

GLYCOLIPIDS OF THE MOUSE PERITONEAL MACROPHAGE

Alterations in Amount and Surface Exposure of Specific Glycolipid
Species Occur in Response to Inflammation and Tumoricidal Activation

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The macrophage participates in a wide variety of important physiological functions, including the recognition and destruction of tumor cells. Since many of these functions are mediated by the cell surface, the structural and functional characterization of macrophage surface components is an area of considerable interest and intense study (1–3). One approach to this study involves analyzing alterations in macrophage surface components that occur in response to inflammation or as a consequence of activation to a tumoricidal state (1–4). Information obtained from studying these alterations has proven useful in identifying specific components that are involved in some of the specialized surface functions characteristic of the inflammatory and the activated macrophage. To date, most work in this area has focused on protein components of the macrophage surface, particularly on receptor proteins. Although much has been learned from these studies, other potentially important components have not been studied in detail.

Glycolipids constitute one class of surface components for which biochemical data are scant. An increasingly large body of evidence indicates that glycolipids are important functional components of the cell surface (5); for this reason, it seems likely that glycolipids may play key roles in macrophage surface phenomena, either by themselves, or in concert with other surface components. The limited data that do exist on macrophage glycolipids, however, are fragmented and contradictory. For example, there are reports that argue both for (6) and against (7) the presence on the macrophage surface of asialo GM₁, a molecule of considerable importance, since it has been implicated as a marker for specific types of cytotoxic cells (8).

In light of the above observations we have carried out a detailed study on the major glycolipid constituents of the resident, inflammatory, and tumoricidally activated mouse peritoneal macrophage. The results presented in this paper demonstrate that the inflammatory and the activated macrophage are character-

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Structures of the glycolipids referred to in this paper are presented in Table I.

ized by alterations in the chemical amount and in the surface exposure of specific glycolipid species relative to the resident macrophage.

Materials and Methods

Mice. Female C57/BL6 mice obtained from The Jackson Laboratory (Bar Harbor, ME) at 7 wk of age were used in all experiments.

Macrophages. Peritoneal macrophages were obtained by lavage of the peritoneal cavity with 10 ml of cold phosphate-buffered saline (PBS; Ca⁺⁺ and Mg⁺⁺ free)¹ containing 1 mM Hepes buffer, pH 7.2. Harvested peritoneal cells were centrifuged at 250 g for 10 min at 4°C and resuspended in MEM containing 10% heat-inactivated fetal calf serum and 2 mM freshly prepared glutamine (normal medium). Aliquots of the peritoneal cell suspension (containing ~10⁷ macrophages) were plated in 60-mm plastic tissue culture plates, and the plates were incubated at 37°C for 4 h. The plates were then washed vigorously with PBS to remove the nonadherent cells, and the macrophages were surface labeled as described below.

Resident macrophages were obtained as described above, from untreated mice. ~2–3 × 10⁶ peritoneal cells were harvested from each mouse, of which 40–50% were macrophages as determined by Wright-Giemsa staining.

Thioglycollate (TG)-elicited macrophages were obtained from mice that had been injected intraperitoneally 4 d before sacrifice with 1.5 ml of a 4% solution of TG broth (Difco Laboratories, Inc., Detroit, MI) prepared according to the manufacturer's instructions. This procedure yielded 2–3 × 10⁷ peritoneal cells, of which 80–90% were macrophages.

In order to obtain bacillus Calmette-Guerin (BCG)-activated macrophages, mice were injected with 10⁷ live BCG (Phipps strain 1022; Trudeau Institute, Saranac Lake, NY) 3 wk before, and subsequently 3 d before, sacrifice. This procedure yielded 5–6 × 10⁶ peritoneal cells, of which 40–50% were macrophages.

Cytolysis Assay. All populations of macrophages were tested for their ability to lyse P815 cells in an 18-h Na₂⁵¹CrO₄ (⁵¹Cr; New England Nuclear, Boston, MA) release assay as described by Russell (9). The assay was carried out in triplicate in 6-mm wells of a 96-well microtiter plate containing 5 × 10⁵ adherent macrophages and 5 × 10⁴ labeled P815 cells. The spontaneous release of ⁵¹Cr from P815 cells during the course of the assay was <25% of the total label incorporated. The percent net cytolysis is defined as: [(Released cpm in experimental wells – cpm spontaneous release)/(total cpm releasable – cpm spontaneous release)] × 100. The cytolysis data are presented in Table II.

