SLAM Family Receptors and Autoimmunity

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1. Introduction
The immune system is responsible for the defense against a wide array of pathogens but without responding to each individual’s (self) antigens. Autoimmune diseases are characterized by a loss of tolerance to self antigens that leads to the appearance of auto-reactive lymphocytes. The main factors that contribute to the development of autoimmunity are genetic susceptibility and infection. Disease susceptibility is the result of the combined action of multiple genes. It has been shown that certain gene polymorphisms can influence the establishment of self-tolerance. The human immune system is a complex machinery involving numerous proteins. Cell-surface proteins expressed by leukocytes are of particular relevance due not only to their participation in the network of interactions that regulate the innate and adaptive immune responses, but also to their potential as excellent targets for diagnostic and therapeutic interventions (Diaz-Ramos et al., 2011). These molecules deliver signals that modulate leukocyte development, activation, survival, clonal expansion, and important effector functions. Some of these cell-surface signaling molecules have the capacity to activate lymphocytes and other leukocytes, while others function as down-modulators of immune responses, playing a key role in the establishment of tolerance to self antigens. Thus, it is not surprising that many of the allelic variants associated with autoimmunity identified, to date, correspond to leukocyte cell-surface molecules (Maier & Hafler, 2009). In this review we will discuss recent observations that point to a key role of signaling lymphocyte activation molecule family (SLAMF) receptors in the development of autoimmunity.

2. Signaling lymphocyte activation molecule family of cell-surface molecules
In recent years, the SLAMF of leukocyte cell-surface molecules has been identified as a group of receptors that modulates the activation and differentiation of a wide array of cell types involved in both innate and adaptive immune responses (Calpe et al., 2008; Detre et al., 2010; Schwartzberg et al., 2009; Vinuesa et al., 2010). The SLAMF, also known as the CD150 family, consists of nine structurally related leukocyte cell-surface glycoproteins that belong to the immunoglobulin (Ig) superfamily, namely: SLAMF1 (CD150 or SLAM), SLAMF2 (CD48), SLAMF3 (CD229 or LY9), SLAMF4 (CD244 or 2B4), SLAMF5 (CD84), SLAMF6 (CD352, NTB-A or Ly108), SLAMF7 (CD319 or CRACC), SLAMF8 (CD353 or BLAME) and SLAMF9 (CD84-H1) (Table 1).
<table>
<thead>
<tr>
<th>Receptor</th>
<th>Aliases</th>
<th>Expression</th>
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<tr>
<td>SLAMF1</td>
<td>CD150, SLAM</td>
<td>B, T, DC, platelet, Mϕ</td>
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<tr>
<td>SLAMF2</td>
<td>CD48</td>
<td>B, T, monocyte, NK, DC, pDC, granulocytes, HSC, MPP</td>
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<tr>
<td>SLAMF3</td>
<td>CD229, LY9</td>
<td>B, T, pDC</td>
</tr>
<tr>
<td>SLAMF4</td>
<td>CD244, 2B4</td>
<td>NK, CD8 and γδ T, monocyte, basophil, eosinophil, mast cell, HSC, MPP</td>
</tr>
<tr>
<td>SLAMF5</td>
<td>CD84</td>
<td>B, T, mast cell, platelet, monocyte, granulocytes, Mϕ, DC, pDC, HSC, MPP</td>
</tr>
<tr>
<td>SLAMF6</td>
<td>CD352, NTB-A (Ly108 in mice)</td>
<td>B, T, NK, neutrophil, pDC</td>
</tr>
<tr>
<td>SLAMF7</td>
<td>CD319, CRACC, CS1</td>
<td>B, T, NK, DC, pDC</td>
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<tr>
<td>SLAMF8</td>
<td>CD353, BLAME</td>
<td>B, DC, monocyte, Mϕ</td>
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<tr>
<td>SLAMF9</td>
<td>CD84-H1, SF2001</td>
<td>B, T, monocyte, DC</td>
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Table 1. Members of the SLAM (CD150) family. The expression data apply largely to human cells. Receptor gene name is shown in bold. B=B cells, DC=dendritic cell, pDC=plasmacytoid DC, HSC=hematopoietic stem cells, Mϕ=macrophages, MPP=multipotent hematopoietic progenitors, NK=natural killer cells, SLAMF=SLAM family, T=T cells.

2.1 Genomic organization of the SLAM locus
Seven of the genes encoding SLAMF members are clustered within a 400-500 kilobase (kb) genomic segment on human chromosome 1q23 and on mouse chromosome 1H3 (Calpe et al., 2008; Engel et al., 2003). However, genes coding CD353 and SLAMF9 (CD84-H1) are located outside of, but in close proximity to, the SLAM locus (Calpe et al., 2008; Veillette et al., 2006). This characteristic implies that those genes encoding the SLAMF members were created by successive gene duplications of a single ancestor gene, raising the possibility that numerous polymorphisms and splice variants of most of the family members have subsequently been formed in this way. The majority of such variations mainly affect their corresponding ectodomains or the length of their respective cytoplasmic tails (Calpe et al., 2008; Veillette, 2010). Human EAT-2 and mouse Eat-2a and Eat-2b genes are also located close to the SLAM locus. Although the SLAMF genes are equally arranged in mouse and human genomes, they differ in its orientation; the genes that in humans are closer to the centromere are situated in mice closer to the telomere.

