Consequence of the SLAM-SAP Signaling Pathway in Innate-like and Conventional Lymphocytes

André Veillette,1,2,3,* Zhongjun Dong,1 and Sylvain Latour4

1Laboratory of Molecular Oncology, Clinical Research Institute of Montréal
2Department of Medicine, University of Montréal
3Department of Medicine, McGill University
Montreal, Québec, Canada H2W 1R7
4Unité INSERM U768, Hôpital Necker Enfants-Malades, Paris 75015, France
*Correspondence: veillea@ircm.qc.ca
DOI 10.1016/j.immuni.2007.11.005

Signaling lymphocytic activation molecule (SLAM) family receptors mediate important regulatory signals in immune cells, as a result of their exquisite ability to associate with members of the SLAM-associated protein (SAP) family of adaptors. As discussed herein, recent findings show that the SLAM and SAP families carry out pivotal functions in innate-like and conventional lymphocytes. They are critically needed for the development of innate-like lymphocytes such as NKT cells. In addition, they influence several of the functions of conventional lymphocytes, including the ability of CD4+ T cells to secrete certain cytokines and mediate B cell help; CD8+ T cell proliferation and cytokine production; NK cell-mediated cytotoxicity; and B cell antibody production. These unique functional properties appear to be facilitated by the ability of SLAM-related receptors to serve as self-ligands during homotypic interactions between immune cells. The importance of the SLAM-SAP pathway in normal immunity is highlighted by the finding that SAP is mutated in humans suffering from the immunodeficiency X-linked lymphoproliferative disease.

Introduction

Cell-cell interactions are critical in the development, differentiation, and activation of immune cells. These interactions are typically heterotypic, involving two cells of different lineages. Classical examples of heterotypic interactions are the contacts between immature T cells and thymic epithelial cells that allow positive selection of conventional T cells in the thymus or those of mature cytotoxic lymphocytes with virus-infected target cells that trigger lymphocyte-mediated cytotoxicity. In some cases, however, cell-cell interactions are homotypic, involving two cells of the same immune lineage, e.g., the interactions between immature double-positive (DP) thymocytes that lead to selection of NKT cells, a T cell subset with innate-like immune cell characteristics.

The receptor-ligand pairs mediating intercellular interactions in the immune system are varied. Most of them involve receptors that bind a heterologous ligand (heterotypic receptor-ligand interactions), including immunoreceptors such as the T cell receptor (TCR), which recognizes an antigen bound to a major histocompatibility complex (MHC) molecule; co-stimulatory molecules and their ligands such as CD80 and CD86, CTLA-4 and B7, and CD40 and CD40 ligand (CD40L); and adhesion molecules and their ligands such as LFA-1 and ICAM-1. These heterotypic receptor-ligand associations usually occur during heterotypic cell-cell interactions. In certain cases, the receptors are self-ligands and, thus, are able to participate in either homotypic or heterotypic cell-cell interactions. This form of interaction is typical of most members of the signaling lymphocytic activation molecule (SLAM) family of receptors, which act in concert with their intracellular signaling counterparts, the SLAM-associated protein (SAP)-related adaptors, to regulate immunity. Here, we review the biochemical and functional characteristics of SLAM and SAP families as well as highlight findings reported in this issue of Immunity on the pivotal roles of the SLAM-SAP pathway in “innate lymphocyte” differentiation (Griewank et al., 2007; Li et al., 2007; Horai et al., 2007). We also discuss the importance of the SLAM-SAP pathway in lymphocyte activation and its role in the immunodeficiency X-linked lymphoproliferative (XLP) disease.

The SLAM Family of Receptors

The SLAM family of receptors is composed of six members named SLAM (CD150); 2B4 (CD244); Ly-9 (CD229); CD84; natural killer, T and B cell antigen (NTB-A; Ly108 in the mouse); and CD2-like receptor activating cytotoxic cells (CRACC; also named CD319 or CS1) (Engel et al., 2003; Veillette, 2006a; Ma et al., 2007). These receptors possess an extracellular segment with two or, in the case of Ly-9, four immunoglobulin (lg)-like domains, a single transmembrane region, and a cytoplasmic domain with several tyrosine-based motifs. These tyrosine-based motifs undergo phosphorylation and recruit SAP-related adaptors. The expression of SLAM-related receptors is restricted to cells of hemopoietic lineages, including
Table 1. Distribution of SLAM Family Receptors on Immune Cells

<table>
<thead>
<tr>
<th></th>
<th>SLAM</th>
<th>2B4</th>
<th>NTB-A (Ly108)</th>
<th>Ly-9</th>
<th>CD84</th>
<th>CRACC</th>
</tr>
</thead>
<tbody>
<tr>
<td>DP thymocytes</td>
<td>++</td>
<td>±</td>
<td>+++</td>
<td>++</td>
<td>++</td>
<td>−</td>
</tr>
<tr>
<td>Mature T cells</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resting</td>
<td>±</td>
<td>−</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>−</td>
</tr>
<tr>
<td>Activated</td>
<td>+++</td>
<td>++ (CD8(^+))</td>
<td>+++</td>
<td>+++</td>
<td>++</td>
<td></td>
</tr>
<tr>
<td>Mature NKT cells</td>
<td>±</td>
<td>ND</td>
<td>±</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>B cells</td>
<td>++</td>
<td>−</td>
<td>+++</td>
<td>+++</td>
<td>++</td>
<td>−</td>
</tr>
<tr>
<td>NK cells</td>
<td>−</td>
<td>+++</td>
<td>+++ (h)</td>
<td>+</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>Mature DCs</td>
<td>+++</td>
<td>+++</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Macrophages</td>
<td>±</td>
<td>±</td>
<td>−</td>
<td>+++</td>
<td>+++</td>
<td>++</td>
</tr>
</tbody>
</table>

Relative expression of SLAM receptors in various immune cell types, based on published and unpublished data (Engel et al., 2003; Veillette, 2006a; Ma et al., 2007; A.V., unpublished data). --, no expression; ±, weak expression; ++, moderate expression; ++++, strong expression; ND, not determined. Abbreviations: CRACC, CD2-like receptor activating cytotoxic cells; DC, dendritic cell; DP, double-positive; h, human; NK, natural killer; NTB-A, natural killer, T and B cell antigen; SLAM, signaling lymphocytic activation molecule.

adaptive and innate immune cells (Table 1). Most, if not all, immune cell types express more than one SLAM family receptor. All six SLAM-related genes are located within a 400–500 kilobase (kb) genomic segment on chromosome 1 in humans and mice. This feature implies that the genes that encode the SLAM family were created by serial gene duplication.

