Defective NKT cell development in mice and humans lacking the adapter SAP, the X-linked lymphoproliferative syndrome gene product

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SAP is an adaptor protein expressed in T cells and natural killer cells. It plays a critical role in immunity, as it is mutated in humans with X-linked lymphoproliferative syndrome (XLP), a fatal immunodeficiency characterized by an abnormal response to Epstein-Barr virus (EBV) infection. SAP interacts with the SLAM family receptors and promotes transduction signal events by these receptors through its capacity to recruit and activate the Src kinase FynT. Because it has been previously established that FynT is selectively required for the development of NKT cells, we examined NKT cells in SAP-deficient mice and in humans with XLP. In the absence of SAP, the development of NKT cells is severely impaired both in mice and in humans. These results imply that SAP is a potent regulator of NKT cell development. They also identify for the first time a defect in NKT cells associated with a human primary immunodeficiency, revealing a potential role of NKT cells in the immune response to EBV.

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SAP (also named SH2D1A) is a small adaptor protein that is composed of a unique SH2 domain with a short COOH-terminal extension. It is expressed in T cells and NK cells where it has been found to be essential for the functions of the SLAM family of immune receptors (1, 2). Through its SH2 domain, SAP associates with tyrosine-based motifs located in the cytoplasmic domain of the SLAM family receptors. SAP couples these receptors to intracellular signaling pathways by its ability to interact simultaneously with the Src-related protein tyrosine kinase FynT (3). This interaction between SAP and FynT is direct and involves a second binding surface in the SH2 domain of SAP and the SH3 domain of FynT (4). The importance of SAP in immunity was provided by the finding that the *SAP/SH2D1A* gene is mutated or deleted in humans with X-linked lymphoproliferative disease (XLP), an inherited fatal immune dysfunction characterized by a defective immune response to EBV infection (5). In most of the cases, affected young boys develop a fulminant infectious mononucleosis with the features of a hemophagocytic syndrome. When children survive this initial episode or are asymptomatic they go on to develop hypogammaglobunemia and aggressive lymphoproliferative disorders such as lymphomas (5). However, the pathophysiology of XLP remains poorly understood although studies using SAP-deficient mice showed that the lack of SAP principally leads to impaired Th2 cell responses and to a defect in long-term humoral responses (6–8).

NKT cells represent a peculiar subpopulation of αβ T cells with immunoregulatory properties by their ability to rapidly secrete large amounts of cytokines such as IFN-γ, IL-4, IL-10, and TGF-β (9–11). NKT cells express in their majority an invariant TCR (Vα14-Jα18/Vβ8 in mouse and Vα24-Jα18/Vβ11 in humans) that reacts with the monomorphic MHC class I–like molecule CD1d. They are also characterized by expression of receptors of the NK lineage, including NK1.1 and NK cell inhibitory receptors. NKT cells recognized glycolipid antigens presented by CD1d (9–11). The sponge-derived glycolipid α-galactosyl ceramide (αGalCer) when presented by CD1d selectively activates NKT cells. In contrast to conventional T cells,

NKT cells are not subjected to negative selection in the thymus but are positively selected by CD1d expressed on $CD4^+$/$CD8^+$ thymocytes in the presence of the self-glycolipid iGb3 (12, 13). It has been proposed that NKT cells might be important for the initiation and the regulation of immune responses by interplaying with innate and adaptive immune responses (9–11).

There is accumulating evidence supporting the notion that some of the signaling pathways driving NKT cell development are unique and differ from those involved in conventional T cells and NK cells (11). The Src kinase FynT has been shown to be required for NKT cell development but not for T cell and NK cell differentiation (14, 15), even though its precise role in NKT cell ontogeny is not known. Because SAP has the capacity to associate and to activate FynT (4), we examined whether SAP is required for NKT cell development. In this study, we report that the NKT cell development is severely impaired both in mice and humans lacking SAP. This is the first report to date describing an inherited fatal immunodeficiency condition in humans in which NKT cells are lacking.

RESULTS AND DISCUSSION

Lack of NKT cells in SAP-deficient mice

We first examined the amount of NKT cells in the different hemopoietic organs of SAP-deficient mice by flow cytometric analysis. The percentages of NKT cells stained by anti-

