NERVES AND NEUROPEPTIDES IN THE REGULATION OF MUCOSAL IMMUNITY

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The subject of mucosal immunity continues to generate considerable interest. It is clear that the presentation of antigen to the mucosa, especially in the form of replicating virus or live attenuated organisms produces a local mucosal immune response. This response is better than if the antigens are presented in other forms, and certainly better than if they are presented via other routes[1-3]. Thus, the experiments performed some fifty years ago on immunization of volunteers against dysentery by oral immunization[4] with Shigella organisms, which produced incomplete but definite protection amongst those vaccinate, clearly hold up.

With the understanding that IgA was the predominant immunoglobulin of secretions have come attempts to explain this phenomenon. Not surprisingly, since 90% of the B cells synthesizing immunoglobulin, e.g. in the intestinal tract, appear to be making IgA, emphasis has been placed on this immunoglobulin molecule. Most studies involved in characterizing immune responses at local mucosal surfaces have focused on IgA, and it has become dogma that this is the most important mechanism of protection at mucosal surfaces. This indeed may be true, but it has not been categorically proven. Indeed, it would be difficult to do so. There are very few clear experiments which support the thesis, by passive transfer of immunoglobulin or other means, that IgA is the predominant form of protection at mucosal surfaces. It is obvious that IgA must play an important role, but whether it is the most important role in terms of potentially invasive organisms, still remains to be established. For example, the cellular component of mucosal immunity has received considerably less attention, and it is less well known and understood how cells may involve themselves in provision of protection, at mucosal surfaces. Furthermore, the interaction between immune cells and the epithelium, and the regulation of so-called non-specific protective mechanisms is both poorly studies and understood.

In view of the fact that mucosal immunity and its principles have been well reviewed and discussed elsewhere, and such reviews are readily available to the interested reader, we will review some potentially interesting more recent developments in this field.[1-3,5,6]

It is well established that lymphoid tissues are innervated, and that large amounts of neuropeptides are found in mucosal tissues.[7] Interest has been elicited by observations of increased levels of neuropeptides such as vasointestinal polypeptide (VIP), in the involved tissue, in inflammatory bowel disease.[8] We ourselves have been interested in a relationship between neurotransmitters, nerves and immunity in mucosal tissues.[9,10]

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In this short review, we will attempt to cover this aspect of the potential regulation of mucosal immune events, since it is an area which has received relatively little attention over the years, and is one which may play an important part in protection. Furthermore, with the recent observations by Ader and Cohen, and subsequently by others, it has become clearer that the central nervous system (CNS) might influence immunity.[11] Other experiments have suggested that the immune system itself might influence the CNS, but we will not discuss this important area herein.

In order to put our studies into a better perspective, while the reader should go elsewhere for details, we will very briefly summarize some principles of mucosal immunity before discussing the possible role of nerves and neuropeptides in this system.

PRINCIPLES OF MUCOSAL IMMUNITY

IgA

As stated before, IgA is the predominant immunoglobulin found in mucosal secretions. It is found in the so-called secretory forms, usually covalently coupled to secretory component, which acts as the transport protein for the dimeric IgA secreted by plasma cells in the tissues, across the epithelial cells to their apical surfaces. At the cell surface, the molecule is cleaved leaving a portion of the secretory component behind in the membrane, and releasing the secretory IgA molecule. The molecule in this form is highly resistant to proteolysis and therefore particularly suited to the potentially degradative surroundings in which it finds itself. The function of the IgA molecule is primarily to interfere with binding to surface sites, and thus prevent bacterial colonization and viral attachment. Once bound, IgA also seems able to prevent complement lysis and furthermore may cooperate with cells (particularly activated macrophages and/or cells of the suppressor/cytotoxic T-cell lineage) to produce antibacterial antibody-dependent cell-mediated cytotoxicity.[12]

Lymphoid Aggregates

The initiation of the IgA response is either in the aggregates of lymphoid tissue in the gut (GALT) or the lung (BALT) or in the draining lymph nodes. Evidence has been adduced to suggest that the epithelium itself, especially if it expresses Ia molecules, as it does when involved in inflammatory events, may process antigen so as to present it in a classical form, to T cells and B cells.[13] The extent of processing by the epithelium as compared to the lymphoid aggregates is undetermined at the present time. There are two not antagonistic views which state that initiation of the immune response therefore can occur in either the lymph node or the lymphoid aggregate, but it is in the lymphoid aggregates particularly that amplification of the system occurs (secondary immune response).

