INFECTION AND IMMUNITY, Sept. 1990, p. 2888–2894
0019-9567/90/092888-07$02.00/0

Vol. 58, No. 9

Altered B-Lymphocyte Membrane Architecture Indicated by Ganglioside Accessibility in C3H/HeJ Mice

HERBERT C. YOHE,* CHARLES S. BERENSON, CAROLE L. CUNY, AND JOHN L. RYAN†

Department of Internal Medicine, Yale University School of Medicine, and Veterans Administration Medical Center, West Haven, Connecticut 06516

Received 13 April 1990/Accepted 18 June 1990

We have analyzed both the total ganglioside composition and the surface accessibility of C3H/HeN B lymphocytes and C3H/HeJ B lymphocytes. Seventeen individual resorcinol-positive moieties were visualized by two-dimensional thin-layer chromatography of the purified gangliosides from both strains. Complete homology between strains was seen in the patterns of total gangliosides purified from the endotoxin-responsive and -hyporesponsive strains, with only minor differences in the relative concentrations of four gangliosides. In comparison, only 12 individual gangliosides were accessible to surface labeling following galactose oxidase treatment in these same strains, suggesting that some gangliosides are masked at the cell surface in both strains. However, labeling of the more polar components was greatly reduced in the endotoxin-hyporesponsive (C3H/HeJ) strain, suggesting that these gangliosides have decreased accessibility to galactose oxidase at the cell surface. Therefore, while the total ganglioside compositions of the two strains were nearly equivalent, there were dramatic differences in ganglioside surface accessibility. These findings indicate that an alteration in membrane structure that is associated with the endotoxin hyporesponsiveness observed in C3H/HeJ B lymphocytes exists.

Studies on the cellular and molecular mechanisms of activity of bacterial endotoxins have been facilitated by the existence of genetically hyporesponsive murine strains. In 1968, Sultzer (28) determined that the C3H/HeJ strain was defective with respect to certain physiologic and pathologic responses to endotoxins. Since that time, a vast amount of investigation has helped unravel the genetic basis of hyporesponsiveness to endotoxins (22, 24). The *Lps* gene has been mapped to a single locus on chromosome 4. The product of this locus has remained undefined. Several years ago, Jakobovits et al. (15) were able to reconstitute endotoxin responsiveness in C3H/HeJ B lymphocytes by fusing them with liposomes containing membrane extracts from syngeneic endotoxin-responsive B lymphocytes. Kuus-Reichel and Ulevitch (17) also obtained partial restoration of endotoxin responsiveness in C3H/HeJ B cells by treating the cells with the proteolytic enzyme trypsin. A recent study (11) has shown that certain rough chemotypes of lipopolysaccharide (LPS) have the ability to elicit responses from C3H/HeJ B lymphocytes. Taken together, these data suggest that the endotoxin-triggering mechanism is intact but may be occluded in the plasma membrane of C3H/HeJ cells.

Gangliosides are sialic acid-containing glycosphingolipids which are located predominantly in the plasma membrane of eucaryotic cells. These amphipathic molecules are anchored in the lipid bilayer by their hydrophobic ceramide group, while oligosaccharide-sialic acid extends toward the external environment. Although glycoproteins usually represent the high-affinity receptors for lymphocyte stimulation, gangliosides have been shown to represent receptors or components of receptor complexes in a variety of ligand-membrane interactions (16). Several bacterial toxins, certain cytokines, viruses, and hormones have been shown to bind to gangliosides (31). Miller et al. (20) have found that an exogenous ganglioside fraction could abrogate B-cell tolerance at levels which had no effect on the induction of antibody synthesis. Also, gangliosides shed by antigen-stimulated T cells inhibited certain B-cell functions (5). The accumulated evidence suggests that B-cell membrane gangliosides may serve as part of a receptor complex or triggering mechanism for immunoregulatory substances in B lymphocytes.

