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Mast cell growth on fibroblast monolayers: two-cell entities

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Summary. Clonal mast cell differentiation occurs when mesenteric lymph node cells from mice immunized with an antigen are grown in its presence on fibroblast monolayers prepared from mouse embryonic skin. Two types of mast cell clones are identified: the first, originates from a precursor present in the lymphoid cell suspension and the second, from a precursor in the fibroblast monolayer. Clones of the first type fail to appear when T cells are eliminated from the suspension; but they grow luxuriantly in the presence of fluid, harvested from cultures containing the antigen-sensitive T cells exposed to the antigen. The two mast cells differ in the clonal size and rate of growth, life span, cell size, shape and size of the granules and numbers of IgE receptors. It is concluded that the rapidly multiplying lymphoid mast cells associate with the mucosae of the respiratory and gastrointestingal tracts and appear in large number in response to immunological stimuli; the long lived mast cells derived from the embryonic skin monolayer are found in the general connective tissue.

INTRODUCTION

Since mast cells were first described by Ehrlich (1877), their origin has been the subject of much debate. Various proposals have been mesenchymal (Asboe-Hansen, 1971), monocyte (Desaga, Parwaresch & Müller-Hermelink, 1971), myeloid (Kitamura, Shimata, Hatanaka & Miyano, 1977) and lymphoid (Ginsburg & Lagunoff, 1967).

Clonal growth of mast cells *in vitro* was demonstrated by Ginsburg & Lagunoff, (1967), Ginsburg (1963), Ginsburg, Nir, Hammel, Eren, Weissman & Naot (1978), in cultures of mouse thymus and lymph nodes, and by Ishizaka, Okudaira, Mauser & Ishizaka (1976), Ishizaka, Adaci, Chang & Ishizaka (1977), in cultured rat thymus. Recently Kitamura, Matsuko & Hatanaka (1979b), induced growth of mast cells in the skin and mesentery of mice of the w/w^v strain, which are congenitally devoid of mast cells, by administration of bone marrow and foetal liver cells. Their results differed from ours, since in Kitamura's study, injection of lymphoid cell suspension failed to induce clonal growth of the mast cells (Kitamura, Go, Shimada, Matsuda, Hatanaka & Seki, 1979a). The present study resolves some of the controversy, by demonstrating that there are in fact two different types of mast cells, the first type—the 'connective' tissue mast cells (CTMC)—proliferating in embryonic skin fibroblast monolayers, and the second—the 'mucosal mast cells' (MMC; Mayrhofer & Fisher, 1979)—originating from precursors in the lymph node and thoracic duct cell suspensions. If T cells are removed, clones of the latter type are totally absent, but appear and grow luxuriantly on addition of the 'T-cell factor' (TCF) to the culture (Ginsburg, Olson, Huff, Okudaira & Ishizaka, 1981).

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MATERIALS AND METHODS

Immunization and tissue culture procedures were as described previously (Ginsburg *et al.*, 1978). Two to four months' old BALB/c mice were immunized by weekly intraperitoneal injections of 0·2 ml of horse serum or ovalbumin (0·1%) for 1 month. Five to twelve days later cell suspensions were prepared, separately, from mesenteric and peripheral lymph nodes.

Embryonic fibroblast monolayers were prepared from (i) skins of 18 days' old embryos and (ii) whole 10–13 days' old embryos, as described previously (Ginsburg *et al.*, 1978; Ginsburg, 1965). Cultures were made by plating 10^7 cells in 100 mm petri dishes in Waymouth's medium plus 10% calf serum. The cultures were passaged by trypsinization, $2–4 \times 10^5$ cells were plated in 35 mm petri dishes in Waymouth's medium plus 10% calf serum. These monolayers were used for plating lymphoid cells. In experiments to examine cell growth in the embyro monolayers in the constant presence of TCF, the cell suspensions obtained from the embryos after trypsin treatment were resuspended in Dulbecco's medium and 15% horse serum. To half of the plates TCF 1:1 was added and 1 ml containing 3×10^5 cells were plated in 16 mm wells (Costar, Cambridge, Mass., catalogue number 3524). The medium was replaced every 4 days with medium containing TCF 1:1. TCF added to the culture was medium harvested from 2 to 8 days' culture of horse serum immune lymph-node cells cultured in the presence of the horse serum.

In order to obtain lymphoid mast cells, 5×10^6 lymph node cells of immunized mice in 2 ml of medium were plated in 35 mm petri dishes containing fibroblast monolayers. TCF 1:1 was added in some cultures. Monolayers devoid of either mast cells or their precursors were selected. This lack of mast cell progenitors could be ascertained after constant incubation of the monolayers with TCF.

T cells were removed from the suspension by incubation with anti-Thy 1 antiserum and guinea-pig complement, and the cell suspension was passed through a Sephadex G10 column (Okudaira & Ishizaka, 1975). The resulting suspension contained 1·5 to 3% Thy 1 positive cells as determined by immunofluorescence staining. Histamine content in the cells and in the culture medium was determined by the automated technique of Siraganian (1974), and the number of clones in a culture by direct count; the total cell number was determined, after trypsin treatment of the culture, from haemocytometer counts, and the percentage of mast cells from a cytocentrifuge preparation made from the same suspension.

