

Natural killer T cells and X-linked lymphoproliferative syndrome

Sylvain Latour

Purpose of review

Recent progress in elucidating the physiopathology of X-linked lymphoproliferative syndrome (XLP) has raised novel and important issues regarding the biology of natural killer T cells. Here I will review this information and discuss the issues involved.

Recent findings

XLP is a rare inherited immunodeficiency characterized by a high susceptibility to severe infection by the Epstein–Barr virus. Mutations in the gene *SH2D1A* (or alternatively *SAP*) underlie 80% of familial XLP (XLP-1) cases. Recently the remaining 20% of familial XLP (XLP-2) cases were shown to harbor mutations in the gene *XIAP* (X-linked inhibitor of apoptosis protein). Both *SAP* and *XIAP* deficiencies are associated with a defect in the development and/or homeostasis of natural killer T cells.

Summary

It can be hypothesized that the susceptibility to Epstein–Barr virus in XLP might result from the defect of natural killer T cells. The role of these cells in viral infection is unclear, but several herpes viruses have developed strategies to escape natural killer T cells. The discovery that *SAP* and *XIAP* deficiency leads to a defect in natural killer T cells has also shed light on novel signaling pathways required for natural killer T cell development and/or homeostasis.

Keywords

Epstein–Barr virus, natural killer T lymphocytes, *SAP*, *XIAP*, X-linked lymphoproliferative syndrome

Abbreviations

AICD	activation-induced cell death
APC	antigen-presenting cell
DP thymocyte	double positive thymocyte
EBV	Epstein–Barr virus
HLH	hemophagocytic lymphohistiocytosis
HSV	herpes simplex virus
IAP	Inhibitor-of-apoptosis family protein
NF-κB	nuclear factor κB
NKT	natural killer T
NOD mice	nonobese diabetic mice
PKCθ	protein kinase Cθ
SAP	signalling lymphocyte activation molecule-associated protein
SLAM	signalling lymphocyte activation molecule
SLAM-R	signalling lymphocyte activation molecule receptor
TCR	T-cell receptor
XIAP	X-linked inhibitor of apoptosis protein
XLP	X-linked lymphoproliferative syndrome

© 2007 Wolters Kluwer Health | Lippincott Williams & Wilkins
1528-4050

Introduction

Natural killer T (NKT) lymphocytes represent a specialized T-lymphocyte subpopulation with unique features distinct from conventional T cells [1*,2*]. NKT cells expressed an invariant (in human) or semi-invariant (in mice) T-cell receptor (TCR) that recognized self and microbacterial glycosphingolipid antigens presented by the major histocompatibility complex (MHC) class I-like molecule CD1d. The development of NKT cells is also distinct from conventional T cells, as NKT cell precursors are positively selected by CD4⁺CD8⁺ cortical thymocytes (called DP thymocytes, for double positive) and the signaling pathways involved differ from those of conventional T lymphocytes. Functionally, when activated, NKT cells very rapidly produce large amounts of T_H1 and T_H2 cytokines that transactivate other immune cells. These properties account for their capacity to participate to both innate and adaptative immune responses. Although NKT cells have been implicated in a broad variety of pathological conditions, the significance of their role is still partly unclear, as discussed below.

X-Linked lymphoproliferative syndrome

X-Linked lymphoproliferative syndrome (XLP) or Purtilo syndrome is a rare inherited immunodeficiency that belongs to the family of genetic diseases causing hemophagocytic lymphohistiocytosis (HLH) [3]. XLP is characterized by a high susceptibility to Epstein–Barr virus (EBV) infection that triggers a severe infectious mononucleosis with the features of an HLH in 60% of cases [4,5*,6]. In contrast to the orchestrated immune

Curr Opin Allergy Clin Immunol 7:510–514.

© 2007 Wolters Kluwer Health | Lippincott Williams & Wilkins.

