The role of direct presentation by donor dendritic cells in rejection of minor H antigen mismatched skin and hematopoietic cell grafts

Eduardo Fernandes[1], Hugh, D. Goold[2], Adrien Kissenpfennig[3,4], Bernard Malissen[3], Julian Dyson[1] and Clare L. Bennett[2]

[1]Section of Immunobiology, Division of Immunology and Inflammation, Imperial College London, Hammersmith Hospital, London.

[2]DC Immunotherapy group, Department of Haematology, Division of Cancer Studies, UCL Royal Free campus, London.

[3]Centre d'Immunologie de Marseille-Luminy, INSERM-CNRS-Université de la Méditerrannée, Marseille, France.

Keywords: Dendritic cells, Langerhans cells, skin, minor H antigen.

Address correspondence to:

Clare Bennett: c.bennett@medsch.ucl.ac.uk

Dept of Haematology, UCL Royal Free Campus, Rowland Hill St., London NW3 2PF.

Julian Dyson: peter.dyson@imperial.ac.uk

Section of Immunobiology, Imperial College London, Commonwealth Building, Hammersmith Hospital, London W12 0NN.

Footnotes

Title

[1]E.F performed experiments, analyzed data, and helped write the paper, H.G provided technical support, A.K and B.M. provided reagents, J.D designed the study and helped write the paper, C.B. designed the study, performed and analyzed experiments and wrote the paper.

[2]This work was supported by a Leukaemia and Lymphoma Research Fellowship to C. Bennett and a British Heart Foundation Clinical Fellowship to E. Fernandes.

[3]The authors declare no conflict of interest.

Authors' current addresses

[1]Section of Immunobiology, Division of Immunology and Inflammation, Imperial College London, Hammersmith Hospital, London.

[2]DC Immunotherapy group, Department of Haematology, Division of Cancer Studies, UCL Royal Free campus, London.

[3]Centre d'Immunologie de Marseille-Luminy, INSERM-CNRS-Université de la Méditerrannée, Marseille, France.

[4]Infection and Immunity Group, Centre for Cancer Research and Cell Biology, School of Biomedical Sciences, Queens University, Belfast, Northern Ireland.

Address correspondence to:

Clare Bennett: c.bennett@medsch.ucl.ac.uk. Tel. +20 77940500 x34063

Julian Dyson: peter.dyson@imperial.ac.uk. Tel. +20 83838252

Abbreviations

APC - antigen presenting cells

DC - Dendritic cells

DT - diphtheria toxin

DTR – diphtheria toxin receptor

LC - Langerhans cells

Minor H antigens - minor histocompatibility antigens

Abstract

Background. The success of transplantation is hampered by rejection of the graft by alloreactive T cells. Donor dendritic cells (DC) have been shown to be required for direct priming of immune responses to antigens from MHC mismatched grafts. However, for immune responses to MHC matched, minor H antigen mismatched grafts the magnitude of the T cell response to directly presented antigens is reduced, and the indirect pathway is more important. Therefore we aimed to investigate the requirement for donor DC to directly present antigen from minor H antigen mismatched skin and hematopoietic grafts.

Methods. Langerhans cell (LC)- or conventional (c)DC-depleted skin or hematopoietic cells from male DC-specific diphtheria toxin receptor (DTR) mice were grafted onto, or injected into, syngeneic female recipients, and survival of the male tissue was compared with non-depleted tissue. Activation of the alloreactive immune response was tracked by the expansion of T cells specific for male HY-derived epitopes.

Results. Our data demonstrate that depletion of donor LC, dermal cDC, or both from skin grafts prolongs their survival, but does not prevent rejection. Extended survival correlates with delayed expansion of HY peptide-specific $CD8^+$ T cells. In addition depletion of donor cDC delays rejection of male hematopoietic cells.

Conclusions. Our results demonstrate for the first time that direct presentation of minor H antigens by donor DC is required for efficient rejection of skin and hematopoietic grafts by $CD8^+$ T cells. But, in the absence of donor DC, indirect presentation of minor antigens is sufficient to mediate the response.

Introduction

Transplantation is the only curative option for end stage organ failure and is an effective approach for the treatment of haematological malignancy. The main barrier to this approach is allograft rejection directed against antigens encoded in the major histocompatibility complex (MHC) (*1*). Transplant rejection also takes place between MHC matched individuals, where the minor histocompatibility (H) antigens, peptides derived from polymorphic self-proteins differing between donor and recipient and presented by MHC class I and II molecules, stimulate an immune response leading to rejection of the allograft (*2, 3*).

