Review

Molecular Basis of Neuroimmune Interaction in an *In Vitro* Coculture Approach

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A team of researchers from Nagoya, Tokyo and Hamilton developed a unique technique for studying neuro-immune interaction with confocal laser scanning fluorescence microscopy several years ago. It relies on guiding immune and nerve cell interaction by creating an adhesive environment using an *in vitro* coculture dish. With their technique, they are able to study details of the mechanism of how nerve cells communicate with immune cells (mast cells and T lymphocytes) and *vice versa*. They showed that nerve-mast cell communication could occur in the absence of an intermediary transducing cell and that the neuropeptide substance P, operating *via* NK-1 receptors, was a soluble factor of this communication. In addition, recently, they showed that ATP which was released from activated mast cells mediated the activation of nerve cells. Further, with their technique, Nagoya's group was able to study details of the molecular mechanism of nerve-mast cell interaction. N-cadherin and CADM1 (cell adhesion molecule 1) appeared to mediate attachment and promoted the communication between mast cells and nerves predominantly. It would lead to new therapeutic modalities for diseases based on neuroimmune interaction such as neurogenic inflammation, intestinal bowel diseases, asthma, and autoimmune disorders. *Cellular & Molecular Immunology*. 2008;5(4):249-259.

Key Words: neuro-immunology, coculture, confocal microscopy, soluble factor, adhesion molecule

Introduction

During last two decades, there has been exponential increase in data illustrating that the nervous and immune systems are not disparate entities (1-9). The nervous system including the brain and the peripheral neurons can stimulate or inhibit activities of the innate and adaptive immune systems through the hormonal and neuronal pathway. In turn, the immune system can influence nervous system activity through the release of immune mediators and cytokines.

Sympathetic nerve fibers innervate the vasculature and parenchyma of primary and secondary lymphoid organs, and other lymphoid tissues (10, 11). Immunohistochemical studies demonstrated a close association of sympathetic nerve terminals with macrophages and lymphocytes. Activation of central nervous system (CNS) by injection of immune stimuli such as lipopolysaccharide (LPS) or proinflammatory cytokines (IL-1β, IL-6, or TNF) results in a rapid increase in both firing rate of the sympathetic nerve innervation of spleen and the release of noradrenaline within spleen. The neuronal mediators released from nerve terminal binds to receptors on immune cells. Sympathetic neurons can stimulate B cells and helper T (Th1) cells through activation of β-adrenergic receptors and support a Th2 cell immune response (12, 13). Noradrenaline seems to inhibit the activity of cells in innate immunity such as macrophages, neutrophils or natural killer (NK) cells through β_2-adrenergic receptors but stimulate it in adaptive immunity involved with B cells and T cells.

Then, the nervous system can also modulate inflammatory response and allergic reaction. The CNS regulates systemic inflammatory responses to endotoxin through humoral pathways (14-16). The vagus nerve, which innervates liver, lung, spleen, kidneys, and gut, has been involved with this regulation, and activation of afferent vagus nerves by endotoxin or cytokines stimulates anti-inflammatory responses through hypothalamus-pituitary-adrenal (HPA) axis (17-19). Tracey's group showed that direct electric stimulation of the efferent vagus nerve inhibited the synthesis of TNF in liver, spleen, and heart, and then attenuated concentration of TNF in serum during endotoxaemia. Efferent activity in vagus nerve leads to release acetylcholine in some organs (20, 21). Acetylcholine interacts with nicotinic acetylcholine receptors on tissue macrophages and inhibits the release of proinflammatory cytokines (22, 23). On the other hand, stimulation of capsaicin-

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sensitive sensory neurons induces neurogenic inflammation by production of inflammatory symptoms such as vasodilation, plasma extravasation, and hypersensitivity (24). The experimental evidence supports that neuropeptides substance P and calcitonin gene-related peptide (CGRP) triggers neurogenic inflammation by interacting with the neurokinin-1 (NK-1) receptor and the CGRP1 receptor, respectively, on endothelial cells, mast cells, immune cells and arterioles (25-32). Destruction of capsaicin-sensitive nerve fibers with neonatal capsaicin depletes the content of these neuropeptides and attenuates neurogenic inflammation.

Further, the involvement of sensory neurons in not only neurogenic inflammation but also the mediation of nociception and pain has been established. Many inflammatory agents can stimulate the primary spinal afferent neurons. Especially, trypsin and mast cell tryptase cleave proteinase-activated receptor (PAR) 2 on the primary spinal afferent neurons and stimulate release of these neuropeptides (33). Moreover, mast cells containing tryptase are in close proximity to nerve fibers containing substance P and CGRP (34-36). Accordingly, Bunnett's group proposed that IgE-mediated mast cell activation results in the release of tryptase from intracellular secretory granules, and the tryptase activates PAR2 on sensory neurons to stimulate the release of substance P and CGRP (33). These neuropeptides induce the further activation of mast cells as well as inflammatory response such as arteriolar vasodilation and increased blood flow.

As described above, mutual association between nerves and immune cells has been observed in normal and pathological conditions. Their association is widely accepted but their precise molecular basis has not been clear yet. Here, we have been able to elucidate the molecular basis of communication between nerves and mast cells by molecular imaging of CADM1 and N-cadherin. We will review in this paper, focusing the current experiments for the molecular basis of neuroimmune interaction in an *in vitro* coculture approach by our group.

Coculture system

Immunohistochemical studies show that there is the innervation of both primary and secondary lymphoid organs with sympathetic nerve fibers (10, 11, 37-39), and that mast cells are also often found in the proximity of nerve fibers containing neuropeptides in a variety of tissues including skin, intestine, and dura mater (34, 40-42). Based on electron microscopic observation, nerve terminals are in direct apposition to T cells and mast cells. In addition, nerve fibers and immune cells seem to demonstrate 'synapse-like' structure such as thickening of pre-synaptic cellular membranes and concentration of vesicles containing neurotransmitters at their junctions. The synaptic clefts in neuronal synapse (neuron-neuronal contact zones) and immunological synapse (antigen-presenting cell-T cell contact zones) are 15-30 nm, while neuroimmune synaptic clefts are only ~6 nm (43).

To understand the nature of this association and its function, Blennerhassett and Bienenstock developed a tissue culture model involving the coculture of isolated nerve cells with immune cells (44, 45). They studied the early events of specific and selective association between sympathetic neurons and rat basophilic leukemia (RBL-2H3) cells (a model of mucosal mast cells). Using time-lapse microscopy they found that neurites extended toward RBL cells, and subsequently made contact with them. They also showed that 60-100% of RBL cells acquired neurite contact within 17 h of cocultures, but not YB2/0 (a rat plasmacytoma cell line) cells as a control experiment (45). Transmission electron microscopy (TEM) showed that there were large dense core neurosecretory granules within the neurite adjacent to RBL cell membrane with less than 20 nm, on average, between apposing membranes. Besides, they found that attached RBL cells ceased to divide and increased numbers of granules and the granule contents (serotonin) compared to those of the cells without contact to the neurites, indicating that attachment of neurons induced the maturation of RBL cells (46). They also showed that activation of peritoneal mast cells had the capacity of altering neuronal physiology by inducing depolarization and decreasing membrane resistance (47). We thought that the *in vitro* coculture model was very useful to investigate the molecular mechanism of direct communication between nerves and immune cells because the possibility that an intermediary cell transduces or modulates the nerve-immune cell interaction was dismissed.

Nerve-mast cell communication

The nerve-mast cell relationship has served as a prototypic association and has provided substantial evidence for bidirectional communication between nerves and immune cells (48). A variety of molecules synthesized by and released from mast cells can influence neuronal activity (49, 50). Tryptase directly activates PARs expressing on sensory neurons (23). Products of arachidonic acid metabolism, such as cysteinyl leukotrienes and prostaglandins, influence the local environment involving nerves (51). Cytokines including TNF-α and growth factors, such as NGF, cause changes in local nerves so as to lower their threshold to activation and promote nerve fiber elongation (52-55). Because nerves release the neuropeptides such as substance P and CGRP on stimulation and mast cells express receptors for many neuropeptides (56-59), nerve activation, in turn, results in mast cell activation, i.e., degranulation or secretion of mediators (60). Thus, it is very important to understand the molecular mechanism in direct nerve-mast cell communication. Their direct functional interplay has been also examined using *in vitro* coculture and confocal microscopy.

