Springer Semin Immunopathol (1990) 12: 191–202

Springer Seminars in Immunopathology

Mast Cells

Jean S. Marshall and John Bienenstock

Department of Pathology, McMaster University, 1200 Main Street West, Hamilton, Ontario, Canada L8N 3Z5

Introduction

Mast Cells in Health and Disease

Because of their unusual fixation and staining properties even the presence of mast cells within the intestinal mucosa was disputed for many years. Nowadays there is an increasing recognition of the importance of these cells in many disease states and their potential role in intestinal homeostasis and immunity. There is well-established evidence for the mast cells having a role in food allergy, anti-parasitic responses and the gastrointestinal manifestations of systemic mastocytosis. More recent evidence is available which suggests that mast cells are of importance in inflammatory bowel disease (IBD), hypereosinophilic gastroenteritis and motility disorders.

Human mucosal mast cells are known to degranulate in vitro via the cross-linking of IgE attached to specific receptors on the cell surface [3]. After passive sensitization of human ileum or colon a local food-antigen-specific reaction can be induced after ingestion or direct application of the antigen [13]. Other stimuli may also stimulate degranulation, however, such as components of the complement cascade, neurotransmitters, osmotic changes and mechanical damage. In the human, as in animal models, certain parasitic infections are associated with raised numbers of mast cells. For example following *Trichinella spiralis* infection an increased number of mast cells are observed [14]. Spirochaetosis of the human rectum is also associated with an intraepithelial mast cell and IgE plasma cell response [12]. Eosinophilic gastroenteritis appears, in some patients, to be associated with IgE-mast cell-mediated reactions. The typical histological findings of local eosinophil infiltration, tissue edema crypta hyperplasia and villus atrophy are reminiscent of the observations in animal models of nematode parasite infection. These changes may well reflect the consequences of sustained mast cell degranulation within the intestine.

For many years it has been suggested that mast cells may play a role in the etiology or pathogenesis of IBD. Mast cell numbers have been shown to be elevated

Offprint requests to: J. Bienenstock

in the bowel wall of ulcerative colitis patients and in studies of Crohn's disease changes in mast cells which may represent activation and degranulation have been reported [21, 31]. There are some anecdotal reports of clinical responses of ulcerative colitis to the mast cell stabilizing drug disodium cromoglycate but these have not been supported by more rigorous clinical trials. However, since this drug may not effectively stabilize the majority of intestinal mast cells in the human this does not exclude mast cells as an important participant in the disease process. Mast cells may also be of importance in gut motility disorders. In a rat model of *Trichinella spiralis* infection mast cell activation by antigen and other secretagogues will induce contraction of longitudinal muscle from the intestinal wall [37]. Similar observations have recently been made using muscle obtained from the intestine of patients with Crohn's disease [38]. These results suggest that not only does mast cell activation have the potential to alter gut motility but also that such a mechanism could contribute to the diarrhea observed in inflammatory bowel diseases. It has also been recently shown that irritable bowel syndrome can be effectively treated with tricyclic antidepressant agents. These drugs are known to have profound mast cell stabilizing effects both in animals and humans. It is possible that this aspect of their activity is important in irritable bowel syndrome treatment.

We should not limit our perception of mast cells as of importance only in disease states, since recent evidence, which will be discussed below, suggests that mast cells may also play an important role in maintaining normal gut function. By interactions with the nervous system and with other arms of the immune response a role for mast cells in maintaining homeostasis within the intestinal micro-environment can be envisaged [24]. In this review we will attempt to update the reader on some of the more important recent findings in the area of mast cell biology with regard to both the potential role of mast cells in maintaining the integrity of intestinal function in the face of antigen challenge and their role in disease processes.