Alloantigen recognition and activation of graft-specific T cells occurs by three mechanisms. In the direct pathway intact donor MHC molecules expressed on graft-associated antigen presenting cells (APC) are recognized by host T cells. The high frequency of naïve T cells specific for allogeneic MHC molecules has led to the suggestion that the direct pathway mediates acute rejection of MHC mismatched grafts (*4, 5*). Alternatively, in the indirect pathway, recipient APC process graft-derived material and present peptides to alloreactive host T cells (*6, 7*). Indirect allospecific T cells have a lower precursor frequency and this response is thought to become more important once donor APC are lost from the graft, thereby mediating chronic rejection of grafted tissue. Finally, in semi-direct allorecognition host APC acquire intact MHC:peptide complexes from donor cells (*6*).

Dendritic cells (DC) are professional APC, required to prime and control T cell responses. The role of donor DC in rejection of MHC mismatched grafts has been explored by grafting tissue into an intermediate host under immunosuppression, followed by re-transplantation of the now passenger cell-depleted graft to a secondary recipient. Using this approach, MHC mismatched kidney grafts were accepted indefinitely (*8*), while heart graft survival was prolonged (*9*). In addition, use of Flt3 ligand deficient donors, which are severely depleted of DC and other immune cells, prolonged survival of MHC mismatched heart grafts (*10*). Transfer of donor DC into such transplant recipients was sufficient to initiate graft rejection (*8*). Therefore, these data suggest that donor DC are required for direct presentation of alloantigens from MHC mismatched grafts.

For MHC mismatched grafts, the high frequency of T cells recognizing allogeneic MHC accounts for the importance of the direct pathway of antigen presentation. In contrast, minor H antigen disparate grafts share MHC molecules with the recipient, that differ only by a limited set of bound self-peptides. Consequently, the magnitude of the direct response is expected to be markedly reduced in comparison with MHC mismatched grafts (*3*). The indirect pathway was first described in the context of minor H antigens (*11*) where it operates efficiently (*12*). It is therefore of interest to investigate whether direct presentation of alloantigen by DC is still required following transplantation of minor H mismatched grafts.

Here, we have investigated the requirement for donor DC for the priming of skin and haematopoietic graft rejection immune responses mediated by the male-specific minor H antigen, HY. Identification of the HY peptide epitopes presented by the $H2^b$ MHC haplotype has facilitated the analysis of this immune response *(12*). In particular, MHC class I HY-specific tetramers can be used to quantify the CD8 T cell response. To examine the role of graft-resident DC we have used DC-specific diphtheria toxin receptor (DTR) mice for *in vivo* ablation of defined DC populations from skin and haematopoietic cell grafts prior to transplantation (reviewed in *13*). In this approach, cell-type specific expression of the high affinity, primate DTR renders these cells uniquely susceptible to killing by diphtheria toxin (DT). Further, the receptor is a fusion protein with GFP allowing identification of expressing cells and monitoring of their depletion by flow cytometry. As mouse cells do not otherwise express a high affinity DTR, cell depletion is restricted to the tissue expressing the DTR gene. Endocytosis of the toxin rapidly blocks protein synthesis initiating apoptosis of both mitotic and terminally differentiated cells. To direct DTR expression to specific DC subsets, the CD11c and Langerin promoters have been used *(13)*. Using this approach, we demonstrate that direct presentation by donor DC is required to elicit the most rapid rejection of minor H antigen mismatched skin and haematopoietic grafts.

Results

Depletion of cDC or Langerin$^+$ DC from skin grafts delays their rejection.

To address the role of donor DC in transplant rejection we grafted skin from male donors, that had or had not been depleted of different DC populations, onto female recipients. Donor skin contains three major populations of DC: Langerhans cells (LC) in the epidermis; and Langerin$^+$ and Langerin^{neg} CD11c$^+$ conventional (c)DC populations in the dermis (*14, 15*). In initial experiments we investigated whether donor dermal cDC were required for the rejection of minor H alloantigen (HY) mismatched skin grafts by using CD11c-DTR mice to specifically deplete CD11c^{hi} cDC. In these transgenic mice a high affinity DTR is expressed from the CD11c promoter, rendering CD11c^{hi} DC sensitive to killing by DT (*13, 16*). Figure 1A shows the splenic, GFP$^+$, CD11c$^+$ cDC present in untreated CD11c-DTR mice. Panel B shows that a single injection of DT routinely depletes 80-90% of systemic cDC, which has previously been shown to include dermal cDC (*17*). DT was injected into wild type (wt) or CD11c-DTR / C57BL/6 male mice and 24 hours later the mice were sacrificed and their skin grafted onto syngeneic female recipients. Female mice receiving grafts from wt males injected with DT rejected the grafts after approximately 30 days as published, (Figure 2A; median survival time for 50% of the grafts 26.5 days (*18*)), demonstrating that treatment of the graft with DT does not alter rejection in this model. However, depletion of cDC from the graft significantly prolonged survival (Figure 2A; median survival for 50% of the grafts 41 days).