The number of macrophages was determined after incubation with carbon particles as follows: the culture was incubated with trypsin solution (1:1 mix with the culture medium). This is in order to allow the carbon particles to reach the macrophages underneath the monolayer. A suspension of carbon particles (india ink) was diluted 1:10 with the culture medium and six drops were added to the plates. After 3 hr incubation the dispersed cells were harvested. In order to detach tightly adhering macrophages which the trypsin failed to remove, sodium azide (100 mM) was added to the plates, and the macrophages were harvested by pipetting after incubation for 1 hr, and added to the general suspension. The carbon particles caused complete blackening of macrophages by filling their cytoplasm, while the mast cells and fibroblasts remained unaffected.

For differential cell count (mast cells, macrophages and fibroblasts) 4/5 total volume of the suspension were mixed with 1/5 volume of fixative solution containing 0·1% toluidine blue, 50% ethanol, 10% formaldehyde and 1% acetic acid (Prouvost-Danon, Silva-Lima & Quiroz Javierre, 1966). Before mixing the cells were resuspended in phosphate-buffered saline (PBS), to prevent precipitation. It was found most important to incubate this mixture for at least 2 hr, to ensure distinctive (red) staining of the mast cell granules.

For microscopic analysis of the cultures the plates were washed with PBS, fixed with absolute methanol, and stained with 0·5% toluidine blue in 50% ethanol or with May-Grunwald Giemsa. When the cultures were allowed to dry after fixation, the macrophage cytoplasm stained deep blue and the mast cells granules deep purple; when staining was applied before the methanol had evaporated, lysosome granules in macrophages were stained red. (These granules were positively stained for acid phosphatase.)

IgE receptors were determined only in the mast cells which did not adhere to the monolayer. In older cultures, or shortly after passaging, there was always some proportion of free-floating mast cells (Ginsburg & Sachs, 1963; Ishizaka *et al.*, 1976) which could be readily harvested as pure suspension (92%–95%). ^{125}I-coupled rat-myeloma IgE was used as described previously (Ishizaka *et al.*, 1976). (In preliminary work we found that binding of rat IgE to mouse mast cells was the same as mouse IgE, and similarly 90% histamine release was obtained by subsequent appli-

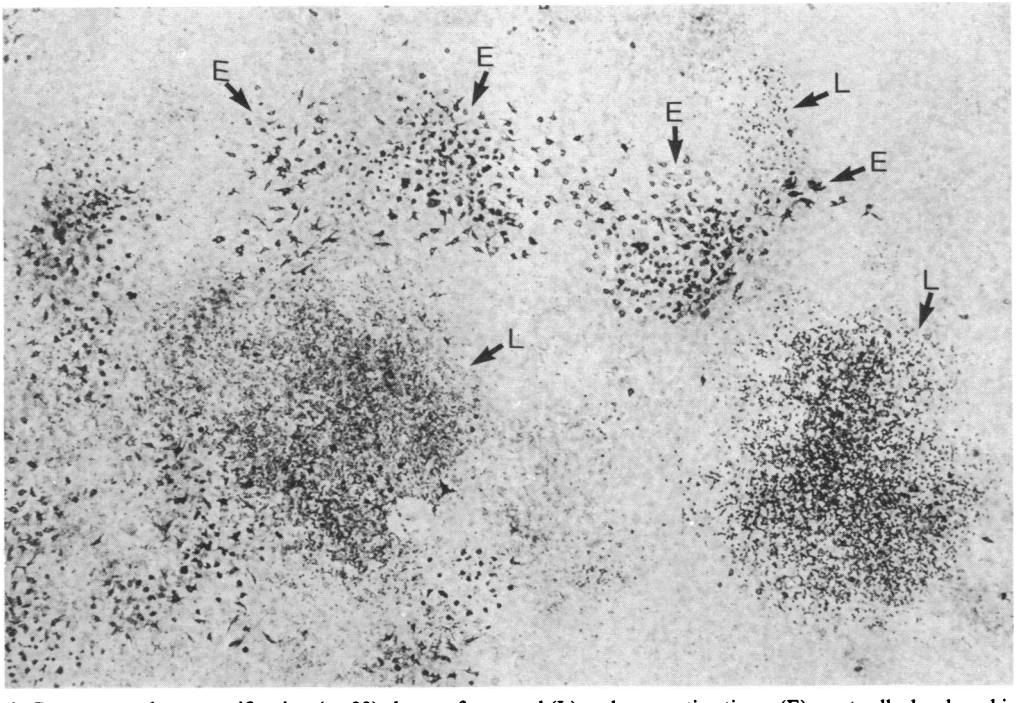

Figure 1. Compares at low magnification (× 33) clones of mucosal (L) and connective tissue (E) mast cells developed in single culture from which T cells were depleted and TCF added at the beginning of the culture. In control cultures maintained without TCF the mucosal mast cell clone were completely absent. Plate was fixed with methanol and stained with 0·5% toluidine blue in 50% ethanol. Mast cells are stained deep red against fibroblast background stained pale blue. Thirteen days' old culture.

cation of goat anti-rat IgE; manuscript in preparation).

RESULTS

Clones of the two mast cell types could develop side by side in the same culture (Fig. 1); they could also be grown separately in different cultures. CTMC developed in some cultures of embryonic skin monolayers maintained with Waymouth medium plus 10% calf serum. Addition of TCF was not required. Pure cultures of MMC were obtained by selecting monolayers that were entirely devoid of mast cells or their precursors. After plating the lymph node cells on such monolayers with TCF in the medium, the appearance of the youngest forms of mast cells, not before 18 hr (submitted for publication) could be followed in the lymphoid cell suspension. Thus, the study has established the lymphoid origin of these types of mast cells.

