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Current Perspective

Direct Interaction Between Nerves and Mast Cells Mediated by the SgIGSF/SynCAM Adhesion Molecule

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Abstract. Accumulating evidence has so far indicated that cross-talk between the nervous and immune systems plays a pivotal role in the pathophysiology of various diseases. As a prototypic demonstration of neuro-immune systems, the interaction between nerves and mast cells has been examined intensively. Anatomically, mast cells are often located in close proximity to nerves. Functionally, both cells communicate with each other in a bi-directional manner. Substance P released from nerves and proteases and cytokines from mast cells have proved to be important mediators in such communication. On the other hand, the molecules involved in membrane-membrane contacts between nerves and mast cells were largely unknown. In 2003, both cells were found to express the identical adhesion molecule, named SynCAM (synaptic cell adhesion molecule) or SgIGSF (spermatogenic immunoglobulin superfamily). Since SgIGSF/SynCAM binds homophilically, its involvement in nerve-mast cell interaction was examined in vitro. Superior cervical ganglia expressed SgIGSF/SynCAM along their neurites. Adhesion to these neurites of mast cells lacking SgIGSF/SynCAM was poor, and this was normalized by ectopic expression of SgIGSF/SynCAM. Moreover, SgIGSF/SynCAM-expressing mast cells were more competent in communicating with the neurites. Further understanding of the adhesion molecule-dependent interaction will be expected to open a new avenue in the field of neuro-immune cross-talk.

Keywords: synaptic cell adhesion molecule (SynCAM), substance P, neurogenic inflammation, mental stress, microphthalmia transcription factor

Introduction

It has long been demonstrated that there is functional cross-talk between the nervous and immune systems, with that between nerves and mast cells being the most intensively studied (Fig. 1). Mast cells are known to be located in close apposition to nerves in a variety of tissues including the skin, intestinal mucosa, and dura mater (1 – 3). Electron microscopy revealed that membrane-membrane contact actually occurred between both cells in vivo (2). Nerves in contact with mast cells often contain neuropeptides such as substance P and calcitonin gene-related peptide (4). Because nerves release these neuropeptides on stimulation, and mast cells express receptors for many neuropeptides (5, 6), nerve activation results in mast cell activation, that is, degranulation or secretion of mediators (7).

Mast cells synthesize and release a variety of molecules, which can, in turn, influence neuronal activity (8). For example, tryptase directly activates proteinase-activated receptors on neurons (9), and tumor necrosis factor-α and nerve growth factor cause changes in local nerves so as to lower their threshold to activation (10). These nerve-mast cell effects are assumed to participate in the promotion and regulation of many inflammatory diseases such as multiple sclerosis, interstitial cystitis, and irritable bowel syndrome (11). In spite of such clinical importance, the molecules that connect mast cells directly with nerves have not been studied intensively so far.

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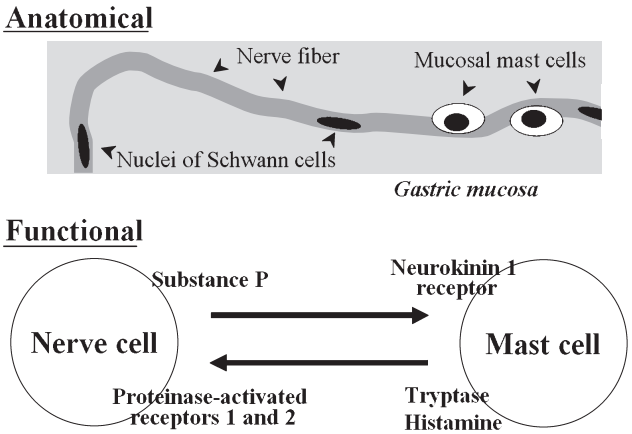

Fig. 1. Schematic demonstration of nerve-mast cell interaction. Anatomically, mast cells are often located in close proximity to nerves in various tissues including skin and intestinal mucosa. Functionally, both cells communicate in a bi-directional manner. Substance P released from nerves acts on neurokinin1 (NK-1) receptors expressed on mast cells. Proteases (tryptase, etc.) and chemical mediators (histamine, etc.) released from mast cells act on proteinase-activated receptor 1 and 2 on nerves.