Direct activation of mast cells by substance P released from nerves

After the coculture of RBL cells with superior cervical ganglia (SCG) neurites for a few days, RBL cells attached to SCG neurites. Addition of scorpion venom or bradykinin, which resulted in dose-dependent SCG neurite activation but

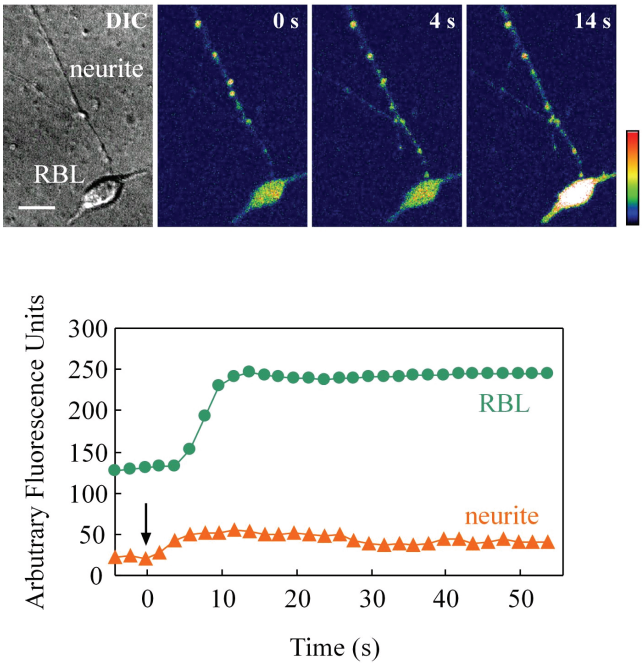

Figure 1. Nerve-induced mast cell activation. (Upper) Representative differential interference contrast (DIC) image and sequential Ca^{2+} images after the addition of bradykinin (time = 0) in the SCG-RBL coculture. Bar = 25 μm. (Lower) Time-courses of Fluo-3 fluorescence intensity changes in RBLs and in SCG neurites. An arrow indicates the time point of bradykinin addition.

not RBL cell activation, to the coculture dishes induced the increase in Ca^{2+} concentration in SCG neurites (Figure 1) (61). The neurite activation was invariably followed by activation of RBL cells in contact with neurites. The number of responding RBL cells increased in accordance with increasing concentration of scorpion venom or bradykinin. RBL cells which did not attach to neurites in the coculture dishes did not responsed directly to scorpion venom or bradykinin. Since it was reported that substance P in isolated SCG neurons in culture showed a 25-fold increase within 48 h *in vitro* (62) and that SCG were actually positive for substance P-like immunoreactivity, the substance P was examined as a neurite-derived mediator responsible for the RBL activation. Inclusion of a neutralizing anti-substance P antibody and an NK-1 receptor antagonist CP99, 994-01, in the SCG-RBL cocultures did not affect the neurite activation evoked by bradykinin, but significantly and dose-dependently inhibited the subsequent RBL cell activation. As for control, the experiments of treatment with an isotype-matched irrelevant antibody and an NK-2 receptor antagonist SR48, 968 were done, but they did not affect either bradykinin-induced neurite activity or the subsequent Ca^{2+} mobilization in RBL cells in cocultures. In addition, bone marrow-derived mast cells (BMMCs), which are thought to be mucosal mast cell couterparts, were cocultured with SCG neurites. BMMCs were shown to express NK-1 receptors in the presence of cytokines IL-4 and SCF, and this expression of NK-1 receptors was demonstrated to be of functional relevance and lead to an increase in the sensitivity to substance P (56, 63, 64). When BMMCs expressing NK-1 receptors in variable level were cocultured with SCG, BMMCs were found to respond in association with activated nerve fibers according to the level of NK-1 receptor expression (65). When NK-1 receptors were not expressed on BMMCs, the response was negligible though the majority of cells were c-kit positive. These data illustrate that nerve-to-mast cell activation can occur in the absence of an intermediary transducing cell and that substance P, operating *via* NK-1 receptors, is an important mediator of this response.

Substance P has been shown to cause mast cell degranulation when used at high doses, whereas the exposure to picomolar concentrations of substance P primes the mast cell, lowering the threshold for degranulaiton to the second stimulus (56, 66). To study whether degranulation in mast cells attached to neurites was induced by neurite activation, we first observed the surface structures of mast cells by an atomic force microscope (AFM) and a scanning electron microscope (SEM). AFM and SEM showed that resting RBL cells in cocultures before stimulation had a spherical and nearly smooth cell body. When SCG were stimulated with bradykinin, RBL cells were spread and flat, and many pores whose diameters were 0.8-1.0 μm derived from exocytosis of intracellular granules existed on the cell surface of RBL cells attached to neurites (67). These drastic morphological changes of the cell surface on RBL cells also occurred after stimulation with antigen (68). In addition, the membrane ruffling and the intracellular granule movement in activated RBL cells were analyzed by a membrane protein CD63. CD63 is located on the basophilic granule membranes in basophils, mast cells, and platelets, and is also on the plasma membranes (69). On activation of mast cells, the increase in surface expression of CD63 was detected (70). To analyze the movement of intracellular granules and the membrane ruffling on degranulation, we prepared a plasmid of chimera protein of CD63 and green fluorescent protein (GFP) and transferred it into RBL cells (71). In cocultures of RBL cells expressing CD63-GFP with SCG, membrane ruffling was observed on RBL pseudopodial extensions in contact with the activated neurites, but not on noncontacting pseudopodia (72). These morphological changes of RBL cells including cell spread, membrane ruffling, and granule movements were suppressed in the pretreatment of RBL cells with NK-1 receptor antagonist. These results indicate that nerve-derived substance P induces not only Ca^{2+} response but also degranulation to mast cells attached with neurites.

Direct activation of neurites by ATP released from mast cells

On the other hand, to investigate the signal from mast cells to nerves, the RBL cells were stimulated with anti-IgE antibodies or antigen in cocultures. Our data illustrated that SCG neurites were activated in response to RBL activation after a lag time (Figure 2) (61, 73). In coculture dishes, anti-IgE antibody- or antigen-induced RBL activation was invariably followed by SCG neurite activation. The

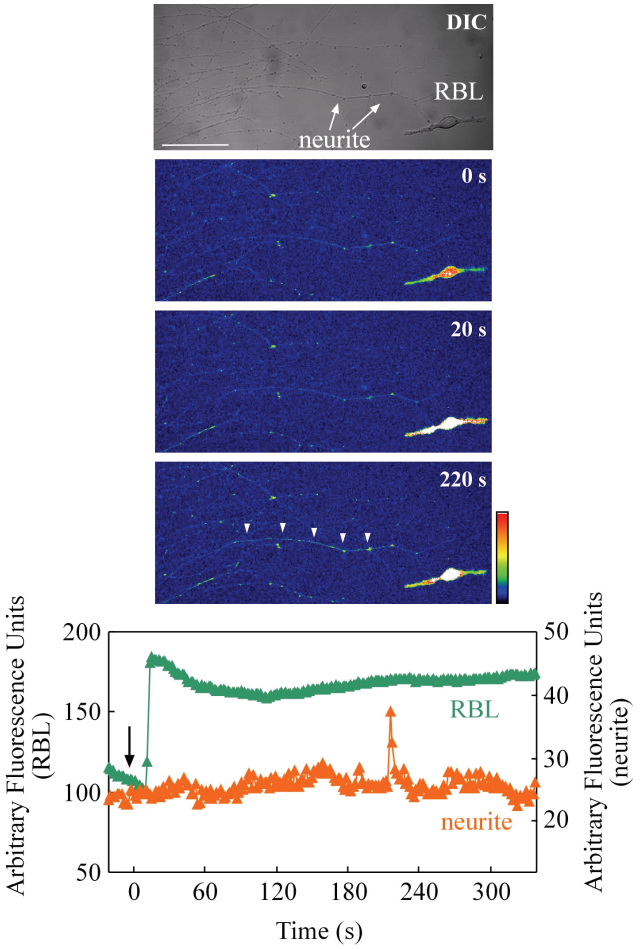

Figure 2. Mast cell-induced neurite activation. (Upper) Representative DIC image and sequential Ca^{2+} images after the addition of antigen (time = 0) in the SCG-RBL coculture. Arrowheads indicate the neurite which is strongly activated. Bar = 50 μm. (Lower) Time-courses of Fluo-3 fluorescence intensity changes in RBLs and in SCG neurites. The fluorescence intensity in the SCG neurite was shown in expanded scale. An arrow indicates the time point of antigen addition.

