (Fig. 2B), help for cytotoxic CD8 T cells can theoretically only be provided by indirect-pathway CD4 T cells. To confirm this, *Rag2*^{-/-} C57BL/6 mice were reconstituted with GzmBCreRosa26YFP CD8 T cells and with TCR75 CD4 T cells that recognize donor H-2K^d alloantigen as self-restricted, processed allopeptide exclusively via the indirect pathway. Whereas irradiated BALB/c grafts were not rejected in *Rag2*^{-/-} C57BL/6 recipients reconstituted with TCR75 CD4 T cells only (Fig. 4F), TCR75 CD4 T cells provided help for differentiating effector cytotoxic CD8 T-cell responses (Fig. 4E) that were responsible for rapid allograft rejection (Fig. 4F).

When coupled to the earlier observation that host DCs are essential for CD4 T-cell independent 2C CD8 T-cell alloresponses (Fig. 2C and D), these latter experiments provide functional support for the three-cell cluster model and presentation of intact MHC class I alloantigen by host cells for generating cytotoxic alloimmunity (Fig. 1C), with linked help provided by indirect-pathway CD4 T cells.

Discussion

The present studies address the important question of how CD4 T-cell help is provided to alloreactive CD8 T cells for the development of destructive cytotoxic responses, and our results help to resolve a long-standing debate on the mechanism by which help is delivered by CD4 T cells with indirect allospecificity. The use of donor mice deleted of hematopoietic cells, allied to ablation of DCs or absence of host SLT enabled us to confirm that in certain circumstances, host DCs were critical for priming cytotoxic CD8 T-cell responses that effected rapid allograft destruction. In doing so, our findings provide a solution to the paradox created by two earlier landmark studies that observed that SLT was required for rejection of a vascularized allograft (18), but that cytotoxic CD8 T cells could mediate allograft rejection when expression of target MHC class I alloantigen was limited to graft parenchyma (17). Moreover, because our model was refined to exclude the contribution of alternative effector mechanisms of graft rejection, we believe our paper is the first to demonstrate the functional relevance of the semi-direct pathway to transplant rejection.

The means by which immune cells acquire or trogocytose membrane proteins from other cells are becoming better understood (12). Most data relate to acquisition of MHC–peptide complexes from the surface of APCs by responding T cells, a process dependent upon downstream signaling of the TCR (25). The immunological consequences of such trogocytosis are still not clear and may include: increasing competition between T cells through reduction in available target MHC/peptide epitope, increased T-cell activation from continued positive signaling via the internalized complexes, and regulatory T cell–T cell interaction. Trogocytosis may hold particular relevance for transplantation, because the ability to transfer allogeneic membrane proteins between donor and recipient immune cells is likely to have more profound consequences than transfer of self-proteins. Nevertheless, few studies have reported functional significance for either solid organ or hematopoietic stem cell transplantation (26, 27) and our findings thus emphasize the potentially important contribution of trogocytosis in initiating adaptive alloimmunity.

As to why host DCs were required in our experiments for generating CD4 T-cell-independent, cytotoxic 2C CD8 T-cell alloresponses to an irradiated heart allograft, this observation presumably reflects that, in the absence of donor DCs, intact MHC class I alloantigen is only presented by host DCs in association with the necessary costimulatory ligands for effector differentiation of the 2C CD8 T cells. This result in turn suggests that the DCs have been activated by acquisition of alloantigen. One

