



Confounding factors complicate conclusions in *aly* model

To the editor—Secondary lymphoid organs are widely understood to play a pivotal role in coordinating adaptive immune responses, though direct demonstrations are few. Most available evidence use as their model the alymphoplastic (*aly/aly*) mouse which lacks peripheral lymph nodes as well as Peyer's patches¹⁻³. Most recently, Lakkis and colleagues demonstrated that splenectomized *aly/aly* mice are rendered immunologically 'ignorant' of their subsequent cardiac allograft, and *aly/aly* mice with spleens in place permanently accept skin allografts. The authors conclude that secondary lymphoid organs are essential for mediating rejection. Although they are undoubtedly necessary in certain responses, it is unclear to us that secondary lymphoid organs play an essential role here. Apart from the absence of lymph nodes, *aly/aly* mice host a variety of other serious immune defects which might confound the authors' findings, including depressed baseline immunoglobulin production and isotype switching, defective T-cell function and faulty homing responses^{4,5}.

Lymphotoxin- α -deficient (*Lta*^{-/-}) and lymphotoxin- β -receptor-deficient (*Ltbr*^{-/-}) mice are similarly devoid of lymph nodes and Peyer's patches, but share few other defects additionally associated with *aly/aly* mice^{6,7}. Their T-cell responses indeed appear unperturbed^{8,9}. *Lta*^{-/-} mice reject allogeneic skin grafts in near

identical tempo to wild-type controls (Fig. 1a), a finding confirmed in *Ltbr*^{-/-} and splenectomized *Ltbr*^{-/-} recipients (MST:14.2d, *n* = 9). Moreover, splenectomized *Lta*^{-/-} and *Ltbr*^{-/-} recipients remain capable of rejecting BALB/c cardiac allografts, sometimes in as few as 21 days (Fig. 1b). The delayed rejection points to the importance but also the dispensability of secondary lymphoid organs for rejection of cardiac allografts. The finding that *Lta*^{-/-} and *Ltbr*^{-/-} recipients reject heart transplants in the absence of secondary lymphoid organs challenges the wider significance of conclusions Lakkis and colleagues draw from their data, and suggests that concomitant intrinsic defects found uniquely in *aly/aly* mice—apart from lymph-node agenesis—may have contributed significantly to their findings. The reciprocal adoptive transfer controls they performed to address these concerns are complicated by issues of homeostatic expansion and survival.

The findings of Lakkis and colleagues in *aly/aly* mice are no doubt important. However, given the pleiotropic effects of the *aly* mutation and in light of conflicting findings in *Lta*^{-/-} and *Ltbr*^{-/-} mice when compared with *aly/aly* mice, a more cautious interpretation of the data might be prudent. It is likely that the aggregate of diverse immune defects in the *aly/aly* phenotype together, rather than lymph node and Peyer's patch agenesis alone,

may be responsible for the immunological 'ignorance' witnessed in *aly/aly* mice. The salient consideration is that other studies of immune responses studied in *aly/aly* mice might overestimate the involvement of secondary lymphoid organs¹⁻³.

ROBERT CHIN¹, PING ZHOU^{1,2},
MARIA-LUISA ALEGRE^{1,2} &
YANG XIN FU^{1,3}

¹Committee on Immunology

²Department of Medicine and

³Department of Pathology

University of Chicago

Chicago, Illinois, USA

Email: yfu@midway.uchicago.edu

Lakkis replies—Using *Lta*^{-/-} and *Ltbr*^{-/-} mice as models to study the role of secondary lymphoid organs in the alloimmune response, Chin *et al.* present data that seem to challenge our conclusion that the immune response to a vascularized organ graft can not be initiated in the absence of secondary lymphoid organs⁴, which is based on a systematic study of allograft rejection in the alymphoplastic (*aly/aly*) mouse. Although the findings of Chin *et al.* are intriguing, they do not refute our conclusion. We carefully excluded in our study the possibility that failure to mount an alloimmune response in splenectomized *aly/aly* mice resulted from an intrinsic abnormality in *aly/aly* T-lymphocyte activation or homing.