The M cells overlying the follicular aggregates have the selective capability of phagocytosing antigen and passing this into the follicles. Lymphocytes migrate from the lymphoid tissue to the draining lymph nodes and through the lymphatics to the blood. There seems to be a selective localization of lymphocytes, derived from mucosal tissues, within mucosal tissues, especially those from which they were originally derived.[14] This has produced the theory of 'a common mucosal immune system' in which cells, for example, of the IgA lineage from the mesenteric lymph node, have a tendency to selectively populate mucosal tissues such as respiratory and GI tracts distal to their point of origin.[15] More recently, it seems that this selective mucosal localization is on the basis of molecules expressed on lymphocyte cell surfaces, with complementary receptors expressed on the endothelium of post-capillary vessels in mucosal tissues.[16] What regulates the expression of these molecules is currently under intensive examination.

T cell Regulation

The lymphoid system of mucosal tissues is under intense immune regulation. On the positive side, this is through the generation of T helper cells which promote isotype-specific immunoglobulin synthesis,[17] or other T cells known as switch cells,[18] which promote the switch at the gene level from expression of IgM to IgA. A number of cytokines are now known to be involved in this process of IgA synthesis involving signalling between T cells and B cells, and IL-4 and IL-5 are particularly involved in this promotion of IgA synthesis.

A third set of T cells appear to be involved in promotion of IgA immune responses. These are a part of a cellular network known as the contra-suppressor pathway.[19] These T cells induce suppressor cells which in turn suppress suppression of IgA, thus rendering help.

Obviously, this positive regulation of the mucosal immune system must be carefully balanced by a negative effect, and down-regulated. This local suppressor concept extends to the whole concept of oral tolerance in which antigen presented orally promotes depression or suppression of the immune response in peripheral systemic tissues, while preserving mucosal responses.[20] While it is generally agreed that this is a very complex event, T suppressor cells generated in the mucosal lymphoid tissue are clearly involved.[21]

Epithelial Leukocytes

Within the epithelium lie a population of leukocytes most of them granulated.[22] These cells are above the basement membrane and have been termed intraepithelial leukocytes or lymphocytes (IEL). They represent an enormous compartment of lymphoid tissue which, in the intestine alone, occupy a volume the size of an average spleen. Many of the cells bear the CD8 phenotype most commonly associated with suppressor/cytotoxic T cells and lack pan-T markers in mice, rats and humans. It is possible that many of these leukocytes are indeed not T cells and some evidence has recently come forward suggesting that they may be more related to the myeloid lineage than to T cells (Croitoru and Ernst, unpublished data). Nevertheless, within this population are lymphocytes which have been shown to possess natural killer activity against tumors and viruses, contain precursor cells for cytotoxic T lymphocytes as well as the mature cells themselves, and mitogen activated cytotoxic cells.[23-25] They also contain a population of progenitor cells which under the influence of IL-3 differentiate into mast cells and have the highest mast cell precursor frequency in the body, higher even than that found in bone marrow.

Mucosal Nerves

The nervous network within mucosal tissues is very extensive.[26] It has been calculated that the number of nerve cell bodies present in the gastrointestinal tract is at least equivalent to that found in the spinal cord. Neurotransmitters are found in very large amounts in these tissues, particularly substance P (SP), VIP and somatostatin (SOM). In addition, nerves predominate in T cell zones of lymphoid aggregates, where they contain both neuropeptides, and especially sympathetic neurotransmitters, such as noradrenaline.[7] The blood vessels are surrounded by nerve plexuses, particularly at the level of post-capillary venules which are sites of lymphocyte traffic out of the circulation. Recently our own group has demonstrated that intestinal mucosal mast cells appear to be associated with enteric nerves lying in very close apposition to them.[27] In view of the network of enteric nerves and the fact that twigs from these nerves pass into the epithelium in respiratory and GI tracts, it is obvious that neuropeptides released at these nerve endings could greatly influence the function and activity of lymphocytes.

