WIADOMOŚCI PARAZYTOLOGICZNE
T. XXXII, NR 3: 1986
PROC. OF ICT NO XIX

MUCOSAL MAST CELLS: ORIGIN, KINETICS AND FUNCTION

JANNIE BUYS [1] and E. J. RUITENBERG [1, 2]

[1] National Institute for Public Health and Environmental Hygiene, Bilthoven, The Netherlands
[2] Department of Immunology, Faculty of Veterinary Medicine, Utrecht, The Netherlands

Introduction

Some helminths, parasitic for man and animals, irritate the intestinal mucosa during their stay in the gut. This irritation is characterized by an influx of inflammatory cells, among which mucosal mast cells (MMC), in the intestinal villi. Two MMC populations are abserved in the gut i.e. the intestinal mast cells (IMC) of the lamina propria and the globule leukocytes (GL) situated among the cells of the epithelial lining. The MMC are the mucosal cunterparts of the mast cells of the connective tissues (CTMC), well known from acute allergies and asthmatic attacks. The MMC differ from the CTMC in several respects the most prominent being the T-cell dependence (Ruitenberg and Elgersma, 1976), the lower histamine content (Befus et al., 1982), the lower number of FcE receptors (Lee et al., 1985), the non-susceptibility to several pharmaceuticals inducers as well as blockers of histamine release (Pearce et al., 1982) and the absence of heparin (Tas and Berndsen, 1977). Mast cells release histamine and the membrane phospholipid derived leukotrienes LTC_4 and LTD_4. These products are responsible for the vasodilatation and smooth muscle contraction. Also LTB_4 is produced, a chemoattractant of eosinophilic and polymorphonuclear granulocytes (Lewis and Austen, 1981; Nijkamp and Sitsen, 1982). It is assumed that MMC also release histamine and LT's although the effect at gut level is unclear. Other questions about relation between the various mast cell subpopulations, their origin, proliferation and maturation will be discussed. Kinetics of mucosal mast cells in the gut and the in vitro growth of mast cells will be presented. Results are based on the experimental model *Trichinella spiralis* in small laboratory rodents.

The MMC response

Normal mice or rats infected with the nematode *Trichinella spiralis* show an increase in MMC during the stay of the worms in the gut. Soon after expulsion of the adult worms, for mice at 12-14 days p.i. and for rats at 16-18 days p.i., the number of MMC returns to normal. Peak counts coincide with completion of worm expulsion (Parmentier et al., 1982). The height of MMC counts depends on the strain of the experimental animals used and is genetically determined (Alizadeh and Wakelin, 1982).

Originally it was assumed that the mesenteric lymphnodes (MLN) played an important role in populating the intestine with MMC. During the intestinal phase, both in the gut and in the MLN an increase in mast cells with MMC staining property was observed. It was suggested that the MLN contained MMC precursor cells which, by way of the thoracic duct and the peripheral circulation, were transported to the gut (Parmentier et al., 1982). However extirpation of $\geqslant$ 90% of the MLN did not influence MMC response (Fig. 1). In contrast after cannulating the thoracic duct for 24 hours during a *T. spiralis* infection MMC numbers dropped to base levels (Buys and Ruitenberg, submitted). (Fig. 2). These results suggested that factors essential for induction and maintenance of high MMC numbers, released by either the antigen or gut cells were drained. The factors were supposed to induce directly or indirectly the proliferation and maturation of MMC precursor cells.

The sites at which proliferation takes place is questionable and may be at either or at both bone marrow and gut levels. Maturation most