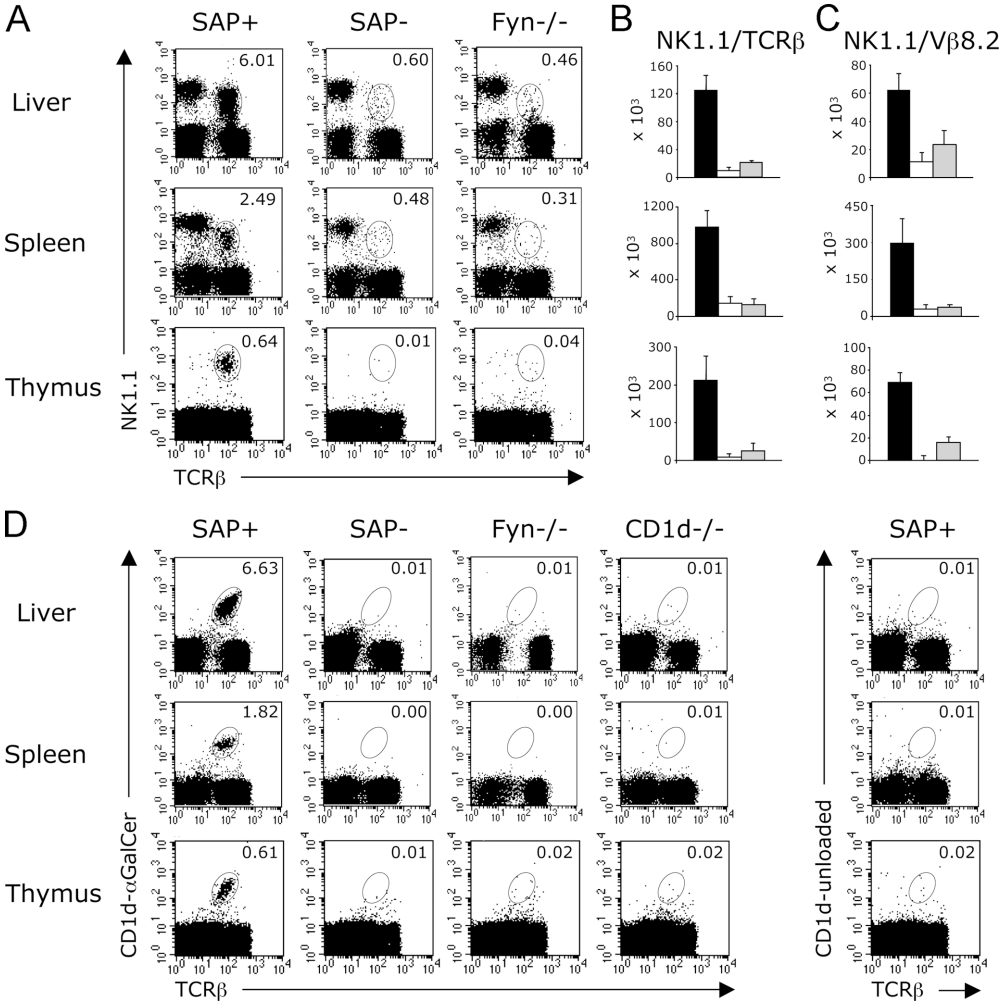

Figure 1. Defect in NKT cells in SAP-deficient mice. NKT cells were analyzed by flow cytometry in the liver, spleen, and thymus of wild-type (SAP^+), SAP-deficient (SAP^-), Fyn-deficient ($Fyn^{-/-}$), and CD1d-deficient ($CD1d^{-/-}$) mice. (A) Liver lymphocytes and splenocytes were stained with anti-CD19 and thymocytes with anti-HSA. After gating on $CD19^-$ or HSA^{low} cells, dot plots were constructed. Representative two-color dot plots show staining with anti-TCRβ versus anti-NK1.1 antibodies. The percentage of NKT cells corresponding to double positive cells in the circle gate is indicated in each plot. The data are representative of at least five mice per group. (B and C) Absolute numbers of NKT cells in the liver, spleen, and thymus mononuclear cells from SAP^+ (black histogram), SAP^- (white histogram), and $Fyn^{-/-}$ mice (gray histogram) stained for NK1.1 and TCRβ (B) or NK1.1 and Vβ8.2 TCR (C). Cells were counted in each organ and absolute numbers of NKT cells were determined based on their proportion (gated on double positive cells). Numbers are mean ± SD of three mice per group. (D) Same experiment as A. Representative two-color dot plots show the staining with anti-TCRβ antibodies versus αGalCer-loaded CD1d tetramers or unloaded CD1d tetramers (right). The percentage of NKT cells corresponding to double positive cells in the circle gate is indicated in each plot. The data are representative of at least five mice per group.

