GUT MUCOSAL MAST CELLS

Origin, Traffic, and Differentiation

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Mast cells are observed in the gut mucosa of normal rats, primarily in the lamina propria (LP)[1] and to a lesser extent in the epithelium. Their number and presence in the epithelium strikingly increase in nematode-parasitized rats, e.g. after infestation by *Nippostrongylus brasiliensis* (Nb) (1). In normal mice, mucosal mast cells (MMC) are nearly absent from the gut and appear in nematode-infested mice, almost exclusively in an intraepithelial location (2).

MMC differ from serosal mast cells (SMC) (see reviews, references 3 and 4) in that they contain smaller and less numerous granules and nonidentical amines and in requiring special fixatives for their certain identification in tissue sections (see Materials and Methods). MMC also differ from SMC by their T cell dependence for in vitro proliferation and differentiation.

In vitro, mouse mast cell lines with the biochemical and cytological properties of MMC and basophils (5), have been obtained mostly from bone marrow cell (BM) cultures. The factor responsible for MMC proliferation and differentiation in these conditions is present in the supernatant of Con A (6–8) or antigen (Nb) (9) stimulated T cells, and is also present spontaneously in large amounts in the supernatant of the mouse Wehi 3 tumor (W3 sup) (10, 11). This factor, named IL-3, has been extensively purified and shown to be identical to the mast cell growth factor (MCGF) and to the "histamine-producing cell stimulating factor" (HCSF) (12). IL-3 also has a colony-stimulating factor (CSF) activity, and may correspond to a "multispecific CSF" (13). In this paper, the name IL-3 will be used to describe the factor(s) present in W3 sup that acts on MMC and their precursors (MMC-P), and induces the continuous growth of MMC culture (MCGF activity) and the release of histamine by BM cells (HCSF activity).

In vivo, the T cell dependence of MMC is well established. Nude mice, which

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[1] *Abbreviations used in this paper:* BM, bone marrow; BM MMC-P, bone marrow mucosal mast cell progenitors; CSF, colony-stimulating factor; HCSF, histamine-producing cell stimulating factor; IE, intraepithelial; IEL, intraepithelial lymphocytes; LN, lymph nodes; LP, lamina propria; MCGF, mast cell growth factor; MLN, mesenteric lymph nodes; MMC, mucosal mast cells; MMC-P, mucosal mast cell precursors; Nb, *Nippostrongylus brasiliensis*; PLN, popliteal lymph nodes; PP, Peyer's patches; SMC, serosal mast cells; TD, thoracic duct; TDL, thoracic duct lymph; W3 sup, Wehi 3 tumor supernatant.

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have normal SMC, do not develop gut MMC when they are infested with nematodes (2). In Nb-infested rats, the increase in gut MMC is correlated with the presence of specific immune T lymphocytes circulating in the thoracic duct lymph (TDL) (14, 15).

In the present work, we studied the properties, frequency, cell traffic, and conditions of differentiation of MMC-P into gut MMC, in normal mice as well as in mice bearing a Wehi 3 tumor, and in mice and rats infested with Nb. In this latter situation, the appearance of gut MMC in large numbers results from a combination of the peculiar traffic properties of the gut T lymphocytes (16) and of their ability to release, upon contact with Nb antigen, unusually large amounts of IL-3.

Materials and Methods

Animals and In Vivo Treatments. C57Bl/6, BALB/c, Nude (Swiss), germ-free mice (Swiss) were obtained from Iffa Credo or CNRS Orléans, France. Lewis rats were raised in our animal house. Animals bearing graft of fetal intestine were grafted subcutaneously with 18-d old fetus gut at least 20 d before utilization (16). Some BALB/c mice were injected subcutaneously or intraperitoneally with 5×10^6 Wehi 3 B tumor cells and sacrificed 15 d later. For immunization by Nb, infective larvae (L3) were inoculated subcutaneously into the flank region of adult female animals (700 L3 in C57Bl/6 mice, 3000L3 in Lewis rats). Selective irradiation of mesenteric lymph nodes (MLN) or spleen were performed with pieces of ^{32}P containing polyvinyl chloride (CEA, Saclay, France) (16). For selective depletion of dividing cells, mice received intraperitoneally, four times over 20 h, 0.06 mg of vinblastine sulfate and were sacrificed 3 h after the last injection. Thoracic duct cannulation was performed in rats according to Ford and Hunt (17) and in mice as previously described (18). Colony-forming unit-spleen assay was performed by injecting various number of cells (10^5 to 7.5×10^5) from BM, spleen, and gut mucosa into lethally irradiated syngeneic mice. Colonies were scored macroscopically and histologically at day 7.

Preparation of Cell Suspensions. Cell suspensions from lymphoid organs were prepared by pressing small fragments through a wire mesh no. 100. BM was obtained by flushing the femurs. Intraepithelial lymphocytes (IEL) were obtained as previously described in normal mice (16): after washing the gut and removing the Peyer's patches (PP), the small bowel was opened and flattened. The epithelium was scraped with a scalpel and the fragments obtained were dissociated with a magnetic stirrer for 5 min in medium 199 (Gibco Europe-Uxbridge UB8 2YG Middlesex, England) with 20% fetal calf serum (FCS) and 1 mM dithiorerythritol for 10 min. The washed suspension was then shaken vigorously on a Vortex mixed for 3 min, and filtered quickly through a buffered glass wool column (1.8 g packed in a 20-ml syringe). Dead cells and debris were eliminated on a Ficoll Isopaque gradient (19). This procedure yields $2–8 \times 10^6$ nonepithelial cells per mouse in a rather pure form. In rats the procedure was similar, but better results were obtained with a smaller glass wool column (0.5 g) and the use of Percoll gradient (20). The average yield varied between 15 and 30×10^6 nonepithelial cells per rat. In Nb-infested animals, particularly during the self-cure, the isolation was more difficult because lymphoid cells stuck to the glass wool and therefore smaller columns were used; however the cell recovery was lower and epithelial cell contamination was unavoidable. In all cases, no cells from the LP were isolated with IEL, since no plasma cells were observed among the isolated cells. Isolation of LP cells was performed in mice by a modification of the procedure of Davies and Parrot (20): the washed small bowel, after removal of PP, was cut in 1-cm long fragments, which were submitted to magnetic stirring in RPMI (Gibco Europe) plus 10% FCS (three times, 15 min each). This treatment removes the villous epithelium but preserves the crypt epithelium, as seen on histological sections. Use of EDTA was avoided in infested mice because it leads to a low yield of LP cells. The fragments were then transferred to flasks containing 15 ml RPMI with 20% FCS and 45 μg/ml collagenase