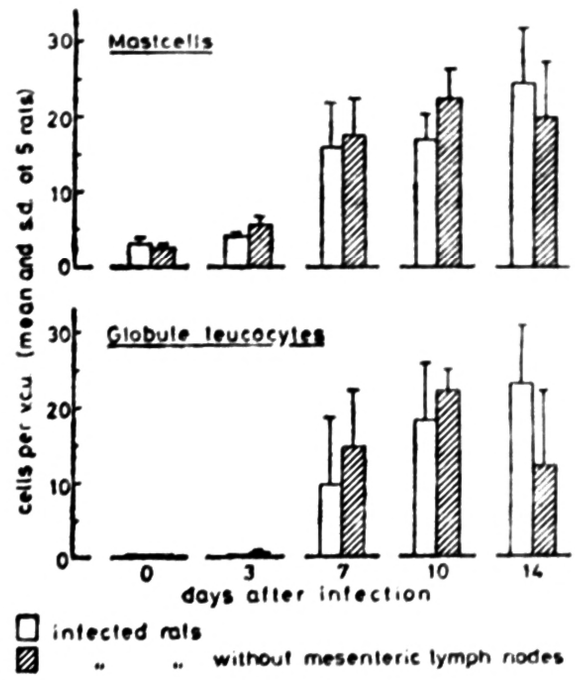

Fig. 1. Effect of extirpation of mesenteric lymph nodes at day -14 on numbers of mast cells and globule leucocytes in jejunum of Wistar rats infected with 2500 *T. spiralis* larvae

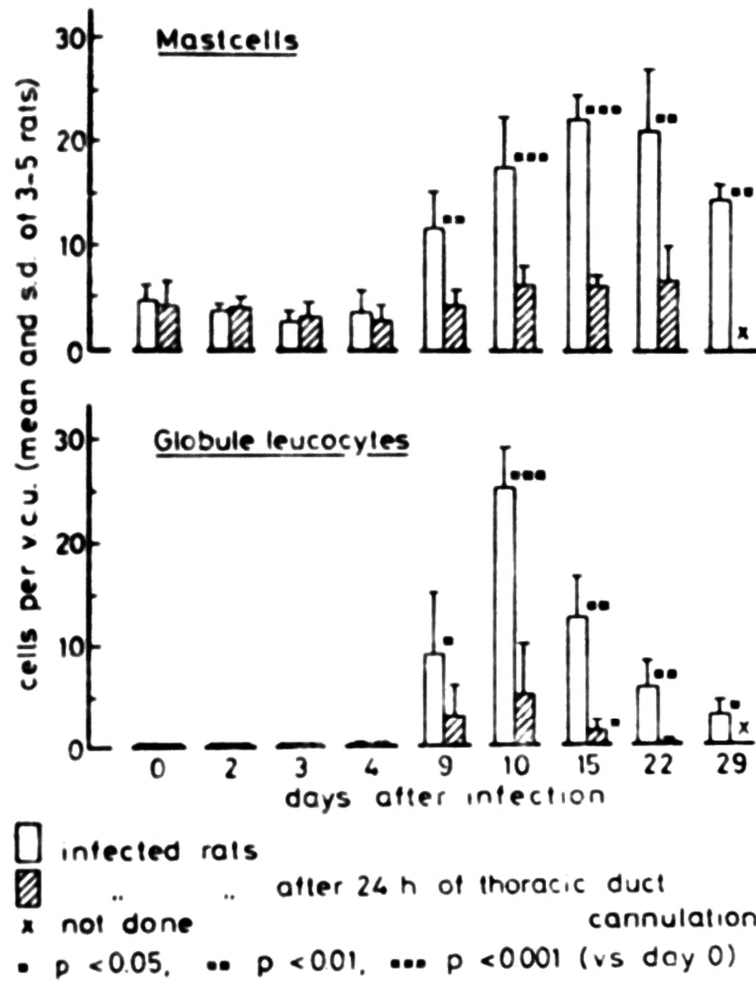

Fig. 2. **Effect of thoracic duct cannulation** on numbers of mast cells and globule leucocytes in jejunum of Wistar rats infected with 2500 *T. spiralis* larvae