TCRβ and anti-NK1.1 antibodies in the liver, spleen, and thymus of SAP-deficient mice were severely decreased when compared with wild-type (SAP^+) mice (Fig. 1 A). This decrease was similar to that found in Fyn-deficient mice (Fig. 1 A). Similar results were obtained with anti-NK1.1 plus anti-Vβ8.2 antibodies (not depicted). However, the percentages of conventional T cells ($TCR\beta^+$ $NK1.1^-$) and NK cells ($TCR\beta^-$ $NK1.1^+$) in SAP-deficient and Fyn-deficient mice were comparable to those observed in wild-type mice (Fig. 1 A). Consistent with the decreased proportions of NKT cells in SAP-deficient mice, absolute numbers of these cells in the liver, spleen, and thymus were found to be severely reduced in SAP-deficient and Fyn-deficient mice relative to wild-type mice (Fig. 1, B and C). Moreover, very low amounts of transcripts encoding the Vα14-Jα18 TCR rearrangement were detected by semiquantitative RT-PCR in the spleen of SAP-deficient and Fyn-deficient animals when compared with wild-type animals (not depicted). Next, we examined CD1d-restricted NKT cells using CD1d tetramers loaded with αGalCer (Fig. 1 D). Although CD1d-restricted NKT cells were easily detected in wild-type (SAP^+) mice, a dramatic reduction of their frequency was observed in SAP-deficient, Fyn-deficient, and CD1d-deficient mice. Importantly, most of the residual $TCR\beta^+$ αGalCer-loaded $CD1d^+$ cells found in SAP-deficient, Fyn-deficient, and CD1d-deficient mice appeared to be nonspecific staining, as a close proportion of these cells were detected with unloaded CD1d tetramers (Fig. 1 D, right, and not depicted). These data indicate that SAP-deficient and Fyn-deficient mice lack CD1d-restricted NKT cells.

NKT cells are known to proliferate and produce IFN-γ and IL-4 upon engagement of their invariant TCR with CD1d-presented αGalCer (11). Stimulation of wild-type splenocytes with αGalCer resulted in a robust cell proliferation and production of IFN-γ and IL-4 (Fig. 2, A–C). By contrast, no significant cell proliferation and no production of IFN-γ and IL-4 were observed with SAP-deficient and Fyn-deficient splenocytes cultured in the presence of αGalCer (Fig. 2, A–C). As control, stimulation with anti-CD3 plus IL-2 induced a strong cell proliferation by wild-type, SAP-deficient, and Fyn-deficient splenocytes (Fig. 2 A). IFN-γ production in these conditions was found to be comparable between wild-type and SAP-deficient splenocytes, whereas it was slightly decreased with Fyn-deficient splenocytes. However, SAP-deficient and Fyn-deficient splenocytes failed to produce IL-4 upon anti-CD3 plus IL-2 stimulation. These data were consistent with recent studies showing that activated T lymphocytes from SAP- and Fyn-deficient mice have a defect in IL-4 production (6, 16). Taken together, these results indicate that the compartment of NKT cells is selectively and severely impaired in the absence of the SAP protein.

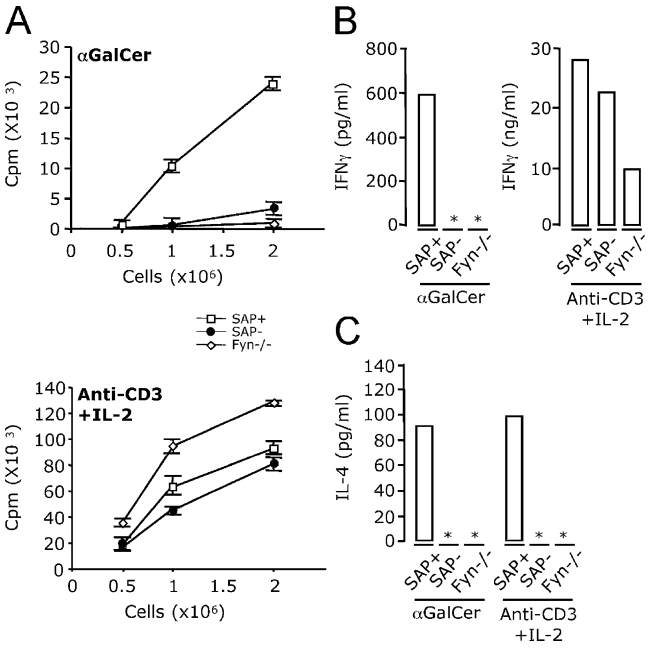

Figure 2. Absence of αGalCer–dependent NKT cell responses in SAP-deficient mice. (A) Proliferation of splenic NKT cells in response to αGalCer or anti-CD3 plus IL-2. Splenocytes of wild-type (SAP^+), SAP-deficient (SAP^-), and Fyn-deficient ($Fyn^{-/-}$) mice were cultured at various cell concentrations with 100 ng/ml αGalCer or in the presence of 3 μg/ml of immobilized anti-CD3 antibodies plus IL-2. Proliferation of cells was assessed by $[^3H]$thymidine incorporation. Spontaneous proliferation in the absence of αGalcer was similar with SAP^+, SAP^-, and $Fyn^{-/-}$ splenocytes. Data are presented as a mean ± SD of one representative experiment out of three. (B and C) IFN-γ and IL-4 production by splenic NKT cells (10^6 splenocytes) in response to αGalCer or anti-CD3 plus IL-2. Cells were stimulated similarly as in A. The presence of IFN-γ and IL-4 in the supernatants was detected by ELISA. In some cases, IFN-γ and IL-4 were not detectable (*). Data are from one representative experiment representative of three.