Mast Cell Heterogeneity: Animal Models

The recognition of mast cell subtypes and the emergence of the concept of mast cell heterogeneity has opened up many new questions concerning the lineage and maturation of mast cells in different tissues. The most studied and best-defined model of mast cell heterogeneity is the rat. In this animal two types of mast cell have been defined; these are the intestinal mucosal mast cell (IMMC) isolated from the small intestines of rats infected with the nematode *Nippostrongylus brasiliensis* (Nb) and the connective tissue mast cell (CTMC) of which the peritoneal mast cell (PMC) is the most frequently examined example.

It is well known that IMMC will lose some of their staining characteristics following prolonged fixation in standard formalin or other aldehyde-based fixatives, while they can be readily visualized following Carnoys or basic lead acetate fixation. [9] The proteoglycan contents of IMMC and CTMC differ substantially, with the majority of CTMC conatining mainly heparin proteoglycan while IMMC contain chondroitin sulfate di-B. Alcian blue/safranin O staining at low pH and

berberine sulfate fluorescence staining are both dependent upon the proteoglycan content of mast cells (see Table 1) to distinguish between mast cell types. The proteglycan content of a given mast cell is not, however, sufficient to class it as CTMC- or IMMC-like. In embryonic development [8], in neonatal animals and during mast cell repopulation of the peritoneal cavity a population of mast cells are observed which are initially Alcian blue positive, berberine negative but become safranin and berberine positive over time. It has been suggested that "immature" CTMC may have a proteoglycan content similar to that of IMMC. In adult PMC small amounts of chondroitin sulfate and heparin are observed [17] and in normal adult rats a subpopulation of Alcian blue-positive, berberine-negative-staining mast cell are observed in CTMC sites such as the tongue and skin. These limitations of proteoglycan markers have been largely overcome by the discovery of specific protease enzymes in mast cells from different tissue sources. Two major proteases have been characterized and cloned. There are rat mast cell protease I (RMCP I) and RMCP II [41]. RMCP I is found only in CTMC and is observed in apparently all mast cells in tissues such as the tongue regardless of their proteoglycan content. To date RMCP I has not been observed in IMMC. RMCP II is found in the IMMC and its release into the serum following mast cell activation has proved to be a useful marker of mast cell degranulation events in vivo [40]. Recently very low levels of RMCP II have been observed in PMC, however, it is not known whether this reflects a low level of RMCP II expression by the majority of PMC or a minor IMMC-like subpopulation within the peritoneal cavity [26].

Functional Studies

The staining characteristics of mast cell subtypes are reflected in functional differences betwen CTMC and IMMC (see Table 2). Of particular interest is the lack of response to IMMC to compound 48/80 and the bee venom peptide 401, both of these agents induce substantial and rapid degranulation of CTMC [19].

Table 1. Granule contents of art mast cells

	Source		
	Peritoneum	Intestinal mucosa	Standard bone marrow culture
Proteoglycan			
Heparin	+++	0	0
Chondroitin sulphate di-B	+	+++	+++
Serine protease			
RMCP I	+++	0	0
RMCP II	+	+++	+++

Data compiled from several sources [19, 26]
RMCP rat mast cell protease

Table 2. Functional differences between rat mast cells

	Source	
Agent	Peritoneum	Intestinal mucosa
Degranulating		
Antigen, anti-IgE	++	++
Compound 48/80	++	0
Bee venom peptide 401	++	0
Ionophores	++	+/++
Substance P	++	+
Somatostatin	++	0
VIP	++	0
Bradykinin	++	0
Neurotensin	++	0
Dynorphin	++	0
β endorphin	++	0
Stabilizing agents		
Disodium cromoglycate	++	0
Quercetin	++	+
Doxantrazole	++	+

Summary of data compiled several references [2, 19]

The recognition that disodium cromoglycate is unable to stabilize IMMC lead to an increased awareness of the potential clinical importance of understanding mast cell heterogeneity. Substance P has been shown to induce degranulation of both CTMC and IMMC [33]; however, some other neuropeptides appear only to affect CTMC. Even within the CTMC population of the rat there may be further functional heterogeneity. If, for example, the response to compound 48/80 of CTMC from the peritoneal cavity is compared with the skin- and lung-derived CTMC, it is found that, while 0.3 μg/ml 48/80 will induce 20% histamine release in PMC 20 μg/ml, is required to induce the same extent of degranulation in the latter two populations. These results may reflect differences in maturity of the effects of isolation procedures and underline the importance of not using functional studies along to define mast cell phenotype.