With the exception of 2B4, all SLAM family receptors are self-ligands (Mavaddat et al., 2000; Romero et al., 2005; Kumaresan et al., 2002; Martin et al., 2001; Falco et al., 2004). Intriguingly, there is a marked variation in the apparent affinity of these self-associations (K_d = 0.5–200 μM), suggesting that the various members of the SLAM family may modulate immune cell functions to different extents. Crystallographic determination of the structure of the NTB-A-NTB-A and CD84-CD84 homodimers indicated that self-association is mediated by the amino-terminal variable (V)-type Ig-like (IgV) domain, which enables head-to-head contact between two monomers (Cao et al., 2006; Yan et al., 2007). The end-to-end distance of the extracellular segments of these dimers is estimated to be between 100 and 140 Å, a distance similar to that proposed for TCR-MHC or CTLA-4-B7. Thus, SLAM family members bearing two Ig-like domains are well fit to colocalize with other receptor-ligand pairs known to have crucial functions during lymphocyte development and activation. Although a structure of the four-Ig-like domain-bearing Ly-9 remains to be determined, mutagenesis studies suggest that Ly-9 also forms homodimers through its amino-terminal IgV domain (Romero et al., 2005). This property implies that the end-to-end distance of Ly-9 dimers may be greater and the topography of Ly-9 distribution at the cell surface may be different, when compared to those of the other members of the SLAM family.

Unlike its relatives, 2B4 is not a self-ligand. It is involved in heterotypic associations with CD48, a glycosylphosphatidylinositol (GPI)-linked member of the CD2 family of receptors broadly expressed on hemopoietic cells (Latchman et al., 1998; Brown et al., 1998). Like 2B4, CD48 bears two Ig-like domains in its extracellular segment. A recent crystallographic analysis showed that, similar to NTB-A and CD84 homodimers, 2B4-CD48 heterodimers interact by way of their IgV-like domains (Velikovsky et al., 2007). Nonetheless, there are features that make this association quite distinct from the NTB-A-NTB-A interaction. In particular, most of the contacts between 2B4 and CD48 involve residues located in loops of the IgV-like domain. In contrast, a majority of the interactions in NTB-A dimers implicate residues in strands of this domain. The estimated size of the extracellular segment of the 2B4-CD48 heterodimer is similar to that of the NTB-A-NTB-A and CD84-CD84 dimers, suggesting that 2B4-CD48 heterodimers can colocalize with SLAM family receptor homodimers at the interface between immune cells.

Considering that all SLAM family receptors except 2B4 are self-ligands and that CD48, the ligand of 2B4, is found on all cell types expressing 2B4, SLAM-related receptors have the potential to regulate immunity in the context of homotypic or heterotypic cell-cell interactions.

The SAP Family of Adaptors

There are three members of the SAP family of adaptors: SAP, Ewing’s sarcoma-activated transcript-2 (EAT-2), and EAT-2-related transducer (ERT; in rodents only) (Engel et al., 2003; Veillette, 2006a; Ma et al., 2007). These adaptors are essentially composed of only a Src homology 2 (SH2) domain, fused to a short C-terminal tail. SAP is expressed in T cells, NK cells, NKT cells, and some B cells, including transformed B cell lines and, perhaps, normal B cells. In contrast, EAT-2 is found in NK cells, dendritic cells (DCs), and macrophages, whereas ERT is present in NK cells. The gene that encodes SAP (SH2D1A in humans or Sh2d1a in mice) is located on the X chromosome in humans and mice, whereas the gene that encodes EAT-2 (SH2D1B in human or Sh2d1b1 in mice) is positioned on chromosome 1 in some B cells, including transformed B cell lines and, perhaps, normal B cells. In contrast, EAT-2 is found in NK cells, dendritic cells (DCs), and macrophages, whereas ERT is present in NK cells. The gene that encodes SAP (SH2D1A in humans or Sh2d1a in mice) is located on the X chromosome in humans and mice, whereas the gene that encodes EAT-2 (SH2D1B in human or Sh2d1b1 in mice) is positioned on chromosome 1 in...
both species, near the SLAM locus. Through their SH2 domain, SAP-related adaptors associate with high affinity and high specificity with tyrosine-based motifs in the cytoplasmic domain of SLAM-related receptors. So far, there is no definitive evidence that SAP family adaptors interact with other classes of molecules by way of their SH2 domains.

SAP family members couple SLAM-related receptors to active biochemical signals (Engel et al., 2003; Veillette, 2006a; Ma et al., 2007). SAP recruits the Src-related protein tyrosine kinase (PTK) FynT to the SLAM family receptors, through a second binding surface centered on arginine 78 in the SAP SH2 domain (Latour et al., 2001, 2003; Chan et al., 2003). This arginine 78-based motif directly interacts with the FynT Src homology 3 (SH3) domain, thereby causing recruitment and enzymatic activation of FynT. The SAP-FynT interaction is specific, given that the arginine 78-based motif of SAP does not seem to bind other members of the Src family. Nevertheless, this motif can also associate with another SH3 domain-containing molecule, PAK-interacting exchange factor (PIX) (Gu et al., 2006). The role of this interaction in SAP-mediated functions is not known.

The aptitude of SAP to recruit FynT enables SLAM family receptors to mediate protein tyrosine phosphorylation signals (Bloch-Queyrat et al., 2005; Chen et al., 2004; Latour et al., 2001, 2003; Simarro et al., 2004; Chan et al., 2003). These signals appear to be pivotal for the function of SAP in immune cells, given that the immune defects caused by SAP deficiency in CD4+ T cells, NK cells, and NKT cells (discussed below) are also encountered in mice lacking FynT (Davidson et al., 2004; Cannons et al., 2004; Bloch-Queyrat et al., 2005; Gadue et al., 1999; Eberl et al., 1999). Moreover, a mouse expressing a SAP mutant unable to bind FynT (SAP<sup>R78A</sup> “knockin” mouse) had the same defect in CD4+ T cell functions as SAP-deficient mice (Davidson et al., 2004). Intriguingly, recent data indicated that some of the functions of SAP may occur through a FynT-independent mechanism. In support of this, it was found that the severe antibody defect noted in SAP-deficient mice was not observed in FynT-deficient mice (Cannons et al., 2006; McCausland et al., 2007). Furthermore, retrovirus-mediated gene transfer of SAP<sup>R78A</sup>, like that of wild-type SAP, was apt at restoring the compromised antibody production in SAP-deficient mice (Cannons et al., 2006). It is not known whether these FynT-independent functions of SAP are mediated by other Src family kinases interacting with SAP through alternative mechanisms or whether they involve as yet unknown effectors of SAP.

Unlike SAP, EAT-2 and ERT do not possess the arginine 78-based motif and, thus, are unable to recruit FynT through this mechanism (Engel et al., 2003; Veillette, 2006a; Ma et al., 2007). Instead, they bear one or two tyrosines in their C-terminal tail that can undergo tyrosine phosphorylation and recruit SH2 domain-containing molecules. This mechanism appears to be crucial for the function of EAT-2 and ERT because mutation of the C-terminal tyrosines abrogated the impact of EAT-2 overexpression in mouse NK cells (Roncagalli et al., 2005). The identity of the downstream effectors of EAT-2 and ERT is not known.