activation of RBL cells affected calcium signals of neurites over quite a long distance (> 150 μm) from the site attached to RBL cells. The lag times from RBL activation to SCG neurite response varied, and it mostly took several minutes (< 4 min) to induce Ca^{2+} response in SCG neurites after RBL stimulation. We surmised that this was consistent with the increased amounts, and variety of mediators, that were released upon mast cell activation/degranulation, as compared with the lower concentrations of mediators that would be released at neuronal synapses or varicosities. We have recently shown that ATP released from activated mast cells is one of important mediators to activate nerves because pyridoxalphosphate-6-azophenyl-2',4'-disulfonic acid (PPADS), a purinergic receptor antagonist or apyrase, an ATP hydrolyzing enzyme, reduced the Ca^{2+} signals in neurites (74). Also we found that the supernatant of RBL cells activated with antigen for 30 min (ATP concentration; ~32 nM) induced a rapid Ca^{2+} response in SCG neurites but not that of resting cells (ATP concentration; ~0.88 nM). The pretreatment of activated RBL supernatant with PPADS or apyrase significantly suppressed the responding rate of SCG neurites. Thus, in terms of bidirectional communication, our data using coculture system and confocal microscopic analysis precisely show that neurite or mast cell activation can result in activation of the reciprocal cell type in the absence of any modulating or trasnducing effects of an intermediary cell.

Adhesion molecules involved in the bidirectional communication between nerves and mast cells

The nerve-mast cell interaction is involved in both homeostatic and pathological regulations in their close apposition. The neurite-mast cell association is found to occur in the coculture dishes using AFM as shown in Figure 3. The AFM image shows that the growth cone covers mast cell surface over approximately 7 μm. However, the molecules that sustain this association were not identified. To investigate the adhesion molecules involved in the interaction, we first observed the distribution of cadherins, which are a family of Ca^{2+}-dependent and homophilic cell adhesion proteins playing a role in the formation of synaptic plasticity, on the nerve-mast cell cocultures (75, 76). Immunostaining analysis showed that both SCG and BMMCs expressed N- and E-cadherins, and that N- and E-cadherins are present mainly in the cytoplasm of BMMCs in the absence of SCG neurites (77, 78). With the association of neurites, however, N-cadherin was localized on the plasma membrane of BMMCs, but E-cadherin was not (78). β-catenin, which is a protein associated with cadherins in the cytoplasm, was also accumulated at the periphery of the plasma membrane in BMMCs attached to neurites, while it was distributed in the cytoplasm without neurites. These suggested that N-cadherin was involved in the contact formation between nerves and mast cells.

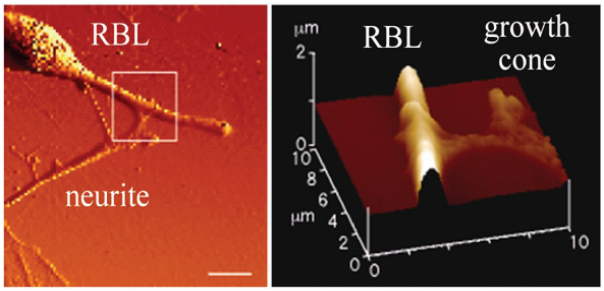

Figure 3. AFM images of SCG neurites and mast (RBL) cells in coculture dishes. (Left) An AFM image of neurites attached to a pseudopodium and a cell body of mast cell. Bar = 10 μm. (Right) The enlarged image of square in the right image. A thin neurite with the growth cone extended over a fiber-like pseudopodium of mast cell. Association between the growth cone and the mast cell occurred over ~7 μm.

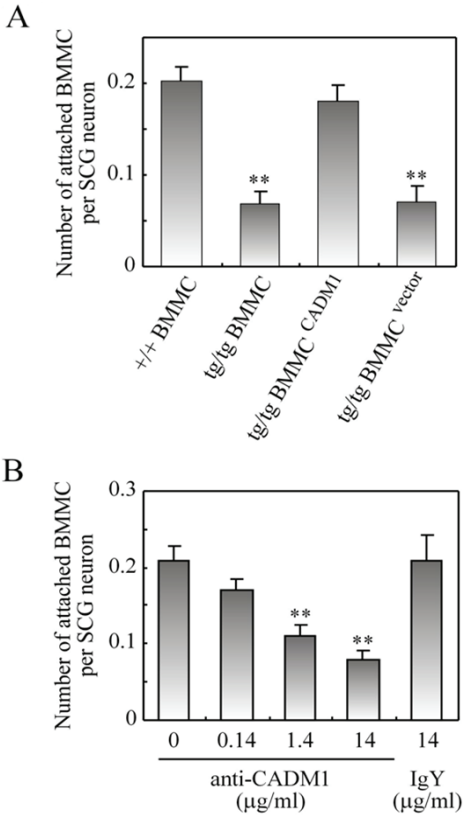

Figure 4. Attachment of mast cells to SCG neurites. (A) Attachment of various types of mast cells (BMMCs) to SCG neurites. The numbers of attached tg/tg BMMCs from MITF-deficient mice, which do not express CADM1, were one-third as large as the number of attached wild type +/+ BMMCs, which express CADM1. Transfection of a vector containing CADM1 cDNA (tg/tg $BMMC^{CADM1}$) improved the poor attachment of tg/tg BMMCs and showed normal attachment to neurites, but an empty vector (tg/tg $BMMC^{vector}$) did not affect. (B) Inhibition of attachment of +/+ BMMC to neurites by the antibody against the extracellular domain of CADM1 (anti-CADM1). Anti-CADM1 antibody inhibited the attachment of +/+ BMMC to neurites in a dose-dependent manner. In contrast, control chicken IgY did not influence the attachment of +/+ BMMCs.

In addition, Biederer et al. and Ito et al. have recently isolated the same adhesion molecule, designated the synaptic cell adhesion molecule (SynCAM) or spermatogenic immunoglobulin superfamily (SgIGSF) in different processes (79, 80). Biederer et al. showed that SynCAM was localized preferentially on both sides of most synapses in the brain and functions as a homophilic adhesion molecule that spaned the synaptic cleft in the nervous system (79). Ito et al. found that SgIGSF mediated the adhesion of BMMCs to fibroblasts and that the expression of SgIGSF in BMMCs was regulated by the microphthalmia transcriptional factor (MITF) (80). Though this identified adhesion molecule is also described in different names such as nectin-like molecule-2 (Necl-2) (81) and tumor suppressor in lung cancer 1 (TSLC1) (82), its name has currently approved as cell adhesion molecule 1 (CADM1) by the HUGO gene nomenclature committee. Because CADM1 is strongly considered to be one of the candidates involved in the attachment and communication between mast cells and nerves, we examined this possibility by coculturing SCG neurons and mast cells with or without CADM1. CADM1 was localized intensively at the contact site between mast cells and neurites. Mast cells lacking CADM1 attached poorly to neurites, and ectopic expression of this adhesion molecule significantly improved their attachment (Figure 4A). The attachment levels of mast cells expressing CADM1 were dose-dependently reduced in the presence of an anti-CADM1 blocking antibody (Figure 4B). These results suggested that CADM1 was the major adhesion molecule that mediated *in vitro* attachment between mast cells and neurites (83).