These cultures enable comparison of the two mast cell entities. A typical clone of MMC is shown in Fig. 2 and its general appearance on monolayers in Fig. 3. Clonal growth of histiocytes occurred in cultures grown without TCF (Figs 6 and 7).

The CTMC increase slowly over a period of 1 to 2 months in cultures and they tend to spread locally after mitosis (Fig. 4). The maximal clone size after 1 month is about 400 cells. The rate of growth and spatial arrangement is identical to that observed by Kitamura *et al.* (1979b), in the mesentery after bone marrow transplantation. In older cultures the cells detach and float freely as pure suspensions or as clumps. Cultures populated with CTMC were kept for at least 6 months without apparent mitosis and without reduction in cell number.

In the lymphoid variant mast cell the kinetics of cell development were observed up to 22 days (Fig. 8). Here peak growth usually occurred at about the eighteenth day and was followed by disintegration; these cultures could not be kept longer than 1 to 2 months at most. It is now evident that the mast cells died off, after the T- and B-cell influence was over, due to lack of TCF in culture. In recent studies it was found

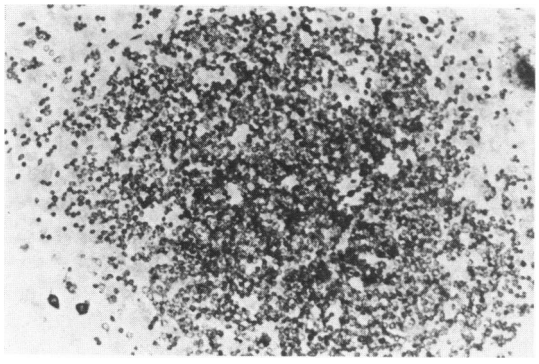

Figure 2. Clone of MMC developed in culture grown in the presence of TCF. Toluidine blue. (Magnification ×82.)

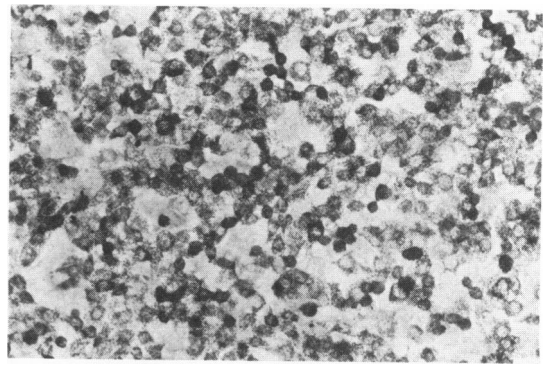

Figure 3. Clone of MMC developed in culture grown in the presence of TCF. Toluidine blue. (Magnification ×206.)

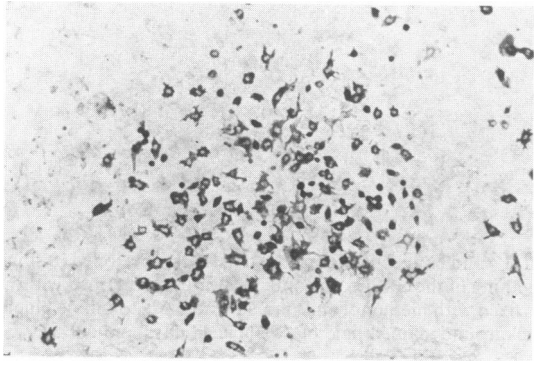

Figure 4. Clone of CTMC developed in skin embryo fibroblast monolayer. Twenty-three days after passage toluidine blue. (Magnification ×82.)

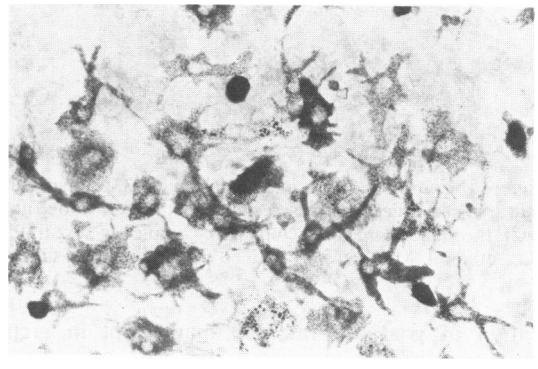

Figure 5. Clone of CTMC developed in skin embryo fibroblast monolayer. Twenty-three days after passage. Toluidine blue. (Magnification ×260.)

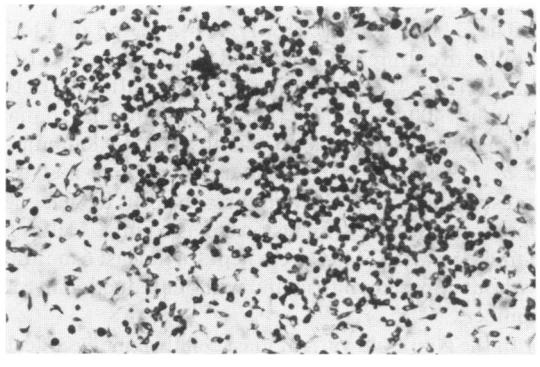

Figure 6. Histiocytes multiplied in T cell-depleted culture maintained without TCF. Culture was fixed at the ninetieth day with methanol and while wet was stained with May-Grunwald giemsa. The cytoplasm turned red due to lysosome granules filling the cytoplasm. When cultures were dried after methanol and then stained (as commonly practised) the cytoplasm turned blue and the granules were invisible. (Magnification ×82.)

