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Cytochemical Analysis of Mast Cell Phenotypes in the Lymph Nodes of Allogeneic Tumor–Grafted Mice and in the Thymus of Preleukemic Mice

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INTRODUCTION

Variations in mast cell (MC) phenotype have been investigated from morphological, biochemical, immunological, and functional points of view (1). Histologically, the MC heterogeneity is easily shown by use of the sequential staining of Alcian blue followed by safranin. This allow to currently discriminate between the alcianophilic (blue) mucosal mast cells (MMCs) and the safraninophilic (red) connective tissue–type mast cells (CTMCs). This staining reaction has been used to study the MC response to fibrosarcoma graft in mice (2). It was shown that MC number was increased in the graft-draining lymph nodes (LNs). However, most of the MCs appeared red in syngeneic tumor-draining LNs, whereas they were blue in allogeneic tumor-draining LNs. To define the phenotypes of these MC subpopulations, we have compared their tinctorial properties to those of peritoneal MCs (CTMCs) and of intestinal MCs (MMCs) by use of several cytochemical reactions. We have also studied the thymus MCs in irradiated mice (lymphoma induction) as in irradiated and bone marrow–grafted mice (lymphoma inhibition).

MATERIALS AND METHODS

C57BL/6 and CBA mice from our breeding colony were used at 3 months of age.

Tissues

The cells of the peritoneal cavity of several mice were harvested by gently washing with cold culture medium (RPMI 1640 Gibco) without serum. Some cell suspensions were incubated for 30 min at 37°C in a solution of 0.5 μg/ml of poly-L-lysine in RPMI in order to induce MC secretion (3).

Pieces of intestine of normal mice were resected.

Tumor pieces of about 10.6 living tumor cells were implanted s.c. in CBA or C57BL/6 mice. The tumor was the transplantable fibrosarcoma T2, originally induced in C57BL/6 mice by MCA and maintained by serial s.c. transplantation of solid tumor fragments. The allogeneic or syngeneic tumor-draining lymph nodes were removed from CBA or C57BL/6 mice every 2 days during 10 days after the graft. Control lymph nodes were removed from normal mice.

C57BL/6 mice were irradiated with four doses of 1.75 Gy applied at weekly intervals. Some of them were grafted with 10.10^6 bone marrow cells immediately after the last irradiation. Mice were sacrificed at days 13 and 20 after the last irradiation and their thymuses were resected.

Histology

The different tissues were fixed for 60 min at 4°C in Carnoy's fluid, in 10% formaldehyde in water, or in 4% glutaraldehyde in phosphate buffer. They were dehydrated in ethanol and embedded in paraffin. Sections of 5 μ were performed.

Histochemistry

Enzymatic Digestion

The sections were incubated for 90 min at 37°C in a solution of trypsin (0.1 mg/ml) in phosphate buffer in order to remove the protein fraction of the mast cell granules.

Biebrich Scarlet Reaction

The sections were stained for 60 min in the staining solution at pH 9.5. This anionic dye is known to selectively demonstrate histones and other basis proteins (4).

Silver Methenamine Reaction

The sections were stained for 90 min at 60°C in the silver basic solution; in these conditions, the silver is known to form mercaptides with the sulfhydryl groups of basic proteins (5).

Alcian Blue–Safranin Reaction

The sections were stained for 60 min in 3% Alcian blue in sulfuric solution (2N), pH 0.2 and counterstained for 5 min in 0.1% safranin O solution in water, pH 6.5 Alcian blue stains sulfate groups ionized at very low pH, whereas safranin strains most of the acid groups ionized at pH near the neutrality (6).

Berberine Sulfate Reaction

The sections were stained for 20 min in 0.02% berberine sulfate solution in 1% citric solution, pH 4. This cationic dye forms strongly fluorescent complexes with heparin. This yellow fluorescence is contrasting with green fluorescence of the nucleoproteins (7).

RESULTS

The results are summarized in Table 1.