Epidermal LC are not depleted from CD11c-DTR mice due to their low level expression of CD11c *in situ* (*13*). Therefore, to address whether LC and Langerin$^+$ DC were required for rejection of male skin grafts we repeated our experiments using skin from male Langerin-DTR mice injected with DT 24 hours previously. The right panel of Figure 1A shows the epidermal GFP$^+$, CD11b$^+$ LC present in untreated Langerin-DTR mice. Figure 1C shows that a single injection of DT efficiently depletes Langerin$^+$ DC from these mice (*19, 20*). Figure 2A demonstrates that depletion of Langerin$^+$ DC also significantly prolongs survival of male syngeneic skin grafts (median survival time for 50% of the grafts 41 days). This was equivalent to the time required to reject cDC-depleted male skin from female recipients.

Collectively these data demonstrate that individual donor DC populations are not required for the rejection minor H antigen mismatched skin, but that activation of the rejection response is less efficient in their absence. The observation that depletion of either CD11c^{hi} cDC or Langerin$^+$ DC delays rejection to the same extent suggested that there was redundancy in the requirement for different skin DC populations to activate an optimal anti-graft response. To address this hypothesis, we transplanted skin from DT-injected male CD11c-DTR x Langerin-DTR mice that were depleted of both cDC and Langerin$^+$ DC (Figure 1B and C). Figure 2B shows that depletion of all cutaneous DC again resulted in prolonged survival of skin grafts (median survival time for 50% for the grafts 44.5 days), but that the grafts were ultimately rejected with the same kinetics as those depleted

of either cDC or Langerin$^+$ DC. Therefore, these data demonstrate that depletion of cutaneous DC from donor skin delays, but cannot prevent, rejection of male syngeneic grafts.

Reduced expansion of donor-specific T cells in mice receiving DC-depleted skin grafts.

The prolonged acceptance of donor DC-depleted skin grafts suggested that male graft-specific T cell responses were not efficiently activated in the absence of donor DC. We have previously defined the *Uty* peptide as a dominant MHC I-restricted minor H antigen that provokes allograft rejection in C57BL/6 male to female grafts (*21*). Therefore, to investigate whether *Uty*-specific T cells were activated in mice receiving DC-depleted grafts we examined expansion of CD8$^+$ HYD^b *Uty* tetramer$^+$ cells in the blood of female recipients at the peak of the T cell response, 2 weeks after grafting (*21*). Figure 3 shows that there is a significant reduction in the expansion of HYD^b *Uty*$^+$ CD8$^+$ T cells in mice that have received grafts depleted of either cDC or LC. Therefore these data demonstrate that prolonged graft survival in mice receiving DC-depleted grafts is associated with a reduction in the priming of allograft-specific T cells.

To investigate whether the delayed rejection of DC-depleted skin grafts reflected altered kinetics for the activation of the male-specific T cell response, we also measured expansion of HYD^b *Uty*$^+$ CD8$^+$ T cells 30 days after grafting. As shown in Figure 4, there is no difference in the accumulation of HYD^b *Uty*$^+$ tetramer$^+$ T

cells at this time point in any of the groups. Collectively these data demonstrate that in the absence of donor cDC or Langerin$^+$ DC, graft-specific T cells are primed with slower kinetics, which results in the prolonged survival but ultimate rejection, of minor H antigen mismatched allografts.

Depletion of cDC delays rejection of hematopoietic grafts.

The role of donor skin DC in direct presentation of graft-derived antigens will depend on the establishment of lymphatic and vascular vessels that allow DC to leave the tissue. Therefore, we investigated whether donor DC were required to prime the rejection response in a hematopoietic transplant model that did not depend on graft vascularization. Wt or CD11c-DTR male mice were injected with DT and 24 hours later splenocytes isolated, labeled with CFSE and injected, along with control female cells labeled with a different dose of CFSE, into female recipients. In this model, the male splenocytes activate the immune response and subsequently act as targets of cytotoxic CD8 T cells (*22, 23*). Figure 5A shows that *in vivo* cytotoxicity is significantly reduced 10 days after immunization of female mice with cDC-depleted male splenocytes. However, at this time, HYD^b *Uty*$^+$ tetramer$^+$ T cells have expanded to the same extent in both groups (Figure 5B). In accord with this observation, by 20 days post-injection, male targets are efficiently killed in both the cDC-depleted and non-depleted groups (Figure 5C). Together these data show that direct presentation of minor H antigens by donor DC contributes to the rejection process by augmenting HY-

specific cytotoxic T cell responses, but is not required for activation of these minor H allospecific T cells.