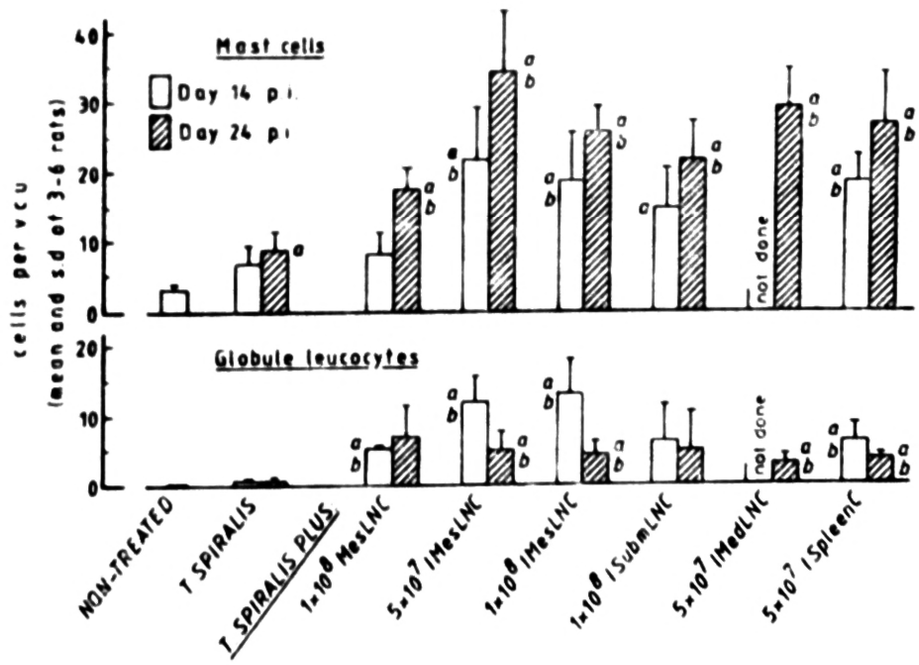

Fig. 3. **Effect of i.v. transfer of immune lymphoid cells from +/*rnu* to *T. spiralis*** infected *rnu/rnu* rats on numbers of mast cells and globule leucocytes in jejunum **(transfer at day 7 p.i.)**

likely occurs in the gut as in the peripheral circulation no mature mast cells have ever been found. The MMC response is dependent on the presence of T-cells as in congenitally thymus deprived animals no MMC response on a *T. spiralis* infection is observed (Ruitenberg and Elgersma, 1976). Immune mesenteric lymph node cells (MLNC) from normal animals can adoptively transfer the MMC response (Parmentier et al., 1982). However, not only MLNC but also the transfer of spleen cells, submandibular and mediastinal lymph node cells restore the response (Fig. 3) (Buys and Ruitenberg, submitted).

It was concluded that MLN contain cells capable of regulating MMC response in the gut. However, they are not essential as in MLN deprived animals MMC response is not impaired and secondly as lymphoid cell populations other than MLNC are capable to restore the response in T-cell depleted animals.

Mast cell growth factor (MCGF) and MCGF target cells

It was shown that MMC response depended on the presence of T-cells. In a series of in vitro studies with rat cells it was investigated which organs contained MCGF producing cells and which organs contained MCGF target cells.

Cell suspensions were prepared from MLN and the spleen from normal animals either non-infected or infected with *T. spiralis*. Cells were cultured in RPMI-1640 with supplements and stimulated with the specific antigen (10 μg/1 × 10^6 cells) or with the pan-T-mitogen concanavalin A (0.31 μg/1 × 10^6 cells). After 3-5 days at 37°C the supernatants were collected. The supernatants or conditioned media (CM) were assayed on cell cultures prepared form the bone marrow, the spleen and the MLN. It was found that CM from both cell populations i.e the MLN and the spleen isolated from infected animals induced mast cell growth in the bone marrow cell (BMC) cultures and, to a much lesser degree, also in the spleen cell cultures. In the MLNC cultures induction of mast cell growth by the different CM was almost nil. CM prepared from spleen cells from non-infected animals also contained MCGF but not the CM prepared from non-immune MLNC. Next, it was assayed if CM prepared at various days p.i. induced mast cell growth in similar ways. For this and further assays only BMC were used as MCGF target cells. CM prepared from MLNC and spleen cells at 11 and 28 days p.i. were added to BMC from non-infected or 11 days previously infected animals. It was found that CM prepared from MLNC from 11 days p.i. induced mast cell growth in the BMC cultures. 80% of the cells were mast cells at 13 days post incubation (p.inc.). However, CM from MLNC from 28 days