Chaby et al. (4) studied the lipids of isolated B lymphocytes from normal and C3H/HeJ mice. They examined the ability of B-cell gangliosides from both strains to react with galactose oxidase and reported that one of five gangliosides present in the LPS-responsive B-cell population was not detectable in the C3H/HeJ strain. There was a concomitant increase in another ganglioside in the C3H/HeJ strain. It was suggested that the loss of a specific ganglioside might be related to the lack of a response to endotoxin. The total ganglioside content and resorcinol-positive thin-layer chromatography (TLC) patterns were not determined. Thus, it remains to be determined whether there is a true alteration in the amounts of specific gangliosides in the B cells of C3H/HeJ mice or whether these B-cell gangliosides have an altered surface accessibility to galactose oxidase. We have therefore compared the total ganglioside patterns and galactose oxidase-susceptible ganglioside patterns of B cells from LPS-hyporesponsive C3H/HeJ mice with the corresponding patterns of B cells from the LPS-responsive C3H/HeN congenic strain of mice to investigate further whether an alteration in the amounts or surface accessibility of specific gangliosides may be involved in the expression of the *Lps* gene.

MATERIALS AND METHODS

Animals. C3H/HeJ female mice were purchased from Jackson Laboratory, Bar Harbor, Maine. C3H/HeN and BALB/c female mice were obtained from the National Institutes of Health facility at Frederick, Md. Endotoxin hyporesponsiveness in the C3H/HeJ mice was documented

* Corresponding author.
† Present address: Merck Sharp & Dohme, Blue Bell, PA 19422.

by testing the ability of protein-free versus protein-containing LPS preparations to induce lymphocyte mitogenesis (23).

Reagents. Media were obtained from Flow Laboratories, Inc., McLean, Va., or Whittaker MA Bioproducts, Walkersville, Md. Fetal calf serum was purchased from Sterile Systems, Logan, Utah.

Solvents used were high-performance liquid chromatography grade. All chemicals were standard analytical reagent quality. Goat anti-mouse immunoglobulin G (mu chain specific) was purchased from Organon Teknika, Malvern, Pa. Fluorescein-conjugated goat anti-mouse immunoglobulin G was also obtained from Organon Teknika.

Cell sources. Total spleen cells were obtained by suspending teased spleen cells in cold Hanks balanced salt solution and filtering the suspension through sterile nylon gauze. The cells were centrifuged at 200 × *g* for 10 min, and erythrocytes were lysed by suspending the pellet in 3 ml of lysing solution (0.15 M NH_4Cl, 0.01 M $KHCO_3$, 0.1 mM EDTA). Cells were washed twice with Hanks balanced salt solution and then suspended in Eagle minimal essential medium containing 10% fetal calf serum. B cells were isolated by the antibody-panning method of Wysocki and Sato (36). Viability was assessed by trypan blue exclusion.

Cells expressing surface immunoglobulin were assessed in all B-cell preparations by direct immunofluorescence with fluorescein isothiocyanate-conjugated goat anti-mouse immunoglobulin G (mu chain specific). No background fluorescence was noted.

Radioactive labeling of cell surface glycoconjugates. In accordance with the method of Chaby et al. (4), B cells of both strains were incubated in medium with 5% CO_2 and 95% humidity at 37°C for 14 h. Cell viability was 50 to 60% following this overnight incubation. Concentrations were adjusted to 5×10^7 viable cells per ml in phosphate-buffered saline (pH 7.0), and cells were labeled by a modification of the procedure of Gamberg et al. (12). Cells were incubated with gentle shaking for 3 h at 37°C with galactose oxidase (type V; Sigma Chemical Co., St. Louis, Mo.) at 5 IU/ml. After being washed with phosphate-buffered saline (pH 7.4), 10^8 cells per ml were gently agitated for 30 min at room temperature in the presence of sodium [^{3}H]borohydride (1 mCi/ml; specific activity, 20 Ci/mmol; Research Products International Corp., Mount Prospect, Ill.) to accomplish labeling. After five rinses with phosphate-buffered saline (pH 7.4), a sample was taken and total incorporated ^{3}H was measured in a liquid scintillation counter. The remaining cells were pelleted for extraction of gangliosides.