(Sigma Chemical Co., St. Louis, MO) at 37°C and incubated with magnetic stirring for 60 min. Disruption was achieved by pumping through a 10-ml syringe. The cell suspension was filtered through a wire mesh, washed, and passed through a glass wool column (0.4 g) and a Ficoll Isopaque gradient as for IEL. The procedure yields ~2×10^6 cells per animal. These cells are not pure LP cells since they include crypt cells, but are mucosal cells devoid of villous epithelium IE cells. They will be referred to as "gut mucosal cells."

Separation of Cell Subsets. Cytotoxicity or panning procedures (or combination of the two techniques) were used. In mice, elimination of Thy-1^+ or Lyt-2^+ lymphocytes was performed by incubating cells with monoclonal rat anti–Thy-1 (AT 83) or anti–Lyt-2 (14.281) (kind gift of F. Fitch, University of Chicago). Low-tox-M rabbit complement (CL 3051, Cedarlane, Hornby, Canada) was then added (final dilution 1/10). For cell separation by panning (21) the same antibodies were used to coat the cells which, after washing, were incubated in dishes coated with goat anti-mouse Ig. In rats, the monoclonal antibody used was MRC Ox 8 (Sera-Lab, Grawley Down, Sussex, England). The purity of the separated cells was verified after each separation by immunofluorescence, Velocity sedimentation at 1 *g* in FCS gradient was performed as described (22).

Immunofluorescence, Histological, and Cytological Procedures, Labeling, and Radioautography. In mice, cells were stained separately or in combination with a rhodamine-labeled rabbit anti–Thy-1 antibody (23), with monoclonal rat anti–Lyt-2 (14.281) and Lyt-1 (AT 105) followed by a fluorescein-labeled mouse anti-rat Ig, with a rhodamine- or fluorescein-labeled goat anti-mouse Ig (Nordic Tilburg, The Netherlands), and with a rhodamine-labeled anti-mouse μ, γ, or α chain rabbit antiserum, as previously described (24). Monoclonal mouse IgE was a generous gift of Dr. I. Böttcher (IgE 14205) (25) and was applied a 1/50 dilution for 3 h for detection of IgE receptors on cultured cells (see below). Controls were incubated with 1/5 diluted normal mouse serum. In the absence of a labeled anti-mouse ϵ chain, IgE was detected, on cultured cells or on isolated MMC, by the positivity of the fluorescence with goat anti-mouse Ig compared to the negativity with anti-μ, -γ, and -α rabbit antisera. In the last experiments of this series, a rabbit anti-mouse ϵ-chain, obtained by immunization with monoclonal IgE (IgEL b4, American type culture collection TIB 141), and made specific by appropriate absorptions, was available and found to give comparable results. In rats, the rabbit anti-mouse α-chains antiserum was used to detect IgA plasma cells. An anti-IgE was prepared by immunization of a rabbit by rat myeloma IgE IR 162 (generous gift of Dr. H. Bazin, University of Louvain) followed by appropriate absorptions. All these antisera were used either on isolated viable cells or, when possible, on tissue sections (frozen or after Carnoy fixation).

Labeling of dividing cells was obtained in vitro as described (16), or in vivo by a single injection of 1 μCi ^{3}H-TdR (CEA Saclay, France) per gram of body weight.

For histological studies, tissues were fixed in Carnoy's fluid. For detection of mouse MMC, tissues were fixed in the fluid described by Ruitenberg and Elgersma (2) which, in our hands, was the most appropriate for good visualization of MMC with toluidine blue (Fig. 1). In rats, tissues were fixed in Carnoy's fluid as described by Enerbäck (26) and MMC were stained by Alcian blue, pH 0.3 and Safranin (27). For autoradiography, sections were stained by Alcian blue, then by anti-α chains antiserum before dipping in photographic emulsion (Ilford K5). Slides without staining of plasma cells were counterstained by Safranin after developing (Fig. 3).

Cytocentrifuged smears were stained as described (16). For detection of MMC raised in cultures, unfixed smears were immediately stained by May Grumwald Giemsa (the use of prior alcohol fixation was found to decrease the number of MMC granules). For studies of cells labeled with fluorescent antibodies, the smears were mounted in tetrahydrofurfuryl alcohol (which preserves granules). For subsequent Giemsa staining of cells labeled with fluorescent antibodies, the exact position of groups of fluorescent cells on the slide was determined with Vernier markings, and the respective position of the fluorescent and nonfluorescent (i.e., detected by phase contrast only) cells recorded by picture or hand drawing. The slide was then stained with May Grumwald Giemsa, and the same groups of cells identified, thus allowing to determine the cytologic characteristic of the fluorescent cells (Fig. 2).

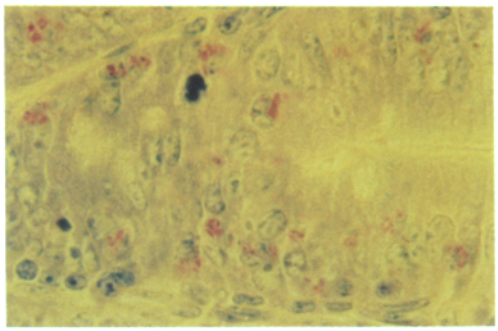

FIGURE 1. Gut tissue section of a mouse bearing a Wehi tumor, toluidine blue stain. Numerous MMC containing red metachromatic granules are located in the crypts and the bottom of the epithelium villous.