Surface Labeling. Populations of macrophages were surface labeled by the galactose oxidase/NaB³H₄ method (10). Adherent macrophages were incubated in 2 ml of PBS containing 30 U of galactose oxidase (Sigma Chemical Co., St. Louis, MO), for 60 min at 4°C. The plates were washed with PBS and subsequently 2 ml of PBS containing 2 mCi NaB³H₄ (New England Nuclear) were added to each plate. The reduction was carried out at 4°C for 60 min. In control experiments, the galactose oxidase incubation was omitted.

Following the NaB³H₄ reduction, the plates were washed with PBS and the macrophages were scraped off in cold PBS with a Teflon scraper. The macrophage suspension was sonicated for 30 s at 4°C with a Branson probe sonicator (model 185; Branson Sonic Power Co.; Danbury, CT) set at the lowest setting. An aliquot from each sonicated suspension was removed for protein assay performed by the method of Lowry (11), and the remaining suspension was centrifuged at 15,000 g for 10 min. The resultant pellet was solubilized in 10 ml of chloroform-methanol (2:1) by probe sonication.

Glycolipid Extraction and Analysis. The chloroform-methanol extracts were dried under N₂, resuspended in distilled water, and desalted on Bond-Elut cartridges (reference 12; Analytical Chemicals, Harbor City, CA). The desalted glycolipids were chromatographed on DEAE-Sephadex A-25 (Pharmacia Inc., Piscataway, NJ) to separate neutral

¹ Abbreviations used in this paper: BCG, bacillus Calmette-Guerin; ⁵¹Cr, Na₂⁵¹CrO₄; HPLC, high pressure liquid chromatography; HRP, horseradish peroxidase; PBS, phosphate-buffered saline; TG, thioglycollate; TLC, thin layer chromatography.

glycolipids from gangliosides (13). Neutral glycolipids were treated with 0.6 N methanolic NaOH for 1 h at room temperature, neutralized with HCl, and desalted. The desalted neutral glycolipids were chromatographed on high performance, thin layer chromatography (TLC) plates (E. Merck, Inc., Darmstadt, FRG) in chloroform-methanol-water, 60:35:8. Gangliosides were also chromatographed on high performance TLC plates in chloroform-methanol-water (55:45:10) containing 0.02% CaCl₂ as described previously (14), and visualized with resorcinol-HCl reagent. TLC plates containing radioactively labeled glycolipids were exposed on ultrafilm (LKB, Bromma, Sweden) for 4–14 d at room temperature.

High Pressure Liquid Chromatography. High pressure liquid chromatography (HPLC; Waters Associates, Milford, MA) analysis was carried out as described (15) on a 2.1 × 50 cm Zipax column (E. I. DuPont de Nemours, Inc., Wilmington, DE) with a 13-min gradient of 1–20% dioxane in hexane. UV detection at 230 nm was employed. The densitometric analysis was performed on the high performance TLC plates using a Zenith soft-laser scanning densitometer (Biomed Instruments Inc., Fullerton, CA).

Gas-Liquid Chromatography. Analysis of the neutral and amino sugar components of gangliosides was performed by gas-liquid chromatography as described (16). Briefly, gangliosides were treated with 0.75 N methanolic HCl extracted in hexane, re-*N*-acetylated, and analyzed as the Me₃Si derivatives on a 3% OV-1 column with a temperature program of 150–250°C.

Sialic acids were analyzed using the same 3% OV-1 column as described (17). Briefly, gangliosides were treated with 0.05 N aqueous HCl for 1 h at 80°C and the products were converted to the Me₃Si derivatives. The column was run isothermally at 220°C. Sugar ratios were determined empirically and compared with the composition of known glycolipids, which were analyzed simultaneously under the same conditions.

