Commentary

SLAM Family Receptors Regulate Immunity with and without SAP-related Adaptors

André Veillette

Laboratory of Molecular Oncology, Clinical Research Institute of Montreal, Montréal, Québec, Canada H2W 1R7
Department of Microbiology and Immunology and Department of Medicine, McGill University, Montréal, Québec, Canada H3G 1Y6
Department of Medicine, University of Montréal, Montréal, Québec, Canada H3C 3J7

Two papers describing mice deficient in signaling lymphocyte activation molecule and 2B4 represent the first accounts of immune phenotypes in animals lacking members of the SLAM family of receptors. The findings provide definitive evidence of the importance of SLAM-related receptors in the regulation of T cell, macrophage, and natural killer cell functions.

The SLAM Family of Immune Cell Receptors. The signaling lymphocyte activation molecule (SLAM) family is a group of six immunoglobulin-like receptors named SLAM (or CD150), 2B4 (or CD244), Ly-9 (or CD229), NK-T-B antigen (NTB-A) (or Ly-108), CD84 and CD2-like receptor activating cytotoxic T cells (CRACC) (1, 2). These receptors are differentially expressed in various immune cell types and are close relatives of the CD2 family. Some, including SLAM, NTB-A, and CRACC, are self-ligands. At least one, 2B4, is implicated in heterotypic interactions with another immune cell receptor, CD48. SLAM is also the immune cell-specific receptor for measles virus in humans. Until now, little was known regarding the physiological functions mediated by the SLAM family. Data from experiments in which cells were treated with antibodies or ligands presumed to stimulate SLAM-related receptors, although at times contradictory, suggested that SLAM-related receptors modulate the immune response.

The notion that SLAM family receptors are immunoregulatory was supported by the observation that they associate, by way of cytoplasmic tyrosine-based motifs, with the small SH2 domain–containing adaptor SAP (2, 3). SAP is expressed in T cells and NK cells and is mutated in X-linked lymphoproliferative (XLP) disease, a human immune disorder characterized by a dysregulated immune response to Epstein-Barr virus (EBV) infection. Since SAP allows SLAM-related receptors to associate with the Src-related protein tyrosine kinase Fyn and to mediate protein tyrosine phosphorylation signals (4–6), it was postulated that XLP may be the result of inadequate SLAM family receptor functions in cells normally expressing SAP. In agreement with this, defects in $CD4^+$ and $CD8^+$ T cell functions were described in SAP-deficient mice (7–9). The impact of SAP deficiency on NK cell functions in mice remains to be examined.

In spite of the mounting evidence that SLAM-related receptors utilize SAP in their signaling mechanism, some data suggest that they can mediate functions in the absence of SAP (1, 2). First, SLAM-related receptors are abundantly expressed in cell types that do not express SAP, including macrophages and DCs. These cell types and NK cells contain a SAP homologue termed EAT-2, which can associate with SLAM-related receptors (1, 2). Whereas the role of EAT-2 in cell signaling is not known, EAT-2 lacks the motif that enables SAP to activate Fyn. On that basis, EAT-2 may have a function distinct from that of SAP. Second, antibody-mediated engagement of 2B4 on NK cells derived from XLP patients was reported by one group to inhibit, rather than activate, NK cell–mediated killing (10). Thus, 2B4 may mediate an alternate signal, leading to NK cell inhibition, in the absence of SAP. Whether this signal is due to EAT-2 is not known. Third, SLAM-related receptors were reported to associate with other molecules (such as the protein tyrosine phosphatase SHP-2) and to mediate certain types of biochemical signals (such as Akt activation) in the absence of SAP. Nevertheless, these data were primarily generated through overexpression studies in nonimmune cells, and their physiological significance is unclear.