**X-Linked Lymphoproliferative Disease: Where SAP Meets XIAP and Innate-like Lymphocytes**

XLP disease is a rare inherited human immunodeficiency primarily characterized by a faulty immune response to Epstein-Barr virus (EBV) infection (Latour and Veillette, 2003; Ma et al., 2007; Morra et al., 2001). In general, boys suffering from XLP present with a massive lymphoproliferative illness and a classical hemophagocytic syndrome, triggered by EBV infection. If untreated, most patients succumb to this illness. However, treatment with immunosuppressive drugs, B cell-depleting CD20 antibodies, and/or bone marrow transplantation can lead to survival. XLP patients also develop hypogammaglobulinemias and malignant lymphomas.

Approximately 50%–70% of cases of XLP are due to mutations of the SH2D1A gene (Coffey et al., 1998; Sayos et al., 1998; Nichols et al., 1998) (Table 2). These mutations range from nonsense and missense point mutations, leading to unstable or nonfunctional SAP proteins, to large deletions in the SH2D1A gene. The precise immune defects responsible for the clinical manifestations of SAP-deficient XLP patients are not elucidated (Latour and Veillette, 2003; Ma et al., 2007; Morra et al., 2001). As will be detailed below, defects involving multiple cell lineages have been described, including altered CD4+ T cell functions, diminished CD8+ T cell cytotoxicity, reduced NK cell cytotoxicity, as well as defective antibody production and memory B cell generation. There is also a near absence of NKT cells. Although several of these immune abnormalities can have grave consequences on health, the marked accumulation of inflammatory cells seen during the EBV-triggered lymphoproliferative illness of XLP is very similar to that seen in other genetic disorders, such as Griscelli syndrome and Chediak-Higashi syndrome, associated with defective cytotoxic immune cell functions (Fischer et al., 2007). This notion led to the early belief that defects in cytotoxic cells, including CD8+ T cells and NK cells, played a major role in the pathophysiology of XLP.

More recently, characterization of the genetic and immunological defects of a subset of XLP patients that has no SH2D1A mutations provided an alternative, perhaps complementary, view (Rigaud et al., 2006). These patients present with clinical syndromes analogous to those of SAP-deficient patients, although distinctions include a propensity to exhibit splenomegaly as an early clinical manifestation and an apparent lack of susceptibility to malignant lymphomas (Table 2). Genetic analyses showed that these patients carry point mutations or deletions of the gene coding for X-linked inhibitor-of-apoptosis (XIAP), a member of the family of inhibitor-of-apoptosis proteins (IAPs) (Vaux and Silke, 2005). In general, these mutations result in an absence of XIAP protein expression (Rigaud et al., 2006). Immunological studies demonstrated that, unlike SAP-deficient humans, XIAP-deficient...
Table 2. Comparison of SAP Deficiency and XIAP Deficiency in Humans

<table>
<thead>
<tr>
<th></th>
<th>SAP Deficiency</th>
<th>XIAP Deficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genetic locus</td>
<td>Xq25</td>
<td>Xq25</td>
</tr>
<tr>
<td>Protein responsible</td>
<td>SAP (SH2 domain-containing adaptor)</td>
<td>XIAP (inhibitor-of-apoptosis)</td>
</tr>
<tr>
<td>Gene responsible</td>
<td>SH2D1A</td>
<td>BIRC4</td>
</tr>
<tr>
<td>Type(s) of mutation found</td>
<td>Null (usually), hypomorphic</td>
<td>Null, ? hypomorphic</td>
</tr>
<tr>
<td>Signaling pathways affected</td>
<td>SLAM family receptors, FynT</td>
<td>Caspases, ? others (TGF-β, Notch)</td>
</tr>
<tr>
<td>Clinical presentations</td>
<td>EBV-triggered HPS, hypogammaglobulinemia, lymphoma</td>
<td>EBV-triggered HPS, hypogammaglobulinemia, splenomegaly</td>
</tr>
<tr>
<td>Immune defects</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD4+ T cells</td>
<td>↓ Th2 priming</td>
<td>↑ AICD (in vitro)</td>
</tr>
<tr>
<td>CD8+ T cells</td>
<td>↓ Cytotoxicity</td>
<td>↑ AICD (in vitro)</td>
</tr>
<tr>
<td>NK cells</td>
<td>↓ Cytotoxicity</td>
<td>? Normal cytotoxicity</td>
</tr>
<tr>
<td>B cells</td>
<td>↓ Antibody production</td>
<td>↑ AICD (in vitro)</td>
</tr>
<tr>
<td>NKT cells</td>
<td>↓ Numbers (developmental block)</td>
<td>↓ Numbers (? peripheral homeostasis defect)</td>
</tr>
</tbody>
</table>

Differences between SAP deficiency and XIAP deficiency in humans. ↓, decreased; ↑, increased. Abbreviations: AICD, activation-induced cell death; EBV, Epstein-Barr virus; HPS, hemophagocytic syndrome; NK, natural killer; Th2, T helper 2; TGF, transforming growth factor; SAP, signaling lymphocytic activation molecule (SLAM)-associated protein; XIAP, X-linked inhibitor-of-apoptosis protein. References: Engel et al., 2003; Veillette, 2006a; Ma et al., 2007; Rigaud et al., 2006; Vaux and Silke, 2005.

patients have grossly normal NK cell functions. However, they have a marked reduction of NKT cell numbers in the periphery, to almost the same extent as SAP-deficient patients. In addition, peripheral blood lymphocytes and EBV-immortalized B cells from these individuals have greatly enhanced sensitivity to apoptotic stimuli such as CD3 antibodies or Fas ligation in vitro. Because the common immunological abnormality seen so far in SAP-deficient and XIAP-deficient patients is a pronounced diminution in the number of NKT cells, it strongly suggests that defects in NKT cells, rather than NK cells or CD8+ T cells, are largely responsible for the EBV-induced lymphoproliferative illnesses and hemophagocytic syndromes observed in XLP.

The mechanisms by which SAP and XIAP control NKT cell numbers may be quite divergent (Table 2). In the case of SAP, studies of SAP-deficient mice showed that there is an early block in the differentiation of NKT cells in the thymus (Nichols et al., 2005; Pasquier et al., 2005; Chung et al., 2005). Similar, albeit slightly less extensive, defects are seen in mice lacking FynT, implying that SAP regulates NKT cell differentiation in large part through its association with FynT (Gadue et al., 1999; Eberl et al., 1999). The situation appears to be different for XIAP. First, unlike XIAP-deficient humans, XIAP-deficient mice seem to have normal NKT cell numbers (Rigaud et al., 2006; Harlin et al., 2001). Second, there is currently no evidence that the biochemical pathways involved in SAP signaling and XIAP signaling intersect at any level. Although SAP operates with SLAM family receptors and FynT, XIAP is primarily known to bind and inhibit caspases (Vaux and Silke, 2005). And third, in keeping with the role of XIAP as an inhibitor of apoptosis, it was observed that mature conventional lymphocytes from XIAP-deficient humans, but not from SAP-deficient humans, are more sensitive to activation-induced cell death (Rigaud et al., 2006).