T Cell Dependence

In general IMMC are referred to as "T cell dependent". Interleukin (IL)-3 derived from T lymphocytes is known to be important in the IMMC hyperplasia induced following Nb infection. Adult athymic "nude" rats do not develop an increased IMMC population immediately following Nb or *Trichinella spiralis* infection while their heterozygous euthymic littermates will do so. However, the term "T cell dependent" could be considered misleading since both the nude rat and mouse have normal numbers of IMMC in the absence of such stimuli.

In vitro models of mast cells are often used for functional and lineage studies. In both mouse and rat, populations of cells can be grown from bone marrow or

other tissue sources in the presence of IL-3 which are more than 90% pure mast cells. Under standard culture conditions these cells are RMCP II positive, Alcian blue positive and Berberine negative. However, additon of dexamethasone or contact with fibroblasts [20] has been reported to induce differentiation into safranin-positive, berberine-positive cells. In vivo in the rat it has still not been clearly shown that an individual entirely RMCP II-positive cell can develop RMCP I positivity or vice-versa. In the mouse elegant experiments by Kobayashi et al. [18] examined the issue of whether IMMC-like cells derived from bone marrow could "trandifferentiate" into CTMC-like cells within the peritoneal cavity and such a change was observed. They also showed that PMC microinjected into the stomach wall could develop alcian blue positivity and lose their berberine positivity. These experiments underline the importance of microenvironmental factors in determining mast cell phenotype, but because of their reliance on proteoglycan markers one cannot rule out a change in this mast cell characteristic without changes in protease content or functional characteristics.

Human Mast Cell Heterogeneity

Although studies in rodents have proved extremely useful one cannot draw a direct parallel from the rat model to the human situation when examining mast cell heterogeneity. In the skin, gut mucosa and lung tissue of man both heparin- and chondroitin sulfate-containing mast cell populations have been described. In each tissue cells have a range of staining characteristics when using berberine or Alcian blue/safranin and they have a range of sensitivities to formalin fixation [23, 36]. Clear biochemical and functional differences between tissue sites have been observed. However, Irani et al. [15] and Schwartz et al. [32] have demonstrated that the protease content of human cells are distributed in a relatively tissue-specific manner. Two major types of mast-cell have been defined [4]. The MC^T mast cell contains only a tryptase enzyme while Mc^{TC} mast cells conatin both typtase and chymase enzymes. A third enzyme with carboxypeptidase activity is found predominantly in M^{TC}. When examining the distribution of these protease markers in various tissues it was found that , although no single tissue contained only Mc^T or Mc^{TC} mast cells, one type of cell would be found preferentially in a given site. For example Mc^T mast cells were found to predominate in the mucosa of the small intestine while mainly Mc^{TC} cells were observed in the skin (Table 3). In the human these markers appear to be much more useful than staining which is dependent upon proteoglycan content, since in both skin and gut mucosa majority of mast cells are Alcian blue positive and berberine negative.

The Mc^T mast cell has been demonstrated to be, at least in part, thymus dependent since patients with acquired immune deficiency syndrome or severe combined immune deficiency both have a significantly reduced number of Mc^T in their small intestine while the number of Mc^{TC} are comparable with controls. The cytokines necessary for human mast cell growth are not well understood. Although human IL-3 has been cloned and is known to support basophil development from human cord blood [16] culture of mast cells from this source has proved

Table 3. Distribution of human mast cell protease enzymes

Source	Percent distribution	
	T	TC
Skin	12	88
Small intestine mucosa	98	2
Small intestine submucosa	13	87
Lung, alveolar lumen	93	7
Lung, bronchi/subepithelium	77	23
Lung, dispersed parenchyma	90	10

From [15]

difficult. Thus, the factors which support mast cell differentiation in the human appear to be separate from IL-3 and are not well characterized.