Isolation and analysis of B-cell gangliosides. For TLC analysis, B-cell pellets (3×10^8 cells) in 50-ml glass centrifuge tubes were extracted with 20 ml of chloroform-methanol (1:1 [vol/vol]). The tubes were placed on a rotating inverter overnight. Cellular debris was removed by filtering the extract through a sintered glass funnel overlaid with a glass fiber mat. The tube and funnel were serially rinsed twice with 5 ml of chloroform-methanol (1:1 [vol/vol]). Gangliosides were isolated by anion-exchange chromatography followed by Iatrobead chromatography as previously described in detail (39).

B-cell ganglioside patterns were examined by two-dimensional TLC on high-performance thin-layer chromatograms (10 by 10 cm; HPTLC Silica Gel 60 plates; E. Merck AG, Darmstadt, Federal Republic of Germany) by the method we have used previously for murine peritoneal macrophage gangliosides (39). The first solvent was comprised of chloroform–methanol–0.25% aqueous KCl (50:45:10 [vol/vol/vol]). After being dried in vacuo over P_2O_5 for 90 min, the plate was rotated 90° counterclockwise and developed in a second solvent comprised of chloroform–methanol–2.5 M NH_4 in 0.25% aqueous KCl (50:40:10 [vol/vol/vol]). Optimal TLC plates were obtained when 4 to 6 μg of B-cell ganglioside sialic acid was used per plate.

For autoradiographic analysis, gangliosides were extracted from 0.4×10^8 to 1×10^8 B lymphocytes. The total lipid extract contained 35×10^4 to 60×10^4 cpm/10^8 cells, and the isolated ^{3}H-labeled gangliosides contained 0.5×10^4 to 5.0×10^4 cpm/10^8 cells. TLC plates of ^{3}H-labeled gangliosides were sprayed with En^{3}Hance spray (New England Nuclear Corp., Westwood, Mass.) and visualized by autoradiography by exposure to hypersensitized XAR-5 film (Eastman Kodak Co., Rochester, N.Y.) for 2 to 3 weeks at −70°C. X-ray film was hypersensitized by the method of Smith et al. (25). Optimal hypersensitization was achieved by treatment with 7% hydrogen–93% nitrogen at 48°C for 16 h. Densitometric analyses of the autoradiographs were performed on a Microscan 1000 (Technology Resources, Inc., Nashville, Tenn.).

Total ganglioside sialic acid quantity and sialic acid types were determined by the gas-liquid chromatographic method of Yu and Ledeen (40).

Individual ganglioside quantification and ganglioside pattern analyses were done with a two-dimensional chromatographic analysis system (Microscan 1000) as previously described for other ganglioside preparations (2, 38). B-cell ganglioside pattern comparison analyses were performed for determining strain differences. Comparisons were initiated by identifying a minimum of three standard spots on each chromatogram. The chromatograms were then brought into registration via nonlinear translation, rotation, and scaling. The basic criteria for homology were distance to neighboring spots, size, shape, and height. Repeat analyses with known quantities of total gangliosides indicated an error of ±5% for spots containing >0.2 μg of sialic acid, with progressively greater error for lower-intensity spots. Calculation of data as relative percentages was influenced by cumulative errors as well as by the variations in the chromatograms of different preparations from the same source. Using brain ganglioside standards for comparison testing, we determined that consistent differences of ±15% for the relative percentages were necessary before differences in major spots were significant. In addition, large differences between chromatograms will affect the comparison of minor spots when data are reported as relative percentages. One means of partially overcoming this difficulty is to normalize the data to spots which have similar intensities in all samples. Data from both TLC pattern analyses and autoradiographs were compared by use of both relative percentages and normalized values. Because of the number of mice necessary for each preparation (50 mice for each strain, 100 per experiment), the number of preparations required to determine significance for minor spots was logistically not feasible. Since 4 μg was the minimum quantity needed for B-cell ganglioside analyses, significant differences in gangliosides containing <6% of the total sialic acid content (i.e., <0.2 μg of sialic acid) were not assigned.