Our experiments involving WT, nontransgenic CD8 T-cell responders demonstrated that CD4 T-cell help was critical for cytotoxic, effector differentiation. We have previously demonstrated simultaneous presentation of intact and processed alloantigen by the same DC (16). Within the SLT, this dual presentation presumably allows the indirect-pathway helper CD4 T cell to license the DC for effective presentation of MHC class I alloantigen to the alloreactive CD8 T cell, which binds either simultaneously, or sequentially (4), to the same DC. Delivery of help by the indirect-pathway CD4 T cell is dependent upon cognate recognition of self MHC class II-allopeptide complexes on the surface of the recipient DC (16), and although not formally tested, this finding suggests that help is mediated principally via costimulatory signaling, rather than via paracrine cytokine release. It is important to acknowledge, however, that our experiments do not provide definitive proof of this three-cell interaction. Our conclusion is based on the observations that host DCs are essential for both helper-cell-independent CD8 T-cell alloresponses against MHC class I expressed on parenchymal graft cells, and, when responder CD8 T-cell numbers are limited, for generating essential indirect-pathway CD4 T-cell help. Hence our experiments do not exclude the possibility that indirect-pathway CD4 T-cell help is provided through interaction with one particular host DC subset, whereas the alloreactive CD8 T cells are activated through interaction with another. This possibility seems unlikely, because it would again represent an unlinked four-cell cluster, and the effectiveness, and control, of CD8 T-cell activation is likely to be much greater if the helper CD4 T-cell and alloreactive CD8 T-cell determinants are expressed on the same host DC. It would be

theoretically possible to visualize this interaction using dynamic intravital microscopy (33), but such an approach would not provide information relating to either cytotoxic differentiation or its functional relevance to graft rejection.

Materials and Methods

Animals. Wild-type C57BL/6 and BALB/c mice were purchased from Charles River Laboratories. Bm12 and C57BL/6 CD11c-DTR mice (21) were purchased from The Jackson Laboratory. *Aly/aly* mice were purchased from CLEA Inc. TCR transgenic *Rag1*^{-/-} 2C TCR mice (20) were gifted by Geetha Chalasani (University of Pittsburgh, Pittsburgh). C57BL/6 *Rag2*^{-/-} mice were gifted by T. Rabbitts (University of Cambridge, Cambridge, UK). C57BL/6-Tg(K^d)Rpb mice that express transgenic H-2K^d (34) and TCR transgenic *Rag1*^{-/-} TCR75 mice, specific for I-A^b restricted H-2K^d₅₄₋₆₈ peptide were gifted by R. P. Bucy (University of Alabama, Birmingham, AL) (35). GzmBCrexRosa26YFP mice (24) were gifted by D. Fearon (University of Cambridge). In experiments involving GzmBCrexRosa26YFP mice or CD8 T cells, 1 mg tamoxifen was administered i.p. to the recipient the day before and every second day after transplant. All experiments were approved by the United Kingdom Home Office under the Animal (Scientific Procedures) Act, 1986.

Cell Purification and Lymphocyte Depletion. Cell subsets were purified using an autoMACS separator. "Activated" 2C TCR CD8 were purified from *Rag1*^{-/-} 2C TCR mice challenged 1 wk earlier with a BALB/c heterotopic heart graft. DCs were purified from recipient mice spleens 4 d after transplantation with

a heart graft and adoptively transferred into naïve secondary C57BL/6 mice as described previously (16). In mice expressing the DTR transgene, DCs were depleted with diphtheria toxin. B cells were depleted with depleting anti-CD20 mAb (18B12, IgG2a). Depletion of the hematopoietic cell fraction in donor mice was achieved by lethal irradiation and treatment with depleting anti-CD4 mAb (rat IgG2b, clone YTS 191.1). Depletion was confirmed by flow cytometric analysis of serum and splenic tissue and the absence "direct-pathway" CD4 T-cell responses.

Statistical Analysis. Data were presented as mean ± SEM where appropriate. The Mann-Whitney *u* test was used for analysis of nonparametric data. Graft survival was depicted using Kaplan-Meier analysis and groups were compared by log-rank (Mantel-Cox) testing. Analysis was conducted using GraphPad 4 (GraphPad Software). Values of *P* < 0.05 were considered significant.

Detailed experimental details can be found in *SI Materials and Methods*.