Functionally a number of differences have been reported between mast cells from different tissue sources [6]. A number of anti-allergic drugs have been examined and their effects compared using dispersed lung bronchoalveolar lavage intestinal and foreskin mast cells. Disodium cromoglycate, which is known to be ineffective in stabilizing rat IMMC, has been shown to have potent stabilizing effects on bronchoalveolar lavage mast cells and a relatively smaller stabilizing effect on intestinal mast cells. Skin mast cells appear not be stabilized by this agent in the human (see Table 4). In dispersed lung, bronchoalveolar lavage and the intestinal mucosa the majority of mast cells are Mc^T and Alcian blue positive berberine negative. In these respects at least one aspect of their functional characteristics does not correlate with their histochemical and protease characterization. Mast cells from different human tissue sources have also been shown to vary in their sensitivity to secretagogues. Substance P, which degranulates both IMMC and CTMC in the rat, will only induce degranulation of human skin mast cells when compared with lung, colon muscle and colon mucosa tissue according to the work of Church et al. [7]. Similar differences in mast cell responses to compound 48/80 have been observed.

Overall data on mast cell heterogeneity in the human could be seen to suggest a wide variety of different cell types depending on the tissue from which they

Table 4. Summary of relative effects of various anti-allergic agents on human mast cell histamine release induced by anti-IgE

Drug	Bronchoalveolar lavage	Dispersed lung	Intestinal mucosa
Cromoglycate	+	0	+/−
Theophylline	0	0	+
Salbutamol	+	+	+/−
Nedocromil	+	+	+/−

Data compiled from several sources [3, 10, 28]

are obtained. However, most of the information currently available could be explained on the basis of two major classes of human cell Mc^T and Mc^{TC} present at varying proportions in tissues. Other apparent changes in responsiveness may be due to maturational factors or the effects of isolation and purification of cell types. Alternatively the mast cell heterogeneity issue in humans may indeed be much more complex than that observed in animal models.

Mast Cell-Nervous System Interactions

An important area of study in the emerging field of psychoneuroimmunology has been the concept of mast cell-nervous system interactions [35]. There is strong morphological evidence that mast cells are associated with nerves in the gastrointestinal tract. Newson et al. [27] have provided ultrastructural evidence suggesting innervation of mast cells in the rat ileum. Stead et al. [34] have shown that following infection with Nb 67% of mast cells in the lamina propria of the rat intestine are associated with subepithelial nerves at the light microscope level. Electron microscopic studies have confirmed these findings and revealed intimate membrane-membrane contact between 8% of the mast cells in infected rats and unmyelinated axons containing 70–170 mm dense-core vesicles. An additional 30% of mast cells were within 250 mm of nerves. In the human nerve mast cell association has been demonstrated in the colonic mucosa of ulcerative colitis patients [42].

There is also functional evidence of communication between nerves and mast cells within the intestine. Histamine release and a decrease in mast cell granule metachromasia is observed following field stimulation of the rat ileum. Preadministration of atropine or tetrodotoxin depressed these responses [1]. Other studies have shown that mast cells in the rat stomach increase in granularity following sectioning of the vagus nerve and that pyloric ligation causes a decrease in tissue histamine levels which can be prevented by vagotomy [11]. This could suggest that the degree of mast cell granularity may be under a tonic influence from the nervous system. In sensitized rats, antigen challenge of jejunum in using chamber studies has been shown to induce mast cell-mediated alterations in ion transport [29]. The nerve blocking drug tetrodotoxin has been shown to reduce these changes and this further suggests that mast cells may act in conjunction with nerves in altering epithelial function.