RESULTS

Characterization of B-cell ganglioside preparations. B-cell preparations from both strains were examined for cell recovery and for purity by direct immunofluorescence for surface immunoglobulin. A yield of 10 to 15% total spleen cells with 94 to 99% surface immunoglobulin-expressing cells was

obtained. No strain difference was noted for either yield or purity. Cell viability was >96%, as estimated by trypan blue exclusion. B cells from both strains were found to contain 1 to 2 μg of ganglioside sialic acid per 10^8 cells. However, B-cell gangliosides showed a difference with respect to the sialic acid composition. Examination of three independent preparations of B cells showed that C3H/HeN gangliosides contained 53 to 55% *N*-glycolylneuraminic acid, while C3H/HeJ gangliosides contained 66 to 70%.

TLC analysis of B-cell ganglioside patterns. Two-dimensional TLC of B-cell gangliosides is shown in Fig. 1. Seventeen separate resorcinol-positive spots were defined. A comparison of the chromatographic mobilities of B-cell gangliosides with those of brain ganglioside standards indicated that B-cell gangliosides 6 and 13 comigrated with the brain gangliosides G_{D1a} and G_{M1}, respectively. The observation that most of the B-cell gangliosides did not comigrate with the brain gangliosides is consistent with the presence of *N*-glycolylneuraminic acid, which is not found in significant amounts in mammalian brain gangliosides (40).

Visual examination of the resorcinol-stained chromatograms indicated little difference between B-cell ganglioside patterns derived from strains C3H/HeN and C3H/HeJ. No gangliosides appeared in one strain but not the other. This observation was supported by pattern comparison analyses, which indicated complete homology between the strains (Table 1). Shifts in intensity (Table 1) were noted between strains for gangliosides 1, 3, 10, and 12. The relative percentages of gangliosides 1, 10, and 12 were higher in C3H/HeJ B cells than in C3H/HeN B cells, whereas the percentage of ganglioside 3 was lower. The greatest and probably only significant difference was seen for ganglioside 3, which represented less than 30% of the ganglioside sialic acid content of B cells in C3H/HeJ mice but 45% of that of B cells in C3H/HeN mice. Together, these four gangliosides (1, 3, 10, and 12) represented over 65% of the B-cell ganglioside sialic acid content for both strains. Differences were not noted for any other specific gangliosides, whether the distribution was examined by relative percentages or normalized to the value for ganglioside 11 (normalized values not shown).

Autoradiographic analysis of surface-labeled B-cell gangliosides. When C3H/HeN and C3H/HeJ B-cell gangliosides were examined for in situ susceptibility to surface labeling by galactose oxidase, profound quantitative differences were noted in most of the gangliosides detected (Fig. 2). Twelve individual gangliosides were labeled. Thus, at least five gangliosides were either totally inaccessible to the enzyme or unable to react with galactose oxidase. Quantitative densitometric analysis of the autoradiographs indicated intensity shifts in virtually all of the susceptible gangliosides (Table 2). The most profound differences between labeled C3H/HeN and C3H/HeJ B cells were in gangliosides 2, 3, 4, 6, and 10. Radiolabel in more slowly moving gangliosides was greatly decreased in C3H/HeJ samples as compared with C3H/HeN samples and, in a duplicate experiment, was nearly absent.

Comparisons between the autoradiographs and the TLC patterns were also made. Spots numbered 1, 2, 5, and 7 on the autoradiographs corresponded to ganglioside spots 1, 3, 9, and 11, respectively, on the thin-layer chromatograms (Fig. 1). Matching of other ^{3}H-labeled moieties to resorcinol-positive spots had a high degree of uncertainty.

^{3}H-labeled gangliosides were prepared from BALB/c B cells and C3H/HeN B cells in separate experiments. Densitometric analysis of the two-dimensional autoradiographic patterns revealed similar density distributions (Table 2). Both patterns showed the same distributional shift when compared with the labeled-ganglioside patterns seen in autoradiographs of C3H/HeJ B-cell gangliosides. Both endotoxin-responsive strains showed a higher percentage of acces-