2.2 Structural characteristics of the SLAMF glycoproteins
2.2.1 Immunoglobulin domains and ligand interaction
SLAMF receptors are composed of an extracellular ectodomain formed by two Ig-like domains; one variable (V)-like lacking disulfide bonds followed by a truncated Ig constant 2 (C2)-like domain with two intradomain disulfide bonds, with the exception of CD229 (SLAMF3), which possesses four Ig-like domains (two tandem repeats of V-Ig/C2-Ig sets). SLAMF molecules are type I transmembrane glycoproteins containing a cytoplasmic tail, with the exception of CD48, which has a glycosylphosphatidylinositol (GPI) membrane anchor (Figure 1) (Calpe et al., 2008; Engel et al., 2003; Ma et al., 2007).
Fig. 1. Structural representation of the human SLAM family members. This structurally-related family of cell-surface receptors is composed by nine members. Their extracellular regions have two, or four in the case of CD229, Ig-like domains.

Excluding CD244, which recognizes CD48 as its ligand, SLAMF members are also characterized by acting as self-ligands through their N-terminal Ig domain (Table 2) (Engel et al., 2003). No interactions with other hematopoietic cell-surface molecules have been described, although CD150 (SLAM) has been reported to be one the major receptors for the measles virus, which accounts for its cell tropism (Tatsuo et al., 2000). Strikingly, mouse CD150 has recently been described as a microbial sensor that positively regulates bacterial killing by macrophages (Berger et al., 2010; Sintes & Engel, 2011). CD150 is able to efficiently recognize the porins OmpC and OmpF of *E. coli*’s outer membrane. Afterwards, the CD150/*E. coli* complex becomes internalized within the macrophage phagosome to govern key processes of bacterial removal machinery such as phagosome maturation and free radical species production by the NOX2 complex (Berger et al., 2010). Moreover, CD48 is known to interact with the Gram-negative lectin FimH in macrophages as well as in mast cells, although counter to CD150 functionality, FimH+ bacteria undergo encapsulation in caveolae rather than becoming internalized within mast cell phagosomes (Baorto et al., 1997; Shin et al., 2000). Whether other SLAMF members might function as bacterial receptors remains to be elucidated.
Table 2. SLAM family ligands and ITSMs. H=human, ITSMs=immunoreceptor tyrosine-based binding motifs, M=mouse, ND=not determined, SLAMF=SLAM family.

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Ligands</th>
<th>ITSMs</th>
<th>SAP/EAT-2 recruitment</th>
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<tbody>
<tr>
<td>SLAMF1</td>
<td>SLAMF1, measles virus, Gram-negative bacteria</td>
<td>2</td>
<td>+</td>
</tr>
<tr>
<td>SLAMF2</td>
<td>SLAMF4, CD2, FimH</td>
<td>None</td>
<td>-</td>
</tr>
<tr>
<td>SLAMF3</td>
<td>SLAMF3</td>
<td>H: 2</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>M: 1</td>
<td></td>
</tr>
<tr>
<td>SLAMF4</td>
<td>SLAMF2</td>
<td>4</td>
<td>+</td>
</tr>
<tr>
<td>SLAMF5</td>
<td>SLAMF5</td>
<td>2</td>
<td>+</td>
</tr>
<tr>
<td>SLAMF6</td>
<td>SLAMF6</td>
<td>2</td>
<td>+</td>
</tr>
<tr>
<td>SLAMF7</td>
<td>SLAMF7</td>
<td>H: 1</td>
<td>EAT-2 (H)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>M: 0</td>
<td></td>
</tr>
<tr>
<td>SLAMF8</td>
<td>ND</td>
<td>None</td>
<td>-</td>
</tr>
<tr>
<td>SLAMF9</td>
<td>ND</td>
<td>None</td>
<td>-</td>
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2.2.2 The immunoreceptor tyrosine-based switch motif and cell signaling

Unlike other cosignaling molecules, the cytoplasmic tails of six of the SLAMF receptors (SLAMF1,3-7) do not contain any ITAMs or ITIMs motifs, but rather possess one or more copies of a unique immunoreceptor tyrosine-based switch motif (ITSM) T-I/V-Y-x-x-V/I (where T is threonine, I is isoleucine, V is valine, Y is tyrosine and x denotes any amino acid), in addition to various tyrosine Y residues (Detre et al., 2010; Engel et al., 2003) (Figure 1 and Table 2). In the same way ITAM or ITIM becomes phosphorylated after receptor ligation, the homophilic engagement of SLAMF members triggers the phosphorylation of Y residues within the ITSM. Subsequently, ITSM serves as a docking site for intracellular adapter molecules and enzymes bearing SH2 domains such as SHP-2, SHP-1, Csk, and SHIP-1 (Mikhali et al., 1999; Parolini et al., 2000; Tangye et al., 1999). The adapter molecules SLAM-associated protein (SAP), EWS/FLI activated transcript-2 (EAT-2) and EAT-2-related transducer (ERT), have high affinity for this unique motif (Calpe et al., 2008; Veillette et al., 2009). Importantly, the SAP-encoding gene (SH2D1A) is mutated in patients with X-linked lymphoproliferative disease.