We also studied the involvement of CADM1 in the communication between mast cells and nerves. Our results showed that there is a significant difference in the proportion responding to neurite activation between mast cells with and without CADM1 (83). Only one-quarter of mast cells without CADM1 responded to neurite activation, whereas more than one-half of mast cells with the adhesion molecule responded. The responding rate of mast cells with CADM1 to neurite activation dose-dependently decreased in the presence of an anti-CADM1 blocking antibody. In addition, the NK-1 receptor antagonist blocked the responding rate to neurite activation in mast cells without CADM1 at much lower concentrations than in mast cells with the adhesion molecule. Thus CADM1 is likely not only to function as simple glue in nerve-mast cell interaction, but also to promote the development of a microenvironment where mast cells have an enhanced susceptibility to nerve activation.

Molecular mechanism of nerve-mast cell interaction

Mast cells are differentiated in various subsets by environmental factors such as surrounding cells and cytokines from a common precursor in bone marrow. All types of mast cells, however, are not activated with substance P. BMMCs cocultured with fibroblasts for several weeks can respond to substance P (84, 85), and BMMCs cultured in the presence of IL-4 and SCF gain sensitivity to substance P by expression of functional NK-1 receptors (56). In addition, the attachment of mast cells to neurons for several days is considered to affect phenotypical and functional characteristics of them because the granule contents and FcεRI expression were found to increase in the cells attached to SCG neurites (46, 86). This transformation of mast cells may occur *via* c-kit on their plasma membrane because SCG express SCF.

In the *in vitro* coculture experiments, it was shown that substance P from activated neurites induced Ca^{2+} mobilization and degranulation in mast cells. While, the exposure of substance P leads to promote the generation of lipid mediators such as PGD_2 and LTC_4 and the production of proinflammatory cytokines including TNF-α and IL-6 through the activation of MAP kinase and NF-κB pathways in mast cells (85, 87). From these results we think the activation of mast cells with nerve cells may promote the cytokine production as well as degranulation.

While most papers support that substance P is an important mediator in nerve-mast cell interaction, there are some reports describing that substance P does not induce mast cell activation (88). As described above, mast cells possess various functional characteristics according to their location and species. We showed here that substance P and ATP are important mediators in nerve-to-mast cell and mast cell-to-nerve, respectively. However, we consider that substance P and ATP are not the sole mediators bearing the bidirectional communication between nerves and mast cells and that there is a wide diversity of circumstances in their communication.

Nerve-T cell communication

Strong evidence that the CNS receives messages from the immune system and *vice versa* messages from the brain modulate immune function has been accumulated (89, 90). Historically, noradrenaline and the sympathetic nervous system have been known to influence the lymphoid organs (27-31). Noradrenaline regulates the function of immune cells that protect the body against pathogen. The presence of sympathetic nerve fibers and the release of noradrenaline within lymphoid organs represent a mechanism by which signals from the CNS influence immune cell function. Adrenergic and opioidergic receptors have been found on lymphocytes, granulocytes, monocytes, macrophages, and NK cells (91-94). The predominant adrenergic receptor expressed on T and B cells is the β_2-adrenergic receptors (90, 95). The β_2-adrenergic receptors are differentially expressed on both resting and anti-CD3-activated murine $CD4^+$ T cell clones, with detectable expression on Th1 cells but not on Th2 cells (96, 97). It was previously reported that following immunization *in vivo*, noradrenaline was released from the sympathetic neurons and was bound to the β_2-adrenergic receptors on lymphocytes (98), which induces the increase in the intracellular concentration of cAMP and Ca^{2+} (99, 100).

Direct activation of Th1 cells by noradrenaline released from nerves

To study whether T cell activation occurs as a direct response to neuronal activation or requires an intermediary cell, we used the *in vitro* coculture approach comprising cultured mouse SCG and T cell hybridomas (K79; possess properties of Th1 cells) (101). When T cells were added to the culture dish where most neurons showed the outgrowth of long neurites at 48 h after initiation of SCG neurons, they moved to SCG neurites and contacted with the neurites within 2 h. Because they did not move and detach from the neurites once they attached, the contact formation between SCG neurites and T cells became very stable after 2 h incubation. These results suggest that some chemotactic factors from SCG neurites should induce the movements of T cells to the neurites. Chemokines acting between T cells and neurons remain to be studied in regard to pathological condition in CNS such as encephalomyelitis and multiple sclerosis (102). T cell-specific chemokines including CCL19, CCL21, and CXCL10 may be candidates for the chemotaxis of T cells to SCG neurites.

Addition of scorpion venom, which resulted in a direct dose-dependent SCG neurite activation but not T cell activation, to SCG-T cell cocultures induced the increase in Ca^{2+} concentration in SCG neurites. This expected neurite activation was invariably followed by activation of T cells in contact with neurites, as indicated by Ca^{2+} increase. The number of responding T cells increased in accordance with increasing concentration of scorpion venom. The inclusion of propranolol, a β-adrenergic receptor antagonist, dose-dependently suppressed the T cell activation after the increase in Ca^{2+} in SCG neurites, but the treatment with phentolamine, an α-adrenergic receptor antagonist, and CP-99, 994-01, an NK-1 receptor antagonist, did not. These indicate that the neurotransmitter noradrenaline released from the activated sympathetic SCG nerve fibers elicits a direct Ca^{2+} mobilization in T cells in contact with neurites *via* β-adrenergic receptors (Ohshiro, Furuno and Nakanishi, to be published). Early studies using unfractionated population of $CD4^+$ T cells showed that β_2-adrenergic receptor stimulation inhibited T cell proliferation by decreasing IL-2 expression and secretion as well as IL-2 receptor expression *via* a cAMP-dependent mechanism (103-108). Recent studies using purified population of T cells proposed that noradrenaline differentially regulates naïve and effector $CD4^+$ T cell activity (109-111). Th1 cells that develop from naïve $CD4^+$ T cells, which were activated in the presence of noradrenaline, produced more IFN-γ per cell. The effect of noradrenaline on Th1 cells directly appeared to depend on the time of stimulation with noradrenaline. When noradrenaline was added to Th1 cells before and after T cell receptor stimulation, the production of IFN-γ was less and more, respectively. Noradrenaline did not affect on Th2 cell activity, as the β_2-adrenergic receptor is absent.

Molecular mechanism of nerve-Th1 cell interaction

We found here that noradrenaline released from SCG neurites induced Ca^{2+} mobilization in Th1 cells, while we still have many subjects to understand the molecular mechanism in nerve-T cell interaction. The neurite-derived chemokines to induce chemotaxis for T cells and the adhesion molecules to be involved in their communication should be identified. In addition, we have not examined whether the transcriptional activity and the cytokine production are enhanced in Th1 cells. It is very interesting to study what kinds of cytokines are produced in neurite-activated Th1 cells for understanding how nerves regulate immune system. It is also attractive to investigate how cytokines produced by Th1 cells influence the function of nerves. The molecular mechanisms in nerve-T cell communication will be demonstrated in the near future.

In vivo relevance

As described above, we have shown that the *in vitro* coculture model is a useful method to study the molecular mechanism in the direct neuroimmune crosstalk. However,

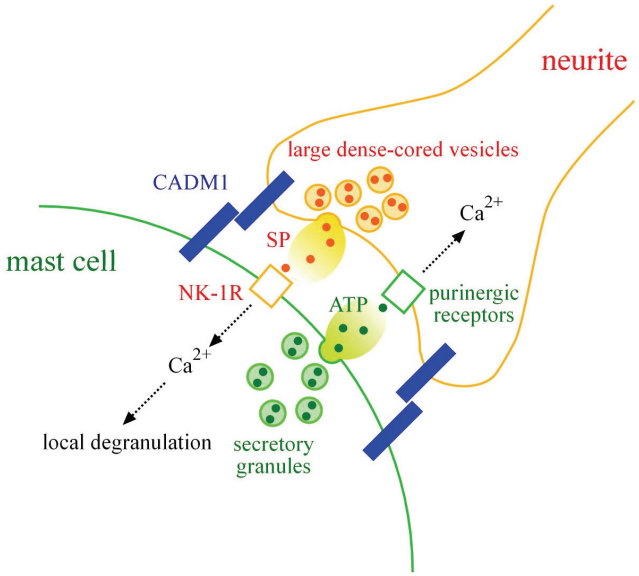

Figure 5. Schematic representation of molecular mechanism in communication between nerves and mast cells. SP, substance P; NK-1R, NK-1 receptor.

because the bidirectional interaction at the neuroimmune junction is complicate in body, the results obtained from *in vitro* cocultures are necessary to be verified by *in vivo* relevance.