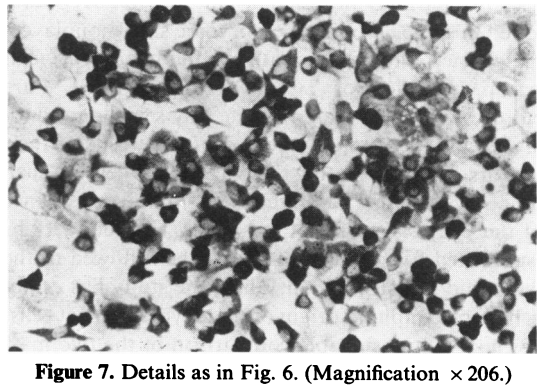

Figure 7. Details as in Fig. 6. (Magnification ×206.)

that the survival and life span of these cells depend on the presence of TCF. MMC clones are much larger and at the thirteenth day in T cell-depleted, with TCF-treated cultures clones were composed of about 2500 to 5000 cells at most (Figs 1 and 2). At later periods of culture the colony arrangement breaks down, the cells disperse, and many of them may float freely. In cultures of MMC processed after 13 days of cultivation, an average number of 63 ± 12 clones was counted, and the total mast cell number was $9{\cdot}18 \times 10^5$ after trypsinization. (Since there are also dispersed or free floating mast cells, the average number in a clone can be estimated only approximately). In Figs 9 and 10, CTMC are seen to be larger, with more abundant cytoplasm completely packed with tiny granules; the MMC are smaller with larger and sparse granules in the cytoplasm (Fig. 9). The granules of these two mast cells are compared with granules of histocytes (Fig. 11).

Histamine content in the mucosal mast cells at peak growth (18 to 22 days) range from 3·92 to 12 μg per 10^6 cells (Table 1); the mean value was about 7 μg. The numbers of IgE receptors on the cells of the two types were determined on pure populations (92%–95%) of free-floating mast cells (Table 1). Surprisingly, the number of receptors in the lymphoid type was found to be six times greater, possibly due to the lymphoid cells having better developed villi or ridges, with the corresponding increase in cell surface for carrying the receptors. Since the mucosal mast cells develop in culture in conditions of immunization, they may be in a state of 'activation', which may account for the variation in both the number of receptors and histamine content. The differences in several parameters of the two mast cell types in culture are summarized in Table 2.

Histiocytes (Figs 6, 7 and 11) developed in the control cultures (i.e. monolayers grown without lymphoid cells, in cultures of lymphoid cells prepared from unimmunized animals, as well as in cultures prepared from immunized animals, and grown in the absence of the antigen). Addition of TCF inhibited macrophage growth (Table 3). The macrophages at maturation in culture reveal extensive lysosome granule formation (Figs 7 and 11).

In May Grunwald Giemsa staining the granules do not show if after methanol fixation the preparations are dried; but they stain red if the cultures are stained immediately after fixation while wet (Figs 6, 7 and 11) or if they are dried in air before staining. The macrophage engulfed carbon particles, whereas mast cells and fibroblast did not.

We have found that the clonal development of the MMC, but not the CTMC, ultimately depends on TCF. Thus TCF may exert an effect on mast cells in the embryonic monolayers. In order to study this

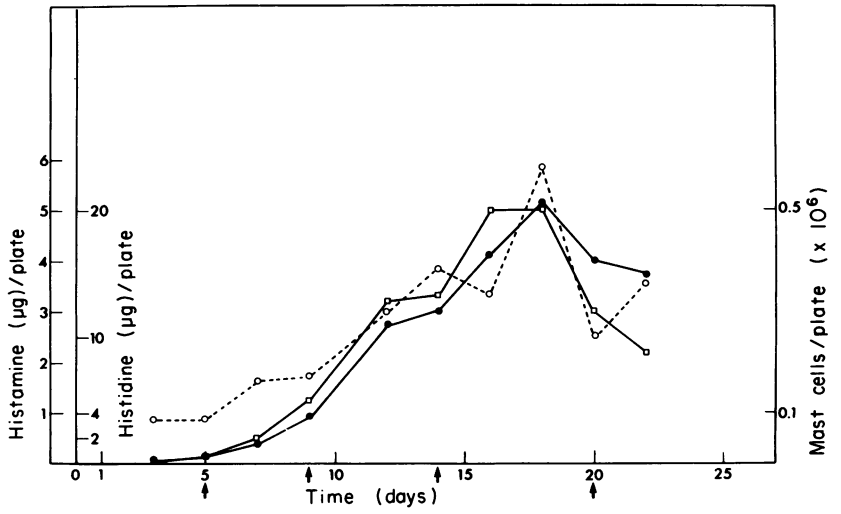

Figure 8. Kinetics of MMC growth (□——□) histamine content in cells (●——●) and histidine content in cells (o- - -o) during a 22 day period of incubation of culture of lymph node cells from immune mice grown on whole embryo fibroblast monolayer. Arrows indicate medium change.