Defects in $CD4^+$ T Cell and Macrophage Functions in SLAM-deficient Mice. SLAM is a homotypic receptor found on T cells, B cells, macrophages, and DCs (1, 2). Its expression is typically low in resting cells and is augmented upon cell activation. A possible role for SLAM was first characterized in human T cells (11). Engagement of SLAM by anti-SLAM mAbs evoked TCR-independent $CD4^+$ T cell proliferation and IFN-γ secretion, but not IL-4 production, by previously activated human T cells. This led to the idea that SLAM was a novel activating receptor on T cells that favored T_H1 cytokine production. Subsequent studies with mouse T cells failed to confirm that antibody-mediated SLAM ligation was sufficient to trigger T

Address correspondence to André Veillette, IRCM, 110 Pine Ave. West, Montreal, Quebec, Canada H2W 1R7. Phone: (514) 987-5561; Fax: (514) 987-5562; email: veillea@ircm.qc.ca

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cell activation (12). Nonetheless, SLAM-specific antibodies moderately enhanced mouse T cell proliferation and IFN-γ secretion in response to low doses of anti-CD3 mAb. This effect was also observed in T cells derived from SAP-deficient mice, indicating that these responses were SAP independent (Fig. 1 A; reference 13). However, a significant limitation of these studies was that it was not known whether the anti-SLAM antibodies were triggering or blocking the function of SLAM.

In this issue, Wang et al. report the phenotype of mice lacking SLAM (14). They found that *Slam*$^{-/-}$ CD4^{+} T cells had a severe defect in TCR-mediated production of IL-4 in vitro. Surprisingly, secretion of IFN-γ was only slightly enhanced. Although the impact of SLAM deficiency on IL-4 provides evidence for the importance of SLAM in immunity, the lack of a substantial effect on IFN-γ is intriguing. Most likely, there is redundancy with other receptors having IFN-γ–inducing functions that compensate for the absence of SLAM. In support of this, it is known that T cells express other SLAM family receptors, including Ly-9 and NTB-A. The small increase in IFN-γ production observed in SLAM-deficient T cells also suggests that SLAM signaling may inhibit rather than augment IFN-γ release. Therefore, the anti-SLAM antibodies used in previous studies were possibly antagonists.

Considering the marked defect in IL-4 production in SLAM-deficient CD4^{+} T cells, the lack of an effect of anti-SLAM antibodies on IL-4 release is perplexing. One interpretation is that anti-SLAM antibodies impinge on only part of the function of SLAM, either by affecting selective SLAM–SLAM interactions or by altering the surface distribution of SLAM. Consequently, anti-SLAM antibodies may influence the coupling of SLAM to pathways regulating IFN-γ without interfering with those controlling IL-4. A clearer understanding of these contradictory observations will likely be provided by a better biophysical and biochemical analysis of SLAM and SLAM-associated signaling pathways in T cells.

Similar to SLAM-deficient T cells, T cells from *Sap*$^{-/-}$ mice have markedly reduced IL-4 production (7, 8). This similarity implies that SLAM regulates IL-4 release through its interaction with SAP (Fig. 1 A). Moreover, it suggests that the defect in IL-4 production in *Sap*$^{-/-}$ T cells is largely due to a block in SLAM receptor signal transduction. However, the IL-4 defect was more drastic in T cells from *Sap*$^{-/-}$ animals than *Slam*$^{-/-}$ animals, raising the possibility that other SLAM family receptors regulate IL-4 through a SAP-dependent pathway. In spite of the clear link between SLAM and IL-4, it must be emphasized that the defects in CD4^{+} T cell help seen in vivo in SAP-deficient mice do not seem to be due to a simple lack of IL-4 production (9). Other as yet unidentified T_H2-related alterations appear to be implicated. Hence, to establish the relative importance of SLAM dysfunctions in SAP-dependent immune defects it will be crucial to assess the extent of functional T cell abnormalities in vivo in *Slam*$^{-/-}$ mice.

SLAM-deficient macrophages were found to exhibit abnormal functions in vitro and in vivo (14). Production of IL-12, TNF-α, and nitric oxide (NO) in response to LPS, with or without IFN-γ, was diminished in *Slam*$^{-/-}$ peritoneal macrophages. Furthermore, IL-6 secretion in response to LPS alone was enhanced. In contrast, IL-12 and TNF-α secretion triggered by Toll-like receptor agonists, such as CpG DNA and peptidoglycan, and the capacity of macrophages to present antigen to T cells were normal. In vivo studies showed that the ability of C57BL/6 mice to clear *Leishmania major* infection was compromised in the absence of SLAM. In this mouse strain, healing of *Leishmania major* lesions is known to rely heavily on macrophage functions, including IL-12, TNF-α, and NO production.