Our results from the *in vitro* cocultures suggested that substance P and NK-1 receptors played an important role in neurogenic inflammation. In skin, contacts between nerves and mast cells were increased in number in both lesional and nonlesional samples of atopic dermatitis and nummular eczema (112). Exposure of stress also increased significantly the number of substance P-positive nerve fibers in the skin, and such nerve fibers contacted mast cells more frequently (113). Mice lacking NK-1 receptors showed a significant increase in the number of mast cells and maintenance of a spatial relationship between mast cells and nerves (114), while they displayed a significantly reduced hapten-induced acute cutaneous allergic inflammatory response (115). The blockade or absence of NK-1 receptors also resulted in a strong reduction in hapten-induced tracheal hyperreactivity and colitis (116, 117). Stress-induced extravassation in the dura mater was absent in *W/W*^{v} mast cell-deficient mice and NK-1 receptor-knockout mice (118). Acute restraint stress resulted dura mater mast cell activation in normal mice but not in NK-1 receptor-knockout mice (118). Substance P and NK-1 receptors are also reported to act as important regulators in the molecular signaling network modulating inflammatory response in alopecia areata and autoimmune hair loss (119-121).

In turn, mast cell mediators are reported to activate neurons. Microiontophoretic application of compound 48/80, an activator of mast cells, in thalamus caused excitation and inhibition on thalamic neurons possibly through mast cell degranulation (122). Moreover, local application of a mast cell mediator cocktail that was released from isolated human intestinal mucosa mast cells stimulated by IgE receptor crosslinking onto individual ganglia evoked an excitatory response consisting of action potential discharge in neurons of guinea-pig and human submucous plexus, and guinea-pig myenteric plexus (123). Mediators of mucosal mast cells from inflammatory bowel disease patients also markedly enhanced the firing of mesenteric nerves (124).

Finally, the adhesion molecule CADM1 was shown to function in mesentery (125). When BMMCs from CADM1-knockout mice were transplanted to mesentery of mast cell-deficient *W/W*^{v} mice, they did not degranulate in response to the mesenteric nerve stimulation whereas trasnfection with CADM1 cDNA to CADM1-deficient BMMC restored those responses to normal level. Thus, mediators and adhesion molecules functioning in nerve-mast cell interaction, which was identified by *in vitro* coculture approach, were shown to be relevant *in vivo*.

Concluding remarks

Considerable evidence existed for a consistent anatomical association between nerves and immune cells in tissue throughout the body. The morphological juxtaposition of nerves and immune cells would, by itself, be of little interest if it were not for evidence of physiological connectivity. The tradition of considering the nervous and immune systems as discrete entities has been replaced with the concept of neuroimmunity, in which strictly neural or immune functions are recognized as opposite ends of a biological spectrum. The neuroimmune system is now widely accepted as an integrated system sharing messenger molecules, receptors, and adhesion molecules allowing for bidirectional communication.

As described in this paper, the molecular mechanism in communication between nerves and immune cells was not highly peculiar in comparison with other cellular interaction. For example, neurotransmitters and neuropeptides released from nerves induced a direct activation of immune cells through the specific receptors on immune cell surface. These neuronal mediators, of course, are functioning as intercellular communication within nervous system. ATP released from activated mast cells by degranulation resulted in a direct activation of neurite through the purinergic receptors. ATP, which is known to be released by various cell types, has recently gained attention as a mediator of intercellular communication through the activation of purinergic receptors (126, 127). CADM1 played an important role in direct nerve-mast cell communication. This adhesion molecule is also known to be involved both in the neural synapse (107, 128) and in the immunological synapse (antigen presenting cell-T cell contacts) (129-131). Contact region between nerves and immune cells in which their direct and efficient communication occurred might be called “neuro-immunological synapse” as shown in Figure 5 (43).

Thus, we are now studying the molecular mechanism in interaction between nerve and immune cells using *in vitro* coculture approach and confocal imaging system. The

technique can be applicable to investigate intercellular communication between other types of cells. The direct interactions of nerve-osteoblastic cells (132) and nerve-pancreatic islet cells (133) have been actually demonstrated using the technique described in this paper. We would add that our data do not dismiss the likelihood of other cell type *in vivo* modulating nerve-immune communication. We hope, however, that a series of results introduced here can contribute to understand the initiation and perpetuation of health states and disordered states such as asthma, inflammatory bowel diseases, eczema, migraines, atopic dermatitis, and multiple sclerosis.

References

1. Ottaway CA. Neuroimmunomodulation in the intestinal mucosa. Gastroenterol Clin North Am. 1991;20:511-529.
2. McKay DM, Bienenstock J. The interaction between mast cells and nerves in the gastrointestinal tract. Immunol Today. 1994;15:533-538.
3. Straub RH, Westermann J, Scholmerich J, Falk W. Dialogue between the CNS and the immune system in lymphoid organs. Immunol Today. 1998;19:409-413.
4. Eskandari F, Sternberg EM. Neural-immune interactions in health and disease. Ann N Y Acad Sci. 2002;966:20-27.
5. Pedotti R, De Voss JJ, Steinman L, Galli SJ. Involvement of both 'allergic' and 'autoimmune' mechanisms in EAE, MS and other autoimmune diseases. Trends Immunol. 2003;24:479-484.
6. Eskandari F, Webster JI, Sternberg EM. Neural immune pathways and their connection to inflammatory diseases. Arthritis Res Ther. 2003;5:251-265.
7. Straub RH. Complexity of the bi-directional neuroimmune junction in the spleen. Trends Pharmacol Sci. 2004;25:640-646.
8. Brogden KA, Guthmiller JM, Salzet M, Zasloff M. The nervous system and innate immunity: the neuropeptide connection. Nat Immunol. 2005;6:558-564.
9. Engelhardt B, Ransohoff RM. The ins and outs of T-lymphocyte trafficking to the CNS: anatomical sites and molecular mechanisms. Trends Immunol. 2005;26:485-495.
10. Felten DL, Felten SY, Bellinger DL, et al. Noradrenergic sympathetic neural interactions with the immune system: structure and function. Immunol Rev. 1987;100:225-260.
11. Felten DL, Felten SY, Bellinger DL, Madden KS. Fundamental aspects of neural-immune signaling. Psychother Psychosom. 1993;60:46-56.
12. Kohm AP, Sanders VM. Norepinephrine: a messenger from the brain to the immune system. Immunol Today. 2000;21:539-542.
13. Elenkov IJ, Wilder RL, Chrousos GP, Vizi ES. The sympathetic nerve - an integrative interface between two supersystems: the brain and the immune system. Pharmacol Rev. 2000;52:595-638.
14. Hu XX, Goldmuntz EA, Brosnan CF. The effect of norepinephrine on endotoxin-mediated macrophage activation. J Neuroimmunol. 1991;31:35-42.
15. Lipton JM, Catania A. Anti-inflammatory actions of the neuroimmunomodulator α-MSH. Immunol Today. 1997;18:140-145.
16. Woiciechowsky C, Asadullah K, Nestler D, et al. Sympathetic activation triggers systemic interleukin-10 release in immunodepression induced by brain injury. Nat Med. 1998;4:808-813.
17. Scheinman RI, Cogswell PC, Lofquist AK, Baldwin AS Jr. Role of transcriptional activation of IκBα in mediation of immunosuppression by glucocorticoids. Science. 1995;270:283-286.
18. Sternberg EM. Neural-immune interactions in health and disease. J Clin Invest. 1997;100:2641-2647.
19. Watkins LR, Maier SF. Implications of immune-to-brain communication for sickness and pain. Proc Natl Acad Sci U S A. 1999;96:7710-7713.
20. Borovikova LV, Ivanova S, Zhang M, et al. Vagus nerve stimulation attenuates the systemic inflammatory response to endotoxin. Nature. 2000;405:458-462.
21. Bernik TR, Friedman SG, Ochani M, et al. Pharmacological stimulation of the cholinergic antiinflammatory pathway. J Exp Med. 2002;195:781-788.
22. Wang H, Yu M, Ochani M, et al. Nicotinic acetylcholine receptor α7 subunit is an essential regulator of inflammation. Nature. 2003;421:384-388.
23. Tracey KJ. Physiology and immunology of the cholinergic antiinflammatory pathway. J Clin Invest. 2007;117:289-296.
24. Jancso N, Jancso-Gabor A, Szolcsanyi J. Direct evidence for neurogenic inflammation and its prevention by denervation and by pretreatment with capsaicin. Br J Pharmacol Chemother. 1967;31:138-151.
25. Holzer P. Local effector functions of capsaicin-sensitive sensory nerve endings: involvement of tachykinins, calcitonin gene-related peptide and other neuropeptides. Neuroscience. 1988;24:739-768.
26. Maggi CA. Tachykinins and calcitonin gene-related peptide (CGRP) as co-transmitters released from peripheral endings of sensory nerves. Prog Neurobiol. 1995;45:1-98.
27. Brain SD, Williams TJ. Substance P regulates the vasodilator activity of calcitonin gene-related peptide. Nature. 1988;335:73-75.
28. Newbold P, Brain SD. The modulation of inflammatory oedema by calcitonin gene-related peptide. Br J Pharmacol. 1993;108:705-710.
29. Holzer P. Neurogenic vasodilatation and plasma leakage in the skin. Gen Pharmacol. 1998;30:5-11.
30. Brain SD, Williams TJ, Tippins JR, et al. Calcitonin gene-related peptide is a potent vasodilator. Nature. 1985;313:54-56.
31. Hughes SR, Brain SD. A calcitonin gene-related peptide (CGRP) antagonist (CGRP8-37) inhibits microvascular responses induced by CGRP and capsaicin in skin. Br J Pharmacol. 1991;104:738-742.
32. Bowden JJ, Garland AM, Baluk P, et al. Direct observation of substance P-induced internalization of neurokinin 1 (NK1) receptors at sites of inflammation. Proc Natl Acad Sci U S A. 1994;91:8964-8968.
33. Steinhoff M, Vergnolle N, Young SH, et al. Agonists of proteinase-activated receptor 2 induce inflammation by a neurogenic mechanism. Nat Med. 2000;6:151-158.
34. Stead RH, Tomioka M, Quinonez G, Simon GT, Felten SY, Bienenstock J. Intestinal mucosal mast cells in normal and nematode-infected rat intestines are in intimate contact with peptidergic nerves. Proc Natl Acad Sci U S A. 1987;84:2975-2979.
35. Naukkarinen A, Harvima IT, Aalto ML, Horsmanheimo M. Mast cell tryptase and chymase are potential regulators of neurogenic inflammation in psoriatic skin. Int J Dermatol. 1994;33:361-366.
36. Metcalfe DD, Baram D, Mekori YA. Mast cells. Physiol Rev. 1997;77:1033-1079.
37. Felten DL, Ackerman KD, Wiegand SJ, Felten SY. Noradrenergic sympathetic innervation of the spleen: I. Nerve fibers associate with lymphocytes and macrophages in specific compartments of the splenic white pulp. J Neurosci Res. 1987;

