Brief Definitive Report

STIMULATION OF MAST CELL CHEMOTAXIS BY INTERLEUKIN 3

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Connective tissue mast cells (CTMC) reside near to blood vessels in connective tissues and are found also in the peritoneal cavity in many species (1). Once thought to be terminally differentiated cells, CTMC are now known to proliferate in response to activated spleen cell-conditioned medium (SCCM) or to a mixture of IL-3 and IL-4 (2, 3). CTMC, which contain large quantities of heparin and histamine, are distinguished from mucosal mast cells (MMC), which are found in the digestive tract, contain little histamine, and have granules that contain chondroitin sulfate E as opposed to heparin proteoglycan. A third related cell type, the circulating basophil, shares some properties with mucosal mast cells but is not considered identical to either MMC or CTMC (1). Although, basophils and basophilic leukemia cells have been shown to migrate toward specific stimuli such as IFN (4), IgE (5), and to undefined factors released from activated lymphocytes (6), little is known concerning the regulation of cell migration in connective tissue mast cells. In the current study, we have tested SCCM as well as purified lymphokines known to stimulate mast cell proliferation for their effects on CTMC migration.

Materials and Methods

Reagents. Recombinant and purified natural murine IL-2, IL-3, and IL-4 and granulocyte-macrophage CSF (GM-CSF) were obtained from Genzyme, Boston, MA. 1 U of IL-2 activity is defined as the amount required to support half-maximal [^{3}H]TdR incorporation by CTLL-2 cells. 1 U of IL-3 activity is described as the amount required to stimulate half-maximal activity of FDC-P2 cells as described by Prestidge et al. (7). GM-CSF activity was established using FDC-P21D cells by the same method. IL-4 activity was defined on the basis of murine B cell proliferation assays according to the method of Grabstein et al. (8). Glycyl-histidyl-lysine was from Calbiochem-Behring Corp. (San Diego, CA), and *N*-formyl-methionyl-leucyl-phenylalanine and endotoxin (*Escherichia coli* LPS) was from Sigma Chemical Co. (St. Louis, MO). PWM (Gibco Laboratories, Grand Island, NY)-stimulated SCCM was prepared according to the method of Nakahata and Ogawa (9). Monoclonal rat anti-mouse IL-3 (IgG2a; 10) was generously provided by Dr. John Abrams, DNAX Research Intitute of Molecular and Cellular Biology, Palo Alto, CA. To neutralize IL-3 activity, the antibody was diluted 1:20 and then incubated with aliquots of IL-3 preparations or spleen cell-conditioned medium for 1 h at 37°C immediately before testing in the chemotaxis assay.

Cells. CTMC were isolated from peritoneal lavages of C57BL/6 mice by centrifugation on metrizamide gradients as described by Hamguchi et al. (2). Purity of the mast cell population was >95% in all cases. The cells were washed and suspended in serum-free Opti-MEM-I

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medium (Gibco Laboratories) containing 1.0% BSA (Boehringer Mannheim Biochemicals, Indianapolis, IN). Peritoneal macrophages were activated in vivo with thioglycollate or proteose peptone 3 d before isolation from peritoneal cavities (11). Cells that were adherent to uncoated plastic culture dishes 1 h after isolation were collected by gentle scraping with a rubber spatula, washed with Opti-MEM-I medium, and used immediately in migration assays.

Migration Assay. Chemoattraction was evaluated using a modified Boyden chamber assay (12) in a 48-well chemotaxis chamber (Neuro Probe, Inc., Cabin John, MD). The agents to be tested were diluted and added to the lower wells of the chamber. The wells were then covered with two polycarbonate membranes, a lower 5-μm porosity membrane and an upper 12-μm membrane that was precoated for 30 min at room temperature in 15 ml of PBS containing 100 μg laminin (Advanced Biotechnologies Inc., Silver Spring, MD) to promote mast cell adhesion to the filter. 5,000 cells in 50 μl of medium were placed in the upper wells of the chamber, and the entire apparatus was incubated at 37°C in a 5% CO_2 environment. After 3 h, cells adhering to the upper surface of the filter were removed by scraping with a rubber blade. Migrating cells that were adherent to the lower side of the 12-μm filter and cells that had passed through the upper filter and were caught on the lower 5-μm filter were incubated overnight in Carnoy's fixative and subsequently stained for 2 min at 25°C in 0.1% toluidine blue (Polysciences, Inc., Warrington, PA). The filters were then mounted between two glass slides in 90% glycerin, and the number of mast cells per well that had migrated across the filter was determined by counting the mast cells remaining on each filter.