mus, CD1d-restricted NKT cell precursors primarily acquire the Vα14-Jα18/Vβ8 TCR that allows their subsequent selection by CD1d-presented self-glycolipid expressed by $CD4^+$ $CD8^+$ thymocytes (12, 13). Then, NKT cell precursors up-regulate CD44 and lastly acquire NK1.1 expression during their final maturation (17). Because CD1d-deficient mice exhibit a profound defect in NKT cell development (18), we ascertained that the defect of NKT cells in the absence of SAP was not caused by a defective CD1d expression. Expression of CD1d by thymocytes (Fig. 3 A) and splenic T and B cells (Fig. 3 A and not depicted) in SAP-deficient mice was found to be equivalent to that of wild-type (SAP^+) and Fyn-deficient mice, excluding that a loss of CD1d expression accounts for the defect of NKT cells observed in SAP-deficient mice. Next, we investigated the expression of CD44 and NK1.1 by thymocytes of SAP-deficient mice that were positive for αGalCer-loaded CD1d tetramers. As shown above in Fig. 1, only a few thymocytes were positive for αGalCer-loaded CD1d tetramers in the SAP-deficient mice in comparison with wild-type (SAP^+) mice. Nonetheless, when examined for CD44 and NK1.1

Early block in NKT cell development in SAP-deficient mice

Next, we examined the developmental steps of NKT cells in SAP-deficient mice. During their development in the thy-

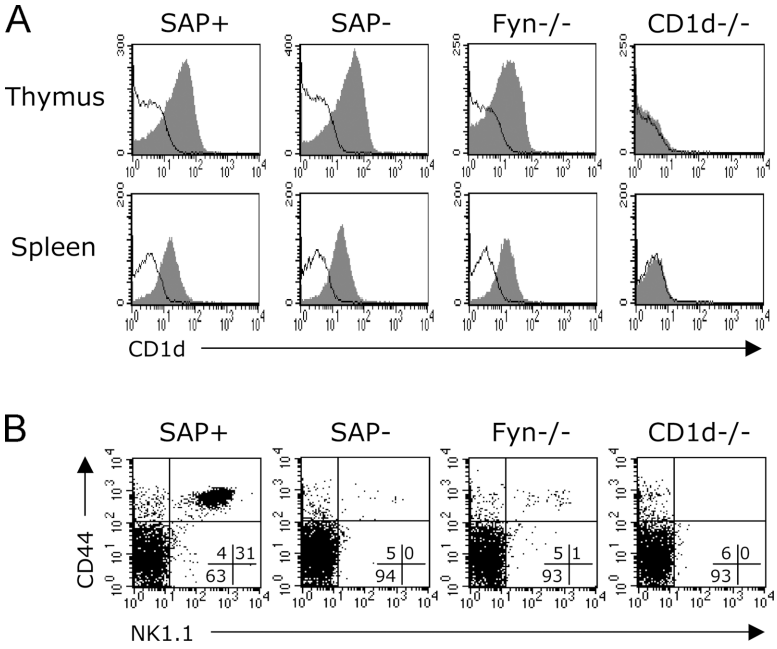

Figure 3. Impaired NKT cell development in SAP-deficient mice. (A) Expression of CD1d on $CD4^+/CD8^+$ thymocytes (thymus) and splenocytes (spleen) of wild-type (SAP^+), SAP-deficient (SAP^-), Fyn-deficient ($Fyn^{-/-}$) and CD1d-deficient ($CD1d^{-/-}$) mice. After gating on $CD4^+/CD8^+$ thymocytes or $TCR\beta^+$ splenic cells, histograms corresponding to CD1d staining (gray histogram) and isotype-matched irrelevant antibodies (white histogram) were constructed. Data are from one experiment representative of three. (B) Expression of CD44 and NK1.1 on NKT cell precursors. Thymocytes were stained with anti-NK1.1, anti-CD44, and αGalCer-loaded CD1d tetramers. After gating on αGalCer-loaded CD1d $tetramer^+$ cells, two-color dot plots corresponding to the staining of CD44 and NK1.1 were constructed. The percentage of cells in each quadrant is indicated.

expression, most of these residual CD1d $tetramer^+$ cells in SAP-deficient mice did not up-regulate CD44 (94%), and none of them expressed NK1.1 in contrast to wild-type (SAP^+) cells (0 vs. 31%, respectively; Fig. 3 B). To confirm the developmental arrest of NKT cells in absence of SAP, thymocytes from SAP-deficient mice were compared with those of CD1d-deficient mice. The proportions of residual CD1d $tetramer^+$ cells that are $CD44^{low}$ were found to be similar in both mice (94 vs. 93%, respectively; Fig. 3 B), suggesting that the block of NKT cell development occurs at a related stage in SAP-deficient and CD1d-deficient mice.