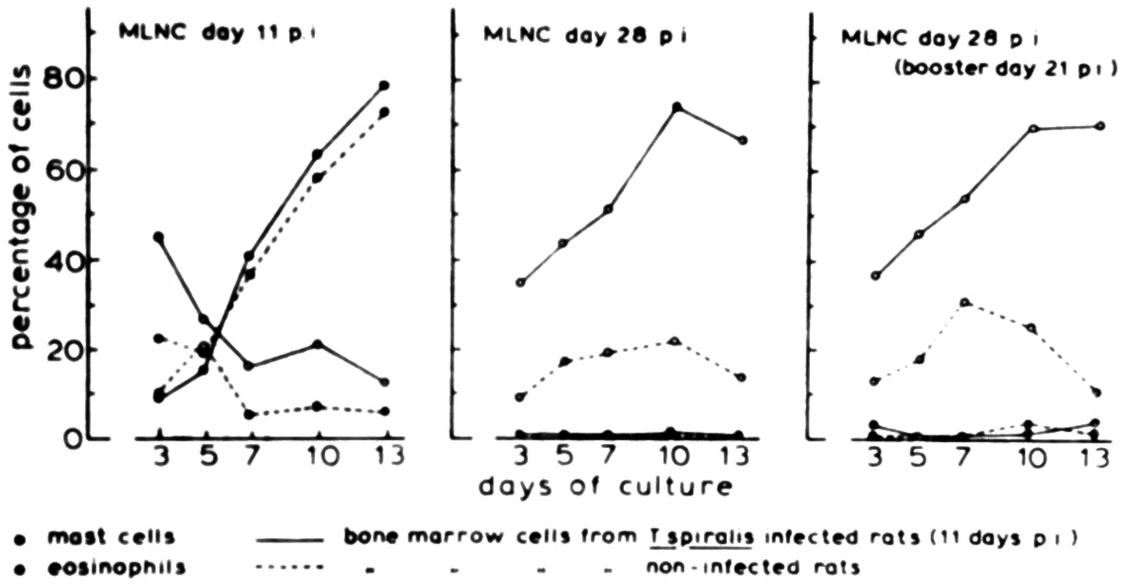

Fig. 4. Bone marrow cells cultured in the presence of conditioned medium prepared of MLNC from *T. spiralis* infected rats and stimulated with specific antigen. Course of relative numbers of mast cells and eosinophils

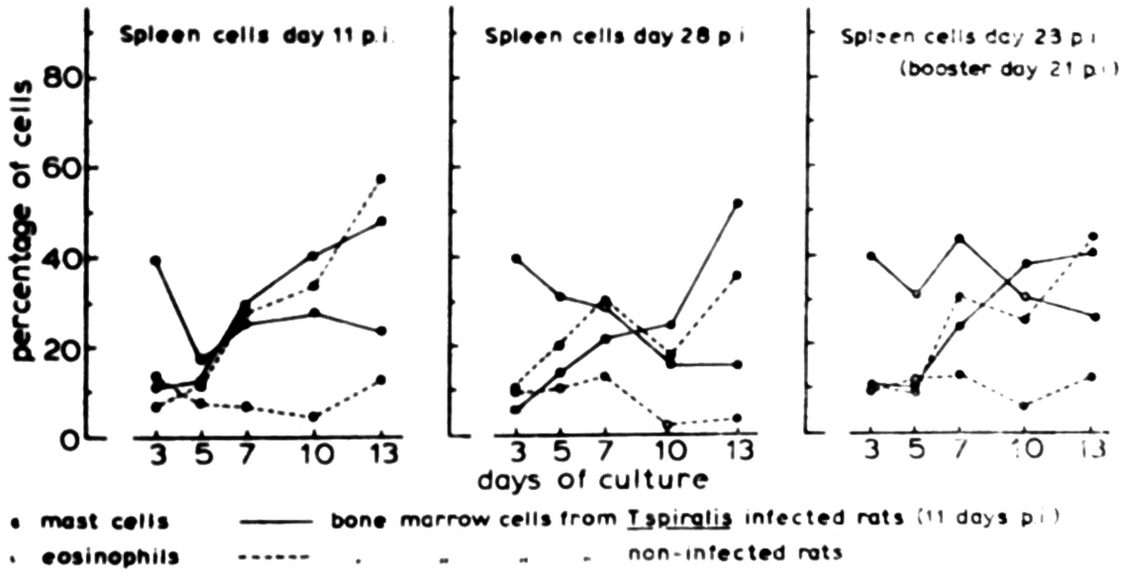

Fig. 5. Bone marrow cells cultured in the presence of conditioned medium prepared of spleen cells from *T. spiralis* infected rats and stimulated with specific antigen. Course of relative numbers of mast cells and eosinophils

p.i. did not induce mast cell growth and the remaining population (at 13 days p.inc.) in the BMC cultures from infected rats consisted mainly of eosinophils (Fig. 4). The CM prepared from spleen cells from both 11 and 28 days p.i. did not show any difference in the induction of mast cell growth (Fig. 5). This experiment was repeated using only the CM prepared form MLNC and the BMC from infected rats. The absolute mast cell and eosinophil numbers were estimated. A net increase of mast cells in the presence of CM from 11 days p.i. was observed until 7 days p.inc. after which the number remained stable during the observation period of 18 days (Fig. 6). Using the CM from 28 days p.i. no absolute increase in eosinophils was observed. Their number remained stable until 9 days p.inc. after which it rapidly decreased (Fig. 6) (manuscript

