Changes in Membrane Gangliosides: Differentiation of Human and Murine Monocytic Cells

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Membrane ganglioside changes in murine peritoneal macrophages and the human promyelocytic leukemia cell line HL-60 have been assessed by two-dimensional thin-layer chromatography. C3H/HeJ mice respond to protein-containing endotoxin but are hyporesponsive to protein-free endotoxin preparations. Compared to unstimulated resident cells, protein-containing endotoxin produced an alteration in the C3H/HeJ macrophage ganglioside pattern whereas protein-free endotoxin did not. In comparison, differentiation of HL-60 cells to a neutrophil-like cell by dimethylsulfoxide gave a ganglioside pattern similar to unstimulated HL-60 cells. However, differentiation of HL-60 cells by phorbol myristate acetate to macrophage-like cells results in a large increase in the monosialo ganglioside Gm1. The evidence presented indicates that discrete ganglioside changes occur in murine monocytes and HL-60 cells upon induction to cells with increased macrophage functions.

INTRODUCTION

Macrophages represent a critical target cell for bacterial endotoxins. Following exposure to endotoxin, the macrophage undergoes differentiation which results in a vast array of pathophysiologic sequelae [1]. In addition to profound structural changes the macrophage is induced to produce various enzymes and cytokines. The result of this differentiation is a cell with potent host defense capabilities. One of the critical cytokines released by activated macrophages is interleukin-1 (IL-1) which contains both endogenous pyrogen activity and lymphocyte-activating activity (reviewed by [2]). The activated macrophage also has the tremendously enhanced ability to produce hydrogen peroxide and oxygen radicals, which play a central role in the armament of the macrophage [3]. Tumor cells and microorganisms can both be destroyed after attack by the activated macrophage. Interaction of monocytes with endotoxin results in differentiation to a macrophage endowed with potent activity. It has also been clearly demonstrated that the interaction of endotoxins with lymphoid cells plays a central role in the mediation of toxic effects on the host observed during the endotoxemia of gram-negative bacteremia [4]. In vivo studies with endotoxins have shown so many physiologic alterations that a clear understanding of even the cellular mechanisms of endotoxin-induced injury has evaded investigators for several decades [5].

Interaction of monocytes with endotoxin results in differentiation to a macrophage

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endowed with potent activity. We are examining biochemical changes seen after endotoxin-induced differentiation of the macrophage in vivo. In particular, our recent work has focused on changes in macrophage glycolipids which are associated with differentiation-induced changes in functional states. Plasma membrane gangliosides represent a minor component of cell surface membranes, but they have critical activities in many systems, including growth regulation, and as receptors for various ligands, including toxins and interferons [6,7].

By comparison, we have also examined the changes in membrane gangliosides seen after differentiation of the human promyelocytic leukemia cell line HL-60. HL-60 cells can be induced to express biochemical and functional characteristics of the human macrophage upon exposure to phorbol myristate acetate (PMA) and characteristics of neutrophils upon exposure to dimethyl sulfoxide (DMSO) [8]. In this paper, we present preliminary evidence on ganglioside changes which occur in murine monocytes and HL-60 cells following differentiation.

MATERIALS AND METHODS

Cell Sources

Murine resident peritoneal macrophages were obtained from C3H/HeJ mice. This strain is hyporesponsive to protein-free endotoxin, but normally responsive to endotoxin with associated protein. Mice were six to eight weeks old, female, and used within two weeks of arrival. They were sacrificed by cervical dislocation and the total peritoneal cells were harvested by lavage with cold BSS* as previously described [9]. One to three million cells were obtained from each animal. About 50 percent of these cells were adherent and were utilized for analysis. Peritoneal macrophages were also activated by the intraperitoneal administration of 10 μg/mouse of E. coli K235 lipopolysaccharide preparations (gifts of D.C. Morrison, Emory University, Atlanta, GA). Macrophages were purified from non-adherent cells by allowing the cells to attach to glass petri dishes. Non-adherent cells were decanted after agitation. The dishes were washed four times with PBS and then washed with 0.31 M pentaerythritol. The gangliosides were extracted as described below. HL-60 cells are maintained in our laboratory. They were grown in RPMI supplemented with penicillin (100 U/ml) and streptomycin (100 μg/ml) in the presence of 10 percent fetal calf serum.