Cell Cultures and Studies of Supernatant Activities. Cell cultures were performed in Dulbecco's supplemented medium (Gibco Europe) with 10% FCS. Limiting dilution assays for detection of MMC-P were carried out by culturing eight appropriate concentrations of tested cell populations in 96 flat-bottom wells (Falcon 3040 Becton Dickinson, Oxnard, CA), 12 wells per cell concentration. Each 200-μl well contained, in addition, 1×10^6 irradiated (4,000 rad) syngeneic spleen cells and an optimal concentration of W3 sup (~5%). After 7–12 d, cells from growing colonies were studied on May Grumwald Giemsa–stained smears. The frequency of MMC-P was determined by using Poisson probability distribution (28).

For the detection of interleukin release, cultures of various lymphoid organs were performed, using 1×10^6 or 10×10^6 cells/ml in the presence of Nb antigen or antigen extracted from L3 larvae, 50 equivalent worms/ml (as described) (29) or of Con A, 5 μg/ml (I.B.F. Villeneuve La Garenne, France) (see text and Tables II and III).

IL-3 was assayed as MCGF (21) and HCSF (30), and IL-2 was assayed on a IL-2–dependent cell line as described (21). In addition, IL-3 was also tested by the induction of MMC in cultures of normal mouse gut mucosal cells. 10^4 cells were plated as for limiting dilution cultures with various dilutions of the supernatant to be tested, and MMC growth detected as described above. Results of IL-3 assays or of W3 sup activity were compared, in some cases, with those obtained with highly purified IL-3 (gift of Dr. Ihle, National Cancer Institute, NIH) (12).

Results

A. *Gut mucosal Mast Cells (MMC) and Their Precursors (MMC-P) in Noninfested Mice*

In the gut of normal mice, MMC are extremely rare and almost exclusively intraepithelial, located within the crypts and at the base of the villi (never more than 5 MMC per 10 crypt villi). MMC-P are defined as those cells that grow and differentiate, when cultured in IL-3–rich medium, into cell populations rich in or composed exclusively of MMC.

1. *Localization and Frequency of MMC-P.* (a) *In Vitro studies, using IL-3–stimulated cultures with limiting dilutions of cells obtained from various locations.*

As a source of IL-3, culture W3 sup were used; in several experiments, identical results were obtained using highly purified IL-3 from W3 sup (12).

As shown in Table I, MMC-P are found in various lymphoid organs, in the BM and among circulating cells as well as among gut mucosa lymphoid cells

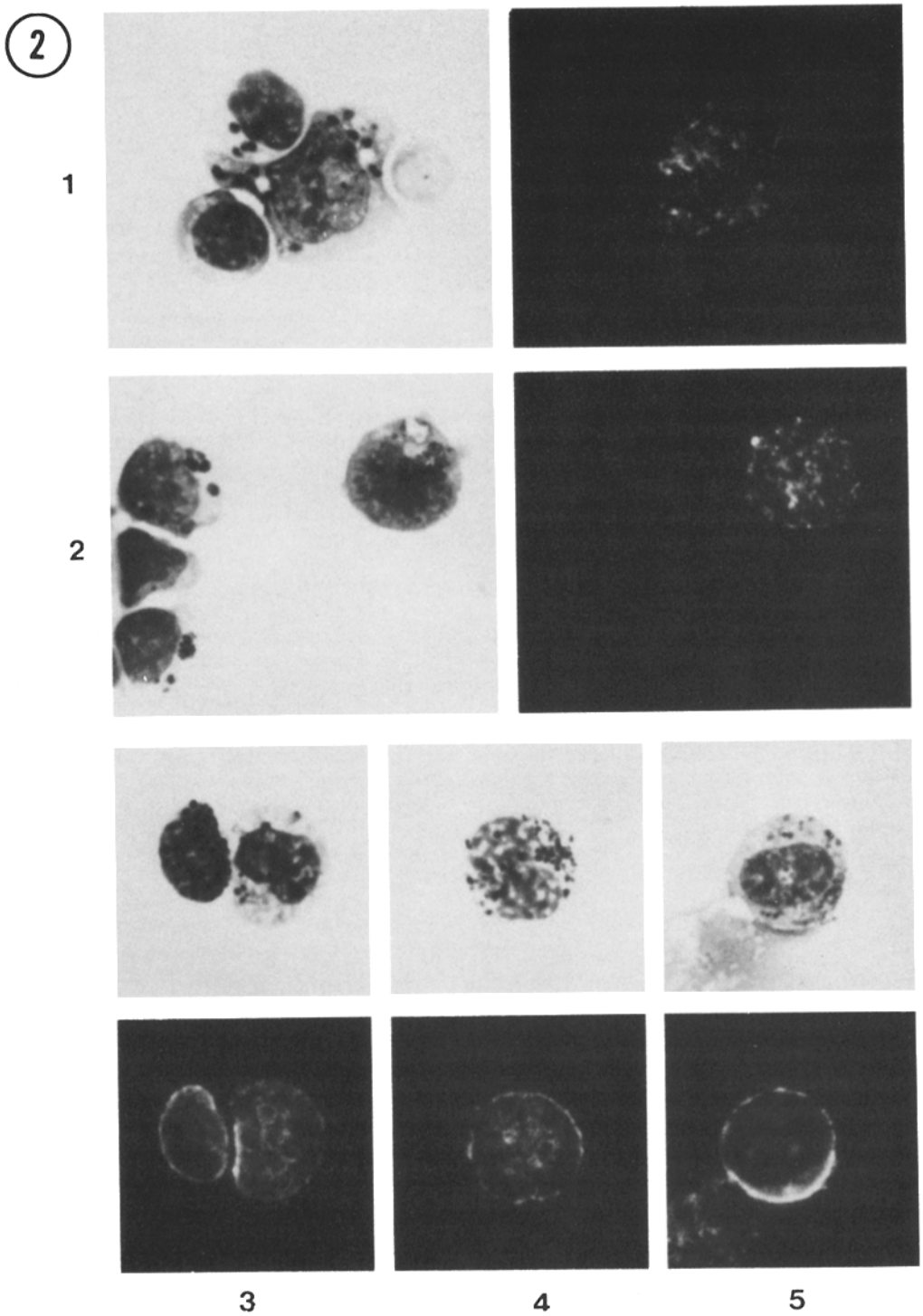

FIGURE 2. Smears of gut intraepithelial cells from Nb-infested mice. May Grumwald Giemsa performed on cells showing membrane IgE by immunofluorescence (early MMC, and granulated lymphocytes, devoid of IgE Fc receptors, and thus unstained by immunofluorescence. 1 and 2: early MMC (day 8, beginning of the self cure, showing IgE fluorescence, and nonfluorescent granulated lymphocytes. 3, 4, 5: Various aspects of MMC observed on day 13; the cells have variable number of grains, but all bear membrane IgE detected by immunofluorescence. Aspects of MMC differentiation in vitro were indistinguishable.