Because mature NKT cells exist in a state of chronic partial activation (presumably by self-lipids) in vivo (Bendelac et al., 2007; Brigl and Brenner, 2004; Godfrey and Berzins, 2007), it is plausible that NKT cell numbers are reduced in XIAP-deficient humans as a consequence of enhanced peripheral destruction of activated mature cells. This elimination may be further enhanced during the widespread immune cell activation caused by EBV infection.

The demonstration that a large proportion of XLP patients carry mutations of the SH2D1A gene provided the first clear indication that SAP-related adaptors and, presumably, SLAM family receptors play important roles in immunity (Coffey et al., 1998; Sayos et al., 1998; Nichols et al., 1998). This hypothesis was further supported by subsequent analyses of SAP-deficient mice (Wu et al., 2001; Czar et al., 2001; Yin et al., 2003; Crotty et al., 2003; Nichols et al., 2005; Pasquier et al., 2005; Chung et al., 2005; Bloch-Queyrat et al., 2005; Chen et al., 2005). More recently, it was cemented by studies of mice lacking individual members of the SLAM family (SLAM, 2B4, Ly108, Ly-9) and mice lacking the SAP-related molecules EAT-2 or ERT (Wang et al., 2004; Davidson et al., 2004; Lee et al., 2004; Howie et al., 2005; Graham et al., 2006; Roncagalli et al., 2005).

The SLAM and SAP Families: Central Players in the Differentiation of Innate-like Lymphocytes

Innate-like lymphocytes are subsets of lymphocytes expressing rearranged antigen receptors, thereby qualifying as antigen-specific lymphocytes (Godfrey and Berzins, 2007; Bendelac et al., 2007; Rodgers and Cook, 2005; Kearney, 2005; Treiner and Lantz, 2006; Berg, 2007).
The developmental characteristics of some of the most studied subsets of innate-like T cells are represented. Abbreviations: Ags, antigens; DCs, dendritic cells; h, human; IEL, intraepithelial lymphocytes; Itk, interleukin-2-inducible T cell kinase; m, mouse; MAIT, The developmental characteristics of some of the most studied subsets of innate-like T cells are represented. Abbreviations: Ags, antigens; DCs, dendritic cells; h, human; IEL, intraepithelial lymphocytes; Itk, interleukin-2-inducible T cell kinase; m, mouse; MAIT, major histocompatibility complex; NKT, natural killer antigens; TCR, T cell receptor; ND, not determined. References: Godfrey and Berzins, 2007; Bendelac et al., 2007; Rodgers and Cook, 2005; Kearney, 2005; Horai et al., 2007; Li et al., 2007; E. Martin, S.L., and O. Lantz, unpublished data; B. Pasquier and S.L., unpublished data.

Table 3. Developmental Characteristics of Some Innate-like T Cells

<table>
<thead>
<tr>
<th></th>
<th>NKT Cells</th>
<th>γδ T Cells</th>
<th>MAIT Cells</th>
<th>IEL CD8αβ T Cells</th>
<th>T-CD4</th>
<th>CD8αβ+ T Cells (Itk-/- Mice)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MHC requirement</td>
<td>CD1d</td>
<td>ND</td>
<td>MR-1</td>
<td>MHC Ia</td>
<td>MHC II</td>
<td>MHC Ia and MHC Ib</td>
</tr>
<tr>
<td>TCR repertoire</td>
<td>Vα8,7/2/Vα14-Jα18 (m); Vα9/Vβ12 (h); Vγ3,4/Vβ1 (m)</td>
<td>Vγ6,8/Vα19-Jα33 (m); Vγ2,13/Vα7,2-Jα33 (h)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Recognized antigen</td>
<td>Glycosphingolipid</td>
<td>Nonpeptidic phospho-Ags (h)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Selecting cells</td>
<td>Thymocytes</td>
<td>B cells or ? DCs</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>IL-15-dependent</td>
<td>Yes</td>
<td>Yes (epithelial/skin)</td>
<td>ND</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>SAP-dependent</td>
<td>Yes</td>
<td>ND</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Other signaling</td>
<td>PKCι, FynT, NFκB</td>
<td>? FynT, ? Lck, Syk</td>
<td>ND</td>
<td>PKCι</td>
<td>CD28</td>
<td>CD28</td>
</tr>
</tbody>
</table>

The developmental characteristics of some of the most studied subsets of innate-like T cells are represented. Abbreviations: Ags, antigens; DCs, dendritic cells; h, human; IEL, intraepithelial lymphocytes; Itk, interleukin-2-inducible T cell kinase; m, mouse; MAIT, major histocompatibility complex; NKT, natural killer T; T-CD4, thymocyte-selected CD4+ T cells; SAP, signaling lymphocytic activation molecule (SLAM)-associated protein; TCR, T cell receptor; ND, not determined. References: Godfrey and Berzins, 2007; Bendelac et al., 2007; Rodgers and Cook, 2005; Kearney, 2005; Horai et al., 2007; Li et al., 2007; E. Martin, S.L., and O. Lantz, unpublished data; B. Pasquier and S.L., unpublished data.

However, unlike conventional lymphocytes, they bear antigen receptors that are usually not polymorphic (sometimes even “invariant”) and that recognize either self-antigens or simple molecular structures from pathogens. Innate-like lymphocytes tend to reside in nonlymphoid tissues and to have markers of memory cells or chronically activated cells, including certain NK cell markers. Lastly, when activated, they exhibit faster and more robust effector functions such as cytokine release, cytotoxicity, or antibody production. Because these properties are typical of innate immune cells, these lymphocytes have been termed “innate-like” lymphocytes. They are thought to serve as a bridge between the rapidly occurring innate immunity and the more slowly occurring adaptive immunity.