Table 1 Tinctorial Properties of Different Mast Cell Subsets

Histochemical reactions	Intestinal MCs	Peritoneal MCs		Lymph node MCs	
		Normal	Post-secretion	Syngeneic tumor graft	Allogeneic tumor graft
Basic protein demonstration					
Biebrich Scarlet (BS)	Orange-red	Orange-red	—	Orange-red	—
Trypsin BS	—	—	—	—	—
Silver methenamine	—	Black	—	Black	—
Acid polysaccharide demonstration					
Alcian blue–safranin (ABS)	Blue	Red	Blue	Red	Blue
Trypsin ABS	Blue	Blue	Blue	Blue	Blue
Berberine sulfate	—	Yellow (fluo)	Yellow (fluo)	Yellow (fluo)	Yellow (fluo)

Intestinal Mast Cells

Glutaraldehyde fixation inhibited the staining of those MCs that appeared alcianophilic after the other fixations. They contained basic proteins demonstrated by the Biebrich Scarlet (BS) but no sulfhydryl groups (silver methenamine negativity); the polyanions were different from heparin, since they were unstained by the berberine sulfate (BbS) reaction.

Peritoneal Mast Cells

Whatever the fixation was, these MCs exhibited the tinctorial properties of the CTMCs. They contained basic proteins (BS^+), they were silver methenamine (SM) positive; the polyanions were heparinlike (BbS^+). They were safraninophilic (red) but became blue (alcianophilic) after a trypsin digestion. Poly-L-lysine treated MC (secretion induced) became BS^-, SM^-, and alcionophilic but remained berberine sulfate positive.

Lymph Node Mast Cells

Syngeneic tumor graft–draining LNs contained numerous MCs that appeared BS positive and SM positive (SH-rich basic proteins). They contained heparin (BbS^+) and were safraninophilic but became blue (alcianophilic) after trypsin digestion. Allogeneic tumor graft–draining LNs contained also numerous MCs that were BS negative, SM negative, contained heparin (BbS^+) but were alcianophilic. Control LNs contained few MCs; some were alcianophilic, other were safraninophilic.

Thymus Mast Cells

Normal mouse thymus sections contained few MCs, generally located outside the capsula. These MCs were safraninophilic or alcianophilic. Irradiated mouse thymuses were

infiltrated with red (safraninophilic) MCs, located in the septa. Irradiated and bone marrow–grafted mouse thymuses contained MCs in their septa; most of these MCs were alcianophilic (blue).

DISCUSSION AND CONCLUSIONS

The mast cells appeared safraninophilic as far as they contained SH-rich basic protein, in normal peritoneal cell suspensions, in syngeneic tumor–draining lymph nodes, or in irradiated thymus.

The mast cells appeared alcianophilic as far as they do not contain SH-rich protein, in secretagogue-treated peritoneal cells, in allogeneic tumor-draining LNs, or in irradiated and bone marrow (BM)–grafted mouse thymus. The blue intestinal MCs do not contained heparin (BbS^-), and were thus different from the other MC subsets studied.

The mast cells in lymph node and thymus would thus be of the connective tissue type.

The s.c. implantation of tumor induced an increase of MCs in the subcapsular space and around the germinal centers in the draining LNs. As the tumor was syngeneic and grew, the MCs contained their usual heparin–basic protein complexes. As the tumor was allogeneic and would be rejected, the MCs did not contain basic proteins anymore but conserved heparin.

Irradiation and irradiation followed by a BM graft induced MC infiltration of the thymus septa. In preleukemic mice (irradiation), the MCs contained basic protein, whereas in nonpreleukemic mice (irradiation + BM) they contained only heparin.

In lymph nodes and in thymus, alcianophilic MCs would thus herald, respectively, allogeneic tumor rejection and thymic lymphoma inhibition. Most probably, this mast cell alcianophily could reflect a previous secreting activity. However, it cannot be excluded that this could represent an early stage in the maturation/differentiation process of CTMCs (8).

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