TABLE I
MMC-P Frequency in Various Organs of Mice*

	BM	Spleen	Gut mucosa‖	PP	MLN	TDL	PLN
Normal C57Bl/6 mice	900	34	700	25‡	6	3	<1.5
Nb-infested C57Bl/6 mice							
day 9–10	900	350	10,500	65‡§	120§	330§	<1.5
day 15		80	1,500		20	12	
Nude mice	450	50	1,700				
Germ-free mice	800	140	2,000				
Normal BALB/c	800	70	3,000		6		<1.5
BALB/c with:							
Wehi i-p	5,000	1,250	5,000				<1.5
Wehi sub-cut					36		

* Average of 2–6 experiments. Number of precursors for 10^6 cells.
‡ Always contaminated by MMC-P present in the adjacent epithelium.
§ On day 9, the total cell number obtained from MLN and from 25 h TD drainage showed a twofold increase, while PP showed a twofold cell decrease.
‖ Gut mucosal cells comprise LP cells and IE cells from the crypts. Pure IE cells coming from the villous epithelium (see Materials and Methods) contain ~100 MMC-P per 10^6 cells; the MMC-P frequency in villous epithelium IE cells cannot be tested in Nb-infected mice, because in these conditions IE cell recovery from the villi is made difficult and poorly reproducible because of the thick layer of mucus covering the gut.

(comprising LP cells and IE cells from the crypts, see Materials and Methods). However, limiting dilution analysis shows that their frequency varies enormously among cells from these different origins: they are most frequent among gut mucosa and BM cells, extremely rare in lymph nodes (LN) and among TDL, and somewhat more common among spleen cells. The results obtained with PP cells may be distorted by contamination by gut mucosal cells (see below). The strikingly high frequency of MMC-P among gut mucosal cells must result from homing to the gut wall of precursors from the BM, since undifferentiated hematopoietic stem cells are absent from the gut mucosa, as found by CFU assay (not shown, see Materials and Methods). This impressive gut-homing of MMC-P, which contrasts with the very small dissemination of these cells cannot be attributed to lymphokine release from antigenically stimulated gut T lymphocytes, since it is observed in athymic nude mice as well as in germ-free mice (Table I), and in sterile gut fetal graft (where it appeared to be similar to that of host gut, although the small number of cells recovered precluded a precise limiting dilution analysis). Furthermore, although their frequency is more difficult to evaluate in the gut of very young mice, because of the possibility of contamination by residual PP, gut MMC-P are easily detectable in 2-, 5-, and 15-d old mice, i.e., at the time when there are no IgA and T cells in the LP, as judged by immunofluorescence. In adult mice, gut MMC-P appear to have a definite, although very small, tendency to pass into the gut draining lymph, judging from the MMC-P frequency in MLN, which is at least four times higher than in popliteal lymph nodes (PLN), and the presence of very few MMC-P in the TD lymph.

(b) *In vivo studies of MMC appearance in BALB/C mice bearing grafted Wehi 3 tumor cells.*

In these mice, which received Wehi 3 cells subcutaneously or intraperitoneally, and whose blood contained detectable levels of IL-3, mature MMC were found in the BM and in the splenic red pulp (which was also strikingly hematopoietic) and, conspicuously, in the gut mucosa. In this last location, they were intraepithelial, mostly within the crypts and the bottom of the villi (85 per 10 crypt villi) (Fig. 1), and also in the epithelium covering the thymic-dependent area of the PP. No MMC were observed in the LN, nor in any of the mucosae examined, i.e., tracheal, bronchial, esophageal, uterine, urinary, and skin. However, the number of serosal MC observed in the dermis was markedly increased. The frequency of MMC-P (certainly including in this situation young proliferating MMC) determined in vitro from the BM, spleen, MLN, and gut LP cells was 6- to 20-fold increased compared to normal mice, while it was unchanged among PLN (Table I).

2. *MMC-P Traffic.* MMC-P appear to home directly from the blood to the gut mucosa, rather than passing via TD lymph, like gut T cells (16) since MMC-P frequency is extremely low in normal TD lymph. To explore more directly MMC-P traffic and compare it to that of gut T cells in normal and Nb-infested mice (see below), TD was cannulated for 2 d. This resulted in a slight increase rather than decrease in gut MMC-P frequency, emphasizing the very low circulation of these cells through lymph. To explore whether blood-borne MMC-P come directly from the BM or pass through the spleen (since this organ contains 5–20 times more MMC-P than other lymphoid organs), gut MMC-P frequency was determined in mice whose spleens had been selectively irradiated for 5 d with a ^{32}P strip, or surgically removed, and was not found to be significantly modified.

3. *Characteristics of the Gut MMC-P and of Incompletely Differentiated MMC.* MMC-P do not bear Thy-1 or Lyt-2 antigens, since prior cytotoxic treatment of gut mucosal lymphoid cells with the appropriate antibodies increased their frequency, as detected by limiting dilution analysis (not shown). This was confirmed with IEL, which contains numerous Lyt-2^+ cells with granules resembling those of mast cells, and whose isolation includes some MMC-P: Lyt-2^+ cell depletion increased MMC-P frequency about 6 times (from 1/21,000 to 1/3,400, average values). Gut MMC-P are not rapidly replicating, since their frequency is not significantly modified after 24 h of in vivo treatment with vinblastine (not shown), which lead to gut epithelial crypt necrosis.