INSERM U768, Hôpital Necker-Enfants Malades, Paris, France

Correspondence to Sylvain Latour, INSERM U768, Hôpital Necker-Enfants Malades, 149 rue de Sèvres, 75015 Paris, France
Tel: +33 1 44 49 50 51; e-mail: latour@necker.fr

Current Opinion in Allergy and Clinical Immunology 2007, 7:510–514

response to EBV seen in normal individuals, affected boys exhibit an uncontrolled T_H1-type immune response with an excessive expansion of polyclonal activated T cells, EBV-infected B cells and activated macrophages. Patients who do not develop HLH could present other aggressive lymphoproliferative and inflammatory manifestations such as lymphoma, lymphoid vasculitis and colitis. The other hallmark of XLP is dysgammaglobulinemia, found in 30% of patients. On a molecular basis, XLP is divided in two distinct diseases, XLP-1 and XLP-2, which represent 80 and 20% of cases of familial XLP respectively. XLP-1 is caused by mutations in the gene *SH2D1A*, or *SAP* [SLAM (signalling lymphocyte activation molecule)-associated protein] whereas mutations in the gene *XIAP* (X-linked inhibitor of apoptosis protein) underlie XLP-2 [5[•]]. Intriguingly, the loci of these two genes are located at nearby positions on chromosome Xq25, suggesting a possible functional link between the genes. XLP-1 and XLP-2 have very similar clinical manifestations, although until now lymphomas have been reported in XLP-1 patients but not in XLP-2 patients. Also, splenomegaly is noticed in XLP-2 patients before they encounter EBV in contrast to XLP-1 patients.

SAP deficiency

The gene *SAP* encodes a small signaling adapter protein that is expressed in T, natural killer (NK) and NKT cells. SAP is composed of a single Src-homology 2 (SH2) domain that binds to tyrosine-based motifs in the intracytoplasmic domain of the SLAM family immunoreceptors, including CD84, Ly-9, CD84, NTB-A, 2B4 and CRACC [4]. The SLAM receptors (SLAM-Rs) are broadly expressed on hematopoietic cells and interact with ligands via homotypic associations with the exception of 2B4. SAP is required for the functions of SLAM receptors by its capacity to recruit the Src-related protein tyrosine kinase Fyn, allowing SLAM receptors to transduce tyrosine phosphorylation signals [7,8]. In T cells, the SLAM–SAP–Fyn signaling module regulates cytokine production by activating at least two pathways: one involves the SH2-containing inositol phosphatase SHIP, the adaptors Dok1, Dok2 and Shc, and the Ras-GTPase-activating protein RasGAP [9], and the other one consists of the protein kinase C θ (PKC θ), Bcl10 and nuclear factor κ B (NF- κ B) [10]. In NK cells, a 2B4–SAP–Fyn module triggers a pathway involving SHIP, Vav-2, c-Cbl and phospholipase C γ 1 that activates cell cytotoxicity [11]. Recently, it was shown that SAP has also the capacity to promote the recruitment of the PAK-interacting exchange factors (PIX), a family of guanine-exchange factors for Rac/Cdc42 small GTPases [12[•]]. This pathway may be required for full activation of nuclear factor of activated T–cells (NFAT) in activated T cells.

To date, it is proposed that dysfunctions of SLAM-Rs in the absence of SAP may underlie the pathophysiology of

XLP and explain most of the clinical manifestations found in XLP [4]. In particular, the appearance of HLH is thought to be caused by impaired 2B4 and NTB-A functions in cytotoxic T and NK cells [3]. However, the susceptibility to EBV infection, which is the triggering event of the disease, is still not clearly understood.