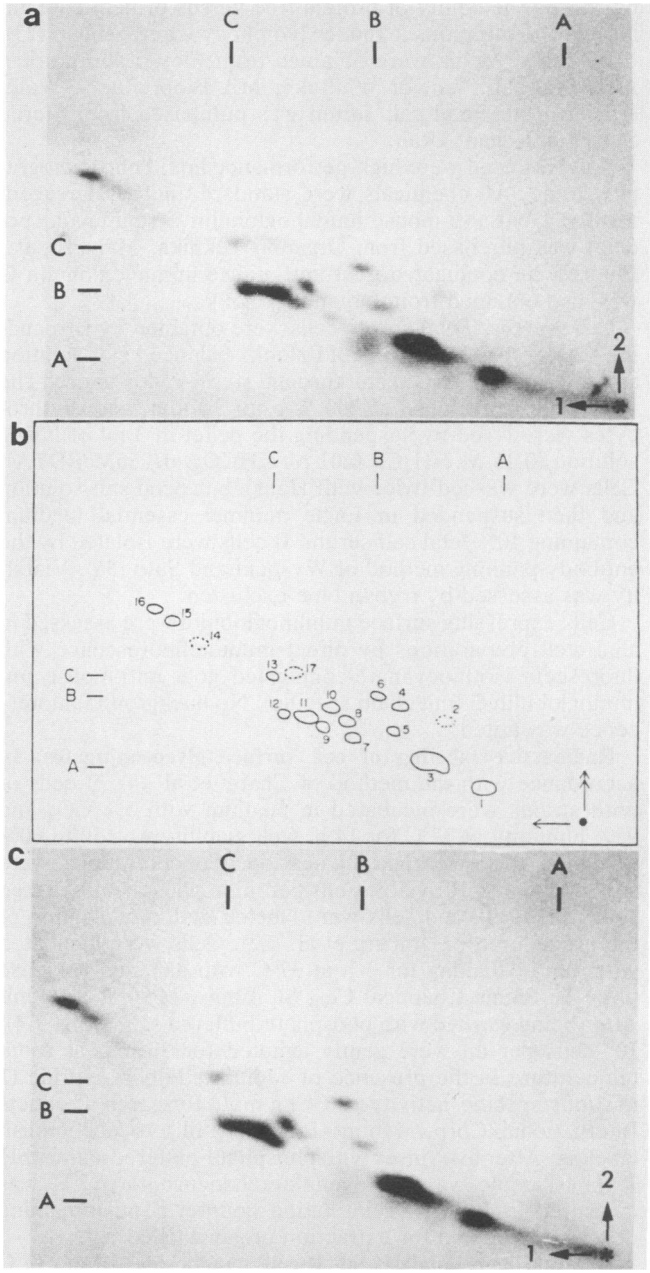

FIG. 1. Thin-layer chromatograms of ganglioside patterns of C3H/HeN (a) and C3H/HeJ (c) B cells. Gangliosides were isolated and separated by two-dimensional TLC as described in the text. The origin is indicated by an asterisk, with the numbered arrows indicating the direction of the first and second solvent runs. Prior to the run in each dimension, brain ganglioside standards were spotted in the margin opposite the sample. Standard ganglioside mobilities were as follows: A, G_{T1b}, IV^{3}NeuAc, II^3(NeuAc)$_2$-GgOSe$_4$Cer; B, G_{D1a}, IV^{3}NeuAc, II^{3}NeuAc-GgOSe$_4$Cer; and C, G_{M1}, II^{3}NeuAc-GgOSe$_4$Cer (30). Identified peaks are indicated in the numbered schematic (b). Broken ovals show spots that consistently represented ≤1% of the total content in both strains (Table 1).

TABLE 1. Relative percentages of resorcinol-positive B-cell gangliosides on thin-layer chromatograms

Peak[a]	Relative percent of total ganglioside content in:	
	C3H/HeN	C3H/HeJ
1[b]	8	14
2	tr[c]	tr
3	45	29
4	1	tr
5	3	2
6	4	4
7	1	1
8	1	2
9	4	5
10[b]	3	8
11	11	12
12[b]	11	15
13	3	1
14	tr	tr
15	1	2
16	4	4
17	tr	tr

[a] Peaks correspond to the schematic in Fig. 1.
[b] Because of the limits of analysis (see Materials and Methods), the differences seen for gangliosides 1, 10, and 12 may not be significant.
[c] tr, Trace (spots representing 0.5% of the total).

sible gangliosides with slower mobilities than did the C3H/HeJ strain, with the BALB/c strain showing the highest percentage in one slow-moving ganglioside (ganglioside 2). This distributional shift was emphasized when the data were normalized to the intensity for ganglioside 7 (Table 2).