Innate-like B cells include B1 cells and marginal zone (MZ) B cells (Kearney, 2005), whereas innate-like T cells include NKT cells, MHC-related 1 (MR-1)-restricted mucosal associated invariant T cells (MAIT cells), H2-M3-restricted T cells, CD8αβ intraepithelial lymphocytes (IELs), γδ T cells, and thymocyte-selected CD4+ T cells (T-CD4 cells) (Table 3) (Godfrey and Berzins, 2007; Bendelac et al., 2007; Rodgers and Cook, 2005; Treiner and Lantz, 2006; Berg, 2007). They also encompass certain populations of CD8αβ T cells that accumulate in mice lacking Tec family kinases such as Itk (Dubois et al., 2006; Brousseau et al., 2006; Atherly et al., 2006). Like conventional T cells, most innate-like T cells arise from DP thymocyte precursors. However, contrary to conventional T cells, which are selected by nonhemopoietic thymic epithelial cells, innate-like T cells are selected by hemopoietically derived thymic cells, most probably other DP thymocytes. Thus, they seem to develop as a consequence of homotypic cell-cell interactions. Although the functions of innate-like T cells are not well understood, there is evidence that they can participate in antimicrobial and antitumor immunity, as well as in immune tolerance (Godfrey and Berzins, 2007; Bendelac et al., 2007; Rodgers and Cook, 2005; Kearney, 2005; Horai et al., 2007; Li et al., 2007; E. Martin, S.L., and O. Lantz, unpublished data; B. Pasquier and S.L., unpublished data.

The prototypical innate-like lymphocytes are the NKT cells (Bendelac et al., 2007; Godfrey and Berzins, 2007; Brigl and Brenner, 2004) (Table 3). Most NKT cells express an invariant Vα24-positive TCR in humans or Vα14-positive TCR in the mouse. They arise from DP thymocytes and are positively selected by self-lipids presented by the class I b MHC molecule CD1d expressed on other DP thymocytes. As discussed above, the development of NKT cells, but not of conventional T cells, is blocked in SAP-deficient humans and mice, providing additional evidence for the unique ontology of these cells (Nichols et al., 2005; Pasquier et al., 2005; Chung et al., 2005). In this issue of Immunity, Griewank et al. pinpointed the block of NKT cell differentiation in SAP-deficient mice to the CD24αCD1d tetramerαCD69β stage, after rearrangement of the canonical Vα14-positive TCR (Griewank et al., 2007). Therefore, it occurs early, at or immediately after the initiation of TCR signaling and positive selection in NKT cell precursors. Considering this, it was proposed that SAP-driven signals may be necessary for the positive selection, proliferation, and/or prevention of negative selection of immature NKT cells. One obvious scenario is that SAP-mediated signals are triggerd by homotypic interactions between SLAM family receptors expressed on DP thymocytes. Compared to other thymocyte subsets, DP thymocytes express the highest amounts of...
SLAM and Ly108. Lower quantities of Ly-9 and CD84 are observed. Importantly, although mice lacking SLAM showed no reduction of NKT cell numbers, mice devoid of Ly108 had a ~50%–70% decrease in NKT cell numbers in thymus, spleen, and liver. Furthermore, using a “pseudo double knockout” of SLAM and Ly108, in which bone marrow cells from SLAM-deficient mice and Ly108- and CD1d-double-deficient mice were coinjected into an irradiated host, evidence was provided that combined lack of SLAM and Ly108 caused an even more severe (~90%) depletion of NKT cells. Hence, SLAM and Ly108 seem to be the predominant SLAM family receptors triggering the function of SAP during NKT cell development.

Another indication of the role of SLAM family receptors in NKT cell differentiation came with the characterization of the Nkt1 locus on mouse chromosome 1 (Jordan et al., 2007). This locus is in part responsible for the reduced NKT cell numbers seen in nonobese diabetic (NOD) mice. Through analyses of congenic mouse strains and RNA expression, it was observed that certain polymorphisms affecting the genes encoding SLAM and Ly108 correlate with the diminished NKT cell numbers. The authors also noted a marked reduction of SLAM expression on DP thymocytes of NOD mice, suggesting that, in the NOD background, SLAM may be largely responsible for the decreased NKT cell numbers.

The involvement of SAP in NKT cell differentiation prompted two groups to examine, in this issue of Immunity, its role in the development of other innate-like lymphocytes. Horai et al. (2007) ascertained the participation of SAP in the development of a population of CD8± T cells prominently found in mice lacking the Tec family kinase, Itk (Dubois et al., 2006; Broussard et al., 2006; Atherly et al., 2006) (Table 3). These cells have features of innate-like lymphocytes, namely constitutive expression of activation or memory markers and rapidly inducible effector functions. Moreover, they are selected in part by class I b MHC molecules expressed on other hemopoietic cells, probably DP thymocytes. Interestingly, by crossing Itk-deficient mice with SAP-deficient mice, Horai et al. determined that SAP was required for selection of these innate-like CD8+ T cells. In a similar way, Li et al. (2007) examined the involvement of SAP in the differentiation of an unusual subpopulation of CD4+ T cells termed T-CD4 (Choi et al., 2005; Li et al., 2005) (Table 3). Although these cells do not exhibit all the characteristics of innate-like lymphocytes, they show the typical fast triggering of effector functions and are selected by MHC molecules expressed on DP thymocytes. Once again, it was found that the development of these cells was critically dependent on SAP. Together, these two provocative reports provide a compelling indication that additional innate-like T cell populations require the SLAM-SAP pathway for their development. One issue with these studies is that the innate-like T cells analyzed were identified in genetically manipulated mice. It remains to be determined whether identical cells exist in normal mice, and whether SAP plays a critical role under a normal setting as well.

It will be important to test the role of SAP in the development of other lymphocyte subpopulations known to have “innate” properties. Nevertheless, in the light of the data available, it is clear that SLAM-related receptors and SAP (SLAM-SAP), as well as, presumably, FynT, carry out a pivotal function in at least some innate-like lymphocytes. The reason for this exquisite dependency is not clearly established. In all likelihood, it relates to the fact that some innate-like lymphocytes are selected at the DP thymocyte stage through interactions with hemopoietically derived cells, probably other DP thymocytes. As a result, their selection may have evolved to rely in a critical manner on homotypic interactions between SLAM family receptors.

A hypothesis for the dependency on SLAM-SAP-FynT may be that this pathway provides signals that are critical for positive selection or survival of these cells. Perhaps, the affinity or avidity of the interactions between their TCR as well as its coreceptors (in some cases, CD4 and/or CD8) and antigen-MHC on hemopoietically derived selecting cells is relatively weak, so that it requires an additional positive signal provided by SLAM-SAP-FynT to drive differentiation or sustain survival. In this model, the SAP-dependent signals would complement quantitatively or qualitatively the TCR-coreceptor-driven signals. An alternative hypothesis is that the SLAM-SAP-FynT pathway mediates signals that inhibit negative selection of these cells. It is plausible that the signals generated by TCR and coreceptor engagement on innate-like T cell precursors are too strong, thereby necessitating an inhibitory signal mediated by SLAM-SAP-FynT to prevent negative selection. Either of these models is consistent with earlier data showing that FynT can have either a stimulatory or an inhibitory impact on antigen receptor signaling in mature conventional T cells (Davidson et al., 2004; Yasuda et al., 2002; Utting et al., 2001; Appleby et al., 1992; Stein et al., 1992).