Discussion

Donor DC are thought to be required for acute rejection of MHC-mismatched transplants, following direct presentation of alloantigens to T cells in lymphoid organs draining the grafted tissue (*24*). However, unlike the high frequency of alloreactive T cells directly recognizing MHC disparate graft antigens, the frequency of T cells specific for directly presented minor H antigens is very low. This will potentially limit the efficacy of the direct pathway, and whether donor DC are important for rejection of minor H antigen mismatched grafts is not known. To investigate this question we have exploited inducible models of DC depletion to transplant male DC-depleted skin or splenocytes to female, DC-replete, recipients. We have shown that neither $CD11c^+$ dermal cDC nor $Langerin^+$ DC are required for the rejection of HY minor H antigen mismatched skin grafts by female recipients. But, in the absence of either or both of these groups of skin DC, graft rejection is significantly delayed due to a reduction in priming of HY-specific $CD8^+$ T cells. The lower frequency of HY alloantigen-specific T cells, and the requirement for recipient DC to take up and process cells released by the graft, indicate that the kinetics of priming of indirect allospecific T cell responses will be slower than that of the direct pathway. Therefore, our results suggest a model in which direct presentation by donor DC is required for the efficient rejection of minor H antigen mismatched grafts, but that indirect priming of $CD8^+$ T cells by recipient DC is sufficient to mediate a delayed response. This model is in agreement with data from McKay *et al*, who demonstrated prolonged survival

of minor H antigen mismatched skin from mice in which donor DC are not activated to migrate out of the graft to directly prime T cells in draining LN (*25*).

An alternative model would suggest that indirect presentation of minor H antigens is the dominant mode of graft rejection, but donor DC migrating out of the graft may provide a more rapid source of alloantigen to be processed by LN-resident recipient DC than other donor cell types. This second model is in accordance with previous data showing that indirect recognition of graft antigens is sufficient to mediate rejection of MHC mismatched skin (*26, 27*). In order to distinguish between the two models we investigated the priming of HY-specific T cells in females receiving male, cDC-depleted haematopoietic cells. In this system alloantigen is abundantly available for indirect presentation by recipient DC within the spleen, and active trafficking of DC from the graft to lymphoid organs is not required to prime the response. Therefore, if donor DC only provided more rapid access to antigens for indirect presentation to LN T cells, depletion of donor DC would not be predicted to alter priming of the T cell response in this second model. We show that transfer of cDC-depleted splenocytes results in expansion of HY-specific $CD8^+$ T cells, but that these T cells do not initially acquire effector function, and male target cells are only partially rejected. However, as with mice receiving DC-depleted skin grafts, rejection of male splenocytes is complete but delayed in mice injected with cDC-depleted cells. Therefore, these data support the first model in which direct presentation by donor DC is an important mechanism for efficient rejection of minor H antigen mismatched transplants.

But, indirect presentation by recipient APC is sufficient to mediate the response and, in the case of skin grafts, we cannot exclude a contribution from migrating DC in providing antigen for indirect presentation by host LN DC. Furthermore, our data demonstrate that the direct pathway is important for the rapid rejection of male to female grafts, despite the reduced precursor frequency of T cells specifically recognizing directly presented minor H antigens.

The LC paradigm states that activated LC migrate from the skin to prime T cell immunity in draining LN. LC which have acquired antigen in the skin migrate to draining LN *(28)*, but recent experiments in virally-infected mice have shown that LC are not required to prime the CD8 T cell response. Consequently the role of LC in skin immunity remains unclear *(*reviewed in *29)*. LC initiate host versus graft reactivity in murine MHC mismatched bone marrow (bm) chimeras *(30)*, and have classically been assumed to prime immune responses to skin grafts *(31)*. However, it has previously been shown that male skin from mice constitutively depleted of epidermal LC is more rapidly rejected from female recipients than wt LC-replete skin showing, in agreement with our data, that LC are not required for rejection of minor antigen mismatched skin grafts *(32)*. However, the authors were not able to demonstrate a difference in T cell priming in this model, probably due to an inability to track antigen-specific T cells. The differences in the kinetics of graft rejection of LC-depleted skin between our data and that of Obhrai *et al* may be due to differences in the LC-depletion model used (*32, 33*). Unlike Langerin-DTR mice, from which LC can be inducibly depleted, the