Together, these results suggest that the absence of SAP leads to a severe defect in the early steps of NKT cell development before they up-regulate CD44. SAP could be involved in the intrinsic maturation of NKT cell precursors, the development of the thymic microenvironment, or both. However, we could not strictly exclude that the defect may occur in more mature cells by activation-induced cell death upon physiological antigen encounter. It is proposed that the main function of SAP is to recruit the Src kinase FynT to SLAM family receptors, allowing their coupling to intracellular pathways (19). Thus, one or several members of the SLAM family receptors might be required for normal NKT cell development. Further studies will be needed to test these possibilities.

Absence of NKT cells in humans with an XLP

Because the lack of SAP is responsible for XLP in humans (1, 2), we investigated whether NKT cells could be normally detected in the blood of patients with XLP. XLP patients carrying different mutations in the *SAP* gene were analyzed and compared with healthy age-matched individuals as well as the mother of one patient. None of the XLP patients except one (patient 4; see Materials and methods) tested in this study expressed the SAP protein in his PBLs as shown by Western blotting of cell lysates performed with anti-SAP antibodies (Fig. 4 B and not depicted). The presence of NKT cells within the PBLs of an XLP patient (patient 5), his mother, and a healthy age-matched donor was assessed by flow cytometry by staining with anti-Vα24 TCR and anti-Vβ11 TCR antibodies (Fig. 4 A, left) or with anti-Vα24 TCR antibodies and αGalCer-loaded CD1d tetramers (Fig. 4 A, middle). In the PBLs from the healthy individual and the mother of the XLP patient, NKT cells were significantly detected with both staining reagents. As a control of specificity, unloaded CD1d tetramers did not identify NKT cells in the healthy individual nor in the mother of the XLP patient (Fig. 4 A, right). In striking contrast, no NKT cells were found in the PBLs of the XLP patient. To confirm this result, PBLs from three additional patients with XLP and six healthy age-matched donors were analyzed. The proportion of NKT cells that were positive for both αGalCer-loaded CD1d tetramers and anti-Vα24 TCR antibodies ranged from 0.08 to 0.18% (0.11 ± 0.04%) in control donors, whereas no detectable NKT cells were observed in XLP patients (0.01 ± 0.01%; $P = 0.001$; Fig. 4 C). Similar results were found by using anti-Vα24 TCR and anti-Vβ11 TCR

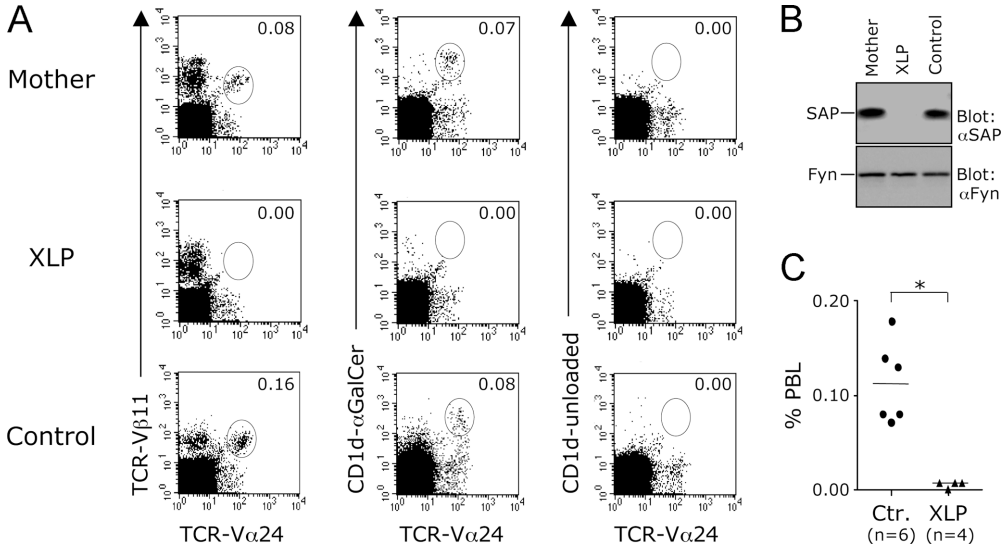

Figure 4. Lack of NKT cells in XLP patients. (A) Representative dot plots showing NKT cells in PBLs from an XLP patient (XLP), his mother, and an age-matched healthy donor (Control). After gating on $CD3^+$ cells, two-color dot plots showing the staining with anti-Vα24 TCR and anti-Vβ11 TCR (left) or anti-Vα24 TCR antibodies and αGalCer-loaded CD1d tetramers (middle) or unloaded CD1d tetramers (right) were constructed. All of the cells that were Vα24 TCR^+ αGalCer-CD1d $tetramer^+$ were also found to be Vβ11 TCR^+. The percentage of NKT cells (double positive cells) in the circle gate is indicated on each dot plot. (B) The absence of SAP protein expression in cells from an XLP patient. Cell lysates from the same individuals represented in A were analyzed by Western blotting with anti-SAP or anti-Fyn antibodies as loading controls. (C) Percentage of NKT cells ($CD3^+$ Vα24 TCR^+ αGalCer-CD1d $tetramer^+$) in the PBLs of blood samples from four patients with XLP (XLP) and six age-matched healthy donors (Ctr.). The bars corresponding to the means of percentages are indicated and * indicates $P < 0.01$.