ACKNOWLEDGMENTS. This work was supported by a British Heart Foundation project grant, the National Institute of Health Research Cambridge Biomedical Research Centre, and the National Institute of Health Research Blood and Transplant Research Unit. S.J.F.H. was supported by an Academy of Medical Sciences/Wellcome Trust starter grant and the European Society for Organ Transplantation Junior Basic Science research grant. J.M.A. and I.G.H. were supported by Wellcome Trust Clinical Research training fellowships and Raymond and Beverly Sackler scholarships.

1. Rocha PN, Plumb TJ, Crowley SD, Coffman TM (2003) Effector mechanisms in transplant rejection. *Immunol Rev* 196:51–64.
2. Le Moine A, Goldman M, Abramowicz D (2002) Multiple pathways to allograft rejection. *Transplantation* 73(9):1373–1381.
3. Rosenberg AS, Singer A (1992) Cellular basis of skin allograft rejection: an in vivo model of immune-mediated tissue destruction. *Annu Rev Immunol* 10:333–358.
4. Ridge JP, Di Rosa F, Matzinger P (1998) A conditioned dendritic cell can be a temporal bridge between a CD4⁺ T-helper and a T-killer cell. *Nature* 393(6684):474–478.
5. Bennett SR, et al. (1998) Help for cytotoxic-T-cell responses is mediated by CD40 signalling. *Nature* 393(6684):478–480.
6. Schoenberger SP, Toes RE, van der Voort EI, Offringa R, Melief CJ (1998) T-cell help for cytotoxic T lymphocytes is mediated by CD40-CD40L interactions. *Nature* 393(6684):480–483.
7. Ali JM, Bolton EM, Bradley JA, Pettigrew GJ (2013) Allorecognition pathways in transplant rejection and tolerance. *Transplantation* 96(8):681–688.
8. Taylor AL, et al. (2007) Pathways of helper CD4 T cell allorecognition in generating alloantibody and CD8 T cell alloimmunity. *Transplantation* 83(7):931–937.
9. Jiang S, Herrera O, Lechler RI (2004) New spectrum of allorecognition pathways: implications for graft rejection and transplantation tolerance. *Curr Opin Immunol* 16(5):550–557.
10. Conlon TM, et al. (2012) Germinal center alloantibody responses are mediated exclusively by indirect-pathway CD4 T follicular helper cells. *J Immunol* 188(6):2643–2652.
11. Lee RS, Grusby MJ, Glimcher LH, Winn HJ, Auchincloss H, Jr (1994) Indirect recognition by helper cells can induce donor-specific cytotoxic T lymphocytes in vivo. *J Exp Med* 179(3):865–872.
12. Dhainaut M, Moser M (2014) Regulation of immune reactivity by intercellular transfer. *Front Immunol* 5:112.
13. Davis DM (2007) Intercellular transfer of cell-surface proteins is common and can affect many stages of an immune response. *Nat Rev Immunol* 7(3):238–243.
14. Herrera OB, et al. (2004) A novel pathway of alloantigen presentation by dendritic cells. *J Immunol* 173(8):4828–4837.
15. Brown K, Sacks SH, Wong W (2011) Coexpression of donor peptide/recipient MHC complex and intact donor MHC: evidence for a link between the direct and indirect pathways. *Am J Transplant* 11(4):826–831.
16. Sivaganesan S, et al. (2013) Copresentation of intact and processed MHC alloantigen by recipient dendritic cells enables delivery of linked help to alloreactive CD8 T cells by indirect-pathway CD4 T cells. *J Immunol* 190(11):5829–5838.
17. Kreisel D, et al. (2002) Non-hematopoietic allograft cells directly activate CD8⁺ T cells and trigger acute rejection: an alternative mechanism of allorecognition. *Nat Med* 8(3):233–239.
18. Lakkis FG, Arakelov A, Konieczny BT, Inoue Y (2000) Immunologic 'ignorance' of vascularized organ transplants in the absence of secondary lymphoid tissue. *Nat Med* 6(6):686–688.
