

Review Article

Role of signaling lymphocytic activation molecule in T helper cell responses

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ABSTRACT

Signaling lymphocytic activation molecule (SLAM; CDw150) is a 70 kDa glycoprotein. Signaling lymphocytic activation molecule is constitutively expressed on memory T cells, CD56⁺ T cells, a subset of T cell receptor $\gamma\delta$ ⁺ cells, immature thymocytes and, at low levels, on a proportion of peripheral blood B cells. Signaling lymphocytic activation molecule is rapidly upregulated on all T and B cells after activation. Engagement of SLAM by F(ab')₂ fragments of an anti-SLAM monoclonal antibody (mAb A12) enhances antigen-specific T cell proliferation. In addition, mAb A12 was directly mitogenic for T cell clones and activated T cells. T cell proliferation induced by mAb A12 is independent of interleukin (IL)-2, IL-4, IL-12 and IL-15, but is cyclosporin A sensitive. Ligation of SLAM during antigen-specific T cell proliferation resulted in upregulation of interferon (IFN)- γ production, even by allergen-specific T helper cell (Th) 2 clones, whereas the levels of IL-4 and IL-5 production were only marginally affected. The mAb A12 was unable to induce IL-4 and IL-5 production by Th1 clones. Co-stimulation of skin-derived Der P 1-specific Th2 cells from patients with atopic dermatitis via SLAM resulted in the generation of a population of IFN- γ -producing cells, thereby reverting their phenotype to a Th0 pattern. Signaling lymphocytic activation molecule is a high-affinity self ligand mediating homophilic cell interaction. In addition, soluble SLAM enhances both T and B cell proliferation. Collectively, these data indicate that SLAM molecules act both as receptors and

ligands that are not only involved in T cell expansion but also drive the expanding T cells during immune responses into the Th0/Th1 pathway. This suggests that signaling through SLAM plays a role in directing Th0/Th1 development.

Key words: allergy, interferon- γ , signaling lymphocytic activation molecule (CDw150), T cell activation, Th2 responses.

INTRODUCTION

T cell activation requires engagement of the T cell receptor (TCR) by antigenic peptide/major histocompatibility complex (MHC) complexes expressed on the antigen-presenting cells (APC). In addition, costimulatory signals mediated by an interaction between CD28 on T cells and its natural ligands CD80 and CD86 expressed on APC are required for cytokine production and the progression of T cell proliferation.¹ However, CD28-deficient mice are still able to generate considerable delayed-type hypersensitivity and antiviral cytotoxic T cell responses, indicating that CD28 is not required for all T cell responses *in vivo* and that, in addition to CD28, other molecules may have costimulatory activity.^{2,3} In addition to the ligand for CD40 (CD40 L),⁴ these molecules may include novel T cell surface molecules that are induced or upregulated early after T cell activation. Here we show that signaling lymphocytic activation molecule is one of the early activation molecules on T cells.⁵ Engagement of SLAM by an anti-SLAM monoclonal antibody (mAb) results in the expansion of activated T cells and interferon (IFN)- γ production. In the present communication, the structure and functions of SLAM, particularly its capacity to redirect allergen-specific Th2 responses into a Th0-like pathway, will be discussed.

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SIGNALING LYMPHOCYTIC ACTIVATION MOLECULE STRUCTURE

Signaling lymphocytic activation molecule is a heavily glycosylated 70 kDa type 1 transmembrane protein composed of a 202 amino acid (aa) extracellular region, a 22 aa transmembrane domain and a 77 aa cytoplasmic tail. The extracellular region of SLAM contains six cysteine residues that are involved in the disulfide bond formation of the two immunoglobulin (Ig) loops characteristic for the Ig superfamily structure and comprises one constant and one variable region domain.