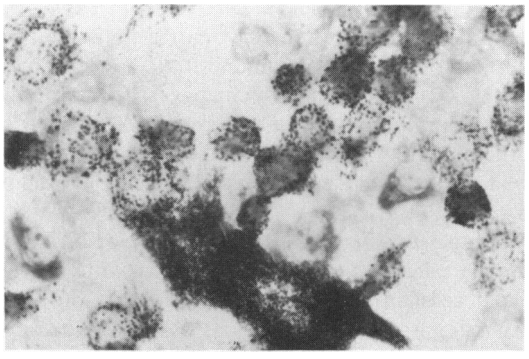

Figure 9. The fine granules at high magnification (× 825) of MMC and CTMC are compared. Two CTMC with cytoplasm loaded with delicate granules are seen at the bottom of the photograph.

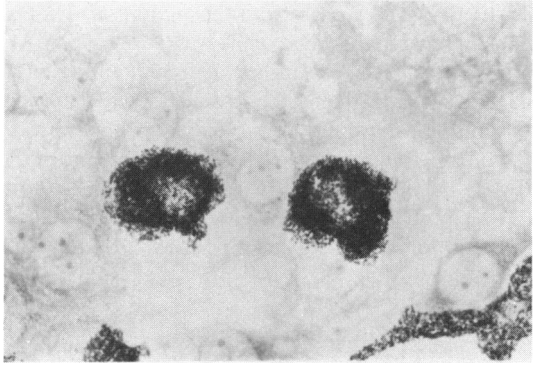

Figure 10. Two CTMC mast cells are shown against the background of the fibroblasts. (Magnification × 825.)

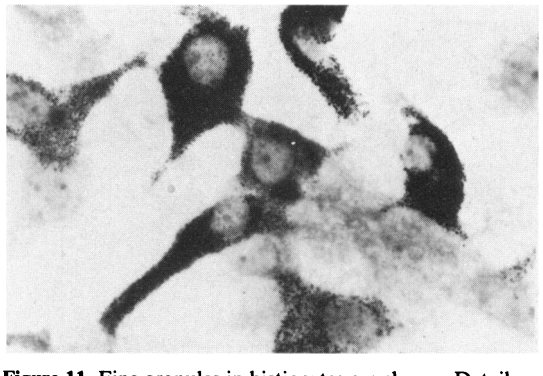

Figure 11. Fine granules in histiocytes are shown. Details as in Fig. 6. (Magnification × 825.)

possibility TCF was added to monolayers at establishment of the culture and constantly thereafter.

Cultures were prepared from two sources (i) from very young whole embryos 10–12 days' old with the intention of obtaining monolayers devoid of mast cell precursors, and (ii) from skins of mature 18–19 days' old embryos. Results with whole young embryo monolayers are shown in Table 3. Surprisingly, substantial mast cell growth of the mucosal mast cell type occurred only in culture maintained with TCF. In one set (Table 3), substantial macrophage growth occurred in culture grown without TCF but failed to grow in the presence of TCF. The effect of TCF on the fibroblasts produced conflicting results. In one culture the number of fibroblasts was about three times greater and in the second the fibroblast population (as well as macrophage) was greater in cultures maintained without TCF. Since we use crude medium as TCF, multiple factors may exert their effect.

In cultures prepared from skin, CTMC were identified but did not increase despite the presence of TCF. In three different experiments with skin monolayers, TCF failed to stimulate CTMC.

DISCUSSION

As early as 1906, Maximow noted that mast cells residing in the mucosa of the rat intestine differ from other mast cells both morphologically and in staining properties, and accordingly termed them 'atypical'. They were later studied extensively by Enerback (1966a, b) who observed that they are smaller, require different fixation for demonstration of the granules and, unlike their connective tissue counterparts, are negative for alkaline phosphatase staining. They stain blue with astra blue-safranin at pH3, whereas those of the dermis stain partly blue and partly red (Enerback, 1966a), probably due to a difference in the degree of sulphation of the heparin and heparan. Similar or identical cells are also known as 'globule leucocytes'; they were first described by Weill (1920), who found them inside the epithelial layer of the intestinal crypts. It is evident from our study that precursors of MMC are present in young embryos as well, but it is unknown whether these precursors are associated with lymphoid primordial tissue. In contrast to MMC, no conclusive results could be obtained so far as to the effect of TCF on the CTMC, fibroblasts and macrophages. Only purified preparations of TCF will permit

Table 1. Number of IgE-receptors, degranulation capacity and histamine content in two independent types of mast cells

Mast cell type	Days in culture	IgE* receptors (*n*)	% Histamine release† (%)	Histamine content (μg per 10^6 mast cells)
CTMC	35	$3{\cdot}1 \times 10^4$	$89{\cdot}9 \pm 2{\cdot}1$	6·6
	63	$3{\cdot}2 \times 10^4$	—	5·9
MMC	30	$1{\cdot}83 \times 10^5$	$74{\cdot}2 \pm 3{\cdot}2$	3·92‡
	52	$2{\cdot}05 \times 10^5$	—	—

* Determination with ^{125}I-rat myeloma IgE as previously described by Ishizaka *et al.* (1976).

† Incubation of 10 μg of rat myeloma IgE in 1 ml of culture medium for 3 hr then goat anti-rat IgE diluted 1:3000.

‡ In many cultures analysed so far histamine content fluctuated and reached maximal of 12 μg per 10^6 cells with average of 7 μg.