SH2D1A is located on the X chromosome in humans and mice (Xq25 and XA5, respectively), which differs from SLAMF receptors and EAT-2 (Calpe et al., 2008). SAP is a small protein (15 kDa) composed of a SH2 domain followed by a 28-amino-acid tail (Figure 2). Human and mouse SAP molecules share 87% of their amino acid sequence, being highly similar in the SH2 domain. SAP is known to be expressed by NK, T cells, NKT cells, eosinophils, platelets and a subset of B cells (Engel et al., 2003). The SAP/SLAMF-receptor interaction occurs between the arginine 32 (R32), located in the SH2-domain of SAP, and the pY-containing ITSMs of SLAMF molecules. Apart from engaging these pY residues, SAP is able to specifically bind to the nonphosphorylated Y281 from one of the CD150 ITSMs. The high avidity shown by SAP to bind to these pY residues explain its ability to block the interaction of other SH2-containing molecules of lesser affinity to the same motif (Finerty et al., 2002; Howie et al., 2002; Lewis et al., 2001; Poy et al., 1999; Sayos et al., 1998).
Human SAP: | VVRRKKIRSTGKLLAATLGLSTLSlageVVSYYLCVLY2G1Y1TYVKSQ8TETGWSAEILAPGVHRRKPRK | 80
Mouse Sap: | VVRRKKIRSTGKLLAATLGLSTLSlageVVSYYLCVLY2G1Y1TYVKSQ8TETGWSAEILAPGVHRRKPRK | 80
Human EAT-2: | HELIKFERTGQHVRLLKRTSPSRLVRGIALLEETFPVSNSLFDVL | 126
Mouse EAT-2a: | HELIKFERTGQHVRLLKRTSPSRLVRGIALLEETFPVSNSLFDVL | 132
Mouse EAT-2b: | HELIKFERTGQHVRLLKRTSPSRLVRGIALLEETFPVSNSLFDVL | 132

Fig. 2. Alignment of SAP and EAT-2 amino acidic sequences. The SH2 domains of SAP and EAT-2 are boxed. Conserved residues among both adaptors are highlighted in yellow, and those specifically conserved for each molecule are shown in bold. In green are the tyrosine residues present in the tail of EAT-2.

One of the particular features of SAP is that its arginine 78 (R78) interacts with the aspartic acid residue 100 (D100) of Fyn, a Src-related protein tyrosine kinase. Such association sequentially mediates Fyn recruitment to the cytoplasmic tail of the CD150 receptor and, following tyrosine phosphorylation, leads to the recruitment of SHIP, docking protein (Dok) 1, and Dok2 (Chan et al., 2003; Chen et al., 2006; Latour et al., 2001; Latour et al., 2003). Therefore, SAP has the ability to simultaneously associate with SLAMF molecules and Fyn, thus forming a trimolecular complex that also reportedly regulates the activation of Vav-1, Casitas B-lineage lymphoma (Cbl), Bcl-10, and protein kinase C-theta (PKC-theta) (PKC-θ)-mediated activation of NF-κB1 in T cells (Cannons et al., 2004; Cannons et al., 2010b; Claus et al., 2008; Zhong & Veillette, 2008). Furthermore, SAP can additionally engage Lck, which phosphorylates CD84, CD150, CD229 and CD244 (Howie et al., 2002; Martin et al., 2005; Nakajima et al., 2000; Tangye et al., 2003). The SAP-SH2 domain has also been described as interacting with the SH3 domain of PAK-interacting protein (β-PIX), a guanine nucleotide exchange factor (GEF) specific for Rac/Cdc42 GTPases (Gu et al., 2006).

Both human and murine EAT-2 genes are located at chromosome 1 (1q23 in humans and 1H3 in mice), in close proximity to SLAMF loci (Calpe et al., 2006). In contrast to human EAT-2, mouse and rat EAT-2 genes are duplicated with an identical genomic organization and encode two similar proteins, namely EAT-2A or EAT-2 and EAT-2B or ERT (Engel et al., 2003). The EAT-2B-encoding gene is a non-functional pseudo-gene in humans. In a manner similar to SAP, human and mouse EAT-2 genes encode small proteins composed of an SH2 domain followed by a short C-terminal tail, but also containing one and two tyrosines, respectively (Y_{120} and Y_{127}) (Figure 2) (Calpe et al., 2006; Roncagalli et al., 2005). EAT-2 is preferentially found not only in NK cells, but also in DC and macrophages, whereas EAT-2B is detected only in NK cells. Human EAT-2 is expressed by NK cells, activated CD4+ and CD8+ T cells, and γδ T lymphocytes (Calpe et al., 2006; Morra et al., 2001; Tassi & Colonna, 2005).