Recent data indicated that MAIT cells, a subset of innate-like T cells presumed to be selected by B cells or, perhaps, DCs, are not lacking in SAP-deficient humans (E. Martin, S.L., and O. Lantz, unpublished data) (Treiner and Lantz, 2006). Also, IELs are not missing in SAP-deficient mice (B. Pasquier and S.L., unpublished data) (Treiner et al., 1992; Utting et al., 2001; Appleby et al., 1992). It will be important to test the role of SAP in the development of other lymphocyte subpopulations known to have “innate” properties. Nevertheless, in the light of the data available, it is clear that SLAM-related receptors and SAP (SLAM-SAP), as well as, presumably, FynT, carry out a pivotal function in at least some innate-like lymphocytes. The reason for this exquisite dependency is not clearly established. In all likelihood, it relates to the fact that some innate-like lymphocytes are selected at the DP thymocyte stage through interactions with hemopoietically derived cells, probably other DP thymocytes. As a result, their selection may have evolved to rely in a critical manner on homotypic interactions between SLAM family receptors.

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of SLAM family receptors, may not be conducive to productive SLAM family receptor signaling. The second possibility is if other molecules, either coreceptors, adaptors, or others, are able to substitute for SLAM-SAP in some innate-like lymphocytes. One example is CD8αα IELs, which reportedly express high amounts of the SAP-related adaptor EAT-2 (Denning et al., 2007). This may also be the case with innate-like B cells such as B1 cells and MZ B cells, which are not known so far to express SAP. These cells may possess EAT-2 or, perhaps, SLAM family receptor-independent mechanisms that enable them to acquire innate properties in the absence of these pathways.

The SLAM and SAP Families: Regulators of Mature Functions in Conventional Lymphocytes

Unlike innate-like lymphocytes, conventional CD4+ T cells, CD8+ T cells, NK cells, and B cells do not require the SLAM and SAP families for their development. However, some of the mature functions of these cells are regulated by the SLAM-SAP pathway.

SAP plays a critical role in CD4+ T cells. It was reported that purified CD4+ T cells from patients with XLP exhibited reduced interleukin-10 (IL-10) release and expression of inducible costimulator (ICOS) upon activation (Ma et al., 2005). They also had an attenuated capacity to provide B cell help in vitro. Likewise, CD4+ T cells from SAP-deficient mice had decreased secretion of T helper 2 (Th2) cytokines IL-4 and IL-13, diminished ICOS expression, and elevated CD40L expression in response to stimulation with antigen receptor-specific antibodies or antigen-MHC in vitro (Wu et al., 2001; Czar et al., 2001; Davidson et al., 2004; Cannons et al., 2004, 2006). These various abnormalities were accompanied by compromised antibody responses to viruses such as lymphocytic choriomeningitis virus (LCMV), γ-herpesvirus 68, and influenza and parasites like Toxoplasma gondii and Schistosoma mansoni (Cannons et al., 2006; Crotty et al., 2003; Chen et al., 2005; Wu et al., 2001; Czar et al., 2001; Kampschroer et al., 2006). Similarly, diminished antibody production was seen in response to immunization with T cell-dependent protein antigens (Hron et al., 2004; Cannons et al., 2006; Davidson et al., 2004; Morra et al., 2005). The relevance of the CD4+ T cell dysfunction to this altered humoral immunity was confirmed by adoptive transfer experiments (Cannons et al., 2006; Crotty et al., 2003; Kampschroer et al., 2006; Morra et al., 2005).

It is currently unclear which component(s) of the CD4+ T cell dysfunction are responsible for the altered B cell responses in SAP-deficient humans and mice. It does not appear to be the reduced Th2 cytokine secretion per se because correction of this defect did not improve the

Figure 1. Differential Involvement of SLAM Family Receptors and SAP in Conventional and Innate-like T Cell Development

A model of the differential involvement of SLAM family receptors and SAP in the maturation of mouse double-positive (DP) thymocytes into conventional T cells (left), NKT cells (middle), or MAIT cells (right) is depicted. The differentiation of DP thymocytes into conventional T cells results from interactions of the TCR (plus its coreceptors CD4 and CD8; not shown) with peptides presented by classical MHC molecules expressed on thymic epithelial cells (TECs). TECs lack SLAM family receptors and, thus, are unable to utilize the SLAM-SAP pathway to promote conventional T cell differentiation. In contrast, the development of DP thymocytes into NKT cells is mediated by interactions between an invariant Vα14-positive TCR and glycolipids presented by the class Ib MHC molecule CD1d expressed on other DP thymocytes. DP thymocytes express high amounts of SLAM and Ly108, which engage in self-associations between DP thymocytes and mediate SAP-dependent signals that promote NKT cell differentiation. Lastly, the maturation of DP thymocytes into MAIT cells is due to interactions between a semi-invariant Vα19-positive TCR that presumably recognizes hydrophilic peptides presented by the class Ib MHC molecule MR1 expressed on B cells or, possibly, DCs. It is possible that the affinity of the MAIT cell TCR for its ligand is such that it enables differentiation in the absence of any need for the SLAM-SAP pathway. Alternatively, MAIT cells may express other receptors or adaptors (e.g., EAT-2), which provide alternatives to SLAM-SAP.
altered antibody responses of SAP-deficient mice (Cannons et al., 2006; Crotty et al., 2003). Moreover, whereas the role of SAP in Th2 cytokine production is FynT dependent, the function of SAP in antibody production seems to be FynT independent (McCausland et al., 2007; Cannons et al., 2006). It is plausible that the altered expression by activated CD4+ T cells of ICOS or CD40L, two molecules required for productive T cell-B cell interactions, is involved (Cannons et al., 2006). Alternatively, other as yet undetermined defects may be implicated, including compromised secretion of other cytokines important for B cell maturation, impaired migration of CD4+ T cells to germinal centers (GCs; a region crucial for T cell-regulated antibody production), or reduced ability of T cells to form stable contacts with B cells.

Some clues are available regarding the identity of the SLAM family members that control SAP-dependent functions in CD4+ T cells. First, activated CD4+ T cells are known to express SLAM, Ly-9, NTB-A (Ly108), and CD84, suggesting that one or more of these receptors are involved (Table 1) (Veillette, 2006a; Ma et al., 2007; Engel et al., 2003). Second, all these SLAM family receptors bind SAP and can mediate SAP-dependent protein tyrosine phosphorylation signals (Veillette, 2006a; Ma et al., 2007; Engel et al., 2003). And third, partial Th2 cytokine production defects were observed in vitro with CD4+ T cells from gene-targeted mice lacking SLAM, Ly-108, or CD84, suggesting that one or more of these receptors are involved (Table 1) (Veillette, 2006a; Ma et al., 2007; Engel et al., 2003). Second, all these SLAM family receptors bind SAP and can mediate SAP-dependent protein tyrosine phosphorylation signals (Veillette, 2006a; Ma et al., 2007; Engel et al., 2003). And third, partial Th2 cytokine production defects were observed in vitro with CD4+ T cells from gene-targeted mice lacking SLAM, Ly-108, or Ly-9 (Wang et al., 2004; Davidson et al., 2004; Howie et al., 2005; Graham et al., 2006). Thus, several members of the SLAM family may cooperate to enable SAP to regulate CD4+ T cell functions.