Immunochromatography. Neutral glycolipids and gangliosides were chromatographed on aluminum high performance TLC plates (E. Merck) in chloroform-methanol-water (60:35:8), dried, then dipped in 0.05% polyisobutyl methacrylate in hexane as described by Brockhaus et al. (18). The plates were then soaked in PBS containing 1% BSA for 2 h before exposure to either a rabbit anti-asialo GM₁ antibody (19), a rat anti-mouse Forssman antigen monoclonal antibody (20), or to horseradish peroxidase (HRP) conjugated to the β subunit of cholera toxin (List Biological Labs, Campbell, CA). HRP-conjugated anti-rabbit or anti-rat immunoglobulins (Accurate Chemical & Scientific Corp., Westbury, NY) were used as second-step antibodies. The plates were developed with 33 mM 4-chloro-1-naphthol in Tris buffer containing 2% methanol and 0.25% H₂O₂.

Results

Populations of adherent resident, inflammatory, and BCG-activated mouse peritoneal macrophages were surface labeled by the galactose oxidase/NaB³H₄ method (10), and glycolipids derived from these macrophages were resolved into individual species by high performance TLC and, in some cases, by HPLC. Comparisons were made among the macrophage populations on the basis of types of glycolipid species present, the relative amounts of these glycolipids (normalized to total cellular protein), and their accessibility to labeling.

In contrast to the standard galactose oxidase/NaB³H₄ protocol (10), the entire procedure was carried out at 4°C to minimize the effects of membrane recycling on the surface labeling pattern (21). No labeling of glycolipids was evident in any of our experiments in the absence of galactose oxidase treatment, establishing that the labeling observed in the presence of galactose oxidase is specifically in carbohydrate. In addition, no differences in the surface labeling patterns were observed for any given population of macrophages plated at different densities. Of the three populations of macrophages, only BCG-activated macrophages are capable of lysing P815 cells during an 18-h ⁵¹Cr- release assay (Table II).

Neutral Glycolipids. Analyses of the neutral glycolipid fraction obtained from the three macrophage populations (Fig. 1A) indicate that CMH, CDH, and asialo GM₁ are the major constituents (see Table I for structures). The neutral glycolipids are present in very small amounts (~6.6 μg neutral glycolipid/mg protein) and, as a result, could not be visualized easily by TLC. They are identifiable by HPLC, however, and the data indicate that the amount of CMH present is considerably greater than that of either CDH or asialo GM₁ in all of the macrophage populations. The HPLC analyses also indicate that the TG-elicited macrophage contains more of these glycolipids than either the resident or BCG-

TABLE I
Structures of Glycolipids

Galactosylceramide (CMH)		Gal-Cer
Glucosylceramide (CMH)		Glc-Cer
Lactosylceramide (CDH)		Gal(β1-4)Glc-Cer
Globotriaosylceramide (CTH)		Gal(α-1-4)Gal(β1-4)Glc-Cer
Globotetraosylceramide (Globoside)	GalNAc(β1-3)Gal(α1-4)Gal(β1-4)Glc-Cer	
Gangliotetraosylceramide (Asialo GM ₁)	Gal(β1-3)GalNAc(β1-4)Gal(β1-4)Glc-Cer	
Forssman	GalNAc(α1-3)GalNAc(β1-3)Gal(β1-Gal(β1-4)Glc-Cer 4)	
GM3		Gal(β1-4)Glc-Cer 3 2 ^α
		NeuAc
GM2		GalNAc(β1-4)Gal(β1-4)Glc-Cer 3 2 ^α
		NeuAc
GM1		Gal(β1-3)GalNAc(β1-4)Gal(β1-4)Glc-Cer 3 2 ^α
		NeuAc
GD1a		Gal(β1-3)GalNAc(β1-4)Gal(β1-4)Glc-Cer 3 2 ^α
		NeuAc
GD1b		Gal(β1-3)GalNAc(β1-4)Gal(β1-4)Glc-Cer 3 2 ^α
		NeuAc(α2-8)NeuAc

* Abbreviations: Gal, D-galactose; Glc, D-glucose; GalNAc, N-acetyl-D-galactosamine; GlcNAc, N-acetyl-D-glucosamine; Cer (ceramide), N-acylsphingosine.