EAT-2 and EAT-2B can bind to the Src-like kinases Hck, Lyn, Lck, and Fgr kinases through their catalytic domains, although neither can directly bind to the SH3 domain of Fyn since both lack the R78 responsible for the association of SAP with Fyn (Calpe et al., 2006; Latour et al., 2003). Nevertheless, EAT-2 and mouse EAT-2A can couple to the SH2 domain of Fyn in NK cells when their C-terminal tyrosines undergo phosphorylation (Clarkson et al., 2007).
Another significant difference between EAT-2 and SAP is that EAT-2-mediated function has not been properly characterized. It was initially believed that these two adapter molecules played opposing roles in leukocyte activation (Ma et al., 2007). Multiple and more accurate studies have clearly confirmed that SAP is a positive regulator of lymphocyte activation, although data concerning EAT-2 activity remains controversial (Clarkson et al., 2007; Cruz-Munoz et al., 2009; Roncagalli et al., 2005; Tassi & Colonna, 2005; Wang et al., 2010b). Roncagalli et al. first described that, unlike SAP, EAT-2 and ERT were inhibitors of NK cell function when they became associated with CD244 (2B4) in 129Sv mice (Roncagalli et al., 2005). On the other hand, this same group demonstrated that mouse CD319 (SLAMF7) acts as a positive regulator of NK cell in a EAT-2A-dependent manner (Cruz-Munoz et al., 2009). Interestingly, a recent paper has shed light on the role played by EAT-2 in C57BL/6 NK cells. Wang and colleagues have shown that both, EAT-2A and ERT positively regulate mouse CD244- and CD84-specific NK cell functions (Wang et al., 2010b). The authors attribute this disparity in mouse EAT-2 functionality to the genetic background used to generate mice lacking or overexpressing EAT-2. Although there is convincing evidence, using mice with a pure genetic background, that EAT-2 acts as a positive modulator of NK cell functions, further experiments are needed to determine the mechanisms underlying EAT-2 downstream signaling.

It is important to keep in mind that SAP and EAT-2 specifically participate in the recruitment of Src-like kinases at the right time and in a precise cell compartment. In addition, since SAP and EAT-2 can bind to the same ITSM, it has been suggested that both adapter molecules may be able to compete for the same docking site. The outcome of this competition can result in the differential recruitment of intracellular kinases or phosphatases, and thus, in variations in the nature and intensity of activation and differentiation processes.

2.3 SLAMF receptors are expressed on hematopoietic cells

SLAMF receptors display a wide-ranging and differential distribution pattern among hematopoietic cells. They can be found on many immune cell types including different subsets of T and B lymphocytes, NK and NKT cells, monocytes, macrophages, DCs, pDCs, platelets, granulocytes, and hematopoietic stem and progenitor cells (Table 1) (Calpe et al., 2008; Engel et al., 2003; Ma et al., 2007). It should be noted that the analyses of SLAMF expression in mouse have thus far not been as exhaustive as in humans, and therefore some species-specific discrepancies may exist. As summarized in Table 1, their heterogeneous, but sometimes overlapping, expression patterns indicate that SLAMF members may play either redundant or specific functions in the regulation of a broad range of both innate and adaptive immune responses.

Kiel and colleagues first discovered that SLAMF receptors are selectively expressed among primitive mouse progenitors in the adult bone marrow in such a way that it is possible to highly purify HSCs using a simple combination of monoclonal antibodies (mAbs) against three of these receptors (CD150, CD244, and CD48) (Kiel et al., 2005). However, the direct combination of mAbs against SLAMF receptors is not suitable for purification of human HSCs (Sintes et al., 2008).

2.4 SLAMF receptors function as regulators of innate and adaptive immune responses

These receptors have been shown to modulate lymphocyte activation processes that are key elements in the initiation and progression of autoimmune diseases, such as the development
of NKT cells, cytokine production in the thymus and periphery, NK- and CD8+ T-cell cytotoxicity, or germinal center (GC)-dependent antibody production (Figure 3 and Table 3) (Ma et al., 2007; Schwartzberg et al., 2009).