staining (control donors: $0.18 \pm 0.08\%$, $n = 8$; XLP patients: 0.00%, $n = 6$; $P = 0.0002$; Fig. 5 A). The six XLP patients tested had clinical manifestations that were diverse but typical of XLP (i.e, fulminant mononucleosis, hemophagocytic syndrome, hypogammaglobunemia, and lymphoma). Whatever these differences, all XLP patients were found to have this common lack of NKT cells.

To point out that the absence of NKT cells is restricted to XLP, we further examined NKT cells from patients affected with other primary immunodeficiencies such as the closely related inherited hemophagocytic lymphoproliferative syndromes, the Chédiak-Higashi syndrome (CHS), and the familial hemophagocytic lymphohistiocytosis syndrome (FHL; reference 20). In these patients, $V\alpha24^+/V\beta11^+$ TCR NKT cells were significantly detectable ($0.09 \pm 0.06\%$, $n = 3$) compared with XLP patients (0.00%, $n = 6$; $P = 0.009$) and were found to be similar or slightly reduced relative to healthy donors ($0.18 \pm 0.08\%$, $n = 8$; $P = 0.12$; Fig. 5 A). Thus, the absence of NKT cells in XLP patients appears to be specific of this immunodeficiency condition. In addition, the absence of SAP seems to selectively impair NKT cell development because the proportions of NK cells in the PB-

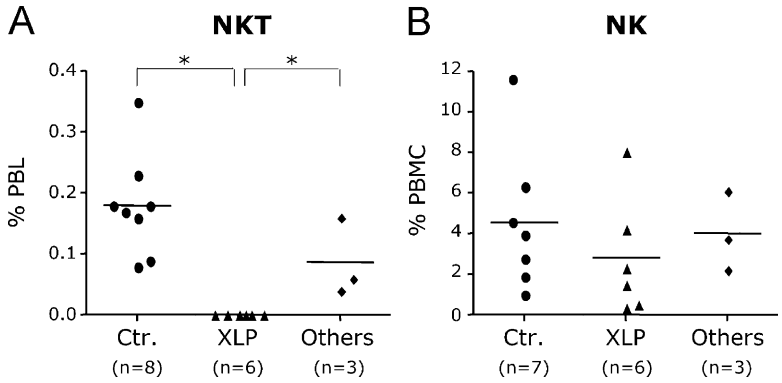

Figure 5. Specificity of the NKT cell defect in XLP. (A) Percentages of NKT cells ($CD3^+$ Vα24 TCR^+ Vβ11 TCR^+) in the PBLs of blood samples from six patients with XLP (XLP) and eight age-matched healthy donors (Ctr.) and three patients with CHS and FHL syndromes (Others). (B) Percentages of NK cells ($CD56^+/CD3^-$) in the PBMCs of blood samples from the same individuals as in A. The bars corresponding to the means of percentages are indicated and * indicates $P < 0.01$.

MCs of XLP patients (2.9 ± 2.8%, $n = 6$) were comparable to those observed in patients with other immune defects (4.0 ± 2.0%, $n = 3$; $P = 0.55$) or in healthy individuals (4.6 ± 3.6%, $n = 7$; $P = 0.34$; Fig. 5 B). This is consistent with the normal development of NK cells found in SAP-deficient mice (Fig. 1).

Altogether, these data indicate that in mice and in humans, SAP is required for normal NKT cell development. NKT cells have been proposed to play critical roles in a variety of immune responses, including host defense against pathogens, regulation of autoimmunity, and tumor surveillance (10, 11, 21, 22). In this report, we showed that patients suffering from XLP are devoid of NKT cells, and because XLP is a severe immunodeficiency characterized by an extreme sensitivity to EBV infection, it is tempting to speculate that NKT cells may play an essential function in the control of EBV infection. Future studies should be aimed at addressing this important issue.

MATERIALS AND METHODS

Patients. XLP patients have been genotyped for *SAP/SH2D1A* and were found to be mutated in *SAP* resulting in the following amino acid changes in SAP: SAP X129R (patient 1), SAP R55X (patient 2), SAP E67G (patient 3), and SAP R55P (patient 4). Patient 5 had a deletion of the third exon of *SAP* and patient 6 had a single nucleotide insertion causing a frameshift that leads to a stop codon. The following are the clinical features of the XLP patients: patient 1 developed hypogammaglobunemia; patients 2, 4, and 6 had a fulminant infectious mononucleosis with a hemophagocytic syndrome; and patients 3 and 5 had lymphoma with hypogammaglobunemia. Two patients with CHS and one patient with FHL who developed hemophagocytic syndrome were also analyzed. Ages of the individuals ranged from 1 to 27 yr old for healthy age-matched donors, 4 to 20 yr old for XLP patients, and 2 mo to 3 yr old for CHS and FHL patients. The mother of patient 5 was 42 yr old. Patients or families provided informed consent for the study in accordance with the Declaration of Helsinki. This study was approved by the INSERM Institutional Review Board.