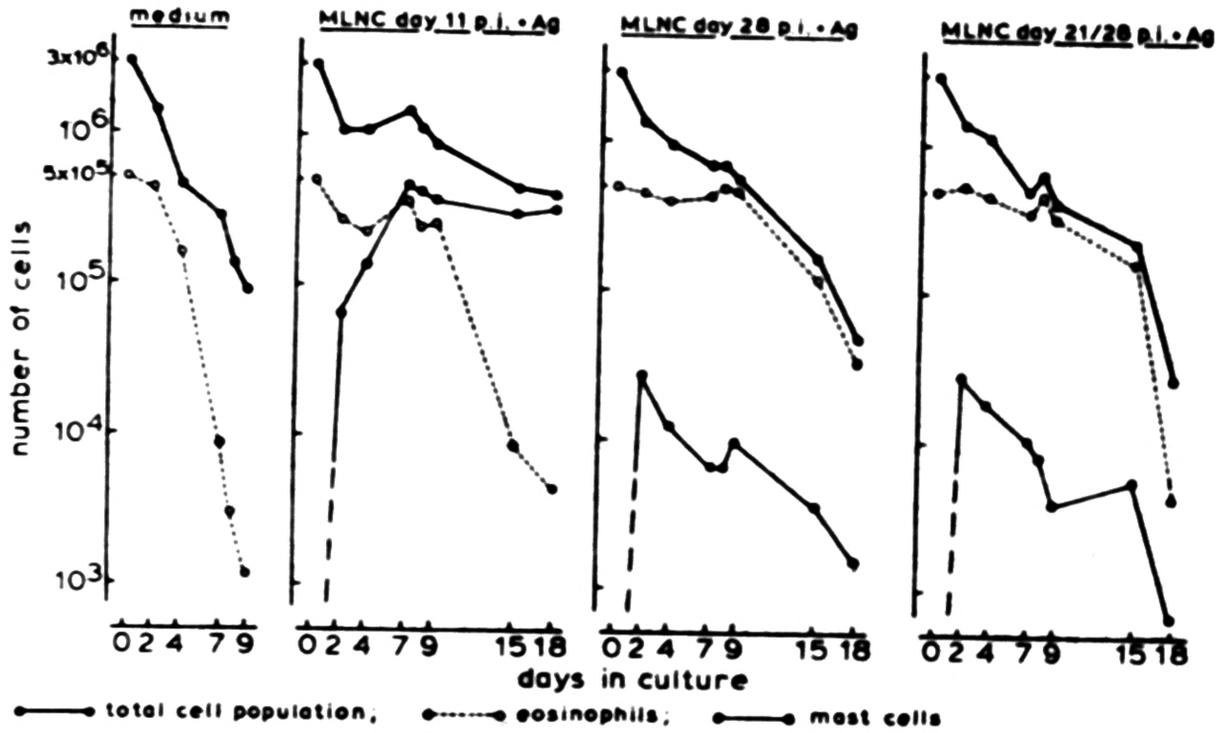

Fig. 6. Bone marrow cells cultured in the presence of conditioned medium prepared of MLNC from *T. spiralis* infected rats at different times post infection