TABLE II
Cytolysis of ⁵¹Cr-labeled P815 Targets by Macrophage Populations*

Macrophage populations [†]	Net cytolysis [‡]
	%
Resident	0
TG-elicited	0
BCG-activated	20.1 (± 1.8)–56.0 (± 6.0)

* 18-h assay performed as described in Materials and Methods.

[†] Macrophage populations were obtained as described in Materials and Methods.

[‡] Values reported are the lowest and highest cytolysis values (±SD) obtained for each macrophage population in several experiments.

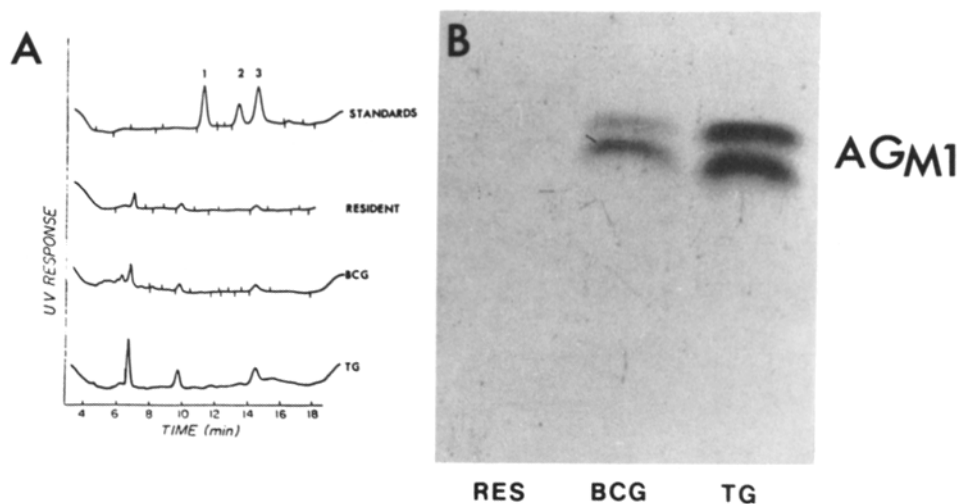


FIGURE 1. Chromatograms of neutral glycolipids derived from mouse peritoneal macrophages. (A) HPLC of neutral glycolipids derived from resident, BCG-activated, and TG-elicited macrophages and detected by UV at 205 nm as the perbenzoyl derivatives. The standard peaks are identified as the derivatives of: (1) globotriaosylceramide (CTH); (2) globotetraosylceramide (globoside); and (3) gangliotetraosylceramide (asialo GM₁). The peaks at 6 and 10 min correspond with ceramide monohexoside (CMH) and lactosylceramide (CDH), respectively. For each sample, the equivalent of 150 μ g of cellular protein was analyzed. (B) Autoradiogram of a high performance thin layer chromatogram of neutral glycolipids derived from resident, BCG-activated, and TG-elicited macrophages surface labeled by the galactose oxidase/ NaB^3H_4 method. Each lane represents the equivalent of 200 μ g of cellular protein.

activated macrophage: the latter two populations contain approximately equal amounts of these glycolipids.

Asialo GM₁ is the predominant neutral glycolipid labeled on the macrophage surface as shown in Fig. 1B. Asialo GM₁ is resolved into two distinct species by TLC (Fig. 1B), and we have confirmed that both of these species are bona fide asialo GM₁ by their ability to bind a rabbit anti-asialo GM₁ antibody (19) as shown by immunochromatography using an HRP-conjugated second antibody (Fig. 2A). Similar experiments with an anti-Forsman monoclonal antibody (20) indicate the presence of Forsman antigen in TG-elicited macrophages (Fig. 2B). Forsman antigen cannot be detected in TG-elicited macrophages by chemical means, including HPLC, presumably because it is present in very small amounts. Other macrophage populations have not been examined for the presence of Forsman antigen.

Although we consistently observed labeling of asialo GM₁ on the surface of BCG-activated macrophages, we have never observed its labeling on the surface of resident macrophages (Fig. 1B) even though, as stated above, asialo GM₁ is present in resident macrophages in amounts equivalent to that found in BCG-activated macrophages. The intensity of labeling of asialo GM₁ on the surface of TG-elicited macrophages is consistent with the increased amount of this glycolipid in these cells (Fig. 1), and it is greater than the labeling of asialo GM₁ on the surface of BCG-activated macrophages.