Results and Discussion

Because PWM-stimulated spleen cells produce factors that promote mast cell proliferation, we investigated whether SCCM also contained mast cell chemoattractant activity. Our results reveal that SCCM stimulates mast cell migration with half-maximal activity at 20% (vol/vol) (Fig. 1). Only cells with toluidine blue–staining granules that had the morphological characteristics of mast cells were scored as positive in the migration assay. Greater than 90% of the mast cells that migrated across the filters remained viable as determined by the ability to exclude trypan blue. Visible signs of mast cell degranulation were not apparent at the end of the 3-h assay.

IL-3 and IL-4 are known to be produced by activated spleen cells. We therefore tested these agents for their ability to stimulate CTMC migration. As shown in Fig. 1 *B*, murine IL-3 stimulates CTMC migration with half-maximal activity at a concentration of 8 U/ml. IL-3 elicits a dose-dependent sigmoidal response characteristic of cellular chemotaxis in which increasing the concentration beyond that producing maximal activity results in a decrease in cell motility. IL-4, in contrast, does not stimulate mast cell migration (Fig. 1 *B*).

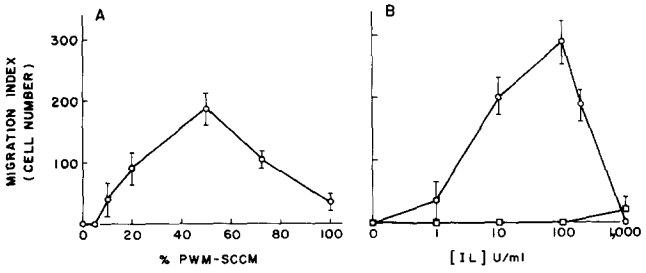

FIGURE 1. Mast cell migration in response to serum-free PWM-SCCM and IL-3 and IL-4. Chemotactic response was defined as the number of cells that traversed the filter in response to a test substance in the lower well, minus the number of cells migrating in response to control medium in both wells. In this experiment, the subtracted background value was 42 cells. Data points are expressed as the mean +/− SEM; n = 3. (*A*) O, SCCM. (*B*) O, IL-3; □, IL-4.

Because mixtures of IL-3 and IL-4 have been shown to produce a synergistic stimulation of CTMC proliferation, we tested the ability of such mixtures to promote CTMC migration. In these experiments, increasing concentrations of IL-4 were added to wells containing limiting concentrations (10 U/ml) of IL-3. As shown in Table I, IL-4 was unable to potentiate the chemoattractant activity of IL-3.

In addition to IL-4, neither recombinant murine IL-2, nor murine GM-CSF had significant mast cell chemoattractant activity (Table I). Both native and recombinant IL-3, however, were active. All of the chemoattractant activity of both natural and recombinant IL-3 preparations was inhibited by a neutralizing antibody (10) to murine IL-3. This antibody also blocked >90% of the activity of the spleen cell-conditioned medium, suggesting that the majority of the mast cell chemoattractant activity produced by activated spleen cells is directly due to the action of IL-3. The antibodies to IL-3 did not cause a nonspecific inhibition of cell motility because they failed to block mast cell migration to Gly-His-Lys, a previously described mast cell chemoattractant (13). The chemotactic activity was not due to contaminating endotoxin as neither endotoxin nor boiled IL-3 were active.

TABLE I

Stimulation of Connective Tissue Mast Cell Migration

Treatment	Chemotactic index*
nm-IL-3	
1 U/ml	37 ± 30
10 U/ml	194 ± 32
100 U/ml	288 ± 35
nm-IL-3 + anti-rm-IL-3	
1 U/ml	16 ± 12
10 U/ml	5 ± 5
100 U/ml	2 ± 1
rm-IL-3	
1 ng/ml	10 ± 9
10 ng/ml	62 ± 16
100 ng/ml	109 ± 14
rm-IL-3 (boiled)	
1 ng/ml	0
10 ng/ml	0
100 ng/ml	1 ± 1
rm-IL-3 + anti-rm-IL-3	
1 ng/ml	10 ± 6
10 ng/ml	0
100 ng/ml	0
rm-IL-4	
1 U/ml	0
10 U/ml	5 ± 6
100 U/ml	3 ± 2
rm-IL-3 (10 ng/ml)	62 ± 16
rm-IL-3 + 1 U/ml IL-4	46 ± 12
rm-IL-3 + 10 U/ml IL-4	66 ± 14
rm-IL-3 + 100 U/ml IL-4	72 ± 29

(*continued*)

TABLE I (*continued*)