in preparation). Summarizing the results spleen cells, when stimulated with an appropriate stimulant, always produce MCGF independent of being immune or non-immune cells. On the contrary MLNC from non-infected rats or from infected rats isolated after encystation of the migrating larvae produce minor amounts of MCGF, whereas the MLNC isolated during the intestinal phase of the infection produce large amounts of MCGF. These results suggest a role for the MLN in the regulation of the MMC response in the gut. It is known that T-suppressor cells possess histamine (H_2) receptors (Rocklin et al., 1979). The histamine released by the MMC activate T-suppressor cells and may thus interfere with the MCGF producing T-cells. MCGF, which is synonymous to interleukin-3 (IL-3) is produced by Thy 1^+, Lyt1^+2^- T-cells (Ihle et al., 1982) and is coproduced with IL-2 and granulocyte stimulating factors (Young et al., 1981). It may well be that under normal conditions the MLN have a major responsibility for the regulation of the inflammatory response in the gut. To test this hypothesis further investigations are needed including the effect on mast cell growth by CM prepared from other lymphoid organs. Also a possible inhibitory effect by MLNC from 28 days p.i. on IL-3 production by MLNC from 11 days p.i. should be assayed. A disadvantage is that standardization of the production of IL-3 from lymphoid cells is difficult. Several factors as the batch of foetal calf serum, donor animals and a number of unknown factors influence the cultures and thus IL-3 production. In general the trend in mast cell growth using different batches of CM is comparable, however, the height in mast cell numbers may vary. The IL-3 dependent mast cells

grown in vitro share a number of properties with MMC in vivo. Both have a histamine concentration of 1.5 pg/cell, the low sulphated glycosaminoproteoglycan and similar morphology (manuscript in preparation). Although it is very suggestive that the grown mast cells are MMC there is no proof that these cells are fully matured. Additional data about specific membrane markers have to be procured for final evidence.

Origin of mast cell precursor cells

As shown before, the bone marrow proved to be the major source from which mast cells could be grown. This, however, applies to the rat model and to the experimental conditions used. The aim was to investigate whether mast cells could be grown from different cell sources and not to procure pure mast cell cultures.

In the past mast cells were grown from various cell sources, i.e. from fibroblasts (Ginsburg et al., 1982) from the bone marrow (Haig et al., 1983), spleen, lymph nodes (Ginsburg et al., 1982), thymus (Ginsburg, 1963), peritoneal macrophages (Czarnetzki and Behrendt, 1981), blood mononuclears (Czarnetzki et al., 1984) and gut intraepithelial lymphocytes (Ernst et al., 1985). The IL-3 used was prepared from either Con-A stimulated spleen or WEHI-3 cells (myeloid cell line). From these results the question arises under which conditions and at which sites mast cell precursor are present. It is quite clear that the progenitor cell resides in the bone marrow. Does proliferation of the stem cell take place in the bone marrow and are the precursors present in the different tissues already committed or are uncommitted precursors already present at sites where they may be needed. In the latter case it is likely that proliferation takes place directly after local activation. Another question is whether mast cell stem cells indeed exist. Czarnetzki succeeded in growing mast cells from rat peritoneal macrophages (Czarnetzki et al., 1981) and human blood mononuclear cells (Czarnetzki et al., 1984). She claims to grow CTMC on basis of morphology and histamine content. CTMC are not dependent on T-cells for their proliferation and the mast cell inducing factor Czarnetzki used, was produced by L-cells, a mutated mouse fibroblast cell line. In our model CM prepared from Con-A stimulated spleen cells from T-cell deprived rats did not induce mast cell growth. Although much progress has been made in knowledge about kinetics of mast cell responses many questions still remain. The identity of the precursor cell is still unknown. A common precursor for CTMC and MMC for which the final differentiation is determined by the micro environment or by different inducing factors activating specific genes belong to the possibilities.

Activation of MMC and their function

Mast cells are activated in different ways. Best known is the interaction of the FcE receptors with IgE and the specific antigen (Ishizaka, 1979; Ishizaka et al., 1981). Activation is followed by release of mediators contained in the granules and the production of membrane phospholipid derived substances (Lewis and Austen, 1981; Nijkamp and Sitsen, 1982). The CTMC have receptors for several allergens and pharmaceuticals, whereas MMC are susceptible to only a few of these substances (Pearce, 1982). The released mediators are, among others, histamine and serotonin, glycosamino proteoglycans, proteases and acid hydrolases (Schwartz and Austen, 1980). Leukotrienes (LT's) and prostaglandin are the phospholipid derived products (Lewis and Austen, 1981; Nijkamp and Sitsen, 1982). Histamine activates different celltypes by way of their histamine (H) receptors. T-suppressor cells are activated by interaction with their H_2 receptors (Rocklin et al., 1979). Endothelial cells of capillaries and smooth muscle cells are activated after interaction with the H_1 receptors causing vasodilatation and muscle contraction respectively. LTC_4 and LTD_4 are the slow reacting substances of anaphylaxis SRS-A) and cause broncho-(muscle) and vasoconstriction (König, 1982). LTB_4, coproduced with LTC_4 and LTD_4 is a chemoattractant of granulocytes, especially eosinophils (König, 1982). Some eosinophil and mast cell products interact i.e. eosinophil-histaminase inactivates mast cell histamine and eosinophil-arylsulfatase inactivates mast cell SRS-A (Wasserman, 1979). These mast cell characteristics were determined using peritoneal mast cells. Whether the mentioned phenomena occur in the gut is likely but not yet proven. Oedema of the jejunum is observed during the intestinal phase, as is the influx of eosinophils.