18:28-36, 118-121.
38. Felten SY, Olschowka J. Noradrenergic sympathetic innervation of the spleen: II. Tyrosine hydroxylase (TH)-positive nerve terminals form synapticlike contacts on lymphocytes in the splenic white pulp. J Neurosci Res. 1987;18:37-48.
39. Bulloch K, Pomerantz W. Autonomic nervous system innervation of thymic-related lymphoid tissue in wildtype and nude mice. J Comp Neurol. 1984;228:57-68.
40. Stead RH, Dixon MF, Bramwell NH, Riddell RH, Bienenstock J. Mast cells are closely apposed to nerves in the human gastrointestinal mucosa. Gastroenterology. 1989;97:575-585.
41. Arizono N, Matsuda S, Hattori T, Kojima Y, Maeda T, Galli SJ. Anatomical variation in mast cell nerve associations in the rat small intestine, heart, lung, and skin. Similarities of distances between neural processes and mast cells, eosinophils, or plasma cells in the jejunal lamina propria. Lab Invest. 1990;62:626-634.
42. Roznieckí JJ, Dimitriadou V, Lambracht-Hall M, et al. Morphological and functional demonstration of rat dura mater mast cell-neuron interactions *in vitro* and *in vivo*. Brain Res. 1999;849:1-15.
43. Tournier JN, Hellmann AQ. Neuro-immune connections: evidence for a neuro-immunological synapse. Trends Immunol. 2003;24:114-115.
44. Blennerhassett MG, Bienenstock J. Apparent innervation of rat basophilic leukaemia (RBL-2H3) cells by sympathetic neurons *in vitro*. Neurosci Lett. 1990;120:50-54.
45. Blennerhassett MG, Tomioka M, Bienenstock J. Formation of contacts between mast cells and sympathetic neurons *in vitro*. Cell Tissue Res. 1991;265:121-128.
46. Blennerhassett MG, Bienenstock J. Sympathetic nerve contact causes maturation of mast cells *in vitro*. J Neurobiol. 1998; 35:173-182.
47. Janiszewski J, Bienenstock J, Blennerhassett MG. Activation of rat peritoneal mast cells in coculture with sympathetic neurons alters neuronal physiology. Brain Behav Immun. 1990;4:139-150.
48. McKay DM, Bienenstock J. The interaction between mast cells and nerves in the gastrointestinal tract. Immunol Today. 1994;15:533-538.
49. Frieling T, Cooke HJ, Wood JD. Serotonin receptors on submucous neurons in guinea pig colon. Am J Physiol. 1991; 261:G1017-1023.
50. Frieling T, Cooke HJ, Wood JD. Histamine receptors on submucous neurons in guinea pig colon. Am J Physiol. 1993; 264:G74-80.
51. Marone G, Galli SJ, Kitamura Y. Probing the roles of mast cells and basophils in natural and acquired immunity, physiology and disease. Trends Immunol. 2002;23:425-427.
52. van Houwelingen AH, Kool M, de Jager SC, et al. Mast cell-derived TNF-α primes sensory nerve endings in a pulmonary hypersensitivity reaction. J Immunol. 2002;168:5297-5302.
53. Kakurai M, Monteforte R, Suto H, Tsai M, Nakae S, Galli SJ. Mast cell-derived tumor necrosis factor can promote nerve fiber elongation in the skin during contact hypersensitivity in mice. Am J Pathol. 2006;169:1713-1721.
54. Leon A, Buriani A, Dal Toso R, et al. Mast cells synthesize, store, and release nerve growth factor. Proc Natl Acad Sci U S A. 1994;91:3739-3743.
55. Suzuki M, Furuno T, Teshima R, Sawada J, Nakanishi M. Soluble factors from rat basophilic leukemia (RBL-2H3) cells stimulated cooperatively the neurite outgrowth of PC12 cells. Biol Pharm Bull. 1998;21:1267-1270.
56. van der Kleij HP, Ma D, Redegeld FA, et al. Functional expression of neurokinin 1 receptors on mast cells induced by IL-4 and stem cell factor. J Immunol. 2003;171:2074-2079.
57. Krumins SA, Broomfield CA. C-terminal substance P fragments elicit histamine release from a murine mast cell line. Neuropeptides. 1993;24:5-10.
58. Cooke HJ, Fox P, Alferes L, Fox CC, Wolfe SA Jr. Presence of NK1 receptors on a mucosal-like mast cell line, RBL-2H3 cells. Can J Physiol Pharmacol. 1998;76:188-193.
59. Bischoff SC, Schwengberg S, Lorentz A, et al. Substance P and other neuropeptides do not induce mediator release in isolated human intestinal mast cells. Neurogastroenterol Motil. 2004;16:185-193.
60. Dimitriadou V, Buzzi MG, Moskowitz MA, Theoharides TC. Trigeminal sensory fiber stimulation induces morphological changes reflecting secretion in rat dura mater mast cells. Neuroscience. 1991;44:97-112.
61. Suzuki R, Furuno T, McKay DM, et al. Direct neurite-mast cell communication *in vitro* occurs *via* the neuropeptide substance P. J Immunol. 1999;163:2410-2415.
62. Kessler JA, Adler JE, Bell WO, Black IB. Substance P and somatostatin metabolism in sympathetic and special sensory ganglia *in vitro*. Neuroscience. 1983;9:309-318.
63. Karimi K, Redegeld FA, Heijdra B, Nijkamp FP. Stem cell factor and interleukin-4 induce murine bone marrow cells to develop into mast cells with connective tissue type characteristics *in vitro*. Exp Hematol. 1999;27:654-662.
64. Karimi K, Redegeld FA, Blom R, Nijkamp FP. Stem cell factor and interleukin-4 increase responsiveness of mast cells to substance P. Exp Hematol. 2000;28:626-634.
65. Furuno T, Ma D, van der Kleij HP, Nakanishi M, Bienenstock J. Bone marrow-derived mast cells in mice respond in co-culture to scorpion venom activation of superior cervical ganglion neurites according to level of expression of NK-1 receptors. Neurosci Lett. 2004;372:185-189.
66. Janiszewski J, Bienenstock J, Blennerhassett MG. Picomolar doses of substance P trigger electrical responses in mast cells without degranulation. Am J Physiol. 1994;267:C138-C145.
67. Ohshiro H, Suzuki R, Furuno T, Nakanishi M. Atomic force microscopy to study direct neurite-mast cells (RBL) communication *in vitro*. Immunol Lett. 2000;74:211-214.
68. Nakamura R, Furuno T, Nakanishi M. Atomic force microscopy for the cellular localization of secretory granules and coated pits in rat basophilic leukemia cells after antigen stimulation. Bioimages. 1998;6:69-75.
69. Metzelaar MJ, Wijngaard PL, Peters PJ, Sixma JJ, Nieuwenhuis HK, Clevers HC. CD63 antigen. A novel lysosomal membrane glycoprotein, cloned by a screening procedure for intracellular antigens in eukaryotic cells. J Biol Chem. 1991;266:3239-3245.
70. Furuno T, Teshima R, Kitani S, Sawada J, Nakanishi M. Surface expression of CD63 antigen (AD1 antigen) in P815 mastocytoma cells by transfected IgE receptors. Biochem Biophys Res Commun. 1996;219:740-744.
71. Amano T, Furuno T, Hirashima N, Ohyama N, Nakanishi M. Dynamics of intracellular granules with CD63-GFP in rat basophilic leukemia cells. J Biochem (Tokyo). 2001;129:739-744.
72. Mori N, Suzuki R, Furuno T, et al. Nerve-mast cell (RBL) interaction: RBL membrane ruffling occurs at the contact site with an activated neurite. Am J Physiol Cell Physiol. 2002;283: C1738-C1744.
73. Suzuki R, Furuno T, Teshima R, Nakanishi M. Bi-directional relationship of *in vitro* mast cell-nerve communication observed by confocal laser scanning microscopy. Biol Pharm Bull. 2001;24:291-294.
74. Suzuki R, Furuno T, Okamoto K, Teshima R, Nakanishi M. ATP

