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In vitro and *in vivo* migratory response of connective tissue mast cells to inflammatory mediators

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Introduction

Connective tissue mast cells (CTMC) have been shown to increase during various pathological processes such as in and around neoplastic proliferations or at sites of inflammatory reactions. Their precursors derive from bone marrow and from blood and peritoneal mononuclear phagocytes [1–3]. *In vitro* maturation occurs under the influence of growth factors that derive from lymphocytes and fibroblasts [2–4]. It has so far not been clarified whether mature CTMC are able to migrate into tissue or whether their precursors move there to develop into mature cells under the influence of growth factors. In the present investigation, we have therefore studied the *in vitro* migratory ability of rat peritoneal exudate cells (RPC = 5–12% CTMC, 2–5% lymphocytes, 80–90% macrophages). Mast-cell depleted peritoneal macrophages (MCD = > 95% pure macrophages) were used for comparison. Furthermore, the effect of subcutaneously injected chemotactic agents, growth factors and ascaris antigen was studied in tissue sections in order to identify factors that cause CTMC enrichment *in vivo*.

Methods

For *in vitro* chemotaxis, a modified agarose microdroplet method was used [5]. Each test substance and buffer alone were examined in 5 wells, and experiments were repeated at least 3 times. Incubations were done for 18 h at 5% CO_2, 37 °C. Ten µl of 0.2% toluidine blue, pH 3.7, were then added to each well, and the distance of migration of cells from the edge of the microdroplet to the leading front was measured at 4 sites, at 90° angles. CTMC within the entire region outside the droplet were counted separately.

Results

In vitro migration of RPC and MCD was comparable with all factors studied. Migration of CTMC was never greater than that of cells in buffer alone. NFMLP (10^{-7} M) and activated serum (1:2 dilution) induced macrophage migration, while LTB_4 (10^{-8} M) and PAF (10^{-6} M) were ineffective. Lymphocyte supernatants (LS) obtained after stimulation of cells with PHA (5–0.05 µg/ml) or Con A (5–0.05 µg/ml) were effective chemoattractants for macrophages over the entire dose range. Fibronection (FN) inhibited macrophage migration at 50 µg/ml and caused some stimulation at 5 to 0.05 µg/ml. Fibroblast growth factor (FGF) was stimulatory at 100 to 0.1 ng/ml and epidermal growth factor (EGF) at 1 µg/ml to 1 ng/ml. Ascaris antigen induced macrophage migration at doses of 10 µl to 0.01 µl/ml, but only in previously sensitized animals. Growth factor-containing preparations such as 15% horse serum (HS) caused no significant chemotaxis, but 20% L-cell supernatants (LCS) were quite effective.

Injections of the same agents that had been studied during *in vitro* chemotaxis were performed in Wistar rats. One ml each of the test substances in

Table 1
Number of CTMC after repeated (every 2 days) subcutaneous injections of 1 ml of the agents shown. The data show mean ±1 SD after 15 different measurements.

Stimulus	CTMC/Micros. Field 10 D
LCS, PHA 0.5 μg/ml	1.1±0.2
LCS, 20%	1.6±0.8
HS, 15% LCS, 20%	3.7±1.4
EGF, 100 ng/ml	0.3±0.3
FGF, 10 ng/ml	2.1±1.1
Ascaris Ag, 10 μl/ml	1.3±0.7
Ascaris Ag, 10 μl/ml*	9.2±4.8

* Sensitized animals.

MEM Dulbecco were injected into 8 different sites on the back. Biopsies were taken 4 h after single injections, and 24 h after multiple injections. CTMC were counted in toluidine blue stained tissue sections in 15 microscopic fields (magn. 400×). No increase of CTMC was seen after injections of chemotactic factors. The data for the remaining factors are shown in Table 1 for a 10 day, q 2 days injection series. The most effective stimuli were a combination of HS, 15% and LCS, 20% and of ascaris antigen, 10 μl/ml in sensitized animals. LS, LCS alone, FGF and ascaris antigen in nonsensitized animals caused a moderate increase, while EGF was ineffective. Similar data were obtained 22 days after repeated injections.

Discussion

These data show that 1. mature CTMC have no *in vitro* chemotactic activity; 2. macrophages which contain CTMC precursors, respond to a broad range of factors varying from common chemoattractants to growth factors; 3. enrichment of CTMC in living tissue depends on immunological stimuli such as ascaris antigen, on fibroblast (L-cell)-derived growth factors and to a lesser extent on lymphocyte-supernatans and FGF. These findings agree with *in vivo* observations on CTMC development and suggest that CTMC increase at tissue sites by a bi-phasic response that is governed by specific mediators: 1. immigration of precursor cells; 2. maturation of precursors at tissue sites.

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