SAP signaling pathways in natural killer T cell development

The observations that SAP-deficient mice and humans are devoid of NKT cells were of a considerable interest because they shed light on unique signaling pathways involved in the NKT cell lineage commitment and development [13–15]. Data obtained with SAP-deficient mice indicate that SAP is strictly required for the positive selection step of NKT cell precursors in the thymus [13]. A similar block of NKT cell maturation is also observed in Fyn-deficient mice [16,17]. As the function of SAP is to couple SLAM-Rs to the protein tyrosine kinase Fyn, one or several SLAM receptors are very likely to be involved in the ontogenesis of NKT cells. The homotypic nature of the SLAM-R ligands/interactions is in favor of specific roles of these receptors during NKT cell development which requires homotypic cellular contacts between cortical DP thymocytes rather than heterotypic interactions with thymic epithelial cells [2[•]]. Different SLAM-R-deficient mice (SLAM, 2B4, Ly-108 and Ly-9) are available, but until now only Ly-9-deficient mice have been analyzed for NKT cells and no obvious deficiency was reported [18]. Recently, a more direct attestation for the importance of SLAM-R in NKT cell development was given by the report that the *slam* locus is a potential candidate for the defect in NKT cell development found in nonobese diabetic (NOD) mice [19[•]]. NOD mice exhibit very low amounts of SLAM in DP thymocytes as well as a two-fold decrease in the expression of Ly-108.

Current studies have established that NF- κ B factors are strictly required for NKT cell development in striking contrast to conventional T cells for which these are only necessary for T-cell activation in the periphery [1[•],2[•]]. Moreover, signals leading to NF- κ B activation in developing NKT cell precursors seem to be less dependent on the TCR and/or different from those in conventional T cells. Indeed, Carma1- and Malt1-deficient mice exhibit normal NKT cell numbers whereas PKC θ - and Bcl10-deficient mice have impaired NKT cell development [20,21]. Thus, the report that the SLAM–SAP–Fyn signaling module activates NF- κ B in conventional T cells through a PKC θ -dependent pathway prompted speculation that SLAM receptors may provide signals for NF- κ B activation required for NKT cell development. The exact nature of the SLAM-R–SAP–Fyn modules involved and the signaling pathways that they trigger during NKT cell differentiation remain to be established.

XIAP deficiency

XIAP encodes an antiapoptotic molecule that belongs to the Inhibitor-of-Apoptosis family proteins (IAPs), which are composed of three BIR protein–protein interaction domains and a C-terminal RING domain with an E3 ubiquitin ligase activity. XIAP is broadly expressed in cells from the hematopoietic system including lymphocytes, myeloid cells and NK cells. The main function ascribed to XIAP is the suppression of apoptosis through its direct interaction with caspases via its BIR domains. XIAP inhibits caspases 3, 7 and 9 in response to various apoptotic stimuli [22–24]. Based on its antiapoptotic function, studies using XIAP inhibitors have indicated that XIAP is a potent target for the treatment of cancer [25]. Therefore, the absence of XIAP may protect patients from cancer, explaining why XIAP-deficient patients do not develop lymphomas in contrast to SAP-deficient patients. However, XIAP is also involved in transforming growth factor- β receptor and Notch signaling as well as pathways that participate in c-Jun N-terminal kinase (JNK) and NF- κ B activation, although the functional implications of these interactions are not clear [23,24,26].

As expected, lymphocytes from XIAP-deficient patients are more prone to CD95- and TRAIL-R (tumor-necrosis-factor-related apoptosis-inducing ligand receptor)-mediated apoptosis [5 $\bullet$]. The excess of lymphocyte apoptosis in XIAP-deficient patients might account for the abnormal immune response to EBV. It is difficult to understand, however, how a defect in a molecule mainly involved in antiapoptotic pathways leads to lymphoproliferative disorders such as HLH and splenomegaly. Signaling pathways other than apoptosis may be affected by the lack of XIAP and might explain the appearance of the hemophagocytic syndrome and splenomegaly. XIAP-deficient mice have been reported with no obvious phenotype, although these mice have not been triggered with a virus [27].