Conditioning studies in our laboratory have provided further evidence that the CNS can influence the mast cell populations of the intestine. Rats primed with ovalbumin (OVA) and infected with Nb were given an audiovisual conditioning stimulus (CS), paired with challenge with a low dose of OVA as unconditioned stimulus (US). A control group received both CS and US but on separate days. This conditioning protocol was repeated on three occasions. Both groups of animals then received the CS alone and the serum levels of RMCP II were followed. One hour after the CS was initiated conditioned animals had a significantly raised serum RMCP II level compared with the unpaired control group [22]. While this mast cell activation may not be directly mediated by nerves within the intestine

these experiments clearly demonstrate that stimulation of CNS can affect the mast cell within the intestinal microenvironment.

The mechanisms by which mast cell-nervous system interactions could occur have been investigated with respect to be effects of neurotransmitters and neuropeptides on mast cell function.

Vasoactive intestinal peptide, bradykinin, neurotensin and acetyl choline have all been reported to induce degranulation of rat CTMC under appropriate conditions. Substance P has been reported to act as an effective secretagogue for both CTMC and IMMC, although only at relatively high concentrations ($\geq 10^{-6}$ M). It is possible that IMMC may respond more effectively within their microenvironment than after the handling and enzyme treatment required for cell isolation. Alternatively the local concentrations of substance P or acetylcholine surrounding a nerve varicosity may be sufficient to induce degranulation. Whatever the mechanism or mechanism involved, potential interactions between the nervous system and mast cells can no longer be ignored when considering the pathology of allergic disease, IBD or irritable bowel syndrome (IBS). Such interactions could also play an important role in homeostasis within the intestine.

Mast Cell Cytokine Production

The ability of mast cells to produce cytokines has been under intensive study over the past few years. The observation of expression of IL-4 by "normal" and transformed mouse mast cell lines aroused interest in this area [5]. Since that time IL-3-dependent mouse mast cell lines have been shown to produce IL-3, IL-4, IL-5 and IL-6 under IgE-dependent antigen stimulation or after treatment with ionophore [30].

Mouse bone marrow-derived mast cells have been shown to produce granulocyte-macrophage, colony-stimulating factor and IL-3 in response to a similar stimulus [39]. These observations indicate a new possible role for the mast cell in vivo. By acting as a source of cytokines in the immediate area of inflammation or infection they could stimulate granulocyte differentiation, the acute phase response and antibody production. Through an autocrine mechanism they could potentially stimulate mast cell hyperplasia. The production of Il-4 by mast cells

Table 5. Cytokine production by mast cells from different sources

Cell type	Cytokines produced
Mouse bone marrow-cultured mast cells	Unstimulated – none known Stimulated – GM CSF, IL-3
IL-3 dependent mouse mast cell lines	Unstimulated – IL-4 Stimulated – IL-3, IL-4, IL-5, IL-6, TNFα
Transformed mouse mast cell lines	Unstimulated – IL-4, IL-3?

Data from several sources [5, 30, 39]
IL Interleukin; *GM-CSF* granulocyte macrophage-colony-stimulating factor; *TNF* tumor necrosis factor

is of particular interest because this cytokine is known to be essential for IgE production in response to infection or challenge in vivo. We have recently shown that mast cell degranulation products from adult rat PMC can induce a mast cell hyperplasia when injected into neonatal rats [25]. One possible explanation of this phenomenon is that cytokines such as IL-3 and IL-4 are produced on degranulation of adult rat PMC.

The potential for mast cells to act as a source of cytokines, allows one to speculate further about their role in the immune response. Production of what has previously been considerd largely T cell-derived factors such as IL-3, IL-4 and IL-5 suggests that mast cells, on activation, could have a role in regulating hemopoesis, antibody production and eosinophilia. IL-6 and tumor necrosis factor production would allow for many other potential effects including induction of the acute phase response.

