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INFLUENCE OF NERVE TISSUE-DERIVED NEUROTRANSMITTERS ON MAST CELL MIGRATION

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ABSTRACT

In vitro culture systems using bone marrow cells from BALB/C mice were set up in medium supplemented with spleen-derived medium. Bone marrow cells grown in spleen-derived medium gave rise to cultures containing >97% mast cells. The cells were used in polarisation chemotaxis assays with the intention of determining the effect of nerve tissue-derived neurotransmitters on mast cell migration. Some available neurotransmitters including (substance-p, histamine, serotonin, Dopa, noradrenaline, acetylcholine, aspartic acid, epinephrine and nerve extracts) were tested. Mast cells showed a significant morphological response to 10^{-3} and 10^{-4} M histamine, 10^{-9} M serotonin, and 10^{-2} M Dopa. Nerve extracts induced some shape changes in mast cells.
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INTRODUCTION

The concept of psychological effects, mediated through the activities of the nervous system on immune functioning has to a large extent been described by several groups of researchers.[1-4] The intimate collaboration between the nervous system and the immune system has changed the view that immune responses are isolated from neural influences. Lymphoid tissue has been shown to be directly innervated by peptide-containing nerve fibers (enteric, sympathetic and parasympathetic nervous system).[5,6] It is well established that mast cells are critical in IgE-dependent hypersensitivity reactions involving the release of multifunctional cytokines including IL1, IL2, IL3, IL4, IL5, IL6, GM-CSF, TNF-α, TGF-β, MIP-1α, MIP-1β, JE, TCA3, and IFN-γ.[7] Also, it is now well established that mast cells are associated with nerves in many tissues[8] in a variety of species, with evidence for mast cells cooperating with the autonomic nervous system.[9] Neuroanatomical associations and close apposition between mast cells and nerves in many normal and diseased organs[10,11] and in several culture models[12] have been studied. The hyperplastic gathering of mast cells around injured and damaged nerves has been observed.[13-15] The negative relationship between mast cells and old nerves and the positive relationship of mast cells with newly regenerating nerves[16] can also be considered as evidence for migration of mast cells toward inflamed tissue as a result of the activity of material being produced by nerves during nerve irritation or regeneration. Based on these observations and the growing list of chemicals, neuropeptides, neurotransmitters and cytokines which are found in autonomic neurons[17-19] and glial cells,[20] and the direct influence of the nerve and neuroendocrine peptides on the immune system,[6] the functional interaction between mast cells and the nervous system[21-23] and histological observations (unpublished observation), the possibility that substances derived from nerve cells might serve to attract mast cells was investigated.

MATERIAL AND METHODS

Bone marrow preparation

Animals were killed in a CO_2 chamber. Under sterile laminar air flow, the legs were disconnected from the body and the feet were cut off. The muscles were then dissected away completely from the femur and tibia in a petri dish containing HBSS solution. Using a 25 gauge needle and a syringe, BSS was flushed through the bone to free the bone marrow. Cells were washed in BSS by centrifugation at 200g

for 10 min.

Spleen cell derived medium

A mixture of 879 mL RPMI-medium, 100 mL (10%) FCS, 1 mL stock 2-mercaptoethanol (50 μM), 10mL stock L-glutamine solution (2 mM), 10 mL non-essential amino acids (0.1 mM), and 0.4 mL stock concanavalin A (2 μg/mL) was produced and pH was adjusted to 7.2 and divided into twenty 75 cm^2 tissue culture flasks, each 50 mL. 5×10^7 nucleated spleen cells from C57B1/6J-C3H male mice were added to each flask and incubated at 37°C in humidified 5% CO_2/95% air for 45 hours. The suspension was certrifuged at 1000 g for 20 min and the supernatant removed, filtered through a 0.45 μm filter, divided into aliquots, and stored frozen until use.

Cell culture for mast cell production

Forty mL of culture medium was put into 75 cm^2 tissue culture flasks and 1×10^5 nucleated bone marrow cells/mL from either male or female BALB/C mice (8-16 weeks old) were added into each flask and incubated at 37°C in humidified 5% CO_2 and 95% air for 7 days. After 1 week the medium was centrifuged at 200 g for 10 min and the pelleted cells resuspended in 1-2 mL medium with a small sample being taken for cell counting and viability determination. 1×10^5 cells /mL were transferred into other flasks containing fresh mast cell culture medium and incubated for another week. At the end of the second week the cells were collected by centrifugation at 200 g for 10 min and used after examination for viability.