First, we showed that *aly/aly* T cells mediate allograft rejection in T-cell-deficient hosts that have normal secondary lymphoid organs⁴. We adoptively transferred T-cell-enriched blood leukocytes from naive splenectomized *aly/aly* mice to recipients of cardiac allografts deficient in T lymphocytes and T-cell receptor- $\beta\delta$. We then observed acute rejection in each recipient albeit at a slower tempo than rejection precipitated by the transfer of naive, wild-type T cells (median graft survival: 20 and 11 d, respectively). These data clearly indicate that the intrinsic defect in *aly/aly* T cells slows the rejection process by a modest degree but does not abolish it.

Second, we showed that transfer of a large number of naive, wild-type T cells ($4-8 \times 10^7$) to splenectomized *aly/aly* mice two days after heart transplantation

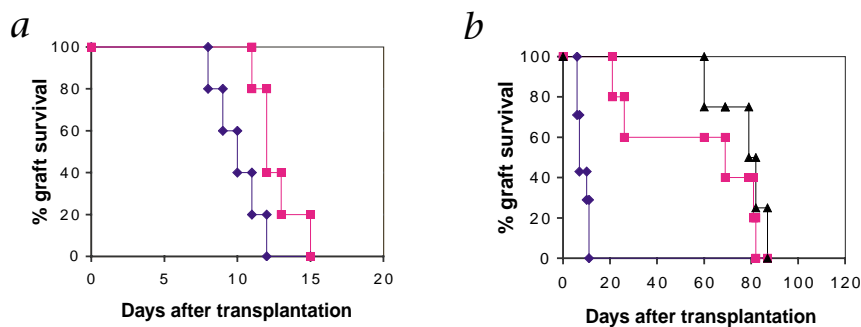


Fig. 1 Limited role of lymph nodes and spleen in allograft rejection. *a*, Lymph nodes are not essential for the rejection of skin allografts. Full-thickness tail skin was transplanted from 5–8-wk-old BALB/c mice to the dorsal flank area of 5–8-wk-old C57BL/6 or B6-*Lta*^{-/-} recipients. Rejection was defined as graft necrosis more than 80%. Time course of rejection of allogeneic (BALB/c) skin grafts is near identical in *Lta*^{-/-} (■; *n* = 5) and wild-type (◆; *n* = 5) mice. Similar results were also obtained when donor abdominal skin was used. *b*, Lymph nodes and spleen are not essential for the rejection of cardiac allografts. Cardiac allograft transplant was performed using BALB/c donors and C57BL/6 (◆; *n* = 7), splenectomized B6-*Lta*^{-/-} (■; *n* = 5) or splenectomized B6-*Ltbr*^{-/-} (▲; *n* = 4) recipients. Allograft survival was assessed by palpation. Rejection was defined as cessation of heart beat. Splenectomized B6-*Lta*^{-/-} and B6-*Ltbr*^{-/-} recipients show delayed, but fully competent rejection.

does not lead to allograft rejection⁴. Therefore, the complete absence of allograft rejection, either by clinical or histological criteria, in splenectomized *aly/aly* mice cannot be attributed to the intrinsic defect (a point mutation in the gene encoding nuclear factor- κ B-inducing kinase¹⁰) present in *aly/aly* mice; rather, it is due to the absence of secondary lymphoid organs.

Chin *et al.* fail to consider the distinct possibility that pre-existing activated/memory T cells, and not primary activated naive T cells, are responsible for cardiac allograft rejection in splenectomized *Lta*^{-/-} or *Ltbr*^{-/-} mice. *Lta*^{-/-} mice, and particularly *Ltbr*^{-/-} mice, have increased numbers of circulating T cells and spontaneously develop massive lymphocytic infiltrates in their peripheral tissues (liver, lungs, pancreas, kidney, fatty tissue and glandular structures)^{6,7}.