Gangliosides. Resorcinol staining of macrophage gangliosides separated by

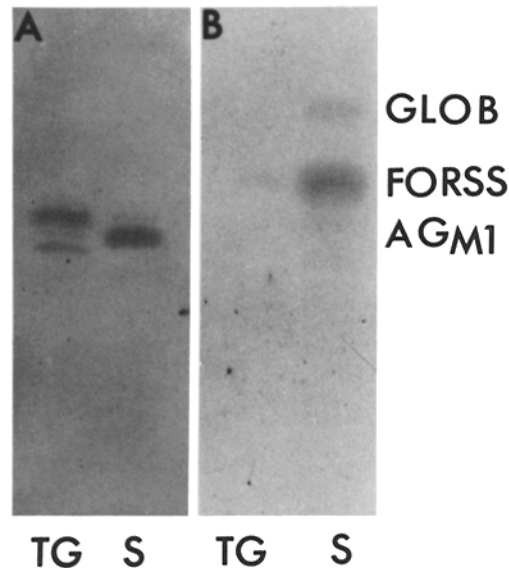


FIGURE 2. Immunochromatography of neutral glycolipids derived from TG-elicited macrophages. (A) High performance, thin layer chromatogram of neutral glycolipids from TG-elicited macrophages (TG) and from a standard mixture of brain glycolipids (S) exposed to a rabbit anti-asialo GM₁ antibody (19) and visualized with an HRP-conjugated anti-rabbit immunoglobulin as a second antibody. (B) High performance, thin layer chromatogram identical to A but exposed to M1/87, a rat anti-mouse monoclonal antibody specific for Forssman antigen (20) and HRP-conjugated anti-rat immunoglobulin. The positions of globoside, Forssman antigen, and asialo GM₁ in the standard mixture are indicated.

TLC (Fig. 3A) indicates that GM₁ is the predominant ganglioside in all three macrophage populations. The amount of ganglioside material in the mouse macrophage (~10 μ g/mg total cellular protein) is considerably greater than the amount of neutral glycolipid.

Three distinct molecular species have been ascribed to GM₁ in our TLC separations (denoted 1, 2, and 3 in Fig. 3). The identification of these three species as GM₁ is based on their co-migration with standard brain GM₁ (which is comprised of only one species) and, more importantly, on their ability to bind the beta subunit of cholera toxin conjugated to HRP (Fig. 4). The beta subunit of cholera toxin has a specific and high affinity for GM₁ (22). Comparison of the resorcinol staining pattern and the cholera toxin-HRP binding pattern indicates that only two species of GM₁ are present in resident macrophages (species 2 and 3). In contrast, three distinct species are present in TG-elicited macrophages. BCG-activated macrophages also appear to contain these three GM₁ species, as indicated primarily by the surface labeling data (see below); the ability of the first GM₁ species in BCG-activated macrophages to bind cholera toxin-HRP, however, is considerably reduced compared with its ability in TG-elicited macrophages (Fig. 4). It is interesting to note that there is a fourth molecular species that migrates with a mobility similar to GM₁ in all three macrophage populations, but that does not bind cholera toxin-HRP. The identity of the molecular species migrating slower than GM₁ in the region of GD_{1a} and GD_{1b} has not been determined.