The differentiation of NKT cells and other innate-like lymphocytes appears to be triggered by SAP/Fyn signaling, which occurs when CD150 (SLAMF1) and Ly108 (SLAMF6) both present on the surface of double positive (DP) thymocytes, though not on thymic epithelial cells, homotypically engage (Griewank et al., 2007; Veillette et al., 2007). Additionally, non-obese diabetic (NOD) mice display diminished NKT cell numbers, which has been linked to a deficiency in CD150 expression during the DP thymocyte stage (Jordan et al., 2007). Supporting this concept, a recent paper has shown that impaired CD150 signaling affects the production of IL-4 and IL-10 by NOD mice NKT cells (Baev et al., 2008). Yet another recent report found that CD1d, CD150, Ly108, and SAP expression in DP thymocytes can be controlled by the transcription factor c-Myb. This regulation seems to be highly selective as other SLAMF members located in the same locus, such as SLAMF2, SLAMF3, and SLAMF5, are not affected (Hu et al., 2010). Despite this data, the generation of double or triple knock-out mice for specific SLAMF molecules would not only aid in comprehensively mapping those cell-surface molecules essential to the development of innate-like lymphocytes such as NKT cells, but would also help to precisely identify the overlapping functions of SLAMF receptors. Although EBV is unable to infect mouse cells, several studies of SAP-deficient mice (SAP−/−) have unraveled the various molecular and cellular mechanisms involved in the
<table>
<thead>
<tr>
<th>Receptor</th>
<th>Functions (self-ligation, Ab stimulation or mutant mice)</th>
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<tbody>
<tr>
<td>SLAMF1</td>
<td>T: ↑ IL-4, IFN-(\gamma) secretion&lt;br&gt;(\text{M}\Phi): ↑ bacterial killing, IL-6, IL-12, TNF-(\alpha) secretion&lt;br&gt;DC: ↑ IL-8, IL-12 secretion&lt;br&gt;Platelet: ↑ aggregates stability</td>
</tr>
<tr>
<td>SLAMF2</td>
<td>T: ↑ proliferation, IL-2 secretion&lt;br&gt;(\text{B, NK, DC}): regulates proliferation and activation</td>
</tr>
<tr>
<td>SLAMF3</td>
<td>T: ↓ IFN-(\gamma) secretion, ERK activation&lt;br&gt;↑ IL-2, IL-4 secretion and T-cell proliferation</td>
</tr>
<tr>
<td>SLAMF4</td>
<td>NK, CD8 T: ↑ cytotoxicity, IFN-(\gamma) secretion&lt;br&gt;(\text{Eosinophil}): ↑ killing, cytokines, peroxidase release</td>
</tr>
<tr>
<td>SLAMF5</td>
<td>T: ↑ proliferation, IFN-(\gamma) secretion, (\text{T}_{\text{FH}}) function, T-B cell adhesion&lt;br&gt;(\text{Mast cell}): ↓ Fc(\gamma)-RI-mediated signalling&lt;br&gt;Platelet: ↑ aggregates stability</td>
</tr>
<tr>
<td>SLAMF6</td>
<td>NK: ↑ cytotoxicity, IL-8, IFN-(\gamma), TNF-(\alpha) secretion&lt;br&gt;CD8 T: ↑ cytotoxicity, IFN-(\gamma) secretion&lt;br&gt;CD4 T: (\text{Th1}) polarized response&lt;br&gt;(\text{Neutrophil}): ↑ bacterial killing, ROS and cytokine production</td>
</tr>
<tr>
<td>SLAMF7</td>
<td>B: ↑ proliferation&lt;br&gt;NK: ↑ cytotoxicity and killing</td>
</tr>
<tr>
<td>SLAMF8</td>
<td>ND</td>
</tr>
<tr>
<td>SLAMF9</td>
<td>ND</td>
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</table>

Table 3. Functions of SLAMF members. Ab= antibody, B=B cells, DC=dendritic cell, IFN=interferon, IL=interleukin, \(\text{M}\Phi\)=macrophages, ND=not determined, NK=natural killer cells, SLAMF=SLAM family, \(\text{T}=\text{T cells, Th1}=\text{Th1 helper cell}\).

pathogenesis of XLP. In contrast to XLP patients, mice lacking SAP exhibit increased levels of CD8\(^{+}\) T cell cytotoxicity compared with their wild-type (\(\text{wt}\)) counterparts (Chen et al., 2005; Crotty et al., 2006; Czar et al., 2001; Wu et al., 2001). After acute infection with lymphocytic choriomeningitis virus (LCMV) mice presented elevated levels of Ag-specific and IFN-\(\gamma\) secreting CD8\(^{+}\) T cells. However, these mice died since were unable to resolve chronic infections (Crotty et al., 2006; Czar et al., 2001; Wu et al., 2001). Concomitantly, SAP\(^{-/-}\) mice can also present compromised antibody responses to viruses such as murine \(\gamma\)-herpesvirus-68 (MHV-68) and influenza, as well as to parasites like \textit{Toxoplasma gondii} and \textit{Leishmania major} (Chen et al., 2005; Czar et al., 2001; Kamperschroer et al., 2006; Wu et al., 2001; Yin et al., 2003).

Multiple studies have clearly demonstrated the existence of a specific defect in CD4\(^{+}\) T cell immunity. As in humans, SAP\(^{-/-}\) mouse CD4\(^{+}\) T cells are afflicted with such a defect; namely, they fail to properly differentiate into Th2 cells, subsequently presenting reduced levels of IL-4 (derived from diminished GATA-3 transcription factor levels), IL-10, and IL-
13. It has also been reported that SAP-mediated IL-4 release is dependent upon Fyn. On the other hand, Th1 cytokines such as IFN-γ typically become elevated (Cannons et al., 2004; Czar et al., 2001; Davidson et al., 2004; Wu et al., 2001). In addition, Wu et al. demonstrated that following infection with the parasite L. major, which is dependent upon Th2 cytokines to induce disease, SAP−/− mice became more resistant to the parasitic infections (Wu et al., 2001). XLP patients exhibit an extreme deficiency in IL-10 secretion by CD4+ T cells, but not in either IL-4 or IFN-γ production (Ma et al., 2005). In this same study Ma et al. reported that upon Ag-stimulation of CD4+ T cells, ICOS (CD278) levels are reduced in XLP patients in the same way that occurs in SAP−/− T cells (Cannons et al., 2006; Ma et al., 2005).