Nerve cell suspension

The brain and large part ot the spinal cord of a female BALB/C mouse was removed under sterile conditions after killing the animal. The tissue was homogenised in a mortar for half an hour and the supernatant collected by centrifugation. The solution was stored in a refrigerator and used within 24h. Serial dilutions were made from the extract of brain and spinal cord as 1/3, 1/9, 1/27, 1/81, 1/243, 1/729, 1/2187, 1/6561, and 1/19683.

Polarisation assay

Hanks' balanced salt solution with MOPS was made freshly. The substances to be tested were prepared in appropriate concentration in 1 mL of HBSS/MOPS and were added into each of the 50 conical capped plastic 15 mL sterile tubes. Mast cells collected from the culture were washed twice with HBSS/MOPS solution centrifuged at 200 g and pelleted cells were resuspended in suitable volumes. An aliquot was taken for cell counting and cell viability examination. 5×10^5 cells were added to each tube and incubated for 30 min at 37°C. 1 mL of 2.5% glutaraldehyde in HBSS/MOPS was added to the tubes and after 15 min the fixed cells were washed twice, centrifuged at 200 g with HBSS/MOPS and resuspended in the remaining HBSS/MOPS and stored at 4°C. About 300-400 cells were examined by phase contrast microscopy under a 40× objective and polarised cells were counted.

RESULTS

The cell polarisation assay was used for investigation of a possible chemoattractant effect of substance-p on mast cells. No significant differences were observed between the experimental and control groups when using the cell polarisation assay. Using the same assay for investigation of a possible chemoattractant effect of histamine on mast cells, a significant percentage of cell polarisation was observed with 10^{-3}, 10^{-4} and 10^{-5}M histamine, with greater differences obtained with 10^{-3} M histamine (Fig. 1).

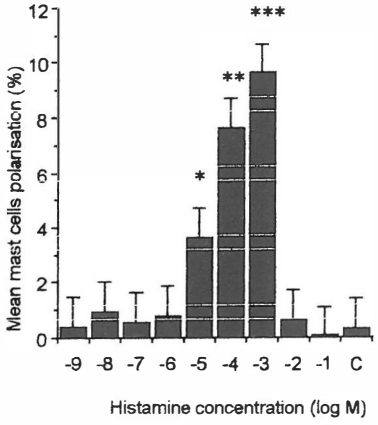

Fig. 1. Dose response studies of the efficiency of mast cell polarisation with different concentrations of histamine.

The cell polarisation assay also showed significant cell polarisation against serotonin and Dopa at 10^{-9}, 10^{-10} and 10^{-2} M (Figs. 2,3), respectively.

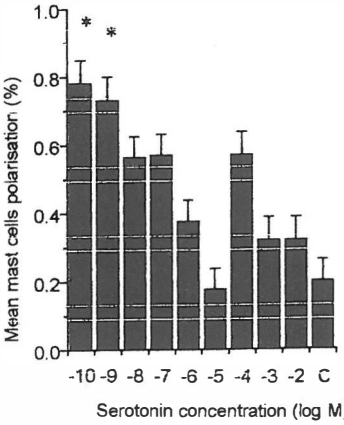

Fig. 2. Dose response studies of the efficiency of mast cell polarisation with different concentrations of serotonin.

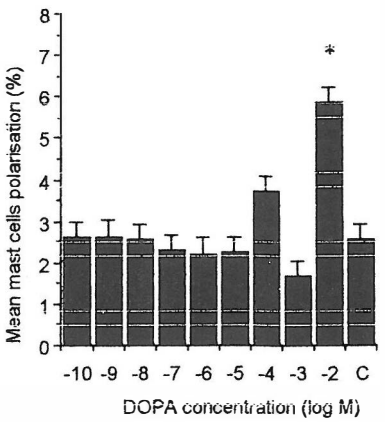

Fig. 3. Dose response studies of the efficiency of mast cell polarisation with different concentrations of Dopa.

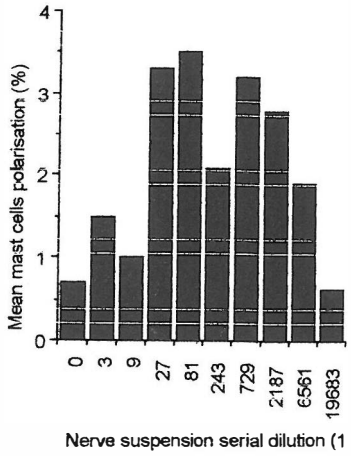

Fig. 4. Dose response studies of the efficiency of mast cell polarisation with different concentrations of nerve suspension.

No significant differences between experimental and control groups were observed regardless of concentrations of noradrenaline, acetylcholine, aspartic acid and epinephrine. Differences were observed between experimental and control groups with different concentrations of nerve suspension (Fig. 4).

The difference between experimental and control groups was found to be significantly different ($*p<0.05$, $**0.001<p<0.01$, $***p<0.001$, using an unpaired t-test, n= 5), each column represents mean±SE.