plays a role in neurite stimulation with activated mast cells. J. Neuroimmunol. 2007;192:49-56.
75. Goda Y. Cadherins communicate structural plasticity of presynaptic and postsynaptic terminals. Neuron. 2002;35:1-3.
76. Tanaka H, Shan W, Phillips GR, et al. Molecular modification of N-cadherin in response to synaptic activity. Neuron. 2000;25: 93-107.
77. Tegoshi T, Nishida M, Ishiwata K, et al. E-cadherin and cadherin-associated cytoplasmic proteins are expressed in murine mast cells. Lab Invest. 2000;80:1571-1581.
78. Suzuki A, Suzuki R, Furuno T, Teshima R, Nakanishi M. N-cadherin plays a role in the synapse-like structures between mast cells and neurites. Biol Pharm Bull. 2004;27:1891-1894.
79. Biederer T, Sara Y, Mozhayeva M, et al. SynCAM, a synaptic adhesion molecule that drives synapse assembly. Science. 2002;297:1525-1531.
80. Ito A, Jippo T, Wakayama T, et al. SgIGSF: a new mast-cell adhesion molecule used for attachment to fibroblasts and transcriptionally regulated by MITF. Blood. 2003;101:2601-2608.
81. Shingai T, Ikeda W, Kakunaga S, et al. Implications of nectin-like olecule-2/IGSF4/RA175/SgIGSF/TSLC1/SynCAM1 in cell-cell adhesion and transmembrane protein localization in epithelial cells. J Biol Chem. 2003;278:35421-35427.
82. Kuramochi M, Fukuhara H, Nobukuni T, et al. TSLC1 is a tumor-suppressor gene in human non-small-cell lung cancer. Nat Genet. 2001;27:427-430.
83. Furuno T, Ito A, Koma Y, et al. The spermatogenic Ig superfamily/synaptic cell adhesion molecule mast-cell adhesion molecule promotes interaction with nerves. J Immunol. 2005; 174:6934-6942.
84. Ogasawara T, Murakami M, Suzuki-Nishimura T, Uchida KM, Kudo I. Mouse bone marrow-derived mast cells undergo exocytosis, prostanoid generation, and cytokine expression in response to G protein-activating polybasic compounds after coculture with fibroblasts in the presence of c-kit ligand. J Immunol. 1997;158:393-404.
85. Okabe T, Hide M, Hiragun T, Morita E, Koro O, Yamamoto S. Bone marrow derived mast cell acquire responsiveness to substance P with Ca^{2+} signals and release of leukotriene B_4 *via* mitogen-activated kinase. J Neuroimmunol. 2006;181:1-12.
86. Suzuki A, Suzuki R, Furuno T, Teshima R, Nakanishi M. Calcium response and FcεRI expression in bone marrow-derived mast cells co-cultured with SCG neurites. Biol Pharm Bull. 2005;28:1963-1965.
87. Azzolina A, Bongiovanni A, Lampiasi N. Substance P induces TNF-α and IL-6 production through NFκB in peritoneal mast cells. Biochim Biophys Acta. 2003;1643:75-83.
88. Bischoff SC, Schwengberg S, Lorentz A, et al. Substance P and other neuropeptides do not induce mediator release in isolated human intestinal mast cells. Neurogastroenterol Motil. 2004;16: 185-193.
89. Elenkov IJ, Wilder RL, Chrousos GP, Vizi ES. The sympathetic nerve-an integrative interface between two supersystems: the brain and the immune system. Pharmacol Rev. 2000;52:595-638.
90. Kohm AP, Sanders VM. Norepinephrine and β2-adrenergic receptor stimulation regulate $CD4^+$ T and B lymphocyte function *in vitro* and *in vivo*. Pharmacol Rev. 2001;53:487-525.
91. Blalock JE. The syntax of immune-neuroendocrine communication. Immunol Today. 1994;15:504-511.
92. Besedovsky HO, del Rey A. Immune-neuro-endocrine interactions: facts and hypotheses. Endocr Rev. 1996;17:64-102.
93. Madden KS, Felten DL. Experimental basis for neural-immune interactions. Physiol Rev. 1995;75:77-106.
94. Carr DJ. Neuroendocrine peptide receptors on cells of the immune system. Chem Immunol. 1992;52:84-105.
95. Bylund DB, Eikenberg DC, Hieble JP, et al. International Union of Pharmacology nomenclature of adrenoceptors. Pharmacol Rev. 1994;46:121-136.
96. Sanders VM, Baker RA, Ramer-Quinn DS, Kasprowicz DJ, Fuchs BA, Street NE. Differential expression of the β2-adrenergic receptor by Th1 and Th2 clones: implications for cytokine production and B cell help. J Immunol. 1997;158: 4200-4210.
97. Ramer-Quinn DS, Baker RA, Sanders VM. Activated T helper 1 and T helper 2 cells differentially express the β2-adrenergic receptor: a mechanism for selective modulation of T helper 1 cell cytokine production. J Immunol. 1997;159:4857-4867.
98. Fuchs BA, Campbell KS, Munson AE. Norepinephrine and serotonin content of the murine spleen: its relationship to lymphocyte β-adrenergic receptor density and the humoral immune response *in vivo* and *in vitro*. Cell Immunol. 1988; 117:339-351.
99. Madden KS, Sanders VM, Felten DL. Catecholamine influences and sympathetic neural modulation of immune responsiveness. Annu Rev Pharmacol Toxicol. 1995;35:417-448.
100. Takemura H, Hatta S, Yamada K, Ohshika H. Regulation of Ca^{2+} mobilization by β-adrenergic receptor in Jurkat human leukemia T-cells. Ann N Y Acad Sci. 1993;707:489-491.
101. Hoshi S, Furutani-Seiki M, Seto M, Tada T, Asano Y. Prevention of TCR-mediated apoptosis by the elevation of cAMP. Int Immunol. 1994;6:1081-1089.
102. Engelhardt B, Ransohoff RM. The ins and outs of T-lymphocyte trafficking to the CNS: anatomical sites and molecular mechanisms. Trends Immunol. 2005;26:485-495.
103. Feldman RD, Hunninghake GW, McArdle WL. β-adrenergic-receptor-mediated suppression of interleukin 2 receptors in human lymphocytes. J Immunol. 1987;139:3355-3359.
104. Mary D, Peyron JF, Auberger P, Aussel C, Fehlmann M. Modulation of T cell activation by differential regulation of the phosphorylation of two cytosolic proteins. Implication of both Ca^{2+} and cyclic AMP-dependent protein kinases. J Biol Chem. 1989;264:14498-14502.
105. Wacholtz MC, Minakuchi R, Lipsky PE. Characterization of the 3',5'-cyclic adenosine monophosphate-mediated regulation of IL2 production by T cells and Jurkat cells. Cell Immunol. 1991; 135:285-298.
106. Krause DS, Deutsch C. Cyclic AMP directly inhibits IL-2 receptor expression in human T cells: expression of both p55 and p75 subunits is affected. J Immunol. 1991;146:2285-2296.
107. Chen D, Rothenberg EV. Interleukin 2 transcription factors as molecular targets of cAMP inhibition: delayed inhibition kinetics and combinatorial transcription roles. J Exp Med. 1994; 179:931-942.
108. Tamir A, Isakov N. Cyclic AMP inhibits phosphatidylinositol-coupled and -uncoupled mitogenic signals in T lymphocytes. Evidence that cAMP alters PKC-induced transcription regulation of members of the jun and fos family of genes. J Immunol. 1994;152:3391-3399.
109. Ramer-Quinn DS, Swanson MA, Lee WT, Sanders VM. Cytokine production by naïve and primary effector $CD4^+$ T cells exposed to norepinephrine. Brain Behav Immun. 2000;14:239-255.
110. Swanson MA, Lee WT, Sanders VM. IFN-γ production by Th1 cells generated from naïve $CD4^+$ T cells exposed to norepinephrine. J Immunol. 2001;166:232-240.
111. Kin NW, Sanders VM. It takes nerve to tell T and B cells what