The profound quantitative labeling differences between C3H/HeN and C3H/HeJ B-cell gangliosides were not mirrored in the total ganglioside patterns visualized by resorcinol. Indeed, the resorcinol-positive density of ganglioside 1 was greater in C3H/HeJ B cells than in C3H/HeN B cells, while the opposite was true in the autoradiograph. Thus, membrane gangliosides from C3H/HeJ B lymphocytes and C3H/HeN B lymphocytes react differently with galactose oxidase.

DISCUSSION

Gangliosides have been implicated as receptors for many immunomodulatory substances, including lymphokines (21), interferons (3, 33) viruses (14, 29), toxins (10), and glycoprotein hormones (16). Recently, Spiegel and Fishman (26) showed that gangliosides have a bimodal function by serving as receptors for signals which can either stimulate or inhibit cell growth. Chaby et al. (4) used galactose oxidase to probe the surface of B lymphocytes and reported that the B cells isolated from C3H/HeJ mice contained one less susceptible ganglioside than did the B cells isolated from a normal strain of mice. Using a similar antibody-panning technique (36), we confirmed the altered surface accessibility of C3H/HeJ B-cell gangliosides as compared with that of congenic C3H/HeN B-cell gangliosides. Autoradiographic density shifts were seen in nearly all of the labeled gangliosides from C3H/HeJ B cells, as compared with those from C3H/HeN B cells. This result is strikingly different from that of Chaby et al. (4). The difference may be partly due to our use of hypersensitized film (25), which permitted a more detailed analysis of the minor gangliosides. In addition, the different solvent system that we used for two-dimensional TLC analysis may account for some of the disparity. We found no