Table 2. Comparison of types of mast cells grown in cultures

Traits	Connective tissue mast cells (CTMC)	Mucosal mast cells (MMC)
Origin in culture	Skin monolayers	Lymphoid suspension
Clone size	Small (maximum 400 cells)	Large (2500–5000 cells)
Rate of clone growth	Slow	Fast
Shape of clone	Diffuse	Denser
Size of cells	Large	Small
Metachromatic granules	Tiny homogenous, densely fill the cytoplasm	Coarsely larger sparse in cytoplasm
Histamine content	6·4 mg/10^6 mast cells	4–12 μg/10^6 mast cells
IgE receptors	3×10^4 per cell	2×10^5 per cell
Degranulation by IgE*	90% histamine release	50%–90% histamine release
Life span	>6 months	<2 months†

* Rat monoclonal IgE plus goat anti-rat IgE or mouse anti-ovalbumin IgE plus ovalbumin.

† Observation based on cultures maintained without TCF. Current study indicates survival and the life span depend on the presence of TCF.

Table 3. Development of mast cells in whole embryo cultures grown constantly with TCF*

Culture	Days in culture	Histamine content (μg/well)		Mast cells per well ($\times 10^3$)		Macrophages per well ($\times 10^3$)				Total cells per well ($\times 10^3$)	
		+TCF‡	−TCF	+TCF	%	−TCF	+TCF	−TCF	%	+TCF	−TCF
a†	20	$0{\cdot}21 \pm 0{\cdot}04$	0	$39{\cdot}8 \pm 8{\cdot}0$	13·3	<1	0	0	—	298± 20	133 ± 31
	29	$0{\cdot}87 \pm 0{\cdot}10$	0	$145{\cdot}7 \pm 38$	24·1	<1	0	0	—	605 ± 110	176 ± 63
b	34	$0{\cdot}09 \pm 0{\cdot}15$	0	$14{\cdot}8 \pm 0{\cdot}2$	10·3	<1	$0{\cdot}4 \pm 0{\cdot}1$	$106{\cdot}2 \pm 12{\cdot}5$	22·5	143± 12	473 ± 44

* Cells obtained after trypsinization of whole embryos were plated in 17 mm wells, 3×10^5 cells in 1 ml medium per well, 8 days later cultures were passaged by plating 10^5 per well.

† a, 11 days' old embryos. b 13 days' old embryos.

‡ TCF, 8 day old medium from horse-serum immune cultures of mouse mesenteric lymph nodes. Mixed 1:1 with fresh medium.

differentiation of effects probably produced by other factors present in the crude culture medium.

Involvement of the mucosal mast cells in helminth infection is a well-established finding (Ruitenberg & Elgersma, 1976; Nawa & Miller, 1979; Befus & Bienenstock, 1979; Olson & Levy, 1976).

In 1967 Ginsburg and Lagunoff showed that when thoracic duct or lymph node cells from immunized mice are grown on fibroblast monolayers in the presence of the antigen, extensive mast cell differentiation occurs, resulting in development of pure populations. In control cultures from unimmunized mice practically pure population of histiocytes developed. This was not explained when first observed, but recent studies of Mayrhofer (1979); Mayrhofer & Fisher (1979); Nawa & Miller (1979), and Befus & Bienenstock (1979), permitted correlation of the *in vitro* and *in vivo* findings and showed a unique cellular system especially adapted to react to helminth and other infections in the gastrointestinal and respiratory tracts (Capron, Rousseaux, Mazingue, Basin & Capron, 1978). The mucosal mast cells in helminth infection derived from precursors in the lymph and the thoracic duct. Like Guy-Grand, Griscelli & Vassali (1978), we found that the mesenteric lymph node is a major source of mast cell precursors, since it has produced, so far, the best mast cell cultures (unpublished data). Miller, (1971) and Murray, Miller & Jarrett (1968), showed that though these mast cells are lymphoid in origin, their proliferation in response to infection takes place in the mucosa. In addition to the lamina propria, they increase in numbers in the epithelium. Ruitenberg & Elgersma (1979) considered the epithelial globule leucocytes as a line of independent mucosal mast cells found in the lamina propria. On the other hand, Murray *et al* (1968) and Miller (1971) maintained that the first derive from the second. Our own examination of histological preparations of the epithelial globule leukocytes in *Nippostrongylus brasiliensis* infection showed that these cells were very similar to the postdegranulation *in vitro* mast cells, which survive degranulation (Ginsburg, Ben-Shahar, Hammel & Ben-David, 1979) and have few granules, some of which are seen as metachromatic clumps (Ginsburg *et al.*, 1978) taking about 5 days to disappear completely. The globule leucocytes are thus most probably cells that had at some time earlier (a few hours to 2 days, by extrapolation from *in vitro* observations) undergone extensive degranulation. This is all the more likely, seeing that IgE, IgG1 and active complement anaphylatoxins are readily available at the site of infection, where the mast cell is exposed to the corresponding antigens. Mayrhofer, Basin & Gowans, 1976, noted that such mast cells, in contrast to their general connective tissue counterparts, contain internal IgE; a possible explanation is that the clumps in postdegranulation mast cells are virtually cavities housing the metachromasia (Lagunoff, 1972).

Another relevant observation in culture is the effect of degranulation on other cells in the monolayer; immediately after degranulation the cytoplasm of the fibroblasts 'opens' numerous 'windows'; after 2–4 hours the fibroblasts stretch back and the monolayer resumes its former texture (Ginsburg *et al.*, 1978, 1979). This 'fenestration' suggests a similar effect in the cells in the mucosa, with the dislocation and 'fenestration' permitting smooth flow of fluids and cells from the interior into the extraneous lumen.