Gut MMC-P appear to differ somewhat from BM MMC-P, since: (*a*) BM mast cell clones proliferate faster, frequently contain lobulated nuclei suggestive of basophils, and, when undifferentiated, are weakly stained with anti–Thy-1 but not anti–Lyt-1 or -2 antibody, suggesting the transient acquisition of Thy-1 before full differentiation, in agreement with observations of Schrader et al. (31); (*b*) BM MMC-P are known to be large dividing cells (32), and their absolute number decreases after in vivo vinblastine treatment (not shown); (*c*) BM cells, within 48 h of exposure to IL-3, synthesize and release large amounts of histamine in the culture medium (33), while no histamine is released by gut mucosal cells in the same conditions (data not shown). Thus, it is likely that gut MMC-P represent a more differentiated precursor than BM MMC-P.

In cultures in which MMC are going to emerge, whatever the source used

(gut, TDL, spleen), their immediate precursors appear to be large blasts, with focal small granules within vacuoles; not all these blasts bear IgE receptors (see Materials and Methods). This indicates that the acquisition of IgE receptors is the earliest event clearly indicating mast cell differentiation, in agreement with other observations (34).

B. *Gut MMC, Their Precursors, and Their Inducers in Nb-infested Mice*

NB expulsion from the gut ("self-cure") takes place, in the mouse, between the 8th and the 12th d after infestation.

1. *Nature of the Dividing Cells in the Gut Mucosa.* Starting around day 8 the gut mucosa contains a high number of dividing "lymphoid" cells whose increase and nature can be best studied after gut mucosal cell isolation from the epithelium. Up to 12% of the isolated cells are labeled after 1 h of culture with ^{3}H-TdR (0.5% in normal mice). These labeled cells contain the following cell types: (*a*) immature MMC (~16%), which are large Thy-1$^-$, Lyt-1$^-$, -2$^-$ blasts, bearing IgE receptors (see Materials and Methods), with small granules containing some histamine (detectable by the OPT reaction) (16), thus identical to the early MMC appearing in culture as described above (Fig. 2); (*b*) Thy-1$^-$ Lyt-2$^+$ blasts, usually containing rather large alcianophilic granules and never bearing IgE receptors (32%); (*c*) Thy-1$^+$ blasts of the Lyt-2$^-$ (9%) and/or Lyt-2$^+$ (43%) phenotype; (*d*) a few Thy-1$^-$, Lyt-2$^-$, and sIg$^-$ blasts. More mature MMC, with large granules and a smaller size, can be isolated or identified on tissue sections around day 10; they are mostly located within the epithelium of the crypts and become especially conspicuous on day 13 (Table IV), at which time they contain more histamine and are no longer dividing. Their number decreases very rapidly in the following days (Table IV).

2. *Localization and Frequency of MMC-P and Proliferating MMC.* As mentioned above for Wehi 3 tumor-bearing mice, limiting dilution analysis of MMC-P in Nb-infested mice also detects proliferating MMC, especially in the gut mucosa; for simplicity, all these cells will be called MMC-P. Table I shows the MMC-P frequency in various organs between day 9 and 10 of infestation. There is a more than 10-fold increase in the gut mucosa, as expected, and most of these cells are dividing, as shown by the observation that 24 h in vivo treatment with vinblastine (day 8) leads to a two- to sevenfold decrease in the absolute number of MMC-P. In three other locations, MMC-P frequency is markedly increased: spleen, MLN, and TDL. The cell recovery of MLN and TDL is about twice, and the percentage of ^{3}H-TdR–labeled cells three times above normal (respectively 6% and 1.8%). Some TDL blasts are Thy-1$^-$, sIg$^-$, but no granules were ever seen. Since the massive increase of circulating MMC-P found in the TDL could explain the increased MMC-P frequency observed in the spleen, this possibility was explored by cannulating the thoracic duct between the 8th and 10th day and then assessing the spleen MMC-P frequency. Since this figure was unchanged (not shown), it appears that the spleen MMC-P increase does not result from the accumulation of cells released in the blood from the thoracic duct (and thus originating in the gut wall and MLN), but from a source of IL-3 present in the spleen.

3. *Localization and Quantification of the Sources of IL-3 Release.* The specific

release of IL-3 in the supernatants of 48-h culture of lymphoid cells in the presence of crude Nb antigen was tested in three ways, as shown in Table II. The "MCGF" test measures the ability of IL-3 to sustain the cell division of a mast cell line established from BM, and the "HCSF" test, its ability to induce the synthesis and release of histamine by normal BM cells in short-term cultures (see Materials and Methods). IL-3 release induced by Nb antigen displayed the expected specificity, since no release was observed using this antigen and cells of normal mice (Table II), and since a different antigen, extracted from larvae instead of adult worms, was without effect (not shown). Specifically stimulated lymphoid cells from the gut mucosa, MLN, and TD lymph, and to a slightly lesser extent, spleen, release IL-3, between days 8 and 10 of infestation, while in contrast PLN or PP cells release very little or no IL-3 (Table II). IL-2 is released concomitantly with IL-3, and in order to compare the respective release of these lymphokines to that of similar sources from normal mice, MLN cells of normal and Nb-infested mice were stimulated by Con A. Table III shows that cells from infested mice released more IL-3 than IL-2, while cells from normal mice gave an opposite pattern. The antigen-specific IL-3-releasing cells are Thy-1^+ Lyt-1^+2^- cells (Table II), apparently exclusively blasts as judged by cell fractionation at 1 *g* velocity sedimentation (not shown). Immediately after the worm rejection, on day 13, antigen-specific lymphokine release disappears, at least in the culture conditions used.

4. *Role of the Traffic of IL-3 Releasing T Blasts in Gut MMC Differentiation.* Since from day 8 to 10 postinfestation, the TD lymph contains many worm-specific T blasts able to secrete IL-3 (Table II). TD cannulation during this period should deplete the gut mucosa of the specific T blasts that would have normally homed

TABLE II

*Lymphocyte Release of IL-3 Under the Influence of Nb Antigen in Mice 8–10 d after Infestation**

	Spleen	Gut mucosa	PP	MLN‡				TDL	PLN
				Untreated	Thy-1^-	Lyt-2^-	Lyt-2^+		
(a) "MCGF assay"§	80	143	0	100	0	63	0	157	0
(b) "HCSF assay"‖	45	80	2.5¶	95	0	90	0	100	5
(c) Induction of MMC in cultures of normal gut mucosal cells**	+	+	0	+	0	+	0	+	0

* 1×10^6 cells stimulated by 48 h of culture with Nb antigen. Each value represents the average of 2–8 experiments. Cultures were performed with 5% human AB serum, since FCS contains histaminase interfering with HCSF assay.