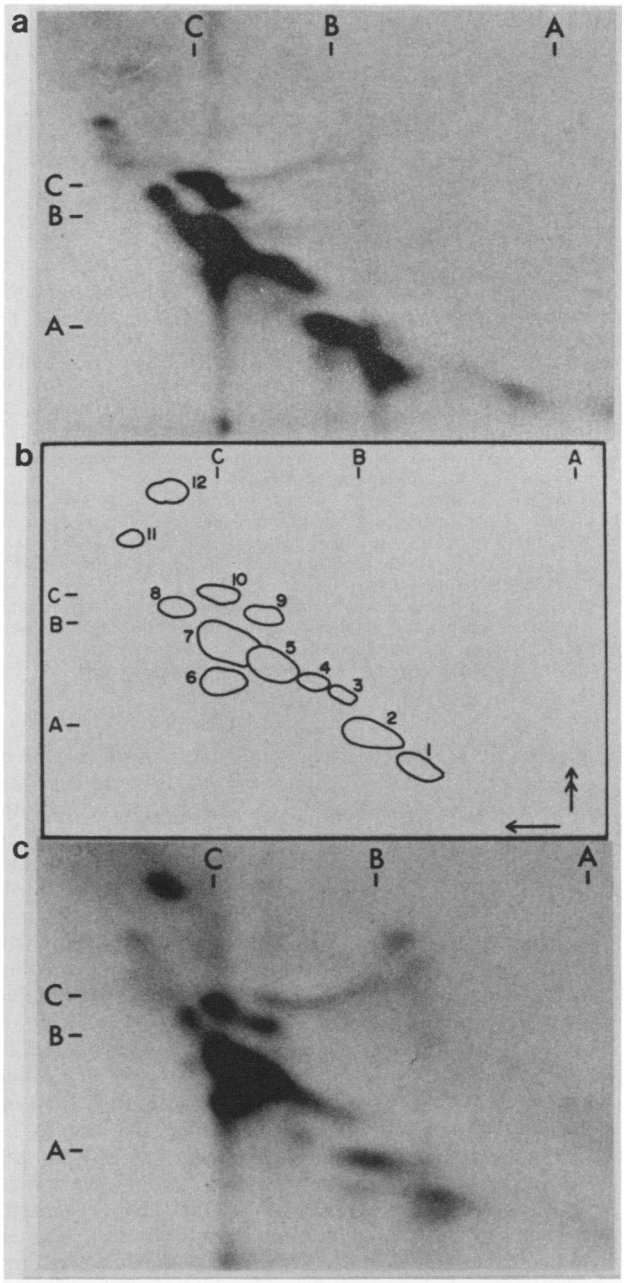

FIG. 2. Autoradiographs derived from thin-layer chromatograms of ^{3}H-labeled gangliosides of C3H/HeN (a) and C3H/HeJ (c) splenic B cells. Identified peaks are indicated in the numbered schematic (b). The orientation is as described for Fig. 1.

ganglioside missing but noted quantitative differences in both major and minor gangliosides between the two congenic strains.

The total ganglioside content and distribution in C3H/HeN and C3H/HeJ B cells were very similar and did not mirror the ganglioside content determined by accessibility to galactose oxidase. This result is not surprising, as it is known from the exogenous neuraminidase studies of Barton and Rosenberg (1) that gangliosides of many cell types are not readily accessible to large molecules. Furthermore, Stein et al. (27) previously noted the presence on murine B cells of glycolipids that are masked to glycolipid antibodies but can

TABLE 2. Distribution of surface-labeled B-cell gangliosides on autoradiographs

Peak[a]	Relative percent (normalized)[b] of total ganglioside content in:		
	C3H/HeN	C3H/HeJ	BALB/c
1	13 (0.9)	8 (0.4)	12 (1.0)
2	20 (1.4)	10 (0.5)	31 (2.8)
3	6 (0.4)	1 (<0.1)	4 (0.4)
4	8 (0.6)	4 (0.2)	5 (0.5)
5	14 (1.0)	18 (0.9)	6 (0.5)
6	7 (0.5)	20 (1.0)	5 (0.5)
7	14 (1)	20 (1)	11 (1)
8	3 (0.2)	3 (0.2)	6 (0.5)
9[c]	6 (0.4)	3 (0.2)	9 (0.8)
10	9 (0.6)	4 (0.2)	10 (0.9)
11	1 (0.1)	tr[d]	tr
12	tr	8 (0.4)	tr

[a] Peaks correspond to the schematic in Fig. 2.

[b] As indicated in Materials and Methods, the expression of quantitative data on relative percentages is influenced by cumulative differences on the chromatograms. Therefore, the density distribution is also reported normalized to ganglioside 7, which appeared similar in all the autoradiographs (numbers in parentheses).

[c] Although spot 9 of each autoradiograph was matched by the densitometer as a homologous peak, small differences in the mobilities of the spots were noted (Fig. 2), suggesting that this match of peaks may be marginal.

[d] Trace (minor peaks whose intensities could not be quantitated).

be exposed by appropriate enzyme treatment. The data of Kuus-Reichel and Ulevitch may relate to this altered surface expression of gangliosides. They found that proteolytic treatment with trypsin enhanced LPS responsiveness (17). In similar fashion, Flebbe et al. reported that certain rough chemotypes of LPS were able to stimulate C3H/HeJ B lymphocytes (11). These chemotypes may be inserted into the membrane to trigger a response because of their hydrophobic properties. An interaction with an occluded ganglioside is one possible event that may be required for effective signal transduction.

There was very little correlation between spot intensities measured by resorcinol staining and autoradiography. Since there were five fewer spots on the autoradiograph than on the TLC plate, several gangliosides appeared not to be accessible to galactose oxidase. Whether these were the same five gangliosides in both strains cannot be determined with certainty. It is clear, however, that the autographs showed major quantitative differences in the surface expression of gangliosides in the endotoxin-responsive and congenic endotoxin-hyporesponsive mice but not in two genetically different endotoxin-responsive strains (C3H/HeN and BALB/c); i.e., the two normal strains had a similar surface expression of gangliosides. The alteration in membrane architecture reflected in these data may relate to the phenomenon of endotoxin hyporesponsiveness in the C3H/HeJ strain.