The mechanism by which SLAM regulates macrophage responses to LPS was not established. Since stimulation with anti-SLAM antibodies was sufficient to trigger IL-12 secretion and inhibit IL-6 release by inflammatory macrophages, it is plausible that SLAM–SLAM interactions between neighboring macrophages induce a signal that influences cytokine release in response to LPS. However, given the similarities between LPS, CpG, and peptidoglycan signaling, it is not clear why the lack of SLAM expression would only affect LPS-triggered responses. Moreover, the nature of the SLAM signals regulating IL-12 and IL-6 synthesis in macrophages remains to be determined. Macrophages do not express SAP but do express EAT-2 (2, 3). As discussed above, EAT-2 may or may not play a role analogous to that of SAP in SLAM family receptor signaling. It is also conceivable that SLAM regulates macrophage function independently of SAP-related adaptors. The characterization of SLAM-induced biochemical signals in macrophages and of the role of EAT-2 in these cells will help clarify these issues.

2B4-deficient Mice Reveal an Inhibitory Role for 2B4 in NK Cells. 2B4 is found on NK cells, CD8^{+} T cells, γδ T cells, and monocytes (1, 2). Its ligand is CD48, a glycophosphatidylinositol (GPI)-linked receptor broadly expressed on immune cells. Previous studies showed that engagement of 2B4 on human or mouse NK cells by anti-2B4 antibodies results in production of IFN-γ (1, 2). Furthermore, anti-2B4 antibodies trigger NK cell–mediated killing in reverse antibody-dependent cellular cytotoxicity assays. These effects were observed to be seriously compromised in XLP-derived NK cells, implying that they rely on the ability of 2B4 to associate with SAP in humans. Engagement of 2B4 expressed on CD8^{+} T cells by CD48 was also reported to enhance TCR-mediated cytotoxicity (15). Thus, together these results led to the belief that 2B4 is an activating receptor, which augments the responsiveness of NK cells and CD8^{+} T cells.

In this issue, Lee et al. report the phenotype caused by lack of 2B4 expression in mice (16). Unexpectedly, they observed that cell-mediated cytotoxicity and IFN-γ secretion was enhanced rather than diminished in *2b4*$^{-/-}$ NK cells. The ability of NK cells to eliminate tumor cells in an intraperitoneal tumor clearance assay was also increased. These altered functions were fully dependent on expression

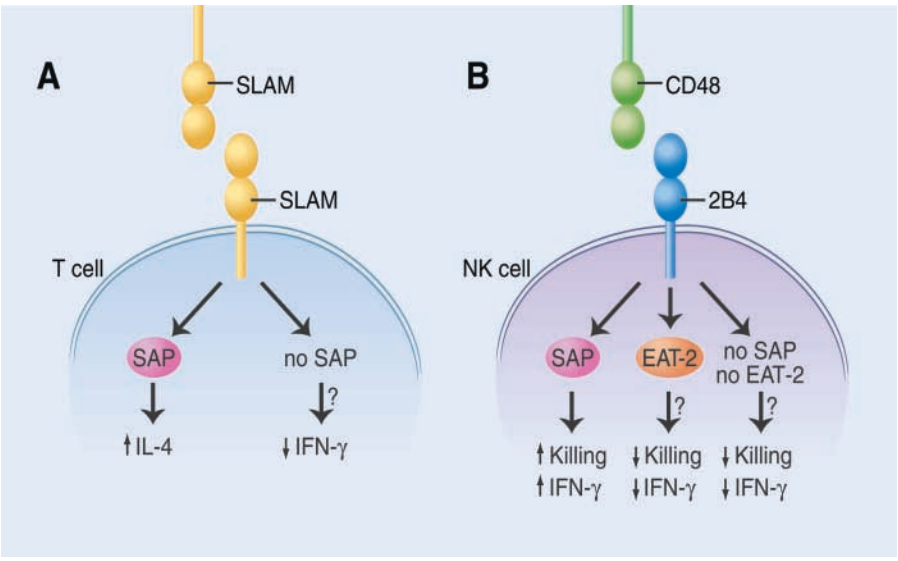

Figure 1. Functions of SLAM family receptors are regulated by differential expression of SAP-related adaptors. (A) Model of SLAM function. In T cells expressing SAP, SLAM–SLAM interactions trigger selective up-regulation of IL-4 secretion in response to antigen receptor stimulation. Although the downstream targets responsible for this activity are not yet understood, they may involve previously described SLAM-SAP effectors, such as Fyn, SHIP-1, Dok-1, Dok-2, Shc, and Ras-GAP (5). In the absence of SAP, SLAM could mediate a different signal, which may lead to down-regulation of IFN-γ secretion. (B) Model of 2B4 function. In NK cells containing SAP, 2B4–CD48 interactions enhance cytotoxicity and secretion of IFN-γ. This response may involve known targets of 2B4-SAP signaling, such as Vav-1, c-Cbl, and SHIP-1 (22, 23). In the presence of EAT-2, 2B4 could induce NK cell inhibition. In the absence of both SAP and EAT-2, 2B4 could trigger yet another signal that inhibits NK cell functions.