XIAP in homeostasis of natural killer T cells

XIAP-deficient patients do not show T-, B- or NK-cell lymphopenia with the exception of NKT cells, numbers of which are markedly reduced. This suggests that NKT cells are more dependent on XIAP for their survival and/or development in contrast to other leukocytes. However, XIAP may have a function in other types of immune cell, as nonrandom X-chromosome inactivation has been observed not only in NKT cells but also in T, B and NK cells of XIAP-mutated female carriers [5 $\bullet$]. The defect in NKT cells in XIAP deficiency is very likely to differ from that in SAP deficiency as few NKT cells are clearly detected in the blood of XIAP-deficient patients and XIAP-deficient mice have normal NKT cell numbers. XIAP might be involved in the peripheral homeostasis and/or in the late stages of NKT cell development.

The homeostatic requirements of NKT cells resemble those of memory CD8 $^{+}$ T cells as NKT cell survival and/or expansion is not dependent on CD1d expression and requires IL-15 [1 $\bullet$,2 $\bullet$]. After TCR-mediated activation, NKT cells rapidly become undetectable due to a rapid down-regulation of surface TCR and not to a massive activation-induced cell death (AICD), as postulated in earlier studies [28,29]. Instead, NKT cells were found to be more resistant to AICD compared to conventional T cells [30]. This feature may be related to the autoreactive nature of NKT cells protecting them to recurrent AICD caused by stimulation by self glycosphingolipids. In the absence of XIAP, NKT cells might progressively disappear by lack of resistance to AICD. Furthermore, this process might be amplified during EBV infection. Whether the NKT cell defect seen in XIAP-deficient patients could in part be a consequence of the infection by EBV remains to be determined.

XIAP-deficient mice have normal numbers of NKT cells and did not show abnormal responses to apoptotic stimuli [5 $\bullet$,27]. One obvious explanation is that the function of XIAP differs between mice and humans. Alternatively, the two XIAP-related proteins, IAP-1 and IAP-2, may compensate for XIAP deficiency in mice but not in humans. However, apoptosis of NKT cells has not been analyzed in these mice. Also, maintenance of mice in free-pathogen conditions might explain the normal numbers of NKT cells found in these mice in contrast to XIAP-deficient humans. Examination of NKT cells following infection with pathogens known to activate NKT cells should help to clarify these issues.

Natural killer T cells in antiviral responses

Recent studies in mice have provided compelling evidence that NKT cells are activated during bacterial infections and participate in the bacterial clearance. Given the importance of this role, it is intriguing that XLP patients do not develop susceptibility to bacterial infections. The role of NKT cells in the control of viral infections is less clear and remains controversial. In mouse models deficient for NKT cells, the outcome of infections with numerous viruses including the murine cytomegalovirus LCMV, Coxsackie virus, encephalomyocarditis virus, influenza A virus and respiratory syncytial virus is not modified, or revealed only a modest effect on the clearance of the virus [31–36]. In spite of the lack of a direct involvement of NKT cells in the control of these viral infections, therapeutic activation of NKT cells by α GalCer – a strong glycosphingolipid agonist derived from marine sponges [2 $\bullet$] – results in enhanced antiviral responses and/or improves the issue of the disease in a number of these models [31–33]. So far, the clearest evidence in support for a role of NKT cells in antiviral responses is given by the recent studies with human herpesviruses. Herpes simplex virus (HSV)-1 and

HSV-2 infections result in a much more severe disease in CD1^{-/-} NKT-deficient mice, with impaired virus clearance [37,38]. Also, supporting the importance of NKT cells in controlling herpesvirus infections, HSV-1 and Kaposi sarcoma-associated herpesvirus have developed immune-evasion strategies that target NKT cells and/or CD1d. HSV-1 strongly reduces CD1d expression on antigen-presenting cells (APCs) by disrupting the CD1d endosomal and/or lysosomal trafficking pathway that is required for loading glycosphingolipids on CD1d [39]. This results in impaired NKT cell activation by APCs. The genome of Kaposi sarcoma-associated herpesvirus encodes two modulator of immune recognition (MIR) proteins that mediate ubiquitylation of a lysine residue in the CD1d intracytoplasmic tail which triggers CD1d endocytosis resulting in inhibition of CD1d expression [40]. In addition, the findings of NKT cell defects in humans with XLP strengthen the hypothesis that NKT cells in humans may have an essential role in antiviral immunity. This is also supported by the report of a patient with disseminated varicella-zoster virus infection to whom a deficiency in NKT cells was detected without any other apparent defects [41].