Another group of defects noted in both SAP−/− mice and XLP patients concern B cell-mediated responses, including the absence of GC formation and deficient humoral responses to T cell-dependent antigen following viral infection or immunization (Cannons et al., 2006; Crotty et al., 2003; Hron et al., 2004). In this regard, diminished numbers of memory B cells in the peripheral blood, as well as long-lived plasma cells, are usually observed in SAP−/− mice. As a consequence, low titers of serum antibodies are detected (Crotty et al., 2003; Czar et al., 2001; Ma et al., 2005; Qi et al., 2008; Yin et al., 2003). It was initially postulated that these alterations might largely stem from defects inherent to B-cell responses, even though it remains unclear whether or not B cells express SAP. However, compelling and abundant evidence indicates that the defective help provided to B cells by SAP−/− CD4+ T<sub>FH</sub> cells is responsible for this impaired GC formation (Cannons et al., 2006; Crotty et al., 2003; Ma et al., 2005). The help that T cells, namely T<sub>FH</sub>, provide to GC B cells is widely known to be essential to the effective production of memory B cells and long lived plasma cells, as well as for successful Ig class switching and antibody affinity maturation (Vinuesa et al., 2005; Vinuesa et al., 2010). This direct role played by T cells was confirmed by adoptive transfer of wt CD4+ T cells to SAP−/− mice, since they were able to abrogate this GC defect (Cannons et al., 2006; Crotty et al., 2003; Morra et al., 2005). Concomitantly, an excellent study from Qi and colleagues revealed that SAP deficiency selectively impairs the capacity of CD4+ T cells to firmly interact with cognate B cells, but not with DCs (Qi et al., 2008). SAP−/− mice exhibit impaired recruitment and retention of T cells within the emerging GC, a defect which abrogates the GC reaction’s sustainability. Along this same line of investigation, this group has recently reported that mouse CD84 and Ly108 are required for long-lasting T-cell:B-cell contact, optimal T<sub>FH</sub> function, and GC formation, although to a lesser extent compared with SAP−/− mice (Cannons et al., 2010a). Nevertheless, how cognate T:B interactions are influenced by SLAMF/SAP-mediated signals has not been fully elucidated.

3. Role of SLAMF receptors in autoimmune disease susceptibility

Multiple cellular and molecular mechanisms are required to maintain self-tolerance, and failure at any of these checkpoints can precipitate tolerance breakdown and lead to autoimmunity. Autoimmune diseases are characterized by variable etiologies and courses of pathogenesis, principally due to the different ways tolerance breakdown occurs. A wide array of genomic association studies suggests that the heterogeneous and alternative contribution of various genetic factors determines to some extent autoimmune disease susceptibility (Vyse & Todd, 1996; Wandstrat & Wakeland, 2001). Interestingly, the functional pathways that are defective in several human and murine autoimmune conditions frequently overlap (Krishnan et al., 2006; Morel, 2010). Chromosome 1 comprises
a large amount of polymorphic genes related to an assortment of autoimmune disorders such as systemic lupus erythematosus (SLE), inflammatory bowel diseases (IBD), rheumatoid arthritis (RA) or multiple sclerosis (Morel, 2010; Tsao et al., 1997; Vyse & Todd, 1996; Wandstrat et al., 2004).

3.1 SLAM locus haplotypes and polymorphisms in mice and humans systemic autoimmunity

A wide array of clinical manifestations are associated with human and mouse SLE, an autoimmune condition in which both environmental factors and a predisposing genetic background contribute to its development. This pathology is clearly marked by a humoral autoimmune component derived from the loss of tolerance to nuclear Ag due to the production of antinuclear antibodies (ANA) such as anti-chromatin and anti-ss or dsDNA. These functional abnormality result in the accumulation of immune complex deposits in the kidney that can ultimately lead to fatal nephritis (Crispin et al., 2010; Fairhurst et al., 2006; Krishnan et al., 2006). Given the important immunoregulatory functions of the SLAMF receptors described above, it is not surprising that there is increasing evidence of their contribution to autoimmune disease susceptibility, particularly for SLE, but also diabetes. In fact, two major susceptibility loci for these two diseases, \( \text{Sle1b} \) and \( \text{Nkt1} \), correspond to the locus on chromosome 1 where the genes encoding for the SLAM receptors are located (Wang et al., 2010a). Genetic and genomic analysis of this locus has revealed a high degree of polymorphism both in mice and humans. Studies in mice have allowed the identification and characterization of two major haplotypes of this locus: the haplotype 1, represented by C57BL/6 and related strains and the haplotype 2 by BALB/c and strains of mice that develop auto-antibodies spontaneously, e.g. NZB/NZW and NZM2410 (Furukawa et al., 2010; Morel et al., 2001; Wandstrat et al., 2004; Wang et al., 2010a). The differences between these two haplotypes are mainly based on: a) genomic structural variations (for example, an increase from one to four in the number of copies of CD244); (b) nonsynonymous mutations in the ligand binding domains of CD229, CD84 and CD48; (c) changes in the levels of transcription of some SLAMF genes; (d) and changes in the expression of isoforms generated by alternative splicing of some members of the family (Wang et al., 2010a). In the complex task of studying SLE pathogenesis, the contribution of mouse models has been extremely helpful due to their ability to closely mimic human SLE (Morel, 2010). In particular, mapping analysis of the autoimmune-prone NZM2410 (NZB x NZW, F1) mouse strain, which bears all the susceptibility Sle loci (\( \text{Sle1, Sle2, and Sle3} \)), revealed that these animals can fully develop SLE. These loci can independently cause a loss of tolerance to chromatin, the extent of which can differ over various serological and cellular phenotypes (Morel et al., 2001). Congenic mice (\( B6.\text{Sle1b} \)), derived from the mouse strains NZM2410 (NZB x NZW/F1) and C57BL/6, that contain the Sle1b locus (haplotype 2) in a haplotype 1 background produce high titers of anti-nuclear antibodies and develop lupus (Figure 4) (Morel et al., 2001; Wandstrat et al., 2004). Thus, if a gene in this region of chromosome 1 is knockout through homologous recombination in 129-derived embryonic stem cells (ES cells) and the resultant mouse is backcrossed with B6, the interpretation of the phenotype of the mutant mouse may be affected by epistatic interactions between the 129 and B6 genomes. This has been recently observed by analysing the phenotype of knockout mice of two SLAMF genes (SLAMF1 and SLAMF2), which were generated with a 129-derived ES cell line. While \( \text{Slamf1}^{-/-} \) and \( \text{Slamf2}^{-/-} \) mice develop features of lupus if backcrossed on to the B6 genetic background [B6.129], \( \text{Slamf1}^{-/-} \) and \( \text{Slamf2}^{-/-} \) mice, backcrossed on the BALB/c
background [BALB/c.129], do not manifest any sign of autoimmune disease (Keszei et al., 2011a).