Animals. SAP-deficient (SAP^-) mice, Fyn-deficient ($Fyn^{-/-}$) mice, and CD1d-deficient ($CD1d^{-/-}$) mice have been described elsewhere (15, 23, 24). Male SAP-deficient mice and male wild-type (SAP^+) littermates were typed by PCR. All mice used in this study were from 8 to 11 wk of age. Mouse studies were performed under the institutional animal care and use guidelines.

Antibodies and reagents. The following mAbs conjugated to FITC, PE, APC, or biotin were used: anti-NK1.1 (PK136), anti-TCRβ (H57-597), anti-Vβ8.1/8.2 TCR (MR5-2), anti-CD4 (RM4-5), anti-CD8 (53-5-8), anti-CD19 (1D3), anti-CD44 (IM7), anti-HSA (M1/69), and anti-CD1d (1B1; all from BD Biosciences). PerCP- or PE-conjugated streptavidin were from BD Biosciences. The mAbs used for experiments in humans were as follows: anti-Vβ11 TCR (C21) and anti-Vα24 TCR (C15) from Beckman Coulter and anti-CD3 (SK7) and anti-CD56 (MY31) from BD Biosciences. CD1d tetramers and αGalCer were provided by A. Bendelac (University of Chicago, Chicago, IL). αGalCer-loaded CD1d tetramers were prepared as described previously (24).

Cell preparation and flow cytometry. Single cell suspensions were prepared from the liver, spleen, and thymus as described previously (14). Human PBMCs were isolated from blood samples by the standard Ficoll-Paque method (Axis-Shield PoC AS; Lymphoprep). Murine single cell suspensions or PBMCs were washed twice in PBS containing 2% FCS and 0.1% NaN_3 before staining with the indicated reagents. For mouse analysis, cells were first preincubated with anti-FcγRII/III antibodies (2.4G2) to block Fcγ receptors before staining. Finally, cells were analyzed using a FACSCalibur and CELLQuest software (Becton Dickinson).

Cell proliferation and cytokine production. Spleen cell suspensions were incubated in complete medium supplemented or not with 100 ng/ml αGalCer or stimulated with 3μg/ml of immobilized anti-CD3 (145-2C11) in the presence of 100 IU/ml of recombinant IL-2. After 36 h in culture, cells were labeled with [^{3}H]thymidine for 12 h, harvested, and counted in a microbetaplate counter (Wallac). Supernatants were collected after 48 h of stimulation with αGalCer and were tested for IL-4 and IFN-γ contents by ELISA according to the manufacturer's instructions (R&D Systems). All assays were performed in duplicate.

Western blot. Immunoblots were performed as described previously (3). Polyclonal antibodies to human SAP were produced by immunizing rabbits with a bacterial fusion protein containing the entire human SAP protein.

Semiquantitative RT-PCR. The transcripts encoding the Vα14-Jα18 TCR rearrangement were detected by RT-PCR. In brief, 5μg of total RNA was reverse transcripted using random hexamers and Superscript II reverse transcriptase (Invitrogen), and the cDNAs were amplified by PCR using specific primers as described previously (18).

Statistical analysis. Student's *t* tests were performed with InStat software.

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REFERENCES

1. Latour, S., and A. Veillette. 2003. Molecular and immunological basis of X-linked lymphoproliferative disease. *Immunol. Rev.* 192:212–224.
2. Engel, P., M.J. Eck, and C. Terhorst. 2003. The SAP and SLAM families in immune responses and X-linked lymphoproliferative disease. *Nat. Rev. Immunol.* 3:813–821.
3. Latour, S., G. Gish, C.D. Helgason, R.K. Humphries, T. Pawson, and A. Veillette. 2001. Regulation of SLAM-mediated signal transduction by SAP, the X-linked lymphoproliferative gene product. *Nat. Immunol.* 2:681–690.
4. Latour, S., R. Roncagalli, R. Chen, M. Bakinowski, X. Shi, P.L. Schwartzberg, D. Davidson, and A. Veillette. 2003. Binding of SAP SH2 domain to FynT SH3 domain reveals a novel mechanism of receptor signalling in immune regulation. *Nat. Cell Biol.* 5:149–154.
5. Gaspar, H.B., R. Sharifi, K.C. Gilmour, and A.J. Thrasher. 2002. X-linked lymphoproliferative disease: clinical, diagnostic and molecular perspective. *Br. J. Haematol.* 119:585–595.
6. Wu, C., K.B. Nguyen, G.C. Pien, N. Wang, C. Gullo, D. Howie, M.R. Sosa, M.J. Edwards, P. Borrow, A.R. Satoskar, et al. 2001. SAP controls T cell responses to virus and terminal differentiation of TH2 cells. *Nat. Immunol.* 2:410–414.
7. Czar, M.J., E.N. Kersh, L.A. Mijares, G. Lanier, J. Lewis, G. Yap, A. Chen, A. Sher, C.S. Duckett, R. Ahmed, and P.L. Schwartzberg. 2001. Altered lymphocyte responses and cytokine production in mice deficient in the X-linked lymphoproliferative disease gene SH2D1A/DSHP/SAP. *Proc. Natl. Acad. Sci. USA.* 98:7449–7454.
8. Crotty, S., E.N. Kersh, J. Cannons, P.L. Schwartzberg, and R. Ahmed. 2003. SAP is required for generating long-term humoral immunity.