Adhesion molecules common to nerves and mast cells

In 2003, nerves and mast cells were found to express the identical adhesion molecule, named synaptic cell adhesion molecule (SynCAM) or spermatogenic immunoglobulin superfamily (SgIGSF), independently by Biederer's group (12) and our own (13). Around this time, other groups reported the expression of SgIGSF/SynCAM in other types of cells, including pulmonary alveolar cells (14), pancreatic secretory cells (15), and spermatogonia (16). This molecule belongs to the immunoglobulin superfamily and structurally resembles neural cell adhesion molecule-1 and -2 (16): it has three immunoglobulin-like motifs in its extracellular domain and an intracellular domain containing a motif sequence that connects to the actin cytoskeleton (Fig. 2) (17).

Biederer and coworkers examined the function of SgIGSF/SynCAM in neurons in detail. They showed that SgIGSF/SynCAM is localized on both sides of most synapses in the brain, and functions as a homophilic adhesion molecule that spans the synaptic cleft (12). More importantly, they demonstrated that SgIGSF/SynCAM drives synapse assembly; that is, synaptic differentiation was induced even in non-neuronal cells at the contact site with neuronal cells when the non-neuronal cells were transfected with SgIGSF/SynCAM and glutamate receptor cDNAs (12).

On the other hand, our group found that SgIGSF/SynCAM was a novel mast-cell adhesion molecule whose expression was critically regulated by the microphthalmia transcription factor (MITF), a member of the basic-helix-loop-helix-leucine zipper family (13). Because the *Mitf*$^{mi\text{-}VGA9}$ mutant allele is practically a null mutation of the MITF gene (18), bone marrow-derived mast cells (BMMC) from homozygous *Mitf*$^{mi\text{-}VGA9}$/*Mitf*$^{mi\text{-}VGA9}$ mice do not express SgIGSF/SynCAM, whereas BMMC from wild-type (+/+) mice express it abundantly. When MITF was expressed in *Mitf*$^{mi\text{-}VGA9}$/*Mitf*$^{mi\text{-}VGA9}$ BMMC exogenously, the mutant BMMC expressed the normal level of SgIGSF/SynCAM (13). In addition, SgIGSF/SynCAM appeared to mediate adhesion of mast cells to fibroblasts because BMMC from *Mitf*$^{mi\text{-}VGA9}$/*Mitf*$^{mi\text{-}VGA9}$ mice were defective in the adhesion and exogenous SgIGSF/SynCAM normalized it (13).

According to the recent biochemical and cell biological studies (18, 19), SgIGSF/SynCAM binds in two ways, homophilic and heterophilic manners. The binding manner is different from cell types that express it and from attachment partner cells. Homophilic binding occurs among lung alveolar cells and among neurons, and heterophilic binding occurs between mast cells and fibroblasts (13) and between spermatogonia

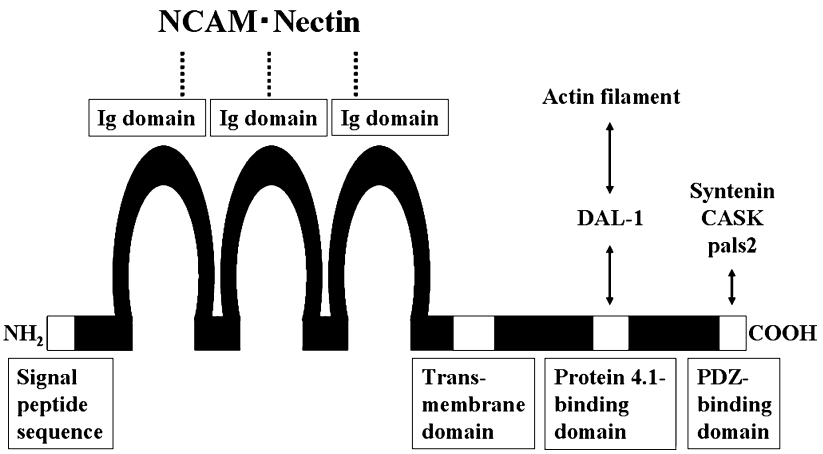

Fig. 2. The structure of SgIGSF. The extracellular domain consists of three immunoglobulin-like domains and the intracellular domain contains several consensus motifs that putatively link with cytoskeletal proteins. The binding partners identified are shown. Structurally, SgIGSF has a significant homology with NCAM (neural cell adhesion molecule) and nectin.