Nonetheless, the results obtained with SLAM family receptor-deficient mice should be considered with some caution, as all these mice were studied in a mixed 129/Sv-C57BL/6 background. There are marked sequence and functional variations in the SLAM family gene loci on chromosome 1 between the two mouse strains, and it is possible that the variations in Th2 cytokine release between wild-type and mutant mice were due to sequence differences between the two strains rather than the gene-targeting events (Veillette et al., 2006). Analyses of mice generated in a pure genetic background will be crucial to resolving this issue.

An involvement of SAP in CD8+ T cells is also documented. In vitro studies revealed that CD8+ T cells from SAP-deficient mice exhibited enhanced proliferation and cytokine production in response to stimulation with antigen receptor-specific antibodies (Chen et al., 2005). Moreover, SAP-deficient mice acutely infected by viruses such as LCMV or γ-herpesvirus 68 showed an increased number of virus-specific CD8+ T cells and a greater interferon-γ production by these cells (Wu et al., 2001; Chen et al., 2005). These effects correlated with more efficient viral clearance. The enhancement of CD8+ T cell numbers and functions in SAP-deficient mice is apparently due to decreased activation-induced cell death (Chen et al., 2007). Interestingly, during chronic LCMV infection, SAP-deficient mice also demonstrated a more severe immunopathology characterized by weight loss, lymphoid organ hypocellularity, and death when compared to wild-type mice (Crotty et al., 2006). This illness was reminiscent of the EBV-induced lymphoproliferative syndrome seen in SAP-deficient humans. Importantly, depletion of CD8+ T cells improved the immunopathology of SAP-deficient mice, implying that hyperactive CD8+ T cells were responsible. Based on these various findings, it was postulated that SAP is an inhibitor of CD8+ T cell activation. Such an activity may be crucial to preventing CD8+ T cell-mediated immunopathologies in response to subacute or chronic viral infections.

A study of cytotoxic T cells (CTLs) obtained from SAP-deficient patients suggested an additional role of SAP in CD8+ T cells (Dupre et al., 2005). It was found that CTLs from SAP-deficient patients had a decrease in lytic activity toward EBV-infected B cells. Furthermore, evidence was obtained that this defect was due to a compromised ability of SLAM-related receptor 2B4 to promote polarization of cytolytic granules when engaged by CD48 on target cells. Thus, when CD48 is expressed on target cells, SAP may be a positive regulator of CD8+ T cell-mediated cytotoxicity.

Several studies have reported that NK cells from SAP-deficient patients have reduced cytotoxicity toward CD48-positive target cells, implying that SAP deficiency interferes with the capacity of 2B4 to promote NK cell activation (Parolini et al., 2000; Nakajima et al., 2000; Tangye et al., 2000; Benoist et al., 2000). Likewise, the ability of NTB-A antibodies to stimulate NK cell-mediated cytotoxicity in reverse antibody-dependent cellular cytotoxicity (rADCC) assays was severely compromised (Bottino et al., 2001). Intriguingly, in the absence of SAP, 2B4 and NTB-A become inhibitory, rather than activating, receptors (Parolini et al., 2000; Bottino et al., 2001). This feature would further compromise NK cell functions in the absence of SAP. In contrast to 2B4 and NTB-A, the ability of CRACC to stimulate NK cell-mediated killing in rADCC assays is not affected by SAP deficiency (Bouchon et al., 2001). Hence, unlike its two relatives, CRACC may be promoting NK cell activation through a SAP-independent mechanism. Altogether, these data imply that SAP is a positive regulator of NK cell activation. This is most likely due to the capacity of SAP to couple 2B4 and, perhaps, NTB-A to FynT, when these SLAM family receptors are engaged by their ligands on target cells.

An impairment of the capacity of 2B4 to enhance NK cell-mediated cytotoxicity has also been shown in NK cells from SAP-deficient mice or mice lacking FynT (Bloch-Queyrat et al., 2005). One caveat of this model is that NK cells from 2B4-deficient mice were found to exhibit increased, instead of diminished, killing toward CD48-positive target cells (Lee et al., 2004). Therefore, the dominant effect of engagement of mouse 2B4 by CD48 on target cells may be NK cell inhibition. Paradoxically, it was later reported that 2B4-deficient NK cells had decreased killing toward CD48-negative targets, implying that 2B4 is mediating an activating signal when it is engaged by CD48 on other NK cells (Lee et al., 2006). These complex findings have raised substantial
questions regarding the function and mechanism of action of 2B4, and whether the function of 2B4 may differ between human and mouse NK cells (Veillette, 2006b; Ma et al., 2007). Currently, a satisfactory resolution of these issues has not been achieved.

The involvement of SAP-related adaptors EAT-2 and ERT in the regulation of NK cell activation has been evaluated (Roncagalli et al., 2005). Analyses for purified NK cells from mice lacking EAT-2 or ERT, or from transgenic mice overexpressing EAT-2 or ERT in NK cells, indicated that, contrary to SAP, EAT-2 and ERT are negative regulators of NK cell-mediated cytotoxicity and cytokine production. Importantly, this function occurred even in the absence of coculture of NK cells with target cells, implying that it is evoked by homotypic NK cell-NK cell interactions. Surprisingly, the inhibitory effect of EAT-2 and ERT is not limited to SLAM family receptors, as it also involves other activating receptors such as NKG2D, CD16, NKR-P1c (NK1.1), and Ly49D. However, all these effects seem to depend on recruitment of EAT-2 and ERT to 2B4. The precise effectors mediating the inhibitory impact of EAT-2 and ERT are not known. Notably, inhibition is dependent on tyrosine phosphorylation in the tail of EAT-2 and, presumably, ERT. Thus, it is possible that, by way of their phosphorylated C-terminal tyrosines, EAT-2 and ERT recruit inhibitory molecules with SH2 domains such as presumably, ERT. Thus, it is possible that, by way of their phosphorylated C-terminal tyrosines, EAT-2 and ERT recruit inhibitory molecules with SH2 domains such as ERT.

In contrast to EAT-2, ERT is also inhibitory in the human system (Roncagalli et al., 2005). Nevertheless, another group showed that the ability of SAP-independent SLAM family receptor CRACC to promote NK cell cytotoxicity in human NK cells correlated with binding to EAT-2 (Tassi and Colonna, 2005). Hence, EAT-2 may have the capacity to transduce activating signals for some SLAM family receptors in human NK cells. This hypothesis is also consistent with a recent report indicating that enforced expression of EAT-2 in a T cell line caused an increase in activation responses (Clarkson et al., 2007). Therefore, it is conceivable that human EAT-2 can have either an inhibitory or an activating function, depending perhaps on the cellular context.