DISCUSSION

Cultured mast cells were used in all the experiments. Perhaps using mast cells collected from other sources would have been more appropriate. The enteric nerve system (ENS) consists of an estimated 10^8 nerve cells, a number equivalent to that in the spinal cord.[24] In addition, the neuronal distribution in the muscular layer of small intestine in some animals, including guinea pigs,[25] rabbits[26] and cats[27] confirms that there is a greater neuronal density in the duodenum than in the ileum. In recent studies, greater numbers of mast cells have been observed in the anterior part of the small intestine than the posterior and more mast cells have been observed in small intestine than in the liver (personal observation). Together, all these observations offer support for the hypothesis that the nervous system may be involved directly in mast cell attraction. This proposition is further supported by the observations that Schwann cell produced stem cell factor (SCF)[28] can cause *in vitro* mast cell migration[29,30] and transforming growth factor-β (TGF-β), which is chemotactic for mast cells,[31] can be produced by astrocytes in disease conditions.[32] In spite of temporary axonal sprouting during nerve regeneration and the innervation of the damaged tissues,[33] the numbers of nerves in peripheral tissues are often supposed to be stable[16] and because mast cells are migratory cells, therefore the innervation of mast cells by nerve fiber does not seem to be highly probable. Using the polarisation assay, mast cells showed a significant morphological response to 10^{-3}, 10^{-4} and 10^{-5} M histamine, 10^{-9} M serotonin and 10^{-2} M L-3-4-dihydroxyphenylalanine (DOPA). Mast cells did not show a significant change of shape at 10^{-1} - 10^{-9} M acetylcholine concentration or at 10^{-4} - 10^{-12} M noradrenaline and aspartic acid concentrations. Preliminary polarisation assays using epinephrine showed that nerve extracts induced some shape changes in mast cells.

Only a few of more than 20 recognised neurotransmitters[34] were examined during this study. The remainder are still to be tested for an effect on mast cell attraction. In spite of these preliminary promising results more experiments need to be carried out. Even if the effect of neurotransmitters on mast cell motility is confirmed, directional cellular mobility must be verified by either the micropore filter, or agarose or collagen gel assay.

It is well established that cytokine profiles are important in mediating the resistance to various infections.[16] T cells are a rich source of cytokines. T cell precursors (CD4+) may terminally differentiate to either the Th_1 subset, producing IL-2 and INF-γ involved in cell mediated inflammatory functions, or differentiate to the Th_2 subset producing IL-4, IL-5, and IL-10 which are involved in antibody production, particularly IgE and eosinophil production and proliferation. IL-4, IL-10 and TGF-β are important down-regulators of Th1 type responses[35,36] and INF-γ and IL-12 are powerful cytokines which can down-regulate Th_2 type responses.[35] Despite our current understanding of the basic effector role of Th_1 and Th_2 on immune responses, the full extent of the pattern of Th_1 or Th_2 selection during immune responses is not yet clear and full of contradictory statements.[36,37] Since mast cells are a source of IL-4, IL-10 and TGF-β together,[38-40] the primary role of mast cells in early Th_2 switching may be important. One hypothesis to explain

why the immune response moves towards Th_1 or Th_2 type is described below.

If the inducing antigen is small or soluble and is taken up by macrophages or B cells, IL-12 production of IFN-γ by Th_1 cells produces macrophage activation and cell-mediated immunity. In contrast, when the foreign particle is coarse or resistant to destruction (pollens, allergens, worms or even a fetus in the uterus [Th_1 response is suppressed systematically during pregnancy] they are more likely to impinge on the nervous system. As a consequence the release of mediators which influence and attract mast cells will result in the involvement of cytokines which favour Th_2-type responses. Any physical, chemical or indeed mechanical stimulation of nerve receptors may cause release of mast cell attracting substances from the nerve cells and consequently local accumulation of mast cells. Later, activation and then degranulation of mast cells by nerve derived substances or by antigen stimulation through IgE on the surfaces of mast cells may result in the release of a large number of mediators including IL-4, IL-10 and TGF-β which cause Th_1 down-regulation and up-regulation of Th_2-type responses. In spite of the clear effects of psychological factors in disease, the contribution of these factors to the process of diseases is not yet clear; recent hypotheses, which point to a functional link between the nervous and immune system might be an acceptable answer to this question. The connection betweennervous system and immune system may be facilitated in part by mast cell migration. The effect of the nervous system upon Th_2 may be a consequence of mast cell degranulation. In the case of parasites, a direct effect of the pathogen may be the initiating stimulus (afferent). In the case of psychological effects the same mechanism may be involved, though the initiating stimulus may be different (efferent).

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