HL-60 cells were induced to differentiate by the addition of either 1.25 percent DMSO for five days or 4 nM PMA for three days. With both differentiation regimens, the cells were smaller in size and had acquired the ability to adhere and spread on glass. Cells were harvested by centrifugation, washed in PBS, and pelleted for ganglioside analysis.

Ganglioside Extraction and Analysis

Gangliosides were extracted from cell samples by a modification of previous methods [10,11]. The total lipid extract was obtained by mixing the cells with chloroform-methanol (1:1, v/v) and filtering the extract through Whatman No. 1 filter paper to remove particulate matter. The total lipid extract was dried and taken up in chloroform:methanol:water (C:M:W) (30:60:8, v/v). The extract was chromatographed on a small (3 ml) DEAE-Sephadex A-25 column to remove neutral lipids. After extensive washing, the acidic lipid fraction (containing gangliosides) was eluted with C:M:0.8 M sodium acetate (30:60:8). After evaporation of the organic solvent,
the acidic lipids were hydrolyzed with 0.1 N NaOH at 37°C for 90 minutes to destroy contaminating phospholipids. After neutralization to pH 4-4.5, the samples were desalted using a reversed phase silica gel column (Sep Pak, Waters Assoc., Waltham, MA). The desalted lipids were removed by eluting the column with methanol followed by C:M (1:2). After drying, the samples were dissolved in C:M (85:15) and gangliosides purified on an Iatrobead 6RS-8060 column as previously described [10]. Ganglioside sialic acid content was determined by resorcinol:HCL [12].

Ganglioside patterns were determined by two-dimensional TLC on HP-TLC silica gel 60 plates (E. Merck, Darmstadt, Germany) [13]. The plates were developed with C:M:0.25 percent KCL (50:45:10) and dried. They were rotated 90° and redveloped with C:M: 2.5 N aqueous NH₃ in 0.25 percent KCL (50:45:10). Ganglioside spots were visualized by resorcinol spray [12].

RESULTS

The ganglioside pattern for C3H/HeJ resident peritoneal macrophages is shown in Fig. 1A. This pattern represents the ganglioside extract from 10⁶ cells. There are approximately 15 resorcinol-positive spots on the pattern with the primary gangliosides appearing in the monosialo and disialo regions. It should be noted that a standard preparation of human brain ganglioside was chromatographed in each dimension (not shown) to facilitate comparison of different plates and to assist in the identification of the sialic acid class of the gangliosides on the two-dimensional plates. The more highly charged (polar) gangliosides migrate more slowly from the origin.

Endotoxins prepared by two different techniques were used to elicit activated macrophages from C3H/HeJ mice. Endotoxin was prepared by cold phenol extraction [14] and butanol extraction [15] from E. coli K235. The phenol-extracted endotoxin (ϕ-LPS) is virtually protein-free (~0.5 percent) and did not elicit a significant change in the ganglioside pattern of macrophages harvested four days after intraperitoneal administration of 10 μg of endotoxin (Fig. 1B). This similarity of the phenol-extracted pattern (Fig. 1B) to the resident pattern (Fig. 1A) in the C3H/HeJ reflects the hyporesponsiveness of this strain to protein-free lipopolysaccharide. The butanol-extracted endotoxin (bu-LPS) contains about 10 percent protein and has been observed to be biologically active in C3H/HeJ mice [16]. When this endotoxin preparation was used to elicit peritoneal macrophages, a change in the ganglioside pattern was observed (Fig. 1C). At least one new ganglioside appeared, reflecting an alteration of sialation of these glycolipids after this differentiation.

In order to investigate further whether cellular differentiation of monomyeloid cells is always associated with changes in the ganglioside pattern, we examined the ganglioside pattern of HL-60 cells after differentiation to macrophage or neutrophil-like cells. Uninduced HL-60 cells had a simple ganglioside pattern (Fig. 2A). Induction of differentiation along the granulocyte pathway with DMSO did not cause the expression of new gangliosides (Fig. 2B). There were only slight differences in the relative intensity of some gangliosides from DMSO-induced HL-60 (Fig. 2B) compared with uninduced HL-60 (Fig. 2A), as has been reported by others [8]. When HL-60 cells were stimulated by PMA along the macrophage pathway a significantly different pattern was observed (Fig. 2C). The spots corresponding to G₃M₃ (arrow in Fig. 2C) increased dramatically with a concomitant decrease in the relative intensity of the more heavily sialated gangliosides. No new major spots were seen.
DISCUSSION