As mentioned earlier, SAP-deficient humans and mice exhibit a marked impairment of antibody production. This is accompanied by severe defects in B cell isotype switching, affinity maturation, and memory B cell generation, as well as by a lack of GCs in lymphoid organs (Ma et al., 2005; Hron et al., 2004; Crotty et al., 2003; Al Alem et al., 2005; Morra et al., 2005). We already discussed that at least part of these defects are due to abnormal CD4+ T cell functions. However, an intrinsic B cell dys-function may also exist. In support of this idea, SAP is expressed not only in T cells but also in some B cells, including perhaps GC B cells (Nichols et al., 1998; Al Alem et al., 2005; Morra et al., 2005). Furthermore, one study indicated that naive B cells from SAP-deficient mice demonstrated diminished Ig switch recombination and secretion in vitro (Al Alem et al., 2005). Finally, another group found that transfer of naive B cells from SAP-deficient animals could recreate the impaired B cell functions seen in SAP-deficient mice (Morra et al., 2005). However, three other groups that used an independent SAP-deficient mouse did not confirm this result (Crotty et al., 2003; Cannons et al., 2006; Kampserschroer et al., 2006). Considering all of these, the role of SAP expression in B cells remains controversial. The creation of a conditionally targeted SAP-deficient mouse, in which SAP expression can be selectively ablated in T cells or B cells, will help resolve this debate.

One striking observation made with genetically engineered SAP-deficient mice is that these mice are fully resistant to pristane-induced lupus, an autoimmune disease believed to mimic systemic lupus erythematosus in humans (Hron et al., 2004). In addition, a spontaneous “revertant” of the autoimmune-prone lpr mouse (named “rpl”) was determined to carry a point mutation of SAP resulting in absent SAP protein expression (Komori et al., 2006). In both cases, the resistance to autoimmunity is likely due to reduced T cell-B cell cooperation as result of SAP deficiency.

SLAM family receptors have also been implicated in the pathophysiology of autoimmunity. The Sle1b locus, which confers enhanced susceptibility to lupus in mouse strains like NZM2410, corresponds to the SLAM family gene locus on chromosome 1 (Wandstrat et al., 2004). In particular, a polymorphism in the Ly108 gene that results in altered expression of two splicing isoforms of Ly108 correlated with greater lupus susceptibility in congeneric mouse strains. Evidence suggested that this polymorphism yielded an increase in the responsiveness of mature T cells and a decrease in the deletion of self-reactive immature B cells (Wandstrat et al., 2004; Kumar et al., 2006). A related polymorphism of the SLAM family locus may be associated with human lupus (Tsao et al., 1997; Moser et al., 1998; Shai et al., 1999).

The SLAM and SAP Families in Other Immune Cell Types

Members of the SLAM and SAP families are expressed not only in lymphocytes but also in other hemopoietic cells including DCs, macrophages, granulocytes, platelets, and hemopoietic stem cells (Table 1) (Engel et al., 2003; Veillette, 2006a; Ma et al., 2007). Consequently, they may regulate the functions of these cells as well. In keeping with this, defects in macrophage activation were reported in mice lacking SLAM (Wang et al., 2004). Likewise, compromised neutrophil functions were observed in mice lacking Ly108 (Howie et al., 2005). Can SLAM family receptors on antigen-presenting cells such as DCs and macrophages provide regulatory cues during antigen
presentation by engaging SLAM family receptors on T cells or B cells? While this is an attractive possibility, it must be pointed out that there is up to now no solid genetic evidence supporting this notion.

Conclusions
Recent studies of SAP-deficient humans and mice, as well as of mice lacking individual members of the SLAM family or the SAP-related adaptors EAT-2 and ERT, have provided undeniable evidence that the SLAM and SAP families carry out critical functions in immune cells. There is indication that SAP and certain SLAM family receptors, in particular SLAM and Ly108, mediate crucial signals during the early differentiation of innate-like T cells like NKT cells and others. Three reports published in this issue of *Immunity*, coupled to previous results, provide very compelling evidence in support of this idea. This function most likely relates to the fact that the differentiation of NKT cells and other innate-like lymphocytes is triggered by interactions between two identical immune cells that express matched complements of SLAM family receptors. It probably relies on the property of SLAM family receptors to engage in self-associations, a feature that would enable most efficient signaling in the context of homotypic cell-cell interactions.

There is also solid evidence that SAP-related adaptors and SLAM family receptors transduce signals that modulate the functions of mature conventional lymphocytes, including CD4+ T cells, CD8+ T cells, B cells, and NK cells. Some of these effects, like those on T cell proliferation and cytokine production, appear to be triggered in the context of homotypic cell-cell interactions, at least in in vitro assays. Whether this is also the case in vivo remains to be established. However, effects on other functions such as target cell-dependent cytotoxicity are probably initiated during heterotypic cell-cell interactions, for example during the contact of a cytotoxic lymphocyte with a target cell. Although additional work is needed to elucidate fully the mechanisms involved in SLAM-SAP-dependent functions in mature lymphocytes, it is reasonable to speculate that the SLAM and SAP families mediate these activities in the context of homotypic or heterotypic cell-cell interactions, depending on the function involved.

Lastly, the identification of *SH2D1A* mutations in humans suffering from XLP, coupled with the recent description of XIAP mutations in another subset of XLP patients, has suggested that one of the most critical activities of SAP in vivo is the regulation of innate-like lymphocyte differentiation. Indeed, so far, the only common immune abnormality seen in SAP-deficient and XIAP-deficient humans is a severe contraction of the NKT cell pool. Although the basis for the NKT cell defect is likely to be dissimilar in the two conditions, this observation suggests that the lack of NKT cells and, perhaps, other innate-like lymphocytes may be largely responsible for the propensity of XLP patients to develop EBV-triggered lymphoproliferative illnesses and hemophagocytic syndromes. It also suggests that NKT cells play a central role in normal anti-EBV immunity.

ACKNOWLEDGMENTS
Work in the Veillette laboratory is supported by grants from the Canadian Institutes of Health Research, the National Cancer Institute of Canada, and the Howard Hughes Medical Institute. Work in the Latour laboratory is supported by the Institut National de la Santé et de la Recherche Médicale, the Agence Nationale de la Recherche, and the Ligue Nationale contre le Cancer-Ille de France (France). A.V. holds the Canada Research Chair in Signaling in the Immune System and is a Howard Hughes Medical Institute International Scholar. Z.D. is supported in part by the Pizzagalli Fellowship from the IRCM. S.L. is a Scientist of the Centre National de la Recherche Scientifique (France).

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