A major limitation of the studies to date examining mast cell cytokine production is that observations are limited to mast cell lines or long-term bone marrow cultures in the mouse. While in many respects these cells resemble the IMMC of the rat, it is possible that similar cytokine production may not occur in cells within the animal. In the case of bone marrow-derived cultured mast cells it may be that contaminating cells are producing GMCSF and/or IL-3. There is as yet no firm information on cytokine production by rat mast cells or "CTMC-like" mouse mast cells. Studies using in situ hybridization techniques will probably be required to confirm the production of cytokines by mast cells in vivo. The regulation of cytokine production, the role of mast cell-derived cytokines in responses to infection, inflammation and maintenance of the intestinal microenvironment are all areas of potential interest and importance.

Conclusions

There is good evidence for mast cell heterogeneity both in the rat model and in the human. The rat and human differ substantially in the characteristics of mast cells found in various tissues and in their functional properties. The human situation is apparently more complex than the relatively simple definitions of CTMC and IMMC available in the rat. Some of the differences between tissues may be explained by mixture of cell types within a given location and by the effects of isolation procedures. The recognition that mast cells interact with the nervous system, by whatever mechanism, allows for CNS influence on mast cell activity. The often-quoted psychological component of diseases such as "food allergy", IBD and IBS could have a functional basis via mast cell activation. The potential role for mast cells in altering gut motility may also be important in this context. To date most of the work on mast cell-nerve interactions has considered the IMMC. Whether similar communication exists between CTMC and the nervous system remains to be elucidated.

The recent observations of cytokine production by mast cells opens up an entirely new set of questions in mast cell biology. The importance of mast cells in immunoregulation and their potential for providing what have traditionally been thought of as T cell-derived factors may prove to be of great importance. This

aspect of mast cell activity has not yet been examined with regard to mast cell heterogeneity. Given the differences in mast cell types with respect to other parameters, it would not be surprising if cytokine production was different in CTMC and IMMC populations. These areas and the issues still to be answered regarding the lineage and regulation of mast cells in different tissues provide major questions for future research.