19. Miyawaki S, et al. (1994) A new mutation, *aly*, that induces a generalized lack of lymph nodes accompanied by immunodeficiency in mice. *Eur J Immunol* 24(2):429–434.
20. Manning TC, et al. (1997) Antigen recognition and allogeneic tumor rejection in CD8⁺ TCR transgenic/RAG(-/-) mice. *J Immunol* 159(10):4665–4675.
21. Jung S, et al. (2002) In vivo depletion of CD11c⁺ dendritic cells abrogates priming of CD8⁺ T cells by exogenous cell-associated antigens. *Immunity* 17(2):211–220.
22. Li H, et al. (2012) Profound depletion of host conventional dendritic cells, plasmacytoid dendritic cells, and B cells does not prevent graft-versus-host disease induction. *J Immunol* 188(8):3804–3811.
23. Bennett CL, Clausen BE (2007) DC ablation in mice: promises, pitfalls, and challenges. *Trends Immunol* 28(12):525–531.
24. Bannard O, Kraman M, Fearon DT (2009) Secondary replicative function of CD8⁺ T cells that had developed an effector phenotype. *Science* 323(5913):505–509.
25. Martinez-Martin N, et al. (2011) T cell receptor internalization from the immunological synapse is mediated by TC21 and RhoG GTPase-dependent phagocytosis. *Immunity* 35(2):208–222.
26. Markey KA, et al. (2014) Cross-dressing by donor dendritic cells after allogeneic bone marrow transplantation contributes to formation of the immunological synapse and maximizes responses to indirectly presented antigen. *J Immunol* 192(11):5426–5433.
27. Chow T, Whiteley J, Li M, Rogers IM (2013) The transfer of host MHC class I protein protects donor cells from NK cell and macrophage-mediated rejection during hematopoietic stem cell transplantation and engraftment in mice. *Stem Cells* 31(10):2242–2252.
28. Curry AJ, et al. (2007) Dendritic cells internalise and re-present conformationally intact soluble MHC class I alloantigen for generation of alloantibody. *Eur J Immunol* 37(3):696–705.
29. Wykes M, Pombo A, Jenkins C, MacPherson GG (1998) Dendritic cells interact directly with naïve B lymphocytes to transfer antigen and initiate class switching in a primary T-dependent response. *J Immunol* 161(3):1313–1319.
30. Obst R, van Santen HM, Mathis D, Benoist C (2005) Antigen persistence is required throughout the expansion phase of a CD4⁺ T cell response. *J Exp Med* 201(10):1555–1565.
31. Bevan MJ, Fink PJ (2001) The CD8 response on autopilot. *Nat Immunol* 2(5):381–382.
32. Kaech SM, Ahmed R (2001) Memory CD8⁺ T cell differentiation: initial antigen encounter triggers a developmental program in naïve cells. *Nat Immunol* 2(5):415–422.
33. Pittet MJ, Weissleder R (2011) Intravital imaging. *Cell* 147(5):983–991.
34. Honjo K, Xu XY, Bucy RP (2000) Heterogeneity of T cell clones specific for a single indirect alloantigenic epitope (I-Ab/H-2Kd54-68) that mediate transplant rejection. *Transplantation* 70(10):1516–1524.
35. Honjo K, Xu XY, Bucy RP (2004) CD4⁺ T-cell receptor transgenic T cells alone can reject vascularized heart transplants through the indirect pathway of alloantigen recognition. *Transplantation* 77(3):452–455.
36. Corry RJ, Winn HJ, Russell PS (1973) Primarily vascularized allografts of hearts in mice. The role of H-2D, H-2K, and non-H-2 antigens in rejection. *Transplantation* 16(4):343–350.
37. Sapozhnikov A, Jung S (2008) Probing in vivo dendritic cell functions by conditional cell ablation. *Immunol Cell Biol* 86(5):409–415.
38. Holmes BJ, et al. (2002) Anti-pig antibody levels in naïve baboons and cynomolgus monkeys. *Xenotransplantation* 9(2):135–147.
39. Callaghan CJ, et al. (2012) Regulation of allograft survival by inhibitory FcγRIIb signaling. *J Immunol* 189(12):5694–5702.