‡ MLN cells were cultured untreated, after treatment with anti-Thy-1 + C (Thy-1^-). Lyt-2^- and Lyt-2^+ cells were obtained by panning of Lyt 2^+-cells (after filtration of MLN cells on nylon wool)

§ Results are expressed as percentage of ^{3}H-TdR incorporation induced by an "optimal" amount of W3 sup on 3 d. bone marrow MMC cultures (average background around 200 cpm; average "100%" stimulation 25.000 cpm); final concentration of supernatants tested: 1/4.

‖ Results expressed in HCSF units in the supernatant of 48 h BM cell culture (units refer to amount of histamine release (30).

¶ This very low activity is observed even after enrichment in T cells by passage through nylon wool.

** See Materials and Methods. In order to allow gut MMC growth in these conditions, supernatants must contain an IL-3 concentration corresponding to at least 25 HCSF U/ml.

TABLE III

*Release of IL-3 and IL-2 my MNL Cells from Normal and Nb-infected Mice, Stimulated by Con A or Nb Antigen**

	Supernatants of cultures performed in the presence of: IL-3‡			IL-2§		
	—	AG	Con A	—	AG	Con A
Normal MLN	0	0	10	0	0	50
MLN from Nb mice (day 9)	0	180	260	1	35	25

* Supernatants obtained from 24-h cultures of 10^7 cells (after nylon wool filtration) in presence of Nb antigen or Con A (see Materials and Methods).

‡ IL-3 was tested by the "MCGF assay" (supernatants tested at dilutions down to 1/64 final concentration, the value presented being the highest before decrease and was observed at 1/16 final concentration) and the results were expressed as in Table II (BKG 140 cpm, "100%": 6,000 cpm).

§ IL-2 was tested with an IL-2–dependent cell line (supernatants tested at dilutions down to 1/64 final concentration, the value presented being the highest before decrease and was observed at 1/16 final concentration) and the results were expressed as percent of maximum ^{3}H-TdR incorporation observed in the presence of a fraction rich in rat IL-2 (BKG 400 cpm, "100%": 4×10^4 cpm).

TABLE IV

*Number of MMC Detectable on Tissue Sections in Nb-infested mice with or without 48 h Interruption of the T Cell Traffic**

Infestation day	Control mice	Cannulated mice	Mice with ^{32}P on MLN
0	0		
10	73	13	
13	136	48	54
15	16	12	

* Gut sections were performed at 5 cm from the pylorus and MMC were numbered by 10 crypt villous units on tissue sections as described (2); results are the average of 2–4 experiments.

there, following the gut T cell cycle previously demonstrated (16). Indeed, on day 10, gut mucosal cells of mice cannulated from day 8 released in the presence of the worm antigen ~10 times less IL-3 (average of 8 HCSF units) than cells from noncannulated mice (average of 80 UCSF units) (Table II). Although it has been shown above that TD drainage on day 8 to 10 does not significantly decrease gut mucosa MMC-P, which at that time are rapidly dividing, TD drainage for 2 d may modify the appearance of histologically detectable gut MMC, if their full differentiation requires constant IL-3 release. That this is indeed the case is shown on Table IV. TD cannulation between day 11 and 13 is less effective in decreasing gut MMC (Table IV), probably because the worm expulsion is well advanced, and the antigenic stimulation of T cells thus decreased. Selective ^{32}P irradiation of MLN for 2 d, which also interrupts the gut T cell traffic cycle, results in a similar decrease in gut MMC (Table IV). At day 15 the gut MMC increase has almost entirely waned, and prior TD cannulation is without effect (Table IV), MMC-P frequency being also almost back to normal. This shows the short life of MMC (which are no longer dividing since day 13) in the absence of IL-3.

Fetal gut grafts of mice grafted before Nb infestation were also examined. While the gut of the host contained histologically detectable MMC in markedly increased amounts, no MMC could be detected in the graft. Since the grafts contained T cells detectable by immunofluorescence, and very probably Nb-specific T cells (16), this strongly suggests that worm antigen leads to local IL-3 release, and was the missing element for MMC differentiation (no worms were found in the grafts of 10 infested animals).

C. *MMC-P Traffic in the Rat*

As mentioned above, in normal conditions gut mucosa MMC-P are mostly nondividing and are not released in the TD lymph. During Nb infestation, these cells start dividing and recirculating in the TD lymph: however, their traffic and homing, after in vitro ^{3}H-TdR labeling and injection in a Nb-infested animal, cannot be adequately studied in the mouse, probably because of extensive cell division and difficulty in identifying early MMC on tissue sections. The situation is different in the rat: mature gut MMC are numerous even in normal conditions, and in newborn rats as well, and are located mostly within the LP where they are easily detectable. These cells are not dividing (few labeled cells 90 min after in vivo ^{3}H-TdR injection), but start rapid replication (40% of labeled cells after ^{3}H-TdR injection) on the 10th day of Nb-infestation (beginning of the self-cure). At the same time, dividing immature MMC, with small granules and IgE receptors, are found among isolated intraepithelial lymphocytes ('globule leucocytes"; references 15, 35). At the end of the self-cure (16th to 18th day) there is about a fourfold increase in gut MMC, and division stops. TD cells, obtained from normal and Nb-infested rats (10th day) were labeled in vitro and injected into Nb-infested rats (16th day). In both conditions, none of the TD blasts had granules. 1 and 2 d after transfer, autoradiographs of gut sections, stained both with Alcian blue and with fluoresceinated anti-IgA serum, were prepared. Labeled MMC, clearly identifiable by Alcian blue (Fig. 3), represented a surprisingly high percentage of the labeled cells present in the gut mucosa, after transfer of TD blasts from both normal or Nb-infested donors, i.e., about one-third of the labeled cells that were not IgA plasma cells (~10% of total labeled cells, average of five experiments). In one transfer experiment, TD blasts were separated by velocity sedimentation, and in a fraction of these blasts, OX 8^{+} cells