References

1. Bani-Sacchi T, Barattini M, Biachi S, Blardina P, Brunelleschi S, Fartozzi R, Mannioni PF, Masini E (1986) The release of histamine by parasympathetic stimulation in guinea pig auricle and rat ileum. J Physiol 371: 29
2. Befus AD, Bienenstock J, Denburg JA (eds) (1986) Mast cell differentiation and heterogeneity. Raven Press, New York, p 1–426
3. Befus AD, Dyck N, Goodacre R, Bienenstock J (1987) Mast Cells from the human intestinal lamina propria. Isolation, histochemical subtypes and functional characterization. J Immunol 138: 2604
4. Bienenstock J, Austen KF, Galli SJ (1989) Nomenclature of mast cells and basophils (1989) In: Galli SJ, Austen KF (eds)Mast cell and basophil differentiation and function in health and disease. Raven Press, New York, p 329
5. Brown MA, Pierce JH, Watson CJ, Falco J, Ihle JN, Paul WE (1987) B cell stimulatory factor-1/Interleukin-4 m RNA is expressed by normal and transformed mast cells. Cell 50: 809
6. Cohan VL, Massey WA, Gittlen SD, Charlesworth EM, Warner JA, Kagey-Sobotka A, Lichtenstein LM (1989) The heterogeneity of human histamine containing cells. In: Galli SJ, Austen KF (eds) Mast cell and basophil differentiation and function in health and disease. Raven Press, New York, p 149
7. Church M-K, Benyon C, Rees PH, Lowman MA, Campbell AM, Robinson C, Holgate ST, (1989) Functional heterogeneity of human mast cells. In: Galli SJ, Austen DF (eds) Mast cell and basophil differentiation and function in health and disease. Raven Press, New York, p 161
8. Combs JW, Lagunoff D, Benditt EP (1965) Differentiation and proliferation of embryonic mast cells of the rat. J Cell Biol 25: 577
9. Enerback L (1987) Mucosal mast cells in the rat and in man. Int Arch Allergy Appl Immunol 82: 249
10. Fox CC, Kagey-Sobotka A, Schleimer RP, Peters SP, MacGlashan DW, Lichtenstein LM (1985) Int Arch Allergy Appl Immunol 77: 130
11. Ganguly AK, Sathiomoorthy SS, Bhatnager OP (1978) Effect of subdiaphragmatic vagotomy on gastric mucosal mast cell population in pylorus ligated rats. Q J Exp Physiol 63: 89
12. Gebbers J-O, Ferguson DJP, Mason C, Kelly P, Jewell DP (1987) Spriochaetosis of the human rectum associated with an intraepithelial mast cell and IgE plasma cell response. Gut 28: 588
13. Gray I, Horten M, Walzer M (1940) Studies in mucous membrane-hypersensitivities IV. The allergic reaction in the passively sensitized mucous membranes of the Ileum and colon in humans. Am J Intern Med 13: 2050
14. Gustowska L, Ruitenberg EJ, Elgersma A, Docieka W (1983) Increase of mucosal mast cells in the jejunum of patients infected with *Trichinella spiralis*. Int Arch Allergy Appl Immunol 71: 304
15. Irani AA, Schechter NM, Craig SS, DeBlois G, Schwartz LB (1986) Two types of human mast cells that have distinct neutral protease compositions. Proc Natl Acad Sci USA 83: 4464
16. Ishizaka T, Saito H, Furitsu T, Dvorak A (1989) Growth of human basophils and mast cells in vitro. In: Galli SJ, Austen KF (eds) Mast cell and basophil differentiaton and function in health and disease. Raven Press, New York, p 39
17. Katz HR, Austen KF, Caterson B, Stevens RL (1986) Secretory granules of heparin containing rat serosal mast cells also possess hightly sulfated chondroithin sulphate proteoglycons. J Biol Chem 261: 133
18. Kobayashi T, Nakano T, Nakahata T, Asai H, Yahi Y, Tsugi K, Komigama A, Akabane T, Kojima S, Kitimura Y (1986) Formation of mast cell colonies in methylcellulose by mouse