Several mechanisms underlying the activation of NKT cells during bacterial infections in mice have been identified. Glycosphingolipids found in the cell wall of Gram-negative lipopolysaccharide-negative bacteria such as *Sphingomonas* and *Ehrlichia* directly activate NKT cells when presented by dendritic cells [42,43]. In the case of Gram-negative lipopolysaccharide-positive bacteria such as *Salmonella*, NKT cell responses are triggered via an autoreactive process that involve endogenous/self glycosphingolipids such as isoglobotrihexosylceramide (iGb3) [43,44]. This response required TLR-signaling and IL-12 production by APCs that amplify the stimulation of NKT cells [2,44]. There is no evidence for virally encoded CD1d ligands, so it is very likely that virus-infected cells also activate NKT cells via an autoreactive mechanism involving self glycosphingolipids. These self glycosphingolipids could be induced by a stress-activated pathway following viral infection. The nature of endogenous glycosphingolipids is probably diverse and remains to be determined, as indicated by the recent studies of Speak *et al.* [45] and Porubsky *et al.* [46]. However, herpes viruses are known to disrupt endosomal/lysosomal trafficking, leading endosomal proteins to be redistributed to the plasma membrane of infected cells. Based on this feature, it is proposed that herpes viruses induce CD1d loaded with self glycosphingolipids to relocate from endosomes to the plasma membrane [47]. This increased CD1d expression on the cell surface might signal the presence of viruses, allowing activation of NKT cells. The balance between virus recognition and virus immune evasion could depend of the viral load, as it was suggested for HSV-1 infection that low titers of virus

increased CD1d expression on APCs while high titers inhibited the expression [48].

On the basis of their innate function, the function of NKT cells during EBV infection might take place at the first steps of the infection to control growth-transforming infection of mucosal B lymphocytes [49]. Besides a role in the early stages of the infection, it should also be considered that NKT cells might participate to the contraction of the pool of activated T cells that normally occur once the triggering pathogen is eliminated. This homeostatic process depends on the cytolytic activity of T cells and it is impaired in patients with XLP [3]. This is suggested by the observations that CD4⁺CD8 $\alpha\alpha$ NKT cells suppress the proliferation and the expansion of activated T cells via CD1d-dependent killing of T cells and APCs [50].

Conclusion

SAP and XIAP deficiencies represent unique models to better understand the function of NKT cells in humans. The physiopathology of XLP favors a significant role of NKT cells in immunity against EBV rather in antibacterial immunity. Further investigations are now needed to determine the exact role of NKT cells in EBV infection: do EBV-infected cells activate NKT cells and what are the ligands and mechanisms involved? Also, the signaling pathways in NKT cells that depend on SAP and XIAP still require molecular and functional characterization.

Acknowledgements

The members of the INSERM 768 laboratory who have been involved in the work discussed in this review are gratefully acknowledged: S. Rigaud, B. Pasquier, M.-C. Fondaneche, N. Lambert, V. Mateo, C. Lenoir, E. Lesteven, S. Siberil, G. de Saint Basile, P. Revy and A. Fischer. S.L. is a Scientist of the Centre National de la Recherche Scientifique (France).

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 588–589).