of CD48 on target cells and were blocked by addition of anti-CD48 antibodies. They were also restored in 2B4-deficient NK cells by retrovirus-mediated expression of the long form of 2B4, but not the short form, which has fewer tyrosine-based motifs in its cytoplasmic domain. These unanticipated results imply that 2B4 is primarily an inhibitory receptor in mouse NK cells and suggest that the ability of anti-2B4 antibodies to trigger NK cell functions in mice, and possibly humans, may reflect their capacity to block 2B4–CD48 interactions.

However, there are still compelling data indicating that expression of CD48 on target cells can enhance the capacity of human NK cells to kill (17). Additionally, one group reported that target cell killing was defective in NK cells from an XLP patient even in the absence of additional anti-2B4 antibodies (18). There is also convincing evidence that the presence of CD48 on $2B4^+$ $CD8^+$ mouse T cells enhances antigen-induced T cell cytotoxicity (15). Therefore, 2B4 may be able to act either as an inhibitory receptor or as an activating receptor (Fig. 1 B).

One scenario is that 2B4 mediates these opposite effects in different subsets of NK cells. In support of this, Moretta et al. reported that anti-2B4 antibody stimulation inhibited NK cell activity in immature human NK cells, whereas it enhanced the function of mature cells (19). These differential effects were postulated to result from expression of SAP in mature but not in immature NK cells. This notion is also consistent with the finding made by the same group that 2B4 ligation was inhibitory rather than activating in NK cells from XLP patients (10). It may also explain the finding of Lee et al. that *Sap* RNA was present in mouse lymphokine-activated killer cells but not in BM-derived mouse NK cells (16). However, since the impact of 2B4 deficiency was similar in these two NK cell populations doubts remain regarding the consequences of differential SAP expression in mouse NK cells.

Other variables could explain a differential capacity of 2B4 to mediate positive or negative signaling (Fig. 1 B), and these variables may be different in human and mouse NK cells. The dominant inhibitory effect of 2B4 in mouse NK cells may relate to their preferential expression of EAT-2 over SAP. Indeed, unlike *Sap* mRNA, *Eat-2* mRNA was detectable both in mouse lymphokine-activated killer cells and in BM-derived mouse NK cells (16). In contrast, as suggested by some of the analyses of XLP-derived NK cells, the apparent activating effect of 2B4 in human NK cells may relate to preferential expression of SAP. It is also possible that 2B4-mediated inhibition relates to the capacity of 2B4 to mediate signals in the absence of SAP and EAT-2. In support of this, transient transfection experiments showed that 2B4 was able to associate with SHP-2 in cells lacking SAP and EAT-2 (20). However, this finding was not confirmed by others (10, 21). A careful analysis of SAP and EAT-2 expression in human and mouse NK cells, in addition to a characterization of 2B4-mediated functions and signals in mice lacking SAP and/or EAT-2, will test these ideas.

Lessons from Mice Lacking SLAM-related Receptors. The reports of Wang et al. (14) and Lee et al. (16) yield several important conclusions. First, they provide firm evidence of the involvement of SLAM-related receptors in immune regulation. Second, they indicate that the previously published studies using mAb stimulation led to some misconceptions regarding the roles of SLAM family receptors in vivo. This may relate to the fact that the antibodies were blocking rather than stimulating the functions of SLAM-related receptors. It is also plausible that the antibodies were affecting only a subset of the functions of SLAM-related receptors, thus exposing a biased view of their roles. And third, these manuscripts coupled with other published findings suggest that a given SLAM-related receptor may have the ability to mediate different, potentially even op-

posite, signals and functions in different cell populations or activation states. This ability may relate to differential expression of SAP-related adaptors (Fig. 1) or to other as yet unappreciated factors. Such flexibility would give SLAM-related receptors an exquisite degree of control during an immune response.

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