and Sertoli cells (16). Recently one of the heterophilic binding partners was identified as Class-I-restricted T-cell-associated molecule (20). Since nerves and mast cells were found to express SgIGSF/SynCAM, both cells might attach to each other through homophilic binding of this molecule. Moreover, considering that this adhesion molecule drives synapse assembly in both neuronal and non-neuronal cells, SgIGSF/SynCAM-mediated adhesion might result in more efficient communication between nerves and mast cells. These possibilities were examined in an in vitro model composed of cocultures of neurite-sprouting murine superior cervical ganglia (SCG) neurons with mast cells (21). In addition to *Mitf*$^{mi\text{-}VGA9}$/*Mitf*$^{mi\text{-}VGA9}$ BMMC, IC-2 cells were used as SgIGSF/SynCAM-negative mast cells because they were an interleukin 3-dependent mast cell line established from BMMC of DBA/2 mice and were lacking SgIGSF/SynCAM expression as revealed by Western blotting and FACS analyses (22).

SCG neurons were cultured in the presence of NGF: they sprouted out numerous neurites in a few days. SgIGSF/SynCAM expression was detected in the neurites along their whole length. SgIGSF/SynCAM did not appear to be specific for synapses but appear to distribute broadly in neurites. This finding was consistent with our previous report in which we detected SgIGSF/SynCAM along peripheral nerve fibers in mouse and human lung (14). However, this conflicted with the observation by Biederer and coworkers who demonstrated synapse-specific localization of SgIGSF/SynCAM in the central nervous system (12). BMMC or IC-2 cells were plated onto the network of neurites sprouting from SCG neurons. In a few hours, considerable numbers of +/+ BMMC attached to the neurites, whereas the number of *Mitf*$^{mi\text{-}VGA9}$/*Mitf*$^{mi\text{-}VGA9}$ BMMC attached was one-third (21). IC-2 cells were also defective in the adhesion to neurites. Exogenous SgIGSF/SynCAM normalized the adhesion level of *Mitf*$^{mi\text{-}VGA9}$/*Mitf*$^{mi\text{-}VGA9}$ BMMC and IC-2 cells (21). A blocking antibody against SgIGSF/SynCAM reduced the number of +/+ BMMC attaching to the neurites by one-third (21). These results suggested that SgIGSF/SynCAM mediated the adhesion between mast cells and neurites probably through its homophilic binding. Consistent with this speculation, SgIGSF/SynCAM was localized intensively at the contact site of both cells (21).

The next question is whether nerves and mast cells communicate with each other via the SgIGSF/SynCAM-mediated contact site. In 2002, Bienenstock, Nakanishi, and their coworkers cocultured SCG neurites with the RBL rat mast cell line and observed the attachment between both cells by electron microscopy (23). Although there were no membrane structures characteristic of synapses, the distance between the plasma membranes of neurites and RBL cells was usually in the order of <20 nm. Indeed, signals were transmitted across this distance from neurites to attached RBL cells, as revealed with Ca^{2+} mobilization monitoring after the neurite activation evoked by scorpion venom (23). This signal transmission appeared to be mediated by SP, which was released from the neurites and acted through neurokinin (NK)-1 tachykinin receptors expressed on RBL cells. The points of contact between neurites and RBL cells were the only sites on mast cell membrane which ruffled on activation of associated neurites, and mast cell granule movement was greatest adjacent to the contact point (23).

The coculture of SCG neurites and IC-2 cells was stimulated with scorpion venom. SgIGSF/SynCAM-overexpressing IC-2 cells responded to scorpion venom-evoked neurite activation twice as efficiently as original IC-2 cells (21). SgIGSF/SynCAM appeared to increase the susceptibility of individual IC-2 cells to neurite activation. Especially, SgIGSF/SynCAM appeared to contribute to SP-mediated communication between SCG neurites and IC-2 cells because the NK-1 receptor antagonist CP-99,994-1 blocked the communication between SCG neurites and intact IC-2 cells at much lower concentrations than the communication between SCG neurite and SgIGSF/SynCAM-overexpressing IC-2 cells (21). However, no plasma membrane ultrastructures characteristic of synapses seemed to develop at the points of contact. This is reminiscent of the past observation by Bienenstock and coworkers. They reported that in the rat intestine, unmyelinated nerves closely apposed to mucosal mast cells but synapse-like specialized junctions were lacking between both (1). The present and past results may collectively allow us to conclude that synapse-like structures do not develop in the apposed plasma membranes of nerves or mast cells. Even though this was the case, SgIGSF/SynCAM appeared not only to function as simple glue in nerve-mast cell interaction, but also to promote the development of microenvironment where mast cells have an enhanced susceptibility to nerve activation. Homophilic binding of this adhesion molecule might result in an increase in the number of NK-1 receptors on mast cells or in the amount of SP released from the neurite.