Signaling lymphocytic activation molecule has a low degree of homology with other Ig supergene family members, such as human Ly 9, CD48 (26%), CD58 (17%), and with the mouse natural killer (NK) cell signaling molecule 2B4 (28%).⁵ Signaling lymphocytic activation molecule also has homology with three putative membrane receptors of the Molluscus Contagiosus Virus (MCV), which is a tumorigenic poxvirus.⁶ The gene encoding human SLAM has been localized on the long arm of chromosome 1 in the region of 1q21.2–1q23 (B Cocks *et al.*, unpubl. obs., 1997). This region contains many genes important in cell–cell interactions, leukocyte adhesion and trafficking, including CD48, Ly9, selectins and myelin P0. The cytoplasmic tail of SLAM contains four potential tyrosine phosphorylation sites, three of which conform to the consensus sequences reported to bind one class of SH₂ domains.⁷

Four alternatively spliced isoforms of human SLAM have been identified. These include a truncated transmembrane form, a secreted form derived by an in-frame deletion of 90 b.p., with the deleted aa encompassing the entire transmembrane region, and an isoform that lacks the leader sequence and that, consequently, is expressed intracytoplasmically.⁵ All SLAM isoforms are present in peripheral blood lymphocytes, CD4⁺ T cell clones, B cells, Epstein–Barr virus (EBV)-transformed B cell lines and fetal thymocytes. The existence of different alternatively spliced isoforms suggests that these may have different functions in different lymphoid populations and during differentiation of these cells.

SIGNALING LYMPHOCYTIC ACTIVATION MOLECULE EXPRESSION

Signaling lymphocytic activation molecule is primarily expressed on lymphoid cells. Signaling lymphocytic activation molecule is constitutively expressed on immature

thymocytes, a proportion of CD8⁺ and CD4⁺ T cells within the CD45 RO⁺ memory T cell population, some TCR $\gamma\delta$ ⁺ T cells, a proportion of B cells, CD56⁺ T cells, T cell clones and EBV-transformed B cell lines. Signaling lymphocytic activation molecule receptors are not present on NK cells, monocytes and granulocytes, most human malignant T cell lines and myeloid cell lines. However, SLAM is rapidly (within 2 h) induced on all CD45 RA⁺ naïve T cells and B cells following activation, indicating that SLAM receptors may play a role in the early stages of T and B cell activation.⁵

ENGAGEMENT OF SLAM RECEPTORS RESULTS IN T CELL EXPANSION

The anti-SLAM mAb A12 used as a surrogate ligand enhances antigen-specific T cell proliferation. In addition, mAb A12 directly induces proliferation of T cell clones, phytohemagglutinin (PHA)-blasts, or T cells that had been activated by specific antigens. Single Fab or F(ab')₂ fragments of mAb A12 were also effective, indicating that cross-linking of SLAM receptors was not required for the induction of T cell proliferation. The direct proliferation-inducing effects of SLAM were dependent on the 'activation state' of the T cells, as the effect decreased in time following activation of the T cells and T cell clones despite the levels of SLAM expression on the surface of these cells remaining unaltered.⁸ These results suggest that stimulation via SLAM requires an activation-induced modification of this molecule or association of SLAM with other activation-induced signaling components.

T cell proliferation induced through stimulation of SLAM-receptors is interleukin (IL)-2, IL-4, IL-12 and IL-15 independent but is cyclosporin A sensitive, indicating that, despite IL-2 not being required, calcineurin and nuclear factor of activated T cell (NFAT) family members seem to be involved in SLAM-mediated signaling.⁸