- 1 Godfrey DI, Berzins SP. Control points in NKT-cell development. *Nat Rev Immunol* 2007; 7:505–518.
Along with [2], this review provides a recent overview of the biology of NKT cells.
- 2 Bendelac A, Savage PB, Teyton L. The biology of NKT cells. *Annu Rev Immunol* 2007; 25:297–336.
Along with [1], this review provides a recent overview of the biology of NKT cells.
- 3 Fischer A, Latour S, de Saint Basile G. Genetic defects affecting lymphocyte cytotoxicity. *Curr Opin Immunol* 2007; 19:348–353.
- 4 Ma CS, Nichols KE, Tangye SG. Regulation of cellular and humoral immune responses by the SLAM and SAP families of molecules. *Annu Rev Immunol* 2007; 25:337–379.
- 5 Rigaud S, Fondaneche M-C, Lambert N, *et al.* XIAP deficiency in humans causes an X-linked lymphoproliferative syndrome. *Nature* 2006; 444:110–114.
Description of the XIAP deficiency: patients with familial XLP without mutations in SAP harbor mutations in the antiapoptotic gene XIAP and have low number of NKT cells similarly to SAP-deficient patients.

- 6 Gaspar HB, Sharifi R, Gilmour KC, Thrasher AJ. X-linked lymphoproliferative disease: clinical, diagnostic and molecular perspective. *Br J Haematol* 2002; 119:585–595.
 - 7 Latour S, Roncagalli R, Chen R, *et al.* Binding of SAP SH2 domain to FynT SH3 domain reveals a novel mechanism of receptor signalling in immune regulation. *Nat Cell Biol* 2003; 5:149–154.
 - 8 Chan B, Lanyi A, Song HK, *et al.* SAP couples Fyn to SLAM immune receptors. *Nat Cell Biol* 2003; 5:155–160.
 - 9 Latour S, Gish G, Helgason CD, *et al.* Regulation of SLAM-mediated signal transduction by SAP, the X-linked lymphoproliferative gene product. *Nat Immunol* 2001; 2:681–690.
 - 10 Cannons JL, Yu LJ, Hill B, *et al.* SAP regulates T(H)2 differentiation and PKC-theta-mediated activation of NF-kappaB1. *Immunity* 2004; 21:693–706.
 - 11 Chen R, Relouzat F, Roncagalli R, *et al.* Molecular dissection of 2B4 signaling: implications for signal transduction by SLAM-related receptors. *Mol Cell Biol* 2004; 24:5144–5156.
 - 12 Gu C, Tangye SG, Sun X, *et al.* The X-linked lymphoproliferative disease gene product SAP associates with PAK-interacting exchange factor and participates in T cell activation. *Proc Natl Acad Sci USA* 2006; 103:14447–14452.
- This article describes a novel SAP-dependent pathway in T cells that is involved in activation of nuclear factor of activated T-cells (NFAT).
- 13 Pasquier B, Yin L, Fondaneche MC, *et al.* Defective NKT cell development in mice and humans lacking the adapter SAP, the X-linked lymphoproliferative syndrome gene product. *J Exp Med* 2005; 201:695–701.
 - 14 Nichols KE, Hom J, Gong SY, *et al.* Regulation of NKT cell development by SAP, the protein defective in XLP. *Nat Med* 2005; 11:340–345.
 - 15 Chung B, Aoukaty A, Dutz J, *et al.* Cutting edge: signaling lymphocytic activation molecule-associated protein controls NKT cell functions. *J Immunol* 2005; 174:3153–3157.
 - 16 Gadue P, Morton N, Stein PL. The Src family tyrosine kinase Fyn regulates natural killer T cell development. *J Exp Med* 1999; 190:1189–1196.
 - 17 Eberl G, Lowin-Kropf B, MacDonald HR. Cutting edge: NKT cell development is selectively impaired in Fyn- deficient mice. *J Immunol* 1999; 163:4091–4094.
 - 18 Graham DB, Bell MP, McCausland MM, *et al.* Ly9 (CD229)-deficient mice exhibit T cell defects yet do not share several phenotypic characteristics associated with SLAM- and SAP-deficient mice. *J Immunol* 2006; 176:291–300.