A finding essential for understanding the overall mechanism of the lymphoid mast cells in the defense against infection is the ultimate dependence of clonal growth on the factor released from antigen-sensitive T cells (Ginsburg *et al.*, 1981). Ruitenberg & Elgersma (1976), and later Mayrhofer & Fisher (1979) showed that the response of the mucosal mast cells to helminth infection is thymus-dependent; the last named authors, however, were unable to determine whether this dependence is due to the T cell as a precursor, as suggested by Guy-Grand *et al.* (1978) or to the TCF. Our own *in vitro* study indicates that the mast cell proliferation depends on the latter, and it could replace the activity of the T cells removed by anti-Thy 1 and complement. These results do not exclude the possibility that the immediate precursor derived from a T cell. Our recent study has shown that young mast cells are absent in the suspensions before plating and that TCF induce 'large lymphocytes' into mast cells differentiation course. The possibility then exists that the immediate precursor which carries receptors for TCF derives from T cells carrying the Thy 1 marker as was shown by Guy Grand *et al.* (1978). This possibility is given strong support from our finding that in culture of lymph node cells of athymic nude mice in the presence of TCF mast cells were totally absent.

Attention should also be drawn to the analogous dependence of IgE synthesis on T cells and to the steep increase in IgE-forming cells and in the IgE level in helminth infection (Ishizaka & Ishizaka, 1978). It could be inferred from data accumulated so far that the initial response of the body to such infection consists of induction of development of antigen-sensitive helper T-cell populations and, through the

latter, the presence of the antigen is a signal for the mast cell and plasma cell lines (otherwise dormant) to proliferate. The large population of mast cells observed after carcinogen application to the skin (Fiore-Donati, DeBenedictis & Chieco-Bianchi, 1962; Chieco-Bianchi & Fiore-Donati, 1963) seems also to be of the lymphoid type. This accumulation is still unexplained and it will be necessary to find out whether it is thymus-dependent.

Our studies *in vitro*, together with those on helminth infection (Ruitenberg & Elgersma, 1979; Nawa & Miller, 1979, Befus & Binenstock, 1979, Olson & Levy, 1976) on the one hand and the work of Kitamura *et al.* (1979a, b) on the origin of mast cells on the other, establish the notion of two independent entities. The connective-tissue mast cells derive from foetal liver and from the bone marrow, and the mucosal mast cells from lymphoid tissue. The differences between the cells reflect adaptation to function in different environments.

Finally, it remains to comment on the relevance of the present findings to allergic situations, in particular, asthma and food allergy, bearing in mind possible involvement of the mucosal mast cells, the hypothesis being that in acute gastrointestinal or bronchial allergy the allergens are the counterpart of helminth infection inducing proliferation of both the IgE-forming cells and MMC; if this proves to be the case, one would expect to find increased numbers of mast cells in the mucosa in the epithelium in such individuals.

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REFERENCES

ASBOE-HANSEN G. (1971) Mast cells and the skin. In: *The Skin* (Ed. by E. B. Helwig and F. K. Mostofi), p. 83. Williams & Wilkins, Baltimore.

BEFUS A.D. & BIENENSTOCK J. (1979) Immunogically mediated intestinal mastocytosis in Nippostrongylus brasiliensis-infected rats. *Immunology*, **38**, 95.

CAPRON M., ROSSEAUX J., MAZINGUE C., BASIN H. & CAPRON A. (1978) Rat mast cell–eosinophil interaction in antibody-dependent eosinophil cytotoxicity to *Schistosoma mansoni* schistosomula. *J. Immunol.* **121**, 2518.

CHIECO-BIANCHI L. & FIORE-DONATI L. (1963) Mast cell reaction and 5-hydroxytryptamine content in skin of syrian hamsters painted with 9:10-dimethyl-1:2-benzanthracene. *Nature (Lond.)*, **199**, 293.

DESAGA J.F., PARWARESCH M.R. & MÜLLER-HERMELINK H.K. (1971) Die Zytochemische Identifikation der Mastzellvorstuffen bei der Ratte. *Z. Zellforsch*, **121**, 292.

EHRLICH P. (1877) Beiträge zur Kenntnis der Anilinfärbung und ihrer Verwendung in der Mikroskopischen Technik. *Arch. Mikros. Anat.* **13**, 263.

ENERBACK L. (1966a) Mast cells in rat gastro intestinal mucosa. *Acta pathol. microbiol. scand.* **66**, 303.

ENERBACK L. (1966b) Mast cells in rat gastro intestinal mucosa. *Acta pathol. microbiol. scand.* **67**, 365.

FIORE-DONATI L., DEBENEDICTIS G. & CHIECO-BIANCHI L. (1962) Development of mast cells reaction during chemical skin carcinogenesis of mouse. *Nature (Lond.)*, **193**, 287.

GINSBURG H. (1963) The *in vitro* differentiation and culture of normal mast cells from the mouse thymus. *Ann. N.Y. Acad. Sci.* **130**, 20.

GINSBURG H. (1965) Growth and differentiation of cells of lymphoid origin on embryo cell monolayer. In: *Methodological approaches to the study of leukemias Philadelphia* (Ed. by V. Defendi) Wistar Institute Press Symposium Monograph No. 4, pp. 21–49.

GINSBURG H., BEN-SHAHAR D., HAMMEL I. & BEN-DAVID E. (1979) Degranulation capacity of mast cells grown in cell culture in the presence of histamine releaser, *Nature (Lond.)*, **280**, 151.