MACROPHAGE SURFACE GANGLIOSIDES

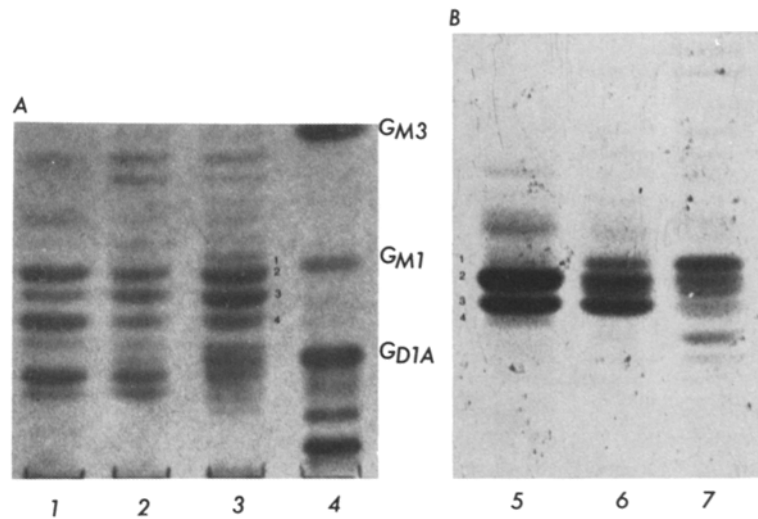


FIGURE 3. High performance, thin layer chromatograms of gangliosides derived from mouse peritoneal macrophages surface labeled by the galactose oxidase/ NaB^3H_4 method. Each lane represents the equivalent of 300 μg of cellular protein. (A) Detection of gangliosides by resorcinol-HCl staining. (B) Autoradiogram of the same chromatogram exposed for 7 d. Lanes 1 and 5 are derived from resident macrophages; lanes 2 and 6 are derived from BCG-activated macrophages; lanes 3 and 7 are derived from TG-elicited macrophages; lane 4 contains standard gangliosides derived from bovine brain and these are indicated in the margin. Individual ganglioside species in the GM_1 region are numbered 1-4.

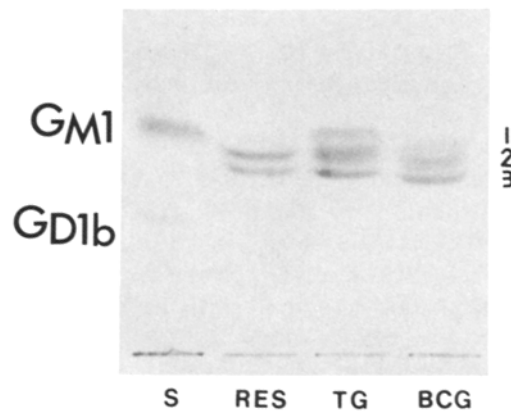


FIGURE 4. Immunochromatography of gangliosides derived from mouse peritoneal macrophages. Gangliosides from resident, BCG-activated, and TG-elicited macrophages were separated by high performance, thin layer chromatography and the chromatogram was exposed to the beta subunit of cholera toxin (cholera genotoxin) conjugated to HRP. The HRP was visualized by carrying out the peroxidase reaction. The individual GM_1 species corresponding to those in Figs. 3 and 5 are indicated at the right.

To obtain additional structural information on the GM₁ species present in TG-elicited macrophages, the region of the TLC plate containing these species was scraped and subjected to additional studies. Gas-liquid chromatographic analysis indicates that they contain galactose, glucose, and *N*-acetylgalactosamine in a ratio of 2:1:1, substantiating the presence of GM₁ (see Table I). Analysis of the neuraminic acid content of these GM₁ species indicates that they contain an approximately equal mixture of *N*-acetyl and *N*-glycolyl neuraminic acid. Such differences in neuraminic acid, as well as differences in ceramide structure, could contribute to the presence of distinct GM₁ species.

Interestingly, alterations in the relative chemical amounts of the GM₁ species exist among the macrophage populations (Fig. 3A). As indicated above, one of the species appears to be totally absent from resident macrophages and it is present only in TG-elicited and BCG-activated macrophages; moreover, this first GM₁ species is more abundant in TG-elicited macrophages than in BCG-macrophages. The other major change involves the third GM₁ species. This species is more abundant in TG-elicited macrophages than in either resident or BCG-activated macrophages. These alterations are readily apparent in the densitometric scan of the corresponding TLC plate (Fig. 5).