Neuropeptide Effects on Lymphocytes

Many different neuropeptides appear *in vitro* to have functional effect on immune cells such as B lymphocytes, T lymphocytes, natural killer cells macrophages and mast cells.[28-32] The neuropeptides which have received the greatest attention are SP, SOM, and VIP.

In vitro these appear to have so-called bidirectional effects, having different effects at very low concentration to those found at higher concentrations, but still within physiological limits. Furthermore, the culture conditions, dose, time of exposure, organ derivation, etc., all appear to exert effect on the responses of such cells to neuropeptides. What is more, these effects appear to be cell-cycle dependent, and this may account for the developing confusion which is currently found when delving into this literature. It should also be noted that if any effects of neuropeptides are found *in vivo*, which reflect those found *in vitro*, this does not necessarily mean that the same events are occurring as *in vitro*, since *in vivo* a multiplicity of signalling events obviously occurs between many organs and cells, which is only reflected, as the balance of these events, in the read-out system.

Neuropeptide Receptors

Specific receptors for SP are seen on human lymphocytes, particularly T helper cells. In the mouse however, high affinity receptors for SP are found both on B and T cells although lymphocytes from Peyer's patches have from 5 to 7 times as many receptors as those from spleen.[31] Receptors for SOM are seen on both murine T and B cells but again a greater percentage of cells from Peyer's patches bind SOM, and here the phenotype is predominantly $Lyt2^+$, indicating the predominance of the suppressor cytotoxic phenotype of the SOM-bearing cell.

Neuropeptide Effects in vitro

Somatostatin inhibits the thymidine uptake of both B and T cells when stimulated by mitogen (Con A, PHA or pokeweed mitogen).[30] This is interesting in view of the $Lyt2^+$ phenotype-bearing cell predominance described above and may explain the mode of action of SOM. In terms of immunoglobulin synthesis, SP tends to stimulate antibody synthesis, while SOM inhibits it, as measured either by a radioimmunoassay or reverse plaque forming assay. Most interestingly, SP produces an apparent organ-specific (Peyer's patches) synthesis of IgA in seven day cultures in the presence of Con A.[30]

Neuropeptide Effects on Cytotoxicity

VIP has been shown *in vitro* to increase human NK activity.[33] This again was bidirectional since preincubation with VIP caused stimulation, whereas coincubation caused an inhibitory effect, not dependent on adenylate cyclase activation. Our own recent observations have shown that SP stimulated the NK activity of IEL, while producing a minimal effect on NK activity of splenic lymphocytes.

Neuropeptide Effects in vivo

In order to obtain some information on whether these *in vitro* phenomena had any *in vivo* significance, we have performed experiments in which substance P was administered for seven days via subcutaneously implanted mini-osmotic pumps. This allowed serum levels of substance P to be attained, at a level two to three-fold higher than normal.[9] Cells freshly isolated from Peyer's patches and spleen from such animals, as opposed to controls, showed an increased proliferative capacity, and furthermore synthesized more immunoglobulin, appropriate to the organ derivation as we had seen

previously in vitro under the influence of Con A. On the other hand, SOM, when infused in a similar fashion had an opposite effect to that previously seen in vitro, namely it now caused proliferation of cells and increased antibody production. This suggests agains that neuropeptides may have dual or bidirectional effects as have been noted before, and extensively documented in the endocrine literature (for example, stimulation versus suppression of secretion).

Infusion of SP appeared to regularly promote the increased NK activity of cells isolated from the IEL compartment compatible with the in vitro findings.