GINSBURG H. & LAGUNOFF D. (1967) The *in vitro* differentiation of mast cells. Culture of cells from immunized mouse lymph nodes and thoracic duct lymph on fibroblast monolayers. *J. Cell Biol.* **35**, 685.

GINSBURG H., NIR I., HAMMEL I., EREN R., WEISSMAN B.-A. & NAOT Y. (1978) Differentiation and activity of mast cells following immunization in cultures of lymph-node cells. *Immunology*, **35**, 485.

GINSBURG H., OLSON E.C., HUFF T., OKUDAIRA H. & ISHIZAKA T. (1981) Enhancement of mast cell differentiation *in vitro* by T-cell factor(s). *Int. Arch. Allergy appl. Immunl.* (In press.)

GINSBURG H. & SACHS L. (1963) Formation of pure suspension of mast cells in tissue culture by differentiation of lymphoid cells from mouse thymus. *J. natn. Cancer Inst.* **31**, 1.

GUY-GRAND D., GRISCELLI C. & VASSALI P. (1978) The mouse gut T-lymphocyte, A novel type of T-cell. *J. exp. Med.* **148**, 1661.

ISHIZAKA T., ADACI T., CHANG T. & ISHIZAKA K. (1977) Development of mast cells *in vitro*. II. Biologic function of cultured mast cells. *J. Immunol.* **118**, 211.

ISHIZAKA K. & ISHIZAKA T. (1978) Mechanisms of reaginic hypersensitivity and IgE antibody response. *Immunol. Rev.* **41**, 109.

ISHIZAKA T., OKUDIRA H., MAUSER L.E. & ISHIZAKA K. (1976) Development of rat mast cells *in vitro*. I. Differentiation of mast cells from thymus cultures. *J. Immunol.* **116**, 747.

KITAMURA Y., GO S., SHIMADA M., MATSUDA H., HATANAKA K. & SEKI M. (1979a) Distribution of mast cell precursors in hematopoietic and lymphopoietic tissues of mice. *J. exp. Med.* **150**, 482.

KITAMURA Y., MATSUDA H. & HATANAKA K. (1979b) Clonal nature of mast-cell clusters formed in w/w^v mice after bone marrow transplantation. *Nature (Lond.)*, **281**, 154.

KITAMURA Y., SHIMADA M., HATANAKA K. & MIYANO Y. (1977) Development of mast cells from grafted bone marrow cells in irradiated mice. *Nature (Lond.)*, **268**, 442.

LAGUNOFF D. (1972) Contributions of electron microscope to the study of mast cells. *J. invest. Dermatol.* **58**, 296.

MAXIMOW A. (1906) Uber die zellformen des lockeren Bindegewebes. *Arch. f. Micr. Anat.* **67**, 680.

MAYROHOFER G. (1979) The nature of the thymus dependency of mucosal mast cells. *Cell. Immunol.* **47**, 312.

MAYRHOFER G., BASIN H. & GOWANS J.L. (1976) Nature of cell binding anti IgE in rats immunized with *Nippostrongylus brasiliensis*. IgE synthesis in regional nodes and concentration in the mucosal mast cells. *Europ. J. Immunol.* **6**, 537.

MAYRHOFER G. & FISHER R. (1979) Mast cells in severely T-cell depleted rats and the response to infestation with *Nippostrongylus brasiliensis*. *Immunology*, **37**, 145.

MILLER H.R.P. (1971) Immune reactions in mucous membranes. III. The discharge of intestinal mast cells during helminth expulsion in the rat. *Lab. Invest.* **24**, 348.

MURRAY M., MILLER H.R.P. & JARRETT W.E.H. (1968) The globule leukocyte and its derivation from subepithelial mast cell. *Lab. Invest.* **19**, 222.

NAWA Y. & MILLER H.R.P. (1979) Adoptive transfer of the intestinal mast cell response in rats infected with *Nippostrongylus brasiliensis*. *Cell. Immunol.* **42**, 225.

OKUDAIRA H. & ISHIZAKA K. (1975) Reaginic antibody formation in the mouse V. Adoptive anti-hapten IgE antibody response of dinitrophenyl heterologous carrier conjugates. *J. Immunol.* **114**, 615.

OLSON C.E. & LEVY D.A. (1976) Thymus-dependency of the mast cell response to *Nippostrongylus brasiliensis* in mice. *Fed. Prof.* **35**, 491.

PROUVOST-DANON A., SILVA-LIMA M. & QUIROZ JAVIERRE M. (1966) Active anaphylactic reaction in mouse peritoneal mast cells *in vitro*. *Life Sci.* **5**, 289.

RUITENBERG E.J. & ELGERSMA A. (1976) Absence of intestinal mast cell response in congenially athymic mice during Trichinella spiralis infection. *Nature (Lond.)*, **264**, 258.

RUITENBERG E.J. & ELGERSMA A. (1979) Response of intestinal globule leucocytes in the mouse during a *Tricinella spiralis* infection and its independence of intestinal mast cells. *Brit. J. exp. Pathol.* **60**, 246.

SIRAGANIAN R.P. (1974) An automated continuous-flow system for the extraction and fluorimetric analysis of histamine. *Anal. Biochem.* **57**, 383–394.

WEILL P. (1920) Etudes sur les leucocytes. I. Les cellules granuleuses des muqueses intestinal et uterine. *Arch. Anat. Micros. Morph. Exp.* **17**, 77.