Nakanishi and coworkers currently identified another candidate molecule that directly mediates membrane-membrane contacts between nerves and mast cells (24). They focused on the fact that +/+ BMMC expressed E- and N-cadherins. These molecules were distributed diffusely in the cytoplasm when +/+ BMMC were not associated with neurites. On association with neurites,

however, N-cadherin changed its localization to the plasma membrane, whereas E-cadherin did not (24). β-Catenin, which is supposed to assist localization of N-cadherin on the plasma membrane, changed its subcellular localization similarly to N-cadherin (24). Since N-cadherin was also expressed along the neurites, homophilic binding was likely to occur between the neurites and +/+ BMMC. Contribution of N-cadherin to nerve-mast cell attachment may explain the substantially reduced but appreciably residual ability for *Mitf*$^{mi\text{-}VGA9}$/*Mitf*$^{mi\text{-}VGA9}$ BMMC to adhere to SCG neurites, because *Mitf*$^{mi\text{-}VGA9}$/*Mitf*$^{mi\text{-}VGA9}$ BMMC expressed normal levels of N-cadherin (13). In the adhesion to neurites, mast cells seemed to utilize SgIGSF/SynCAM and N-cadherin as independent systems. To date, there has been no data indicating the involvement of N-cadherin in nerve-mast cell functional communication.

Whether or not SgIGSF/SynCAM promotes nerve-mast cell interaction also in vivo remains to be addressed in the future. If this was the case, these molecules might be therapeutic targets in various pathologic conditions. Mast cells are not only major effectors in allergic reactions, but recent studies demonstrated that they are also involved in a variety of non-infectious inflammatory diseases such as multiple sclerosis, migraines, atopic dermatitis, interstitial cystitis, and irritable bowel syndrome (11). As the condition of the diseases gets worse, nerve-mast cell interaction is proposed to grow stronger. Clinically, psychological stress often worsens the symptoms. In the intestinal mucosa of irritable bowel syndrome, the number of activated mast cells in close proximity to nerves is reported to correlate with severity and frequency of abdominal pain and/or discomfort (25). Usage of the drugs or antibodies that interfere with the SgIGSF/SynCAM-mediated nerve-mast cell interaction may have therapeutic efficacy against these pathophysiologic conditions (Fig. 3).

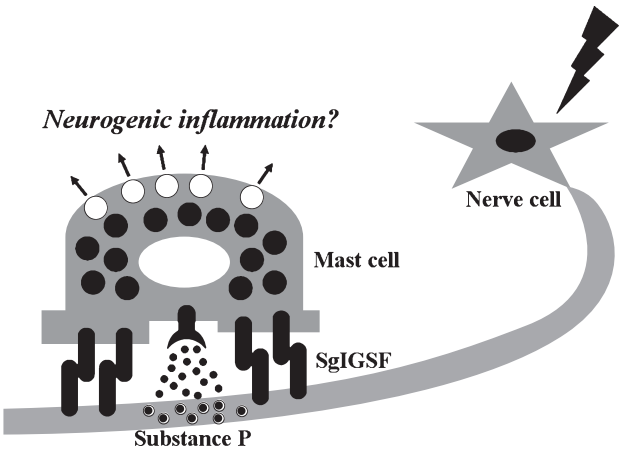

Fig. 3. Is SgIGSF the key molecule in neurogenic inflammation? Our hypothesis is that SgIGSF, which is expressed in both neurites and mast cells, plays an important role in signal transduction between both cells through binding homophilically.

Concluding remarks

During the last decade, there has been a marked increase in the evidence indicating that the nervous and immune systems are not disparate entities. The nerve-mast cell interaction is of importance as a prototype and has provided substantial evidence for bi-directional communication between nerves and immune cells. One of the most important mediators in nerve-to-mast cell communication seems to be substance P, which acts on NK-1 receptors expressed on mast cells. The opposite-direction communication seems to be mediated by proteases and cytokines released from mast cells on stimulation, which act on some types of receptors on nerves. SgIGSF/SynCAM may promote the development of membrane-membrane contacts between closely locating nerves and mast cells and consequently promote the development of a microenvironment suitable for the bi-directional communication. Further characterization of SgIGSF/SynCAM and isolation of other novel adhesion molecules involved in nerve-mast cell interaction will provide us with a deeper insight into the molecular basis underlying the linkage between nervous and immune systems.

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