Langerin-DTA mice used by Obhrai *et al* constitutively lack LC from birth. These mice tend to display hyperimmune responses that are not seen when LC are transiently depleted from Langerin-DTR mice (*28, 34*). An alternative explanation is that we deplete both epidermal LC and dermal Langerin$^+$ DC from our donor skin compared to only LC in the DTA model *(32)*. Our observation that skin depleted of all cutaneous DC was rejected with the same kinetics as skin depleted of either Langerin$^+$ DC or cDC suggests that a single donor DC population does not dominate priming of the skin graft rejection response. Furthermore, these data demonstrate that either the low precursor frequency for T cells recognizing directly presented minor H antigens limits the importance of the direct immune response, which will always be dominated by indirectly primed T cells, and/or that the reduced efficiency of depletion of cDC and LC from CD11c-DTR x Langerin-DTR mice compared to the single DTR mice (Figure 1), results in persistence of sufficient donor DC to directly prime a sub-optimal T cell response.

In conclusion, we have demonstrated that removal of donor DCs from skin and hematopoietic cell grafts weakens the host versus graft (HvG) CD8$^+$ T cell response to minor H antigens. For HLA matched bm transplants, minor H antigens are main targets of both the graft versus host (GvH) and HvG responses. To maintain the benefit of the graft versus leukemia (GvL) effect, the component of the GvH response with specificity for hematopoietic cells *(35)*, it is important to develop practical strategies that target solely the HvG response. In

similar fashion to our findings with mouse hematopoietic cell grafts, depletion of DCs from human bm grafts would be expected to reduce the severity of the HvG response by limiting antigen presentation via the direct pathway without influencing the GvL response. Weakening the intensity of the HvG response by DC depletion may form a useful adjunct for strategies designed to induce graft specific tolerance such as intranasal *(22)* or sub-cutaneous *(36)* delivery of recipient specific minor H antigen peptide epitopes.

Materials and Methods.

Mice and DC depletion models

Animals were used under protocols approved by local institutional research committees, in accordance with U.K. Home Office guidelines. C57BL/6 female mice (6-8 weeks) were purchased from Harlan Orlac (Bicester, UK). CD11c-DTR mice were bought from Jackson Laboratories (USA). Langerin-DTR mice were kindly provided by A. Kissenpfennig and B. Malissen (*37*). Both lines were bred in the Comparative Biology Unit at UCL. Both CD11c-DTR and Langerin-DTR mice have been back-crossed onto C57BL/6 backgrounds for more than 10 generations. 24 hours before sacrifice of mice for grafting, CD11c-DTR and Langerin-DTR males were injected i.p. with 100ng or 500ng of DT (Sigma) in PBS, to deplete cDC or Langerin$^+$ DC respectively (*16, 19*). Control C57BL/6 males were also injected with 100ng DT. To deplete both cDC and Langerin$^+$ DC CD11c-DTR mice were crossed with Langerin-DTR mice for one generation. Langerin-DTR x CD11c-DTR mice were injected with 100ng DT.

Flow cytometry and antibodies

The following antibodies were used: CD11c-PE (N418; Miltenyi Biotec.); CD11b-APC (M1/70; eBiosciences), CD8-PerCP or CD8-APC (53.6-7; BD Pharmingen or eBiosciences). Samples were acquired using a FACSCalibur flow cytometer (BD Bioscience), and analyzed using FlowJo software (Treestar).

Skin grafting

Skin grafting was conducted as described previously *(38)* and after removal of the plaster casts, the grafts were observed every two days. Grafts were read in coded groups and were scored as being rejected when <10% viable tissue was visible.

Tetramer staining

Peripheral blood lymphocytes (PBL) were prepared by collection of tail blood into heparin, followed by brief lysis of red blood cells in water. Samples were resuspended in 50μl RPMI/10% FBS in a 96-well plate and incubated with 0.25μl HY^{Db} *Uty*-PE tetramer (Proimmune, Oxford) for 20 min. at 37°C. Anti-CD8 antibody was subsequently added to the wells and the cells incubated at 4°C for 15 minutes, before washing and resuspension in PBS/1% FBS for analysis.