The role of plasma membrane gangliosides has not been well defined in extraneural systems. There is evidence that gangliosides are receptors for a variety of bacterial toxins and some interferons [6,7]. Additionally, they appear to compose part of the receptor complex for peptide hormones [17]. There have also been studies linking gangliosides to growth regulation in cultured cells, and specific gangliosides have been shown to represent markers for lymphoid populations [18]. There have been, however, few studies on the gangliosides of macrophages. Our studies have focused on the

FIG. 1. Thin-layer chromatographs of gangliosides from the peritoneal macrophages of C3H/HeJ mice. Gangliosides were obtained from (A) resident cells, (B) φ-LPS activated cells, and (C) bu-LPS activated cells. Gangliosides (2-4 μg ganglioside sialic acid) plated are from 10⁶ resident cells (A) and 6 × 10⁷ activated cells (B,C), accounting for the lighter spots seen in B and C compared to A. The gangliosides were chromatographed from the origins (asterisks) in the first direction indicated as “1” followed by chromatography in the second direction “2,” using the solvent systems described in Methods. The pattern seen in “B” is very similar to that seen in “A” while in “C” a new major spot (arrow) is evident.
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FIG. 2. Thin-layer chromatographs of gangliosides from HL-60 cells. Gangliosides were obtained from (A) uninduced HL-60 cells, (B) DMSO-induced cells (granulocyte-like), and (C) PMA-induced cells (macrophage-like). Gangliosides plated are from 6 x 10^7 cells for (A) and (B) and 4 x 10^7 cells for (C). The origins (asterisks) and conditions of two-directional chromatography are the same as in Fig. 1. The pattern seen in "B" is very similar to that seen in "A" while in "C" a marked increase in G_{M1} (arrow) is evident.

ganglioside content of mononuclear cells, as mononuclear phagocytes represent a critical arm of the host defense system, particularly for intracellular pathogens known to generate toxins.

We have shown that protein associated with bacterial endotoxin is capable of altering the expression of gangliosides on murine peritoneal macrophages from lipid A hyporesponder mice. The independent biologic activity of lipid A-associated protein has been well documented [16,9]. In addition, the work of others shows that butanol-extracted endotoxins, but not phenol-extracted endotoxins, are capable of
activating C3H/HeJ macrophages [19]. The observations reported here corroborate and extend these previous works in that butanol-extracted endotoxin produced an alteration in C3H/HeJ macrophage gangliosides whereas phenol-extracted endotoxin did not. Identification of the specific ganglioside induced in the hyporesponder macrophage by the butanol-extracted endotoxin is under way in our laboratory.

The recent studies on HL-60 cells [8] using a one-dimensional TLC system have revealed that ganglioside alterations occur in this cell line when it is induced to differentiate into macrophage-like cells. Using our two-dimensional system, we have confirmed the observation that there is a profound increase in the ganglioside $G_{M3}$. Our system can allow a more complete analysis of the relative quantities of the gangliosides by densitometric scanning. These studies are currently under way in our laboratory.

We have presented preliminary evidence in two systems that the differentiation of monocytic cells with the acquisition of altered functional properties also results in ganglioside changes either in quantity or quality.

The biologic significance of these ganglioside changes remains unknown. It is tempting to speculate that the gangliosides observed in macrophages are associated with receptor complexes for different ligands, but further experimentation will be needed to investigate this possibility. A chemical analysis of the neutral sugar and sialic acid types contained in macrophage gangliosides will be needed in order to provide some correlations of ganglioside structure with alterations in macrophage function.

When presented with endotoxin-containing gram-negative organisms, the host attempts protection through the induction of several immunologic mechanisms, including the activation of macrophages. Unfortunately, products of activated macrophages, such as the pyrogenic cytokine IL-1, have the potential for detrimental as well as beneficial effects. Studies on the molecular changes induced in monocytes by endotoxins may help define mechanisms which may be pharmacologically controlled in order to interrupt the damaging effects while preserving the beneficial effects of the host response.

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