peritoneal mast cells and differentiation of these cloned cells in both the skin and gastric mucosa of W/WV mice. Evidence that a common precurser can give rise to both connective tissue type and mucosal mast cells. J Immunol 136: 1378
19. Lee TDG, Sweiter M, Bienenstock J, Befus AD (1985) Heterogeneity in mast cell populations. Clin Immunol Rev 4: 143
20. Levi-Schaffer F, Austen KF, Gravallese PM, Stevens RL (1986) Coculture of interleukin-3-dependent mouse mast cells with fibroblasts results in a phenotypic change of the mast cells. Proc Natl Acad Sci USA 83: 6485
21. Lloyd G, Green FHY, Fox H, Mani V, Turnberg LA (1975) Mast cells and immunoglobulin E in inflammatory bowel disease. Gut 26: 861
22. MacQueen G, Marshall JS, Siegal S, Perdue MH, Bienenstock J (1989) Conditioned secretion of rat mast cell protease II release by mucosal mast cell. Science 243: 83
23. Marshall JS, Ford GP, Bell WB (1987) Formalin sensitivity and differential staining of mast cells in human dermis. Br J Dermatol 117: 29
24. Marshall JS, Bienenstock J, Perdue MH, Stanisz AM, Stead RH, Ernst PB (1989) Novel cellular interactions and networks involving the intestinal immune system and its microenvironment. APMIS 97: 383
25. Marshall JS, Nielson L, Stead RH, Bienenstock J (1989) The effects of nerve growth factor degranulating agents and degranulation products on mast cells in the neonatal rat. FASEB J (in press)
26. Miller HRP, Huntley JF, Newlands GFJ, Mackellar A, Irvine J, MacDonald A, Lammas AD, Wakelin D, Woodbury RG (1989) In: Galli SJ, Austen KF (eds) Mast cell and basophil differentiation and function in health and disease. Raven Press, New York, p 81
27. Newson B, Dahlstrom A, Enerback L, Ahlman H (1983) Suggestive evidence for a direct innervation of mucosal mast cells. An electron microscopic study. Neuroscience 10: 565
28. Pearce FL, Flint KC, Leung KBP, Hudspith BN, Seager K, Hammond MD, Brostoff J, Geraint-James D, Johnson NM (1987) Some studies on human pulmonary mast cells obtained by bronchoalveolar lavage and by enzymic dissociation of whole lung tissue. Int Arch Allergy Appl Immunol 82: 507
29. Perdue MH, Gall DG (1985) Transport abnormalities during intestinal araphylaxis in the rat. Effect of anti-allergic agents. J Allergy Clin Immunol 76: 498
30. Plaut M, Pierce JH, Watson CJ, Hanley-Hyde J, Nordan RP, Paul WE (1989) Mast cell lines produce lymphokines in response to cross-linkage of Fcϵ R1 or to calcium lonophores. Nature 339: 64
31. Sanderson IR, Leung KBP, Pearce FL, Walker-Smith JA (1986) Lamina propria mast cells in biopsies from children with Crohn's disease. J Clin Pathol 39: 279
32. Schwartz LB, Irani AA, Roller K, Castells MC, Schechter NM (1987) Quantitation of histamine, tryptase and chymase in dispersed human T and TC mast cells. J Immunol 138: 2611
33. Shanahan F, Denburg JA, Fox J, Bienenstock J, Befus AD (1985) Mast cell heterogeneity. Effects of neuroenteric peptides on histamine release. J Immunol 135: 1331
34. Stead RH, Tomioka M, Quinonez G, Simon GT, Felton SY, Bienenstock J (1987) Intestinal mucosal mast cells in normal and nematode infected rat intestines are in intimate contact with peptidergic nerves. Proc Natl Acad Sci USA 84: 2975
35. Stead RH, Marshall JS, Tomioka M, Bienenstock J (1989) Interaction of the muscosal immune and peripheral nervous systems. In: Ader R, Felton DL, Cohen N (eds) Psychoneuroimmunology II. Academic Press, New York (in press)
36. Strobel S, Miller HRP, Ferguson A (1981) Human intestinal mucosal mast cells: evaluation of fixaton and staining techniques. J Clin Pathol 34: 851
37. Vermillion DL, Ernst PB, Scicchitano R, Collins SM (1984) Antigen induced contraction of jejunal smooth muscle in the sensitized rat. Am J Physiol G701–G708
38. Vermillion DL, Collins SM (1989) Mast cells contract human intestinal muscle via the release of P.A.F. and Leukotriene D^4 in patients with or without Crohn's diseaese. Gastroenterology (in press)
39. Wodnar-Filipowicz A, Heusser CH, Moroni C (1989) Production of the haemopoietic growth factors GM-CSF and interleukin-3 by mast cells in response to IgE receptor-mediated activation. Nature 339: 150
40. Woodbury RG, Miller HRP, Huntley JF, Newlands GFJ, Paliser AC, Wakelin D (1984) Mucosal

mast cells are functionally active during spontaneous expulsion of primary intestinal nematode infections in the rat. Nature 312: 450
41. Woodbury RG, LeTrong H, Cole K, Neurath H, Miller HRP (1989) Rat mast cell proteases. In: Galli SJ, Austen KF (eds) Mast cell and basophil differentration and function in health and disease. Raven Press, New York, p 71
42. Yonei Y (1987) Autonomic nervous alterations and mast cell degrenulation in the exacerbation of ulcerative colitis. Jn J Gastroenterol 84: 1045