Treatment	Chemotactic index*
Spleen cell CM	
10%	42 ± 29
20%	92 ± 14
50%	179 ± 26
Spleen cell CM + anti-rm-IL-3	
10%	12 ± 9
20%	21 ± 11
50%	13 ± 9
Gly-His-Lys	
50 μg/ml	6 ± 11
100 μg/ml	54 ± 14
200 μg/ml	18 ± 5
Gly-His-Lys + anti-rm-IL-3	
50 μg/ml	10 ± 10
100 μg/ml	62 ± 8
200 μg/ml	32 ± 14
rm-IL-2	
1 U/ml	1 ± 1
10 U/ml	3 ± 3
100 U/ml	0
rm-GM-CSF	
1 U/ml	1 ± 2
10 U/ml	8 ± 7
100 U/ml	8 ± 10
Endotoxin	
1 ng/ml	7 ± 7
10 ng/ml	0
100 ng/ml	0

* Chemotactic index is the number of cells that migrate across the filter in response to an agent placed in the bottom well of the chemotactic chamber, minus the number of cells that cross the filter in the absence of any chemotactic agent. This background is due to spontaneous random cell migration and varies from 40 to 100 cells/well. Data are expressed as mean number of migrating cells over background (± SEM; $n = 3$). nm, natural murine; rm, recombinant murine.

To determine whether the ability to respond to the chemotactic activity of IL-3 was a general property of leukocytes, murine macrophages were isolated from mouse peritoneal cavities and tested in the multiwell chemotaxis assay. These cells migrated in response to the bacterial chemoattractant *N*-formyl-methionyl-leucyl-phenylalanine (f-MLF), but failed to respond to IL-3 (Table II). This result demonstrates that the chemoattractant response to IL-3 shows specificity and is not shared by all cell types.

Stimulated cell migration may be either directional (chemotaxis) or random (chemokinesis). These can be distinguished by varying the concentrations of chemoattractant on each side of the filter in the assay chamber, as originally described by Zigmond and Hirsch (14). Results of this type of "checkerboard" analysis, shown in Table III, demonstrate that IL-3 primarily stimulates CTMC chemotaxis with a minor chemokinetic component.

TABLE II

Cellular Responsiveness to IL-3

	Cell type	
	CTMC	PM
rm-IL-3		
1 U/ml	10 ± 9	2 ± 2
10 U/ml	62 ± 16	1 ± 1
100 U/ml	109 ± 14	7 ± 3
fMLF		
10^{-8}	0	65 ± 4
10^{-7}	0	86 ± 7
10^{-6}	4 ± 3	39 ± 7

CTMC and peritoneal macrophages (PM) were isolated from mouse peritoneal cavities as described in Materials and Methods. Migration assays were performed as described in Materials and Methods, except that, for PM migration, a non-coated 8-μ filter was used in place of the 12-μ upper filter used in the mast cell assay. Subtracted background values for unstimulated migration were 42 for CTMC and 30 for PM.

TABLE III

Checkerboard Analysis of IL-3-induced Mast Cell Migration

IL-3 in	IL-3 (ng/ml) in upper well			
lower well	0	1	10	100
ng/ml				
0	33 ± 9	33 ± 17	33 ± 6	40 ± 2
1	33 ± 8	74 ± 7	69 ± 5	60 ± 19
10	78 ± 18	87 ± 35	88 ± 7	70 ± 19
100	134 ± 26	95 ± 36	68 ± 18	85 ± 17

Checkerboard analysis of CTMC migration. The indicated concentrations of rm-IL-3 were added to the upper and lower wells of the multiwell chemotactic chamber. Cell migration was measured as described in Materials and Methods.

Our current results demonstrate that IL-3 released by spleen cells can induce CTMC chemotaxis. This activity may, in part, account for the increase in cell number associated with certain immune reactions. The recent observation that activated mast cells can be induced to produce IL-3 (15) further suggests that mast cells may be capable of autoregulation of both growth and motility by altering the environmental concentration of this essential lymphokine.

Summary

PWM-activated spleen cell-conditoned medium (SCCM) and a variety of purified hematopoietic growth factors were tested for their ability to stimulate chemotaxis of mouse connective tissue mast cells (CTMC). Of the agents tested, only IL-3 and SCCM promoted mast cell chemotaxis. Neither IL-2, IL-4, GM-CSF, nor endotoxin had any significant mast cell chemotactic activity. Neutralizing antibodies to mouse IL-3 blocked >90% of the chemotactic activity of SCCM, suggesting that IL-3 is the predominant mast cell chemotactic factor produced by activated spleen cells. Our results demonstrate that mature connective tissue type mast cells are capable

of moving toward a gradient of spleen cell–derived IL-3 and suggest that movement of mature mast cells toward lymphokines may influence the accumulation of mast cells at sites of inflammatory or immune reactions.

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