Immunization and *in vivo* cytotoxicity assays

Splenocytes were isolated from wt C57BL/6 and CD11c-DTR male mice that had been injected with DT 24 hours previously. Control splenocytes were also isolated from female C57BL/6 mice. Target male cells were labeled with low dose (0.5μM) CFSE and mixed at a 1:1 ratio with female control cells labeled with high dose (5μM) CFSE. A total of 10^7 cells were injected i.v. into female C57BL/6 recipients. At defined time points PBL or splenocytes were analyzed by flow cytometry for the presence of the 2 populations of CFSE-labeled cells.

Specific lysis was calculated, in comparison to the ratio of male:female cells injected into recipient mice according to the equation:

$$\left[1 - \left(\frac{\text{(female:male injection mix)}}{\text{(female:male imm. recipient)}}\right)\right] \text{x100}$$

Statistical analysis

The Mann-Whitney U test was used for most statistical analyses. Statistical analysis of graft survival was by the log-rank method. A p value of ≤ 0.05 was regarded as significant.

Acknowledgments

We thank the CBS staff for excellent animal care, Mr David Coe for skin grafting, Dr Diane Scott for helpful discussion and Prof. Elizabeth Simpson for reviewing the manuscript.

References

1. Issa F, Schiopu A, Wood KJ. Role of T cells in graft rejection and transplantation tolerance. *Expert Rev Clin Immunol* 2010; 6 (1): 155.

2. Simpson E, Roopenian D. Minor histocompatibility antigens. *Curr Opin Immunol* 1997; 9 (5): 655.

3. Simpson E, Scott D, James E, et al. Minor H antigens: genes and peptides. *Transpl Immunol* 2002; 10 (2-3): 115.

4. Lindahl KF, Wilson DB. Histocompatibility antigen-activated cytotoxic T lymphocytes. II. Estimates of the frequency and specificity of precursors. *J Exp Med* 1977; 145 (3): 508.

5. Suchin EJ, Langmuir PB, Palmer E, Sayegh MH, Wells AD, Turka LA. Quantifying the frequency of alloreactive T cells in vivo: new answers to an old question. *J Immunol* 2001; 166 (2): 973.

6. Jiang S, Herrera O, Lechler RI. New spectrum of allorecognition pathways: implications for graft rejection and transplantation tolerance. *Curr Opin Immunol* 2004; 16 (5): 550.

7. Bolton EM, Bradley JA, Pettigrew GJ. Indirect allorecognition: not simple but effective. *Transplantation* 2008; 85 (5): 667.

8. Lechler RI, Batchelor JR. Restoration of immunogenicity to passenger cell-depleted kidney allografts by the addition of donor strain dendritic cells. *J Exp Med* 1982; 155 (1): 31.

9. Roussey-Kesler G, Brouard S, Ballet C, et al. Exhaustive depletion of graft resident dendritic cells: marginally delayed rejection but strong alteration of graft infiltration. *Transplantation* 2005; 80 (4): 506.

10. Wang Z, Castellaneta A, De Creus A, Shufesky WJ, Morelli AE, Thomson AW. Heart, but not skin, allografts from donors lacking Flt3 ligand exhibit markedly prolonged survival time. *J Immunol* 2004; 172 (10): 5924.

11. Bevan MJ. Cross-priming for a secondary cytotoxic response to minor H antigens with H-2 congenic cells which do not cross-react in the cytotoxic assay. *J Exp Med* 1976; 143 (5): 283.

12. Millrain M, Scott D, Addey C, et al. Identification of the immunodominant HY H2-D(k) epitope and evaluation of the role of direct and indirect antigen presentation in HY responses. *J Immunol* 2005; 175 (11): 7209.

13. Bennett CL, Clausen BE. DC ablation in mice: promises, pitfalls, and challenges. *Trends Immunol* 2007; 28 (12): 525.

14. Bursch LS, Wang L, Igyarto B, et al. Identification of a novel population of Langerin+ dendritic cells. *J Exp Med* 2007; 204 (13): 3147.

15. Nagao K, Ginhoux F, Leitner WW, et al. Murine epidermal Langerhans cells and langerin-expressing dermal dendritic cells are unrelated and exhibit distinct functions. *Proc Natl Acad Sci U S A* 2009; 106 (9): 3312.

16. Jung S, Unutmaz D, Wong P, et al. In vivo depletion of CD11c(+) dendritic cells abrogates priming of CD8(+) T cells by exogenous cell-associated antigens. *Immunity* 2002; 17 (2): 211.

17. McLachlan JB, Catron DM, Moon JJ, Jenkins MK. Dendritic cell antigen presentation drives simultaneous cytokine production by effector and regulatory T cells in inflamed skin. *Immunity* 2009; 30 (2): 277.