SIGNALING LYMPHOCYTIC ACTIVATION MOLECULE IS A HIGH-AFFINITY SELF LIGAND

Determination of the ligand for SLAM using a SLAM–Ig fusion protein identified SLAM as a self ligand. The fluorescent-labeled SLAM–Ig fusion protein bound only to cells expressing SLAM, including SLAM-transfected L cells. This homophilic interaction between SLAM molecules was confirmed by measuring plasmon resonance. Both the recombinant soluble forms of the extracellular domain of SLAM and the naturally occurring soluble form

of SLAM (in which only the transmembrane region is deleted) showed strong homophilic interactions with an affinity of approximately 0.1 nmol/L. These high affinity SLAM–SLAM interactions had functional consequences, as BAF-3 cells transfected with SLAM molecules immediately form cell aggregates whereas the wild-type cells grow in single cell suspension (B Cocks *et al.*, unpubl. obs., 1997). Similar results were observed with EL-4 cells, whereas EL-4 cells transfected with chimeric SLAM molecules consisting of the extracellular domain of CD8 and the cytoplasmic domain of SLAM failed to induce adhesions of EL-4 cells. Collectively, these results indicate that homophilic SLAM interactions play an important role in inducing cell adhesion. Furthermore, the soluble recombinant forms of SLAM induce, in a dose-dependent fashion, proliferation of pre-activated B cells⁹ and (to a lesser extent) of CD4⁺ T cell clones (B Cocks *et al.*, unpubl. obs., 1997).

The identification of SLAM as a self ligand is not unexpected, as other molecules that share sequence homology with SLAM interact similarly. Human Ly 9 and myelin P0, which are mapped on the same chromosomal region as SLAM, and other Ig-supergene family members, such as CD31 and CD56, interact in a homophilic fashion.

The observation that many other members of the Ig-supergene family (such as CD28 and CD80), CD28, CD86, CD2, CD58, TCR and MHC interact in a heterophilic fashion suggests an evolution of Ig-supergene family members by gene duplication from homophilically interacting precursors.¹⁰

SIGNALING LYMPHOCYTIC ACTIVATION MOLECULE ENGAGEMENT INDUCES IFN- γ PRODUCTION AND REDIRECTS Th2 RESPONSES

T lymphocytes are important producers of cytokines and different CD4⁺ helper subsets have been identified based on their cytokine production profile. T helper 1 cells produce, among other cytokines, IL-2, IFN- γ and lymphotoxin but not IL-4 and IL-5, whereas Th2 cells produce IL-4, IL-5 and IL-13 but not (or only very low levels of) IFN- γ . Both T cell subsets produce IL-3, granulocyte–macrophage colony stimulating factor (GM-CSF) and tumor necrosis factor (TNF)- α . A third subset, the Th0 cells, produces both Th1 and Th2 cytokines. The differences in cytokine production patterns of the various Th cell subsets are also reflected in their functions. Th1 cells are generally involved in cellular immune responses, such as delayed-type hypersensitivity reactions and monocyte

activation, whereas Th2 cells predominantly provide help for B cells in antibody production.¹¹ It is well documented that allergen-specific T cells derived from atopic patients generally belong to the Th2 subset.^{12–14} Interleukin-4 and IL-13 produced by these cells following activation induce IgE synthesis by B cells, whereas IFN- γ produced by Th1 and Th0 cells inhibits IL-4- and IL-13-driven IgE synthesis.^{15,16} Interestingly, engagement of SLAM during antigen-specific T cell responses strongly enhances IFN- γ production, even by allergen-specific Th2 clones.

In contrast, costimulation through SLAM failed to induce IL-4 or IL-5 production by Th1 clones and only marginally affected the production of these cytokines by Th0 and Th2 clones.⁵ Thus, SLAM engagement directs T cell responses towards a Th1 pathway. This notion was confirmed by the observation that the responses of Der p 1-specific Th2 cells obtained from skin biopsies of patients with atopic dermatitis following a local challenge with Der P 1 could be redirected to a Th0 response.¹⁷

Engagement of SLAM during allergen-specific stimulation of these Th2 cell populations resulted in the generation of a stable population of IFN- γ -producing Th1 and Th0 clones. In addition, the production of IL-4 and, particularly, IL-13 was downregulated in these cultures. This had important biological consequences, in that supernatants obtained from the Der p 1-specific T cell population cultured in the presence of anti-SLAM F(ab')₂ fragments, in contrast with their control counterparts, failed to induce IgE synthesis by B cells activated by anti-CD40 mAb.¹⁷ The capacity to induce IgE synthesis could be partly restored by neutralizing anti-IFN- γ mAb, indicating that the quantities of IFN- γ produced following ligation of SLAM are sufficient to completely counteract the IgE production-inducing activity of the remaining IL-4 and IL-13 in these supernatants. In addition, these results indicate that the SLAM-signaling pathway may provide a novel way to intervene in Th2 cell-mediated allergic diseases.