to do. J Leukoc Biol. 2006;79:1093-1104.
112. Järvikallio A, Harvima IT, Naukkarinen A. Mast cells, nerves and neuropeptides in atopic dermatitis and nummular eczema. Arch Dermatol Res. 2003;295:2-7.
113. Peters EM, Kuhlmei A, Tobin DJ, Muller-Rover S, Klapp BF, Arck PC. Stress exposure modulates peptidergic innervation and degranulates mast cells in murine skin. Brain Behav Immun. 2005;19:252-262.
114. D'Andrea MR, Saban MR, Gerard NP, Wershil BK, Saban R. Lack of neurokinin-1 receptor expression affects tissue mast cell numbers but not their spatial relationship with nerves. Am J Physiol Regul Integr Comp Physiol. 2005;288:491-500.
115. Scholzen TE, Steinhoff M, Sindrilaru A, et al. Cutaneous allergic contact dermatitis responses are diminished in mice deficient in neurokinin 1 receptors and augmented by neurokinin 2 receptor blockage. FASEB J. 2004;18:1007-1009.
116. van der Kleij HP, Kraneveld AD, Redegeld FA, Gerard NP, Morteau O, Nijkamp FP. The tachykinin NK1 receptor is crucial for the development of non-atopic airway inflammation and hyperresponsiveness. Eur J Pharmacol. 2003;476:249-255.
117. Rijnierse A, van Zijl KM, Koster AS, Nijkamp FP, Kraneveld AD. Beneficial effect of tachykinin NK1 receptor antagonism in the development of hapten-induced colitis in mice. Eur J Pharmacol. 2006;548:150-157.
118. Kandere-Grzybowska K, Gheorghe D, Priller J, et al. Stress-induced dura vascular permeability does not develop in mast cell-deficient and neurokinin-1 receptor knockout mice. Brain Res. 2003;980:213-220.
119. Peters EM, Arck PC, Paus R. Hair growth inhibition by psychoemotional stress: a mouse model for neural mechanisms in hair growth control. Exp Dermatol. 2006;15:1-13.
120. Arck PC, Slominski A, Theoharides TC, Peters EM, Paus R. Neuroimmunology of stress: skin takes center stage. J Invest Dermatol. 2006;126:1697-1704.
121. Siebenhaar F, Sharov AA, Peters EM, et al. Substance P as an immunomodulatory neuropeptide in a mouse model for autoimmune hair loss (alopecia areata). J Invest Dermatol. 2007; 127:1489-1497.
122. Kovács P, Hernádi I, Wilhelm M. Mast cells modulate maintained neuronal activity in the thalamus *in vivo*. J Neuroimmunol. 2006;171:1-7.
123. Schemann M, Michel K, Ceregrzyn M, Zeller F, Seidl S, Bischoff SC. Human mast cell mediator cocktail excites neurons in human and guinea-pig enteric nervous system. Neurogastroenterol Motil. 2005;17:281-289.
124. Barbara G, Wang B, Stanghellini V, et al. Mast cell-dependent excitation of visceral-nociceptive sensory neurons in irritable bowel syndrome. Gastroenterology. 2007;132:26-37.
125. Ito A, Hagiyama M, Oonuma J, Murakami Y, Yokozaki H, Takaki M. Involvement of the SgIGSF/Necl-2 adhesion molecule in degranulation of mesenteric mast cells. J Neuroimmunol. 2007;184:209-213.
126. Abbracchio MP, Burnstock G. Purinergic signalling: Pathophysiological roles. Jpn J Pharmacol. 1998;78:113-145.
127. Novak I. ATP as a signaling molecule: the exocrine focus. News Physiol Sci. 2003:18:12-17.
128. Sara Y, Biederer T, Atasoy D, et al. Selective capability of SynCAM and neuroligin for functional synapse assembly. J Neurosci. 2005;25:260-270.
129. Galibert L, Diemer GS, Liu Z, et al. Nectin-like protein 2 defines a subset of T-cell zone dendritic cells and is a ligand for class-I-restricted T-cell-associated molecule. J Biol Chem. 2005;280:21955-21964.
130. Boles KS, Barchet W, Diacovo T, Cella M, Colonna M. The tumor suppressor TSLC1/NECL-2 triggers NK-cell and $CD8^+$ T-cell responses through the cell-surface receptor CRTAM. Blood. 2005;106:779-786.
131. Arase N, Takeuchi A, Unno M, et al. Heterotypic interaction of CRTAM with Necl2 induces cell adhesion on activated NK cells and $CD8^+$ T cells. Int Immunol. 2005;17:1227-1237.
132. Obata K, Furuno T, Nakanishi M, Togari A. Direct neurite-osteoblastic cell communication, as demonstrated by use of an *in vitro* co-culture system. FEBS Lett. 2007;581:5917-5922.
133. Koma Y, Furuno T, Hagiyama M, et al. CADM1 is a novel pancreatic islet cell adhesion molecule involved in interaction with nerves and in hormone secretion. Gastroenterology. 2008; 134:1544-1554.