Nature. 421:282–287.
9. Bendelac, A., M.N. Rivera, S.H. Park, and J.H. Roark. 1997. Mouse CD1-specific NK1 T cells: development, specificity, and function. *Annu. Rev. Immunol.* 15:535–562.
10. Taniguchi, M., M. Harada, S. Kojo, T. Nakayama, and H. Wakao. 2003. The regulatory role of Valpha14 NKT cells in innate and acquired immune response. *Annu. Rev. Immunol.* 21:483–513.
11. Kronenberg, M., and L. Gapin. 2002. The unconventional lifestyle of NKT cells. *Nat. Rev. Immunol.* 2:557–568.
12. Gapin, L., J.L. Matsuda, C.D. Surh, and M. Kronenberg. 2001. NKT cells derive from double-positive thymocytes that are positively selected by CD1d. *Nat. Immunol.* 2:971–978.
13. Zhou D., J. Mattner, C. Cantu III, N. Schrantz, N. Yin, Y. Gao, Y. Sagiv, K. Hudspeth, Y.P. Wu, T. Yamashita, S. Teneberg, D. Wang, et al. 2004. Lysosomal glycosphingolipid recognition by NKT cells. *Science*. 306:1786–1789.
14. Eberl, G., B. Lowin-Kropf, and H.R. MacDonald. 1999. Cutting edge: NKT cell development is selectively impaired in Fyn-deficient mice. *J. Immunol.* 163:4091–4094.
15. Gadue, P., N. Morton, and P.L. Stein. 1999. The Src family tyrosine kinase Fyn regulates natural killer T cell development. *J. Exp. Med.* 190:1189–1196.
16. Davidson, D., X. Shi, S. Zhang, H. Wang, M. Nemer, N. Ono, S. Ohno, Y. Yanagi, and A. Veillette. 2004. Genetic evidence linking SAP, the X-linked lymphoproliferative gene product, to Src-related kinase FynT in T(H)2 cytokine regulation. *Immunity*. 21:707–717.
17. Benlagha, K., T. Kyin, A. Beavis, L. Teyton, and A. Bendelac. 2002. A thymic precursor to the NK T cell lineage. *Science*. 296:553–555.
18. Chen, Y.H., N.M. Chiu, M. Mandal, N. Wang, and C.R. Wang. 1997. Impaired NK1+ T cell development and early IL-4 production in CD1-deficient mice. *Immunity*. 6:459–467.
19. Latour, S., and A. Veillette. 2004. The SAP family of adaptors in immune regulation. *Semin. Immunol.* 16:409–419.
20. de Saint Basile, G., and A. Fischer. 2003. Defective cytotoxic granule-mediated cell death pathway impairs T lymphocyte homeostasis. *Curr. Opin. Rheumatol.* 15:436–445.
21. Mars, L.T., J. Novak, R.S. Liblau, and A. Lehuen. 2004. Therapeutic manipulation of iNKT cells in autoimmunity: modes of action and potential risks. *Trends Immunol.* 25:471–476.
22. Smyth, M.J., N.Y. Crowe, Y. Hayakawa, K. Takeda, H. Yagita, and D.I. Godfrey. 2002. NKT cells–conductors of tumor immunity? *Curr. Opin. Immunol.* 14:165–171.
23. Yin, L., U. Al-Alem, J. Liang, W.M. Tong, C. Li, M. Badiali, J.J. Medard, J. Sumegi, Z.Q. Wang, and G. Romeo. 2003. Mice deficient in the X-linked lymphoproliferative disease gene sap exhibit increased susceptibility to murine gammaherpesvirus-68 and hypo-gammaglobulinemia. *J. Med. Virol.* 71:446–455.
24. Benlagha, K., A. Weiss, A. Beavis, L. Teyton, and A. Bendelac. 2000. In vivo identification of glycolipid antigen-specific T cells using fluorescent CD1d tetramers. *J. Exp. Med.* 191:1895–1903.