SIGNALING LYMPHOCYTIC ACTIVATION MOLECULE SIGNALING

Stimulation of EL-4 (mouse thymoma) or Jurkat (human T leukemia) cells transfected with SLAM via the TCR resulted in a strong reduction in the phosphorylation of many tyrosine kinases. In contrast, no effects on tyrosine kinase activity were observed in EL-4 or Jurkat cells that were transfected with chimeric SLAM molecules consisting of the extracellular part of CD8 and the cytoplasmic portion of SLAM. This is consistent with the notion that

SLAM, in contrast with CD8, is a high-affinity self ligand. Preliminary studies revealed that, among others, the phosphorylation of ZAP 70 and cbl was strongly inhibited by SLAM. The same was observed for CD3 ϵ .

The strong reduction in tyrosine kinase activation following SLAM–SLAM interactions of EL-4 and Jurkat cells could be partially restored by pervanadate, suggesting that tyrosine phosphatases, probably recruited by SLAM, play an important role in downregulating phosphotyrosine kinase activation (C Terhorst *et al.*, unpubl. obs., 1997). These data suggest that cell-bound SLAM–SLAM interactions confer a negative signal to activated T cells, which is relieved by anti-SLAM mAb and probably, but to a lesser extent, by monomeric soluble SLAM molecules.

CONCLUDING REMARKS

The present information indicates that SLAM plays a role in Th cell expansion following antigen-specific activation. In contrast to CD28, SLAM does not seem to be involved in the initiation of T cell activation, but once T cells are activated, engagement of SLAM results in a direct, IL-2- and IL-4-independent proliferation of these cells. Together with its capacity to induce preferentially IFN- γ production, these observations indicate that ligation of SLAM receptors directs the expanding T cells into the Th1 pathway, suggesting that SLAM may play a general role in Th1 cell differentiation.

Signaling through SLAM can even reverse allergen-specific Th2 cell responses, as it resulted in the generation of a stable population of IFN- γ -producing Th1 cells, thereby redirecting the allergen-specific Th2 responses into the Th0/Th1 pathway. Whether this induction of IFN- γ production by Th2 cell populations indeed reflects induction of IFN- γ production by IL-4-producing allergen-specific Th2 cells or differentiation of IFN- γ -producing Th1 cells from non-IL-4-producing T cells present in these skin-derived T cell populations remains to be determined. However, these results suggest that modulation of the SLAM signaling pathway may provide a novel way to intervene in allergic diseases. Although the SLAM signaling pathway remains to be elucidated, preliminary findings suggest that SLAM–SLAM interactions between cell-bound forms of SLAM may confer a 'negative signal', as judged by a general inhibition of tyrosine kinase phosphorylation following TCR activation. This notion is consistent with the observation that all anti-SLAM-mAb generated thus far ($n = 20$) have 'agonistic' activity, which probably should be interpreted as reflect-

ing neutralization of inhibitory signals mediated by cell-bound SLAM–SLAM interactions. Because soluble SLAM also enhances T cell proliferation, it can be concluded that it probably has similar effects. Release of these negative signals (probably mediated by tyrosine phosphatases) results in T cell expansion and IFN- γ production by these cells. Characterization of the various components involved in the SLAM signaling pathway will give a better insight into the mechanisms underlying this novel 'negative' signaling pathway in T cells, which play a role in T cell expansion and IFN- γ production by these cells.

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