18. Baruah P, Simpson E, Dumitriu IE, et al. Mice lacking C1q or C3 show accelerated rejection of minor H disparate skin grafts and resistance to induction of tolerance. *Eur J Immunol*; doi 10.1002/eji.200940158.

19. Bennett CL, van Rijn E, Jung S, et al. Inducible ablation of mouse Langerhans cells diminishes but fails to abrogate contact hypersensitivity. *J Cell Biol* 2005; 169 (4): 569.

20. Poulin LF, Henri S, de Bovis B, Devilard E, Kissenpfennig A, Malissen B. The dermis contains langerin+ dendritic cells that develop and function independently of epidermal Langerhans cells. *J Exp Med* 2007; 204 (13): 3119.

21. Millrain M, Chandler P, Dazzi F, Scott D, Simpson E, Dyson PJ. Examination of HY response: T cell expansion, immunodominance, and cross-priming revealed by HY tetramer analysis. *J Immunol* 2001; 167 (7): 3756.

22. Chai JG, James E, Dewchand H, Simpson E, Scott D. Transplantation tolerance induced by intranasal administration of HY peptides. *Blood* 2004; 103 (10): 3951.

23. Robertson NJ, Chai JG, Millrain M, et al. Natural regulation of immunity to minor histocompatibility antigens. *J Immunol* 2007; 178 (6): 3558.

24. Lakkis FG, Arakelov A, Konieczny BT, Inoue Y. Immunologic 'ignorance' of vascularized organ transplants in the absence of secondary lymphoid tissue. *Nat Med* 2000; 6 (6): 686.

25. McKay D, Shigeoka A, Rubinstein M, Surh C, Sprent J. Simultaneous deletion of MyD88 and Trif delays major histocompatibility and minor antigen mismatch allograft rejection. *Eur J Immunol* 2006; 36 (8): 1994.

26. Auchincloss H, Jr., Lee R, Shea S, Markowitz JS, Grusby MJ, Glimcher LH. The role of "indirect" recognition in initiating rejection of skin grafts from major histocompatibility complex class II-deficient mice. *Proc Natl Acad Sci U S A* 1993; 90 (8): 3373.

27. Fangmann J, Dalchau R, Fabre JW. Rejection of skin allografts by indirect allorecognition of donor class I major histocompatibility complex peptides. *J Exp Med* 1992; 175 (6): 1521.

28. Bennett CL, Noordegraaf M, Martina CA, Clausen BE. Langerhans cells are required for efficient presentation of topically applied hapten to T cells. *J Immunol* 2007; 179 (10): 6830.

29. Kissenpfennig, A, Malissen, B. Langerhans cells – revisiting the paradigm using genetically engineered mice. Trends Immunol 2006; 27 (3): 132.

30. Merad M**,** Hoffmann P**,** Ranheim E, et al. Depletion of host Langerhans cells before transplantation of donor alloreactive T cells prevents skin graft-versus-host disease. *Nat med* 2004; 10 (5): 510.

31. Larsen CP, Steinman RM, Witmer-Pack M, Hankins DF, Morris PJ, Austyn JM. Migration and maturation of Langerhans cells in skin transplants and explants. *J Exp Med* 1990; 172 (5): 1483.

32. Obhrai JS, Oberbarnscheidt M, Zhang N, et al. Langerhans cells are not required for efficient skin graft rejection. *J Invest Dermatol* 2008; 128 (8): 1950.

33. Kaplan DH, Kissenpfennig A, Clausen BE. Insights into Langerhans cell function from Langerhans cell ablation models. *Eur J Immunol* 2008; 38 (9): 2369.

34. Kaplan DH, Jenison MC, Saeland S, Shlomchik WD, Shlomchik MJ. Epidermal langerhans cell-deficient mice develop enhanced contact hypersensitivity. *Immunity* 2005; 23 (6): 611.

35. Li JM, Giver CR, Lu Y, Hossain MS, Akhtari M, Waller EK. Separating graft-versus-leukemia from graft-versus-host disease in allogeneic hematopoietic stem cell transplantation. *Immunotherapy.* 2009; 1(4):599.

36. Apostolou I, von Boehmer H. In vivo instruction of suppressor commitment in naive T cells. *J Exp Med.* (2004) ;199 (10): 1401.

37. Kissenpfennig A, Henri S, Dubois B, et al. Dynamics and function of Langerhans cells in vivo: dermal dendritic cells colonize lymph node areas distinct from slower migrating Langerhans cells. *Immunity* 2005; 22 (5): 643.