NEUROPEPTIDES, NERVES AND MAST CELLS

Since we and others have shown that neuropeptides can cause degranulation of mast cells,[34] and since mast cells are involved in axon reflexes in the generation of so-called neurogenic inflammation,[35] we have examined relationships between mast cells, nerves, and neuropeptides. Extensive work has shown that only substance P causes degranulation of isolated mucosal mast cells, which differ in a number of ways from their conventional connective tissue counterparts. A very careful morphometric study furthermore has revealed that mucosal mast cells lying immediately under the epithelium are apparently selectively associated with enteric nerves and that this is not a random finding.[9] Ultrastructural observations have shown very frequent intimate membrane/membrane contact between nerves and mast cells, and similar but not so careful studies have demonstrated such contacts before in a variety of tissues.[37]

We have now performed very extensive observations in vitro on mast cell/nerve interactions involving Ussing chambers. Electrophysiological measurements have shown that mast cells are involved with nerves in the physiological regulation of epithelial cell function and integrity. Other observations have led us to the conclusion that this seems to apply also in vivo since capsaicin treatment at birth, which depresses or ablates the sensory afferent system which contains substance P, has profound effects in these model systems. We have concluded elsewhere that mast cells and nerves are involved as regulatory units in a variety of local regulatory events in the mucosal tissues of the lung and intestine.[9,10]

CONCLUSIONS

There is a large body of literature which suggests that there are neuroendocrine influences on the immune system and this idea is by no means new.[10,38] Considerable evidence exists for hormonal influence on many different aspects of the immune response. Corticosteroids have been well characterized in this respect, and it is known that sex hormones, independent of the gender of the animal, appear to have a significant influence on the synthesis and secretion of IgA in the eye.[39] Secretion of intestinal IgA has been shown in other experiments to be modulated by cholecystokinin (CCK) infusions which were blocked by atropine or CCK antagonists.[40] The well known effects of some of these hormones and neuropeptides on post-capillary venules,[14] on lymphoblast migration[41] and expression of secretory component[42] in sex hormone-dependent situations should also be emphasized.

It should hardly surprise us therefore that neuropeptides have profound effects on lymphocytes, as well as their circulation and migration. It now becomes even more clear that an examination of how neuropeptides regulate these events, and how these can be harnessed to promote mucosal immunity, must become the target for further investigation. It is reasonable to hope at the present time that these types of studies will allow the development of some new approaches to the regulation of mucosal immunity.