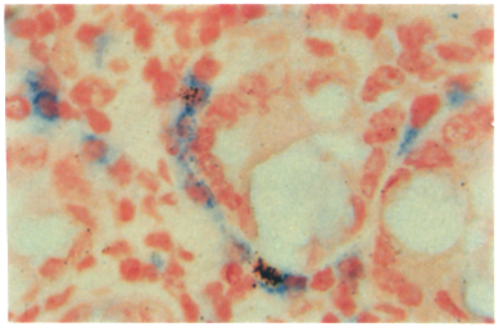

FIGURE 3. Gut tissue section of 17-d Nb-infected rat, 24 h after transfer of ^{3}H-TdR–labeled thoracic duct blasts from normal rats. Two labeled alcian blue positive MMC are observed.

(equivalent of Lyt-2^+ cells in mice) were removed by panning. Labeled MMC were found in increased percentage in the recipient of OX 8^- blasts (48% vs. 25% of the IgA^- labeled cells), which, in contrast, was nearly devoid of labeled IEL. This indicates that TD circulating MMC-P are OX 8^- (as MMC-P are Lyt-2^- in the mouse), and that the TD blasts that migrate to the gut epithelium are OX 8^+.

When a fetal gut is grafted to normal and Nb-infested rats, MMC are present in the grafts of both types of recipients. Although the accuracy of cell counts is questionable, MMC were slightly increased in LP and no MMC were found in the epithelium (contrary to what is observed in the recipient's own gut), as reported by McDonald et al. (35).

Discussion

The discussion will be focused on two main points: the nature and properties of the gut MMC-P; the mechanisms leading to the production of large amounts of gut MMC during Nb rejection.

Gut mucosal and BM cells are the populations where the MMC-P are by far the most frequent, in agreement with Crapper and Schrader (36). The following features of the gut MMC-P emerge from the present observations: (1) Origin: gut MMC-P originate from the BM, since there are no stem cells in the gut mucosa, and since local splenic irradiation or splenectomy do not modify their amount. However, they differ somewhat from their BM progenitors (BM MMC-P): (*a*) in contrast to BM MMC-P (reference 32 and present obervations), they do not divide unless stimulated, as shown by their lack of decrease after in vivo vinblastine treatment, (*b*) in culture, BM MMC-P have a faster growth and transiently acquire Thy-1 antigen (reference 31 and present observations), (*c*) gut MMC-P do not release histamine in the presence of IL-3, while BM cells, probably BM MMC-P, do it on a large scale. All these features suggest that gut MMC-P represent a more differentiated form of precursor than BM MMC-P, perhaps as the result of the influence of a "gut factor" discussed below; (2) surface phenotype: gut MMC-P bear neither Thy-1 nor Lyt-2 antigens, in agreement with Schrader et al. (37), (3) traffic properties: (*a*) the striking gut-homing property of BM-derived circulating MMC-P is not related to any form of T cell activity or antigenic stimulation, since the high frequency of gut MMC-P is observed in nude, germ-free, and newborn (i.e., before the gut seeding by IgA plasma cells and T cells) mice, as well as in sterile grafts of fetal gut. The attraction of MMC-P to the gut results from unknown factor(s), probably released in situ and perhaps responsible for the further differentiation of gut MMC-P compared to BM MMC-P, as well as for the full MMC differentiation observed in normal rat (see below); when stimulated by IL-3, gut MMC-P not only proliferate, but also migrate in the efferent lymph, as witnessed by their enormous increase in frequency in TDL. Increased production and release by the BM probably require direct BM IL-3 stimulation, since a marked increase in BM MMC-P, associated with the emergence of BM mast cells, is observed only in mice carrying the Wehi 3 tumor (which have detectable circulating IL-3), but not during Nb infestation, when IL-3 release is local, (*b*) at least part of the circulating TDL MMC-P remains endowed with gut-homing properties, so that

the whole length of the gut mucosa will receive these cells, which, if the local stimulus to differentiation persists, can rapidly be transformed into MMC (as shown by the rat TD cell transfer experiments). This gut cell cycle closely resembles that followed by stimulated T blasts and IgA-bearing blasts (although these arise, as previously shown, in the PP) (16), in allowing the dissemination to the whole mucosa (by unknown attractive factors) of the progeny of cells that were stimulated to divide locally in the gut. Even though activated, none of the cells undergoing this cycle and found in the TDL have the features of early granulated mast cells; (4) fate of gut MMC-P: under the induction of local (Nb infection) or systemic (mice with Wehi 3 tumor) IL-3 release, proliferating MMC-P are transformed into characteristic early MMC (easily identifiable in gut mucosa cell suspension as blasts with few granules and little histamine, but bearing IgE receptors), then into fully differentiated MMC. In spite of the large increase in MMC induced by local or systemic IL-3 release, it seems likely that not all stimulated MMC-P are transformed in situ into MMC since, at least in the MLN and the spleen, the increase in MMC-P far exceeds that of MMC. The numerous MMC-P that do not reach the stage of differentiated MMC are clearly short-lived, since they disappear very quickly after the cessation of IL-3 stimulation, and this is also obviously true for mast cells, at least in the mice.

