Short Communication

Histamine Synthesis by Bone Marrow-derived Macrophages

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Histamine may be involved in the regulation of hematopoiesis. However, it remained obscure what kind of cells are responsible for its synthesis in bone marrow (BM). In this study, a pure population of macrophages were raised from BM of W/W" mice, which are genetically deficient in mast cells. The addition of Escherichia coli lipopolysaccharide (LPS) or murine recombinant granulocyte/macrophage colony-stimulating factor (mGM-CSF) alone had essentially no effect on histamine synthesis. However, the latter rendered the cells responsive to LPS, leading to a marked increase in HDC-dependent histamine synthesis.

Histamine exerts a variety of immunoregulatory actions.\(^1\) In addition, evidence is accumulating to indicate that it is involved in the regulation of hematopoiesis; the amine stimulates multipotent stem cell (CFU-S) cycling,\(^2\) and induces granulocyte differentiation.\(^3\) However, it remained uncertain what kind of cells are responsible for the histamine production in bone marrow (BM), in spite of profound investigations.\(^4\)\(^-\)\(^6\) We have shown that histamine is produced by peritoneal macrophages through induced histidine decarboxylase (HDC).\(^7\) This study was aimed to examine whether macrophages participate in histamine synthesis in BM. For the purpose, essentially pure populations of macrophages were raised from BM and used.

Mice of both sexes of the genetically mast cell-deficient WBB6/F1 (W/W") strain were used when 2–3 months of age. BM cells were obtained by flushing femurs and tibias. BM-derived macrophages (BMDM) were raised from the BM cells by the method of Esparza \textit{et al.}\(^8\) with some modifications. Briefly, fibroblasts in the BM cells were removed by passing through a Sephadex-G10 column. The remaining cells were cultured at a concentration of \(4 \times 10^6\) cells/ml in petri dishes in 3 ml of RPMI 1640 medium with L-glutamine (2 mm), streptomycin (100 \(\mu\)g/ml), penicillin (100 IU/ml), 5% heat-inactivated horse serum, 15% heat-inactivated fetal calf serum, and 20% L cell-conditioned medium at 37°C in a humidified 5% CO\(_2\). After 12 days, cultured BMDM, thus raised, \((1 \times 10^6\) cells/dish, >98% Mac-1, macrophage-specific antigen, and non-specific esterase-positive) were washed extensively and then stimulated with murine (m) recombinant (r) granulocyte/macrophage colony-stimulating factor (mGM-CSF, 10 ng/ml, kindly donated by the Kirin Brewery Co., Ltd., Tokyo) with or without 1–10,000 ng/ml of Westphal preparation of lipopolysaccharides (LPS) from 

\[\text{Escherichia coli 055: B5 (Difeo Laboratories, Detroit, MI) in a serum-free synthetic medium (GIT, Nippon Pharmaceutical Co., Ltd., Tokyo).}\]

The cells were incubated for another 48 h. After incubation the medium was removed, and the BMDM were washed and scraped by a rubber policeman and collected by centrifugation. The cell pellets were resuspended with 0.02 M sodium phosphate buffer (pH 6.2), containing 0.02 M pyridoxal 5'-phosphate and 0.2 M dithiothreitol, and disrupted by sonication. The cells and the medium were assayed for HDC activity and histamine, respectively.\(^7\)

The culture of the BMDM raised from W/W" mice

\[\text{of Escherichia coli Lipopolysaccharides (LPS) on Histidine Decarboxylase (HDC) Activity (a) and Histamine Production (b) in Bone Marrow-derived Macrophages.}\]

The cells were cultured for 48 hr in the presence (\(\bullet\)) or absence (\(\bigcirc\)) of mGM-CSF (10 ng/ml) with or without various concentrations of LPS. The values represent the means of the triplicate samples.

\[\text{Fig. Effects of Escherichia coli Lipopolysaccharides (LPS) on Histidine Decarboxylase (HDC) Activity (a) and Histamine Production (b) in Bone Marrow-derived Macrophages.}\]
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synthesized essentially no HDC-associated histamine (Figs. (a), (b)). Addition of mrGM-CSF or LPS alone had no effect on the histamine synthesis. However, treatment with mrGM-CSF rendered the cells responsive to LPS; LPS induced a marked dose-dependent increase in HDC activity and histamine synthesis by these cells. More than 98% of the cells obtained from the long-term culture of the BM cells were positive for nonspecific esterase and expressed Mac-1 antigen weakly on their surfaces. Expression of Mac-1 on these cells increased markedly after 2 days of culture in the presence of mrGM-CSF and LPS. Mac-1 has been reported to be expressed weakly on progenitor cells of the macrophage lineage and strongly on mature macrophages. It is highly likely, therefore, that the cells that synthesize histamine in BM are macrophages. The next problem to be solved is to define when the histamine-producing activity is acquired during differentiation of the progenitor cells of the macrophage-lineage. We demonstrated previously that mature macrophages synthesize histamine; the response of mouse peritoneal macrophages to LPS resulted in induction of HDC, and consequently, of histamine production. As shown here, BMDM, which had been freshly raised from BM, synthesized essentially no histamine, while treatment of these cells with mrGM-CSF and LPS for 2 days induced a HDC-associated histamine synthesis with a concomitant increase in expression of Mac-1 antigen on their surface. These results indicate that histamine-forming activity may be acquired by the progenitor cells during differentiation to macrophages. A study is now being done in our laboratory to clarify this problem using promyelocytic cell lines that can differentiate to macrophages.

References