REFERENCES

1. J. Bienenstock and A.D. Befus, Mucosal immunology, Immunology 41: 249-270 (1980).
2. J. Bienenstock and A.D. Befus, The gastrointestinal tract as an immune organ, in: "Gastrointestinal Immunity for the Clinician", R.G. Shorter and J.B. Kirsner, eds., Grune and Stratton, New York, pp 1-22 (1985).
3. In: "Local Immune Responses of the Gut", T.J. Newby and C.R. Stokes, eds, CRC Press, Florida, pp 1-248 (1984).
4. A. Besredka, Local Immunization, Williams and Wilkins, Baltimore, MD, p 1 (1927).
5. C.O. Elson, M.F. Kagnoff, C. Fiocchi, A.D. Befus and S. Targan, Intestinal immunity and inflammation: recent progress, Gastroenterology, 91: 746-768 (1986).
6. J. Mestecky and J.R. McGhee, Immunoglobulin A (IgA): Molecular and cellular interactions involved in IgA biosynthesis and immune response, in: "Advances in Immunology" Vol. 40, F.J. Dixon, ed., Academic Press, pp 153-245 (1987).
7. D.L. Felten, S.Y. Felten, S.L. Carlson, J.A. Olschowka and S. Livnat, Noradrenergic and peptidergic innervation of lymphoid tissue, J. Immunol., 135: 755-765s (1985).
8. A.E. Bishop, J.M. Polak, M.G. Bryant, S.R. Bloom and S. Hamilton, Abnormalities of vasoactive intestinal polypeptide-containing nerves in Crohn's disease, Gastroenterology 79: 853-860 (1980).
9. R. Stead, J. Bienenstock and A.M. Stanisz, Neuropeptide regulation of mucosal immunity, Immunol. Rev. 100: 333-359 (1987).
10. J. Bienenstock, M. Perdue, A. Stanisz and R. Stead, Neurohormonal regulation of gastrointestinal immunity, Gastroenterology 93: 1431-1434 (1987).
11. R. Ader and N. Cohen, CNS-immune system interactions: conditioning phenomena, Behavioural and Brain Sci. 8: 379-395 (1985).
12. A. Tagliabue, L. Nencioni, L. Villa, D.F. Keren, G.H. Lowell and D. Boraschi. Antibody-dependent cell-mediated antibacterial activity of intestinal lymphocytes with secretory IgA, Nature 306: 184-186 (1983).
13. L. Mayer and R. Shlien, Evidence for function of Ia molecules on gut epithelial cells in man, J. Exp. Med. 166: 1471-1483 (1987).
14. J. Bienenstock, A.D. Befus, M. McDermott, S. Mirski and K. Rosenthal, Regulation of lymphoblast traffic and localization in mucosal tissues with emphasis on IgA, Fed. Proc. 42: 3213-3217 (1983).
15. M.R. McDermott and J. Bienenstock, Evidence for a common mucosal immunologic system. I. Migration of B-immunoblasts into intestinal respiratory and genital tissues, J. Immunol. 122: 1892-1898 (1979).
16. E.C. Butcher, The regulation of lymphocyte traffic, in:"Current Top. Microbiol. Immunol." Springer-Verlag Berlin-Heidelberg, vol. 128, pp 85-122 (1986).
17. C.O. Elson, J.A. Heck and W. Strober, T-cell regulation of murine IgA synthesis, J. Exp. Med. 149: 632-643 (1979).
18. H. Kawanishi, L.E. Saltzman and W. Strober, Mechanisms regulating IgA class-specific immunoglobulin production in murine gut-associated lymphoid tissues. I. T cells derived from Peyer's patches that switch sIgM B cells to sIgA B cells in vitro, J. Exp. Med. 157: 433-450 (1983).
19. I. Suzuki, K. Kitamura, T. Kurita, D.R. Green and J.R. McGhee, Isotype specific immunoregulation: Evidence for a distinct subset of T contra-suppressor cells for IgA responses in murine Peyer's patch, J. Exp. Med. 164: 501 (1986).
20. P.B. Ernst, R. Scicchitano, B.J. Underdown and J. Bienenstock, Oral immunization and tolerance, in: "Immunology of the Gastrointestinal Tract and Liver", A.L. Jones et al., eds., In press, Raven Press, N.Y. (1988).