Recently it was shown that mast cells were activated by a T-cell derived factor. In delayed type hypersensitivity (DTH) an early and a late component form part of the reaction. The early reaction (2 hour) is caused by a mast cell dependent T-cell factor, which activates the cell to release its amines. The late reaction is dependent on the early reaction (van Loveren et al., 1983). This phenomenon is true for the CTMC. Investigations into a similar phenomenon occurring in the gut are presently being performed. An MMC dependent lymphokine activating mediator release is an attractive thought as production of lymphokines is a temporary and a local matter. MMC activation and inactivation could thus be controlled very efficiently.

If activated MMC have a direct effect on worm expulsion is a point of discussion. W/W^v mice, which are congenitally mast cell depleted, do expulse *T. spiralis* adult worms although expulsion is delayed by a few-days (Ha et al., 1983). However, normal mice which are rapid expellers

of *T. spiralis* adult worms are also high MMC responders. Genetic involvement was demonstrated using strains of mice sharing the H-2 locus and their recombinant (inbred) strains. Thus, it was found that two loci of the H-2 complex are involved, one in the I region and one unidentified locus in between S and D regions. Mice having T-cells bearing molecules encoded for by the I-A gene were more resistant to a *T. spiralis* infection as compared to mice bearing I-E molecules on the T-cells. Using the recombinant strains it was found that also non-H-2 linked genes are involved in the MMC response (Wakelin, 1985).

Some investigators demonstrated the presence of I-A antigens on the MMC membrane. This finding introduced the possibility that MMC, have apart from other functions, have also an antigen presenting role. This aspect is currently being studied.

From these considerations it is clear that some of the properties attributed to MMC are rather speculative and require further study for full elucidation.

Otrzymano: 16 IX 1985

LITERATURE

1. Alizadeh, H., Wakelin, D.: Genetic factors controlling the intestinal mast cell response in mice infected with *Trichinella spiralis*. — *Clin. Exp. Immunol.*, 49, 331, 1982.
2. Befus, A. D., Pearce, F. L., Gauldie, J., Horsewood, P., Bienenstock, J.: Mucosal mast cells. I. Isolation and functional characteristics of rat intestinal mast cells. — *J. Immunol.*, 126, 2475, 1982.
3. Buys, J., Ruitenberg, E. J.: Factors that initiate intestinal mast cell proliferation during a *Trichinella spiralis* infection in rats are contained in lymphoid tissues proximal to and distant from the gut. — Manuscript submitted.
4. Czarnetzki, B. M., Behrend, H.: Studies on the in vitro development of rat peritoneal mast cells. — *Immunobiol.*, 159, 256, 1981.
5. Czarnetzki, B. M., Figdor, C. G., Kolde, G., Vroom, T., Aalberse, R., Vries, J. E. de: Development of human connective tissue mast cells from purified blood monocytes. — *Immunol.*, 51, 549, 1984.
6. Ernst, P. B., Petit, A., Befus, A. D., Clark, A. D., Rosental, K. I., Ishizaka, I., Bienenstock, J.: Murine intestinal intraepithelial lymphocytes. II. Comparison of freshly isolated and cultured intraepithelial lymphocytes. — *Eur. J. Immunol.*, 15, 216, 1985.
7. Ginsburg, H.: The in vitro differentiation and culture of normal mast cells from mouse thymus. — *Ann. NY. Acad. Sci.*, 103, 20, 1963.
8. Ginsburg, H., Ben-Shahar, D., Ben-David, E.: Mast cell growth in fibroblast monolayers: two-cell entities. — *Immunol.*, 45, 371, 1982.
9. Ha, T. Y., Reed, N. D., Crowle, P. K.: Delayed expulsion of adult *Trichinella spiralis* by mast cell-deficient W/W^v mice. — *Inf. Immunity.*, 41, 445, 1983.