 - 19 Jordan MA, Fletcher JM, Pellicci D, Baxter AG. Slamf1, the NKT cell control gene Nkt1. *J Immunol* 2007; 178:1618–1627.
- This article identified *Slamf1* (SLAM) and *Slamf6* (Ly-108) as potential gene candidates for the NKT cell defects in NOD mice. They showed that SLAM expression is strongly reduced in the DP thymocytes of NOD mice.
- 20 Schmidt-Suppran M, Tian J, Grant EP, *et al.* Differential dependence of CD4+CD25+ regulatory and natural killer-like T cells on signals leading to NF-kappaB activation. *Proc Natl Acad Sci USA* 2004; 101:4566–4571.
 - 21 Stanic AK, Bezbradica JS, Park JJ, *et al.* Cutting edge: the ontogeny and function of Va14Ja18 natural T lymphocytes require signal processing by protein kinase C theta and NF-kappa B. *J Immunol* 2004; 172:4667–4671.
 - 22 Eckelman BP, Salvesen GS, Scott FL. Human inhibitor of apoptosis proteins: why XIAP is the black sheep of the family. *EMBO Rep* 2006; 7:988–994.
 - 23 Salvesen GS, Duckett CS. IAP proteins: blocking the road to death's door. *Nat Rev Mol Cell Biol* 2002; 3:401–410.
 - 24 Vaux DL, Silke J. IAPs, RINGs and ubiquitylation. *Nat Rev Mol Cell Biol* 2005; 6:287–297.
 - 25 Schimmer AD, Dalili S, Batey RA, Riedl SJ. Targeting XIAP for the treatment of malignancy. *Cell Death Differ* 2006; 13:179–188.
 - 26 Liu WH, Hsiao HW, Tsou WI, Lai MZ. Notch inhibits apoptosis by direct interference with XIAP ubiquitination and degradation. *EMBO J* 2007; 26:1660–1669.
 - 27 Harlin H, Reffey SB, Duckett CS, *et al.* Characterization of XIAP-deficient mice. *Mol Cell Biol* 2001; 21:3604–3608.
 - 28 Crowe NY, Uldrich AP, Kyparissoudis K, *et al.* Glycolipid antigen drives rapid expansion and sustained cytokine production by NK T cells. *J Immunol* 2003; 171:4020–4027.
 - 29 Wilson MT, Johansson C, Olivares-Villagomez D, *et al.* The response of natural killer T cells to glycolipid antigens is characterized by surface receptor down-modulation and expansion. *Proc Natl Acad Sci USA* 2003; 100:10913–10918.
 - 30 Harada M, Seino K, Wakao H, *et al.* Down-regulation of the invariant Valpha14 antigen receptor in NKT cells upon activation. *Int Immunol* 2004; 16:241–247.
 - 31 Johnson TR, Hong S, Van Kaer L, *et al.* NK T cells contribute to expansion of CD8(+) T cells and amplification of antiviral immune responses to respiratory syncytial virus. *J Virol* 2002; 76:4294–4303.
 - 32 Exley MA, Bigley NJ, Cheng O, *et al.* CD1d-reactive T-cell activation leads to amelioration of disease caused by diabetogenic encephalomyocarditis virus. *J Leukoc Biol* 2001; 69:713–718.
 - 33 van Dommelen SL, Tabarias HA, Smyth MJ, Degli-Esposti MA. Activation of natural killer (NK) T cells during murine cytomegalovirus infection enhances the antiviral response mediated by NK cells. *J Virol* 2003; 77:1877–1884.
 - 34 Benton KA, Misplon JA, Lo CY, *et al.* Heterosubtypic immunity to influenza A virus in mice lacking IgA, all Ig, NKT cells, or gamma delta T cells. *J Immunol* 2001; 166:7437–7445.
 - 35 Huber S, Sartini D, Exley M. Role of CD1d in coxsackievirus B3-induced myocarditis. *J Immunol* 2003; 170:3147–3153.
 - 36 Roberts TJ, Lin Y, Spence PM, *et al.* CD1d1-dependent control of the magnitude of an acute antiviral immune response. *J Immunol* 2004; 172:3454–3461.