Genetic linkage and association studies of families containing SLE patients as well as case-control studies of populations have identified several linkage regions, including one at 1q23, which contains multiple susceptibility genes, such as those present in the SLAM locus (Tsao et al., 2002). Indeed, the 1q23 locus has been identified in several genome-wide scans in humans and it has been replicated in subsequent linkage studies that have targeted this region (Moser et al., 1998; Shai et al., 1999).

Furthermore, as already mentioned, the syntenic region in mice has also been related to different mouse models of spontaneous lupus (Morel et al., 2001). Interestingly, SAP−/− mice (129SvJ background) are resistant to experimentally pristane-induced lupus. A deficiency in Sh2d1a abrogates the development of hypergammaglobulinemia, autoantibodies including anti-dsDNA, and renal disease (Hron et al., 2004). However, the mechanisms by which this SAP deficiency protects mice against lupus remain to be elucidated.

A family-based association study of UK and Canadian families with SLE has revealed multiple polymorphisms (SNPs) in the promoter and coding region of two members of the SLAMF, SLAMF3 (CD229) and SLAMF7 (CD319) (Cunninghame Graham et al., 2008). The authors of this study found that the strongest association was with a nonsynonymous SNP (rs509749) in exon 8 of SLAMF3 (CD229). This Val602Met change in the cytoplasmic tail lies within the consensus binding site for SAP and may therefore affect downstream signaling events of SLAMF3. The risk allele of this variant was found associated with decreased numbers of CD4+ naïve T cells and activated T cells and with increased numbers of CD8+ memory T cells. According to the authors, the skewing in the T-cell populations may indicate a state of chronic T-cell activation (Cunninghame Graham et al., 2008). Despite of these data, the association of this polymorphism with SLE has not been replicated in independent cohorts of SLE patients both of Japanese and European origin (Suarez-Gestal et al., 2009; Suzuki et al., 2008). Polymorphisms in another member of the SLAMF, namely SLAMF4 (CD244), have also been found associated with rheumatoid arthritis (RA) and SLE.
In one large-scale, case-control association study, two SNPs (rs3766379 and rs6682654) were found associated with increased susceptibility to RA in two independent cohorts from Japan. Interestingly, the genotype distribution of these SLAMF4 (CD244) SNPs in a SLE cohort was similar to that in the RA cohorts, suggesting that these polymorphisms in SLAMF4 (CD244) increase the risk for developing RA as well as SLE (Suzuki et al., 2008). In a recently published report, the SNP (rs3766379) in the SLAMF4 (CD244) gene was also found significantly associated with the susceptibility to SLE in another cohort of individuals of Japanese origin. This association was preferentially observed in subsets of SLE patients with nephritis and neuropsychiatric lupus (Ota et al., 2010). Taken together, and despite some conflicting results, these studies clearly indicate a high degree of polymorphism among the SLAMF genes and suggest the contribution of some of them in conferring susceptibility to autoimmunity. Further investigations are needed to determine the precise role and mechanism of these cell-receptors and their variants in increasing the risk to develop autoimmune diseases.

### 3.2 SLAMF spliced variants and their role in autoimmunity

As mentioned above, polymorphisms of the SLAMF genes also result in the differential expression of isoforms generated by alternative splicing. These variations in splice isoform expression are likely to have functional consequences and have also been implicated as candidates for other autoimmune susceptibility loci (Evsyukova et al., 2010; Gillett et al., 2009; Muschen et al., 1999; Ueda et al., 2003).