Because only activated/memory (CD44^{hi}) but not naive (CD44^{lo}) T cells migrate into non-lymphoid tissues in the absence of inflammation¹¹, it is likely that CD44^{hi} T cells accumulate to abnormally high levels in *Lta*^{-/-} and *Ltbr*^{-/-} mice. Coupled with the knowledge that activated/memory CD44^{hi} T cells exert effector functions^{11,12} and can reject an

organ transplant⁴ (G. Chalasani *et al.*, unpublished data) independent of secondary lymphoid tissues, it is likely that pre-existing activated/memory CD44^{hi} T cells, and not *de novo* activated naive T cells, mediated acute rejection in the models used by Chin *et al.* Therefore, their data do not refute our conclusion that the initiation of the alloimmune response is dependent on secondary lymphoid organs.

The importance of the findings by Chin *et al.* lies in pointing out the subtleties of interpreting data in gene-knockout and mutant mice that lack secondary lymphoid tissues rather than in proving or refuting whether secondary lymphoid organs are essential for initiating the immune response to a foreign antigen.

FADI G. LAKKIS
 Yale University School of Medicine
 Sections of Nephrology and Immunobiology
 New Haven, Connecticut, USA
 Email: fadi.lakkis@yale.edu

1. Miyawaki, S. *et al.* A new mutation, *aly*, that induces a generalized lack of lymph nodes accompanied by immunodeficiency in mice. *Eur. J. Immunol.* **24**, 429–434 (1994).
2. Macpherson, A.J. *et al.* Primitive T cell-independent mechanism of intestinal mucosal IgA responses to

- commensal bacteria. *Science* **288**, 2222–2226 (2000).
3. Karrer, U. *et al.* On the key role of secondary lymphoid organs in antiviral immune responses studied in alymphoplastic (*aly/aly*) and spleenless (*Hox11^{-/-}*) mutant mice. *J. Exp. Med.* **185**, 2157–2170 (1997).
4. Lakkis, F.G. *et al.* Immunologic 'ignorance' of vascularized organ transplants in the absence of secondary lymphoid tissue. *Nature Med.* **6**, 686–688 (2000).
5. Fagarasan, S. *et al.* Alymphoplasia (*aly*)-type nuclear factor κ B-inducing kinase (NIK) causes defects in secondary lymphoid tissue chemokine receptor signaling and homing of peritoneal cells to the gut-associated lymphatic tissue system. *J. Exp. Med.* **9**, 1477–1486 (2000).
6. Banks, T.A. *et al.* Lymphotoxin- α -deficient mice. Effects on secondary lymphoid organ development and humoral immune responsiveness. *J. Immunol.* **155**, 1685–1693 (1995).
7. Futterer, A. *et al.* The lymphotoxin β receptor controls organogenesis and affinity maturation in peripheral lymphoid tissues. *Immunity* **9**, 59–70 (1998).
8. Fu, Y.X. *et al.* Lymphotoxin- α -dependent spleen microenvironment supports the generation of memory B cells and is required for their subsequent antigen-induced activation. *J. Immunol.* **164**, 2508–2514 (2000).
9. Fu, Y.X. *et al.* Development and maturation of secondary lymphoid tissues. *Annu. Rev. Immunol.* **17**, 399–433 (1999).
10. Shinkura, R. *et al.* Alymphoplasia is caused by a point mutation in the mouse gene encoding NF- κ B-inducing kinase. *Nature Genet.* **22**, 74–77 (1999).
11. Reinhardt, R.L., Khoruts, A., Merica, R., Zell, T. & Jenkins, M.K. Visualizing the generation of memory CD4 T cells in the whole body. *Nature* **410**, 101–105 (2001).
12. Masopust, D., Vezys, V., Marzo, A.L. & Lefrancois, L. Preferential localization of effector memory cells in nonlymphoid tissue. *Science* **291**, 2413–2417 (2001).

LETTERS TO THE EDITOR

We want to hear from you! *Nature Medicine* is the forum for the latest, best, and most original biomedical research, news, and opinion. As such, we welcome letters from readers wishing to address topics reported on in previous issues, or subjects of interest to the biomedical research community at large. Letters should be brief and concise (no more than 500 words), and sent to *Nature Medicine*, 345 Park Avenue South, New York NY 10010, USA, or sent by fax (212.683.5751) or email to medicine@natureny.com.