21. G.L. Asherson, M. Zembala, M.A.C.C. Perera, B. Mayhew and W.R. Homas, Production of immunity and unresponsiveness in the mouse by feeding contact sensitizing agents and the role of suppressor cells in the Peyer's patches, mesenteric lymph nodes and other lymphoid tissues, Cell. Immunol. 33: 145-155 (1977).
22. P.B. Ernst, A.D. Befus and J. Bienenstock, Leukocytes in the intestinal epithelium: an unusual immunological compartment, Immunol. Today 6: 50-55 (1985).
23. S. Targan, L. Britvan, R. Kendal, S. Vimadalal and A. Soll, Isolation of spontaneous and interferon inducible natural killer like cells from human colonic mucosa lysis of lymphoid and autologous epithelial target cells, Clin. Exp. Immunol. 54: 14-22 (1983).
24. P.S. Carman, P.B. Ernst, K.L. Rosenthal, D.A. Clark, A.D. Befus and J. Bienenstock, Intraepithelial leukocytes contain an unique subpopulation of NK-like cytotoxic cells active in the defense of gut epithelium to enteric murine coronavirus, J. Immunol. 136: 1548-1553, 1986.
25. J.R. Klein and M.F. Kagnoff, Non-specific recruitment of cytotoxic effector cells in the intestinal mucosa of antigen-primed mice, J. Exp. Med. 160: 1931-1936 (1984).
26. H.J. Cooke, Neurobiology of the intestinal mucosa, Gastroenterology 90: 1057-1081 (1986).
27. R.H. Stead, M. Tomioka, G. Quinonez, G.T. Simon, S.Y. Felten and J. Bienenstock, Intestinal mucosal mast cells in normal and nematode infected rat intestine are in intimate contact with peptidergic nerves, Proc. Natl. Acad. Sci. USA 84: 2975-2979 (1987).
28. D.G. Payan, J.P. McGillis and E.J. Goetzl, Neuroimmunology, Adv. Immunol. 39: 299-323 (1986).
29. M.S. O'Dorisio, Neuropeptides and gastrointestinal immunity, Am. J. Med. 81: 74-82 (1986).
30. A.M. Stanisz, D. Befus and J. Bienenstock, Differential effects of vasoactive intestinal peptide, substance P and somatostatin on immunoglobulin synthesis and proliferation by lymphocytes from Peyer's patch, mesenteric lymph node and spleen, J. Immunol. 136: 152-156, (1986).
31. A.M. Stanisz, R. Scicchitano, P. Dazin, J. Bienenstock and D.G. Payan, Distribution of substance P receptors on murine spleen and Peyer's patch T and B cells, J. Immunol. 139: 749-754 (1987).
32. C.A. Ottaway and G.R. Greenberg, Interaction of vasoactive intestinal peptide with mouse lymphocytes specific binding and the modulation of mitogen responses, J. Immunol. 132: 417-423 (1984).
33. M. Rola-Pleszczynski, D. Bolduc and S. St. Pierre, The effects of vasoactive intestinal peptide on human natural killer cell function, J. Immunol. 135: 2569-2573 (1985).
34. F. Shanahan, J.A. Denburg, J. Fox, J. Bienenstock and D. Befus, Mast cell heterogeneity: effects of neuroenteric peptides on histamine release, J. Immunol. 135: 1331-1337 (1985).
35. J. Foreman, Peptides and neurogenic inflammation, Brit. Med. Bull., In press (1988).
36. J. Bienenstock, M. Tomioka, H. Matsuda, R.H. Stead, G. Quinonez, G.T. Simon, M.D. Coughlin and J.A. Denburg, The role of mast cells in inflammatory processes: Evidence for nerve/mast cell interactions, Int. Archs. Allergy appl. Immunol. 82: 238-243 (1987).
37. G. Skofitsch, J.M. Savitt and D.M. Jacovowitz, Suggestive evidence for functional unit between mast cells and substance P fibres in the rat diaphragm and mesentery, Histochemistry 82: 5-8 (1985).
38. D.J.J. Carr and E. Blalock, A molecular basis for bi-directional communication between the immune and neuroendocrine system, in: Progress in Immunology VI, B. Cinader and R.G. Miller, eds., Academic Press, Florida, pp 619-28 (1986).

39. D.A. Sullivan, Endocrine control of the ocular secretory immune system, in: "Hormone and Immunity", I. Berczi and K. Kovacs, eds., MTP Press, pp 54 (1987).
40. S. Freier, M. Eran and J. Faber, Effect of cholecystokinin and of its antagonist, of atropine and of food on the release of immunoglobulin A and immunoglobulin G specific antibodies in the rat intestine, Gastroenterology 93: 1242-1246 (1987).
41. P. Weisz-Carrington, M.E. Roux, M. McWilliams, J.M. Phillips-Quagliata, and M.E. Lamm, Hormonal induction of the secretory immune system in the mammary gland, Proc. Natl. Acad. Sci, USA 75: 2928-2932 (1978).
42. D.A. Sullivan and C.R. Wira, Hormonal regulation of immunoglobulins in the rat uterus: uterine response to multiple estradiol treatments, Endocrinology 114: 650-658 (1984).