One of the strongest candidates of the SLAMF linked to lupus susceptibility in mice is Ly108 (CD352, SLAMF6). The polymorphism in Ly108 results in the expression of two alternatively spliced isoforms which differ exclusively in their cytoplasmic region (Wandstrat et al., 2004). These two isoforms, Ly108-1 and Ly108-2, are differentially expressed between normal mice and mice susceptible to lupus: whereas the expression of Ly108-1, with two motifs ITSM, is increased in the B and T cells of lupus-prone mice, Ly108-2, with three motifs ITSM, is increased in these cells in normal animals (Kumar et al., 2006). The higher expression of the isoform Ly108-1 in lymphocytes of lupus–prone mice is associated with increased survival rates and ill-suited elimination of autoreactive B cells, resulting in increased autoantibody production (Kumar et al., 2006). Regardless of the fact that it bears an ITSM less than Ly108-2, Ly108-1 is more apt than Ly108-2 to trigger SAP-mediated tyrosine-phosphorylation signals, which involve Vav-1 and c-Cbl in T cells (Zhong & Veillette, 2008). Recently, Ly108 has been reported to promote long-lived stable T:B cell contacts (Cannons et al., 2010a). Since functional defects in both T and B lymphocytes are required for ANA production, it is possible that dysregulation of Ly108 isoform downstream signaling (derived from T:B engagement) might lead to disruption of peripheral tolerance and triggering of the autoimmune process in SLE. A third protein isoform, Ly108-H1, which is absent in two lupus-prone congenic animals has been recently identified (Keszei et al., 2011b). Ly108-H1 is encoded by a splice variant of Ly108 that lacks both exons 7 and 8. Transgenic mice expressing Ly108-H1 isoform present a dramatic reduction of CD4+ T cell–dependent autoimmunity in congenic B6.Sle1b mice, demonstrating that an immune response–suppressing isoform of Ly108 can regulate the pathogenesis of lupus. Nonetheless, how Ly108 isoform-mediated signals are able to breach this tolerence remains to be clarified. Interestingly, SLAMF6-driven co-stimulation of human peripheral T cells is defective in SLE T cells (Chatterjee et al., 2011).
Spliced variants of SLAMF receptors have been also studied in humans. Human activated T cells express, in addition to membrane-form of SLAMF1, mRNA encoding a soluble secreted form of SLAMF1 (sSLAMF1) lacking 30 amino acids (aa) encompassing the entire 22-aa transmembrane region (Cocks et al., 1995). This soluble isoform may play a role in immunomodulation since sSLAM induces proliferation of purified B cells, but also Ig synthesis by these cells (Punnonen et al., 1997).

Most importantly, an altered expression of two SLAMF receptors in humans, SLAMF4 (CD244) and SLAMF7 (CD319), as well as a differential expression of isoforms of these molecules has been described in PBMCs from patients with SLE (Kim et al., 2010). Two different splice variants of human SLAMF4 (CD244), h2B4-A and h2B4-B, with different functional roles in human NK cells, had been previously identified by the same authors (Kumaresan & Mathew, 2000; Mathew et al., 2009). While both isoforms share the same intracellular domain, h2B4-B has five additional amino acids between the V and the C2 regions and is differentially regulated in SLE patients. In contrast, the two SLAMF7 (CD319) isoforms described, CS1-L and CS1-S, have identical extracellular domains but differ in their cytoplasmic tail. CS1-S lacks the two ITSM required for intracellular signaling and while CS1-L functions as an activating receptor, CS1-S does not show any signaling function in NK cells (Lee et al., 2004). Whereas healthy individuals express three-to sevenfold higher levels of CS1-L over CS1-S, this expression ratio is altered in SLE patients. This differential expression of both isoforms in PBMCs of SLE patients is reminiscent of Ly108 expression in lupus-prone mice (Kim et al., 2010).

Thus, an emerging concept derived from these and other studies, is that the differential expression of SLAMF receptor isoforms may contribute to susceptibility to break self-tolerance. In addition to the findings described above, cDNAs enoding SLAMF receptors that lack an extracellular domain, part of the cytoplasmic tail or the transmembrane segment, have been found in different databases (Ensemble, NCBI, EC gene). All these cDNAs, generated by alternative splicing, are mainly based on ESTs (Expressed Sequence Tags) and require experimental validation. Although it is not yet known if they are expressed as proteins, their expression would clearly have functional consequences, as it has been demonstrated in the case of Ly108 in mice. Indeed, the lack of an extracellular Ig domain can directly affect the recognition and the binding to the ligand, and changes that affect the length of the cytoplasmic tail can dramatically affect signal transduction. Preliminary data from our laboratory confirm the existence at the protein level of some of the isoforms predicted for CD84 (SLAMF5) and CD229 (SLAMF3) molecules (unpublished results). Altogether, these data suggest a critical role of aberrant expression of SLAMF spliced variants in conferring susceptibility to autoimmune diseases, in particular to SLE.

4. Conclusion

Lessons from genetic studies in mice have been key to support the hypothesis that SLAMF receptors function as disease modifiers and/or susceptibility factors of systemic autoimmunity. These studies are especially relevant since the phenotype of genetically manipulated mice is very similar to that in SLE patients, with the production of autoantibodies as well as multiorgan involvement, including severe nephritis. Although the interpretation of the phenotypes of knockout mice of the SLAMF receptors has been complicated by issues related to genetic background, all the data clearly underscore that these receptors play a critical role in the development of autoimmune diseases. An emerging
concept is that aberrant alternative splicing plays an important role in the pathogenesis of autoimmune diseases. Studies reviewed in this chapter show that SLAMF isoforms expression appears to be altered in lupus patients. We believe that the study of the interplay between SLAMF isoforms in SLE patients will help identify pathways regulated during autoimmune processes, giving further insight into mechanisms underlying disease susceptibility and possible therapeutic approaches.

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6. References


The present edition entitled "Autoimmune disorders - Pathogenetic aspects" aims to present the current available evidence of etiopathogenetic insights of both systemic and organ specific autoimmune disorders, the crossover interactions among autoimmunity, cardiovascular morbidity and malignancy as well as novel findings in the exciting fields of osteoimmunology and immunology of pregnancy.

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