38. Billingham RaM, PB. The technique of free skin grafting in mammals. *J Exp Biol* 1951; 28: 385.

Figure legends.

Figure 1. Depletion of DC from donor mice.

Donor male wt, CD11c-DTR and Langerin-DTR mice were injected with DT 24 hours prior to harvesting skin for grafting. Depletion of the relevant DC population was determined by flow cytometry. GFP expression is shown on the X axis and staining for CD11b and CD11c as indicated on the Y axes. A. Left panel shows the splenic GFP^+, $CD11c^+$ cDC population of a representative untreated CD11c-DTR mouse. The right panel shows the epidermal GFP^+, $CD11b^+$ LC population of a representative untreated Langerin-DTR mouse. The boxed regions show the gating strategy applied in B and C for GFP^+ cDC or LC from CD11c-DTR or Langerin-DTR mice respectively. B. The left plot shows the splenic $CD11c^+$ population (cDC) of a representative wt C57BL/6 mouse injected with DT. The middle and right plots show depletion of cDC from the spleens of representative CD11c-DTR and CD11c-DTR x Langerin-DTR mice. C. The left plot shows the skin epidermal $CD11b^+$ population (LC) of a representative wt C57BL/6 mouse injected with DT. The middle and right plots show depletion of LC from the spleens of representative Langerin-DTR and CD11c-DTR x Langerin-DTR mice.

Figure 2. Depletion of either cDC or LC delays rejection of syngeneic male grafts from female mice.

Graft survival of syngeneic male grafts placed on C57BL/6 females. A. Donor male wt, CD11c-DTR and Langerin-DTR mice were injected with DT 24 hours

prior to harvesting skin for grafting. The Kaplan-Meier plot is shown from 1 of 2 independent experiments: wt n=6, CD11c-DTR n=9, Langerin-DTR n=9. Log-rank analysis p = <0.0001. B. Grafts from CD11c-DTR x Langerin-DTR mice that had been depleted of both cDC and Langerin$^+$ DC 24 hours before grafting. Kaplan-Meier plot: wt n=7, CD11c-DTR x Langerin-DTR n=8. Log-rank analysis p = 0.0013.

Figure 3. Reduced expansion of HYD^b *Uty*-specific CD8$^+$ T cells in mice grafted with cDC- or Langerin$^+$ DC-depleted skin.

Expansion of male-specific HYD^b *Uty*-specific CD8$^+$ T cells in the blood of female mice 13-19 days after receiving control skin grafts (+ DT), or grafts depleted of cDC or Langerin$^+$ DC. Data are pooled from 2 independent experiments. Symbols represent individual mice, bars show the mean percentage of CD8$^+$ HY^{Db} *Uty*$^+$ cells. ** p = <0.01, * p = <0.05.

Figure 4. Expansion of antigen-specific CD8 T cells at later time points in mice grafted with DC-depleted skin.

Expansion of male-specific HYD^b *Uty*-specific CD8$^+$ T cells in the blood of female mice 30 days after receiving control skin grafts (+ DT), or grafts depleted of cDC or Langerin$^+$ DC. Data show one representative experiment of 2. Symbols represent individual mice and bars show the mean percentage of CD8$^+$ HY^{Db} *Uty*$^+$ cells. There is no statistically significant difference between groups.

Figure 5. Activation of male-specific T cell responses is delayed in female mice receiving cDC-depleted hematopoietic grafts.

A. Specific lysis of male target cells in the blood of female C57BL/6 mice 10 days after receiving splenocytes from wt or CD11c-DTR B6 males injected with DT 24 hours previously. Histograms are representative of the injection mix, and subsequent lysis of CFSE-labeled male (CFSE low) and female (CFSE high) splenocytes injected into non-depleted and cDC-depleted recipients. The graph shows summary specific lysis data pooled from 2 independent experiments. ** $p = < 0.01$. B. Expansion of male-specific HYD^b *Uty*-specific $CD8^+$ T cells in the blood of female C57BL/6 mice 10 days after receiving splenocytes from wt or CD11c-DTR B6 males injected with DT 24 hours previously. Data are pooled from 2 independent experiments. Bars show the mean percentage of $CD8^+$ HY^{Db} Uty^+ cells ± s.e.m. C. Specific lysis of male target cells in the spleen of female recipients 20 days after injection. Data are representative of 2 independent experiments. Symbols represent individual mice and bars show the mean specific lysis of male target cells.

n.s.= not statistically significant.