The galactose oxidase/NaB³H₄ labeling of surface gangliosides (Fig. 3B) indicates that the three species of GM₁ are the predominant gangliosides labeled on the macrophage surface, and also points out interesting differences between the relative amounts of these species and their accessibility to galactose oxidase/NaB³H₄ labeling. The differences are visualized easily by comparing the densi-

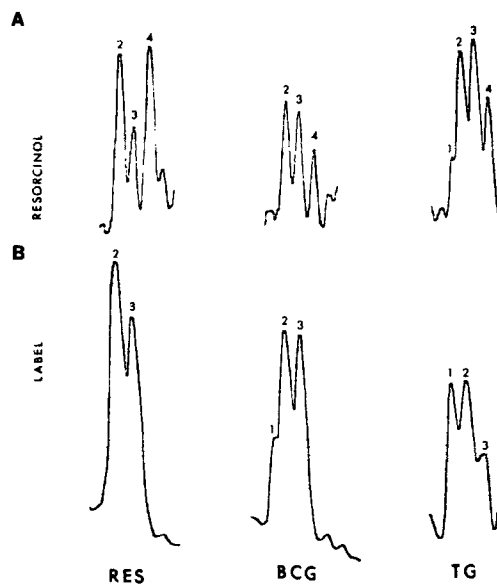


FIGURE 5. Densitometric scan of the GM₁ region of the high performance, thin layer chromatogram shown in Fig. 3. (A) Scan of the resorcinol-HCl stained chromatogram (Fig. 3A). (B) Scan of the autoradiogram of this chromatogram (Fig. 3B). The numbered peaks correspond to the numbered GM₁ species in Fig. 3. The densitometric analysis was performed using a Zenith soft-laser densitometer.

tomeric scans of the resorcinol stain and autoradiogram of the same TLC plate (Fig. 5). A striking contrast is observed in the labeling of the third GM₁ species between TG-elicited and BCG-activated macrophages. This GM₁ species is labeled intensely on the surface of BCG-activated macrophages and only faintly on TG-elicited macrophages even though, as stated above, it is more abundant in TG-elicited macrophages. Both of the GM₁ species present in resident macrophages are labeled intensely on the surface. No labeling of the first GM₁ species is evident on resident macrophages, providing additional evidence that it is absent from this population of macrophages. The first GM₁ species is labeled on the surface of BCG-activated macrophages, even though it is barely detectable by resorcinol staining and cholera toxin binding. It is labeled prominently on the surface of TG-elicited macrophages.

Discussion

An understanding of the mechanism by which macrophages acquire the capacity for the recognition and subsequent lysis of tumor cells is one of the key unresolved problems in macrophage cell biology. Recent studies have focused, in part, on alterations in surface proteins that accompany tumoricidal activation and on their potential functional significance (1-4). In contrast, before our work, little information that dealt with macrophage surface carbohydrates was available. The results presented in this paper are concerned with a major class of surface carbohydrates, the glycolipids. Comparison of populations of resident, TG-elicited, and tumoricidally activated macrophages indicates significant differences in the chemical amount and accessibility to galactose oxidase/NaB³H₄ labeling of their glycolipid constituents. Therefore, we conclude that a chemical and spatial reorganization of macrophage surface glycolipids occurs in response to tumoricidal activation and inflammation.

It is well established that the accessibility of surface glycolipids to external probes provides an indication of their degree of surface exposure (5, 23). The two most widely used probes for measuring glycolipid exposure are vectorial galactose oxidase/NaB³H₄ labeling and antibody binding. Galactose oxidase/NaB³H₄ labeling was the method of choice in this study, since it detects a broader spectrum of glycolipids and it is not limited by technical considerations such as antigen concentration. In the present work, we minimized the possibility of labeling internal membrane compartments by performing the labeling procedure at 4°C (21). It is worth mentioning, however, that we did observe similar results in the experiments in which the macrophages were labeled at 37°C.

The biochemical basis for differences in glycolipid exposure has not been elucidated in detail for any system, although proteins (5) and other carbohydrates (24) have been implicated as factors influencing the degree of glycolipid exposure. Differences in the glycolipid structure itself may also affect exposure (23). The alterations in surface exposure observed in the present study do not appear to result from a nonspecific increase in accessibility to galactose oxidase/NaB³H₄ labeling in TG-elicited and tumoricidally activated macrophages. This is evidenced by the observation that GM₁ is labeled on the surface of resident macrophages while asialo GM₁, although present, is not accessible to labeling on these macrophages. Rather, our results provide evidence for a selective exposure

of specific glycolipids. The differences in exposure may relate to alterations in surface polypeptides (25) and fatty acids (26, 27) that have been reported to occur in activated macrophages.