The reason why some form of gut antigenic stimulation such as that represented by Nb worms leads to a local immune response characterized, in contrast to most other responses, by a massive accumulation of gut MMC, reflects the capacity of these antigens to elicit an unusual T cell response. The T cell response observed in the gut during Nb infection appears similar to what we have described in other conditions with respect to origin, traffic cycle, and homing along the gut mucosa of TD circulating T blasts, but different in one respect: the unusual extent of IL-3 release by Lyt-1^+2^- T blasts upon contact with their specific antigen. T cells present in the gut as the result of usual antigenic stimulation, such as those observed in normal mice, release under Con A stimulation more IL-2 than IL-3, while the converse is true in the case of Nb infestation. The mechanism of this peculiar relationship between the nature of the antigenic stimulus and its effect on lymphokine release is not known. It may take place as soon as T cells are stimulated in the PP. The lack of IL-3 release by PP cells stimulated in vitro by the Nb antigen probably indicates that T cells are immature and migrate as soon as specifically stimulated (the size and cell number of the PP is surprisingly decreased in Nb-infested mice). That IL-3 released by stimulated T blasts present in the mucosa after their cycle through the TD is decisive in the appearance of gut MMC has been clearly shown by the effects of interruption of this cycle by 48-h TD cannulation or selective MLN irradiation. Such interruption leads to a profound decrease in both gut T blasts releasing IL-3 after exposure to Nb antigen and gut MMC (while MMC-P frequency in the gut is not modified). When massive antigenic stimulation abruptly stops, i.e., after worm rejection, IL-3 releasing specific T blasts as well as gut MMC and circulating MMC-P all disappear very rapidly.

The spleen is the only extra-intestinal location where increased numbers of Nb-specific T blasts and MMC-P are found during Nb rejection. T blasts may

reach the spleen through the TD and the blood, since transfer experiments of TD blasts have shown their homing in the spleen and not only in the gut; the increased frequency of MMC-P cannot, however, solely result from the same type of traffic, since TD drainage does not modify it significantly. It seems therefore more likely that some antigen reaches the blood and the spleen, leading to some IL-3 release by specific T blasts (as previously shown, reference 30) and thus to MMC-P increase in the spleen; although local IL-3 concentration is obviously not sufficient to induce massive MMC transformation, some MMC are indeed observed in the spleen during Nb rejection.

The situation of gut MMC in the rat appears somewhat different, and might shed some light on the various differentiation steps leading from BM MMC-P to fully differentiated gut MMC. Since MMC are always present in appreciable numbers in the gut LP of normal rats, the normal level of IL-3 might be higher in the rat, or the differentiation of LP MMC might be helped by an increased level of factor(s) released by the gut mucosa itself, such as the "gut factor" postulated above to explain the attraction of BM-released, circulating MMC-P to the mouse gut in the absence of IL-3 release (e.g. germ-free and nude mice). This last situation seems more likely since MMC appear in fetal gut grafts, in normal rats (while there is no detectable MMC, but only MMC-P, in such grafts in mice, even during Nb infection). An increased level of this postulated "gut factor," enhancing the level of differentiation of MMC-P, might also explain a high frequency of MMC-P among the TD blasts of normal rats, as suggested by the results of the transfer experiments using TD blasts. In this hypothesis, the transition of MMC-P to MMC would entail several steps (influenced by IL-3 and a "gut factor") which may be schematized as follows: (*a*) BM MMC-P, released in the circulation; (*b*) gut MMC-P, sessile; (*c*) activated gut MMC-P, dividing and circulating; (*d*) early gut MMC, dividing; (*e*) fully mature gut MMC, short-lived.

Finally, the relationship between gut MMC and the gut intraepithelial lymphocytes with small mast-cell–like granules should be discussed. We had previously speculated that the latter could be an "aborted" variant of the former, in conditions where the stimulation for full MMC differentiation was insufficient. We had noticed that the most granulated of these IE lymphocytes had weak or no Thy-1 antigen, and found subsequently that most of these cells are Lyt-2^+, and OX 8^+ in the rat and, as detected by double immunofluorescence, mostly Thy-1^-, Lyt-2^+ (38, 40), in agreement with the recent observations of other workers (37, 39). It is clear for the present and Schrader's et al. experiments that these cells do not behave as MMC-P. While we found that these cells undergo modifications (increase in proliferation rate and granule size) during Nb rejection, we never observed evidence for a transition towards MMC, for instance the simultaneous presence of Lyt-2 antigens and IgE receptors. It is apparent that these cells, like most gut T cells, are the progeny of TD blasts (they decrease concomitantly with gut T cells when the cycle is interrupted by ^{32}P MLN irradiation in contrast with the absence of effect of ^{32}P spleen irradiation) and that they acquire their characteristics under a local gut influence, which is increased during NB rejection. Their nature is the subject of further study.

Summary

Gut mucosal mast cells (MMC), which are nearly absent in normal mice are abundant during nematode infection. In normal mice, study of MMC precursors (MMC-P: cells giving rise to MMC colonies in the presence of IL-3) show that: (*a*) their frequency, judged by limiting dilution is very high in bone marrow (BM) and gut, and very low in most lymphoid organs and thoracic duct lymph (TDL); (*b*) gut MMC-P are Thy-1$^-$ Lyt-1$^-$2$^-$ and are not rapidly replicating; (*c*) they are the progeny of less differenciated BM MMC-P which are attracted from the blood to the gut mucosa by local factor(s), other than antigen and T cell factors (since normal amounts of gut MMC-P are found in germ-free, nude, and newborn mice). In mice bearing the Wehi 3 tumor (which releases enough IL-3 to produce detectable blood levels) spleen and mesenteric lymph nodes (LN) show increased MMC-P frequency, the greatest increase being in the gut and BM, where numerous differentiated MMC are found. In *Nippostrongylus brasiliensis* (Nb)-infested mice (known to develop a large, T cell–dependent, gut MMC infiltration), gut MMC-P proliferation is induced by IL-3 released from gut mucosal Thy-1$^+$ Lyt-2$^-$ cells, whose in vitro IL-3 release capability is much higher than that of similar cells from normal mice. Both Nb-stimulated T blasts and proliferating MMC-P undergo cyclic traffic, migrating into the TDL and then seeding the whole length of the gut (a process which allows a widespread immune defense after a local antigenic stimulus). Experiments using 2-d interruption of this traffic and fetal gut grafts, suggest that the continuous homing of T blasts back to the gut which leads to permanent Nb-stimulated IL-3 release, is essential for the full maturation of MMC. Transfer experiments in the rat show that TDL circulating MMC-P rapidly mature into MMC when they home back to the Nb-infested gut. It is proposed that gut MMC arise after several stages of progressive differentiation of MMC-P, influenced both by IL-3 and unidentified gut factor(s).

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