10. Haig, D. M., Mc. Menamic, C., Gunneberg, C., Woodbury, R., Jarrett, E. E. E.: Stimulation of mucosal mast cell growth in normal and nude rat bone marrow cultures. — *Proc. Natl. Acad. Sci., USA*, 80, 4499, 1983.
11. Ihle, J. N., Rebar, L., Keller, J., Lee, J. C., Hapel, A. J.: Interleukin-3: Possible roles in the regulation of lymphocyte differentiation and growth. — *Immunol. Rev.*, 63, 5, 1982.
12. Ishizaka, T.: Membrane events in the triggering of mast cells. In: The mast cell, its role in health and disease. — Ed. Pepys, J. and Edwards, E. M. The Pitman Press, Bath, Gr. Br., 1979.
13. Ishizaka, T., Hirata, F., Sterk, A. R., Ishizaka, K., Axelrod, J.: Bridging of IgE receptors activates phospholipid methylation and adenylate cyclase in mast cell plasmamembranes. — *Proc. Natl. Acad. Sci. USA*, 78, 6812, 1981.
14. König, D.: Die Arachidonsäure: Informationsträger bei der Entzündungsreaktion zwischen Mastzelle, Neutrophielen und Eosinophielen. — *Allergology*, 5, 151, 1982.
15. Lee, T. D. G., Sterk, A., Ishizaka, T., Bienenstock, J., Befus, A. D.: Number and affinity of receptors for IgE on enriched populations of isolated rat intestinal mast cells. — *Immunology*, 55, 363, 1985.
16. Lewis, R. A., Austen, K. F.: Mediation of local homeostasis and inflammation by leukotrienes and other mast cell dependent compounds. — *Nature*, 293, 103, 1981.
17. Loveren, H. van, Meade, R., Askenase, P. W.: An early component of delayed-type hypersensitivity mediated by T-cells and mast cells. — *J. Exp. Med.*, 157, 1604, 1983.
18. Nijkamp, F. P., Sisten, J. M. A.: Leukotrienes, allergy and inflammation. — Pharmaceutisch Weekblad, Scientific Ed., 4, 165, 1982.
19. Parmantier, H. K., Ruitenberg, E. J., Elgersma, A.: Thymus dependence of the adoptive transfer of intestinal mastocytopoiesis in *Trichinella spiralis* infected mice. — *Intern. Archs. Allergy Appl. Immunol.*, 68, 260, 1982.
20. Pearce, F. L., Befus, A. D., Gauldie, J., Bienenstock, J.: Mucosal mast cells. II. Effects of anti-allergy compounds on histamine secretion by isolated intestinal mast cells. — *J. Immunol.*, 126, 2481, 1982.
21. Rocklin, R. E., Greineder, D. K., Melmon, K. L.: Histamine-induced suppressor factor (HSF): Further srudies on the nature of the stimulus and the cell which produces it. — *Cell. Immunol.*, 44, 404, 1979.
22. Reitenberg, E. J., Elgersma, A.: Absence of intestinal mast cell response in congenitally athymic mice during *Trichinella spiralis* infection. — *Nature*, 264, 258, 1976.
23. Schwartz, L. B., Austen, K. F.: Enzymes of the mast cell granule. — *J. Invest. Dermatol.*, 74, 349, 1980.
24. Tas, J., Berndsen, R. G.: Does heparin occur in mucosal mast cells of the rat small intestine. — *J. Histochem. and Cytochem.*, 25, 1058, 1977.
25. Wakelin, D.: Genetic control of immunity to helminth infections. — *Parasitology Today*, 1, 17, 1985.
26. Wasserman, S. J.: The mast cell and the inflammatory response. In: The mast cell, its role in health and disease. — Ed. Pepys, J. and Edwards, E. M. The Pitman Press, Bath, Gr. Br., 1979.
27. Yung, Y. P., Eger, R., Tertian, G., Moore, M. A. S.: Long-term in vitro culture of murine mast cells. II. Purification of mast cell growth factor and its dissociation from TCGF. — *J. Immunol.*, 127, 794, 1981.