An intriguing observation is that TG-elicited and tumoricidally activated macrophages contain a third GM₁ species, or subtype, apparently not present in resident macrophages. The possible functional implications of this additional subtype are unclear. We are currently in the process of obtaining additional structural information on these individual GM₁ species, which should provide insight into the biochemical basis of the alterations in glycolipid composition.

The biochemical and immunological data presented in this study demonstrate convincingly that asialo GM₁ is present in the mouse peritoneal macrophage, and that it is accessible to labeling on the surface of TG-elicited and tumoricidally activated macrophages but not on resident macrophages. Asialo GM₁ has received considerable attention as a surface marker, particularly since it has been reported to be a selective marker for natural killer cells (8). The presence of asialo GM₁ on the surface of macrophages has been a matter of dispute (e.g., 6, 7), and no data before this study had been available on its surface distribution among populations of macrophages differing in their state of activation. One inherent problem in many of these studies is that their conclusions have been derived largely on the basis of immunological data, using antibodies that have not been adequately characterized. The anti-asialo GM₁ antibody used in our work has been well characterized and is known to react with asialo GM₁ on the surface of mouse lymphocytes (19).

In recent studies on macrophage activation, a major strategy undertaken has involved the identification of components that distinguish the surfaces of inflammatory and tumoricidally activated macrophages from resident macrophages (1–4). Our results suggest that surface differences among macrophage populations need not necessarily be qualitative, but may also reside in the spatial organization of surface components. This possibility is supported by the differences in ganglioside exposure observed between BCG-activated and TG-elicited macrophages, as well as by the selective exposure of asialo GM₁ on the surface of BCG-activated and TG-elicited macrophages. Moreover, as will be reported elsewhere (Mercurio, A. M., G. A. Schwarting, and P. W. Robbins, manuscript in preparation) a spatial reorganization of surface glycolipids appears to accompany the *in vitro* acquisition of tumoricidal capacity by TG-elicited macrophages in the presence of gamma interferon and LPS. It is interesting to speculate that a spatial reorganization of surface components could serve to increase the accessibility of those macrophage components required for effective tumor cell interactions.

In summary, the results presented in this paper demonstrate that the glycolipid constituents of the mouse peritoneal macrophage are dynamic structures exhibiting specific chemical and spatial alterations in response to inflammation and tumoricidal activation. Moreover, taken together with our other recent work on protein-bound carbohydrates (28, 29),² the present data substantiate the hypoth-

² Mercurio, A. M., and P. W. Robbins. 1984. Marked structural alterations in a specific class of protein-bound surface carbohydrates—the lactosaminoglycans—occur in response to inflammation and tumoricidal activation of mouse peritoneal macrophages. Manuscript submitted for publication.

esis that macrophage activation is accompanied by widespread alterations in surface carbohydrates.

Summary

We have characterized the major glycolipid constituents of the mouse peritoneal macrophage, and have demonstrated that alterations in the amount and in the accessibility of specific glycolipid species to galactose oxidase/ NaB^3H_4 labeling, an indicator of glycolipid surface exposure, occur in response to inflammation and as a consequence of activation to a tumoricidal state. The key findings are: (a) Asialo GM_1 , a major neutral glycolipid constituent of all macrophage populations examined, is accessible to galactose oxidase/ NaB^3H_4 labeling on the surface of TG-elicited and BCG-activated macrophages but not on resident macrophages; (b) GM_1 is the predominant ganglioside constituent of the mouse macrophage. Resident macrophages contain two distinct GM_1 species, as determined by cholera toxin binding, while TG-elicited and BCG-activated macrophages contain an additional GM_1 species. Differences in the relative amounts of these GM_1 species, as well as in their accessibility to galactose oxidase/ NaB^3H_4 labeling, exist among the macrophage populations. These observations suggest that both a chemical and spatial reorganization of surface glycolipids occurs in response to inflammation and tumoricidal activation.

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