 - 37 Grubor-Bauk B, Simmons A, Mayrhofer G, Speck PG. Impaired clearance of herpes simplex virus type 1 from mice lacking CD1d or NKT cells expressing the semivariant V alpha 14-J alpha 281 TCR. *J Immunol* 2003; 170:1430–1434.
 - 38 Gill N, Rosenthal KL, Ashkar AA. NK and NKT cell-independent contribution of interleukin-15 to innate protection against mucosal viral infection. *J Virol* 2005; 79:4470–4478.
 - 39 Yuan W, Dasgupta A, Cresswell P. Herpes simplex virus evades natural killer T cell recognition by suppressing CD1d recycling. *Nat Immunol* 2006; 7:835–842.
- This paper, with [48*], demonstrated that infection with HSV-1 reduced expression of CD1d on APCs by interfering with the trafficking of endocytosed CD1d from the lysosome to the plasma membrane. This down-regulation of CD1d inhibited NKT cell activation.
- 40 Sanchez DJ, Gumperz JE, Ganem D. Regulation of CD1d expression and function by a herpesvirus infection. *J Clin Invest* 2005; 115:1369–1378.
 - 41 Levy O, Orange JS, Hibberd P, *et al.* Disseminated varicella infection due to the vaccine strain of varicella-zoster virus, in a patient with a novel deficiency in natural killer T cells. *J Infect Dis* 2003; 188:948–953.
 - 42 Kinjo Y, Wu D, Kim G, *et al.* Recognition of bacterial glycosphingolipids by natural killer T cells. *Nature* 2005; 434:520–525.
 - 43 Mattner J, DeBord KL, Ismail N, *et al.* Exogenous and endogenous glycolipid antigens activate NKT cells during microbial infections. *Nature* 2005; 434:525–529.
 - 44 Brigl M, Bry L, Kent SC, *et al.* Mechanism of CD1d-restricted natural killer T cell activation during microbial infection. *Nat Immunol* 2003; 4:1230–1237.
 - 45 Speak AO, Salio M, Neville DC, *et al.* Implications for invariant natural killer T cell ligands due to the restricted presence of isoglobotrihexosylceramide in mammals. *Proc Natl Acad Sci USA* 2007; 104:5971–5976.
- This paper shows that the isoglobotrihexosylceramide (iGb3), which has been proposed to be the selecting ligand involved in NKT cell development in the thymus, is not detected in the mice or human thymus or dendritic cells, suggesting that it is not a physiologically relevant ligand for NKT cells. This is also supported by the data of [46*].
- 46 Porubsky S, Speak AO, Luckow B, *et al.* Normal development and function of invariant natural killer T cells in mice with isoglobotrihexosylceramide (iGb3) deficiency. *Proc Natl Acad Sci USA* 2007; 104:5977–5982.
- This paper outlines how the development and function of NKT cells are normal in mice with isoglobotrihexosylceramide (iGb3) deficiency.
- 47 Hegde NR, Johnson DC. A seek-and-hide game between Cd1-restricted T cells and herpesviruses. *J Clin Invest* 2005; 115:1146–1149.
 - 48 Raftery MJ, Winau F, Kaufmann SH, *et al.* CD1 antigen presentation by human dendritic cells as a target for herpes simplex virus immune evasion. *J Immunol* 2006; 177:6207–6214.
- This article showed that infection with high titers of HSV-1 reduced the expression of CD1d on APCs. This down-regulation of CD1d results in an inhibition of NKT cell activation. By contrast, low titers of HSV-1 increased CD1d expression on APCs.
- 49 Hislop AD, Taylor GS, Sauce D, Rickinson AB. Cellular responses to viral infection in humans: lessons from Epstein-Barr virus. *Annu Rev Immunol* 2007; 25:587–617.
 - 50 Ho LP, Urban BC, Jones L, *et al.* CD4(–)CD8αα subset of CD1d-restricted NKT cells controls T cell expansion. *J Immunol* 2004; 172:7350–7358.