One ganglioside (ganglioside 1) on both the TLC plate and the autoradiograph was more concentrated in C3H/HeJ B cells than in C3H/HeN B cells (14 versus 8%) but appeared less accessible (8 versus 13%) by enzyme labeling. No individual ganglioside in either autoradiograph pattern represented more than 20% of the total ganglioside content, while ganglioside 3 represented 45% of the total ganglioside content on the C3H/HeN TLC plate. Thus, it is evident from these data that there is no loss of a major ganglioside to explain the endotoxin hyporesponsiveness in C3H/HeJ mice. There is, however, profoundly altered surface expression of individual gangliosides between the two strains. Differences in autoradiographic patterns were reproducible in several experiments, but small differences were often noted between experiments. Often the separation of the major ganglioside intensities was not distinct, probably because of differences in enzyme batches, TLC conditions, or autoradiographic conditions. Thus, all comparisons reported are from simultaneously labeled and extracted gangliosides from each strain. We were unable to define resorcinol-positive spots on the plates used in the autoradiographs because of the relatively smaller amount of ganglioside used to prepare these plates. This result underscores the far greater sensitivity of autoradiography in detecting small quantities of gangliosides.

An additional observation from these studies is the difference in sialic acid composition between gangliosides of the two strains. There was about 10% less NeuAc in C3H/HeJ B cells than in C3H/HeN B cells. Similar differences were noted in our earlier study of T cells and thymocytes from these strains (38). In both cases, there was a significant decrease in NeuAc in the C3H/HeJ cells. In our earlier report on murine macrophage gangliosides, the difference in sialic acid composition was less pronounced, but we found smaller amounts of NeuAc in resting macrophages than in lymphocyte populations (37, 39). One explanation for the difference in sialic acid composition between the two strains may be relative alterations in quantities of specific NeuAc-containing gangliosides. More detailed structural analyses are needed to assign structures to each of the major gangliosides in both macrophages and lymphocytes.

The biochemical basis of the C3H/HeJ B-cell hyporesponsiveness to LPS has been postulated to be due to the lack of an LPS receptor in the plasma membrane (6); others have failed to find such a receptor (34). Several groups have used derivatized LPS to probe for surface proteins interacting with endotoxin. Lei and Morrison (18, 19) recently identified an 80-kilodalton protein which interacts with LPS in a wide variety of lymphocytes, including cells from hyporesponsive and responsive murine strains. This result suggests that the simple presence of an LPS-binding protein does not confer LPS responsiveness on B lymphocytes. Other groups (13, 32) have found different proteins which bind endotoxin, but it is not yet clear which of these are functionally significant.

Since LPS is a natural detergent, this amphipathic molecule, upon insertion into the plasma membrane, perturbs the endogenous lipids. The effect of such an insertion depends not only on the amphipathic nature of the endotoxin but also on the composition and surface accessibility of the natural lipids. Support for the critical importance of the interaction of LPS with the plasma membrane has come from recent studies with liposomal lipid A. As compared with free lipid A, liposomal lipid A has profoundly decreased activity in eliciting interleukin-1 and tumor necrosis factor from murine macrophages (8, 9). These data are consistent with the need for LPS to interact directly with the plasma membrane to activate the transduction of a signal across the membrane.

Our recent preliminary studies of the structure of murine macrophage gangliosides (7) have shown that most of the gangliosides have a gangliotetraose structure. Thus, most of the gangliosides should be susceptible to galactose oxidase, and the inability to label significant moieties is more likely related to accessibility than to lack of reactivity. This theory, of course, requires formal proof, since murine B lymphocytes may contain structurally distinct gangliosides.

The accessibility of specific lymphocyte gangliosides to LPS is at this time unknown. We have begun using an

affinity-labeled, photoactivatable LPS derivative (35) which allows radioactive tagging of molecules in contact with the LPS derivative. These studies may help verify whether gangliosides play a role in the transduction of LPS-mediated responses.

These data show that there are profound differences in membrane architecture between C3H/HeN and C3H/HeJ B lymphocytes, as reflected in surface-accessible gangliosides. This result is emphasized by the data for the genetically distinct but normal BALB/c B lymphocytes, whose ganglioside surface accessibility was similar to that of the C3H/HeN B lymphocytes. The biochemical cause of this altered architecture is not clear but may be related to the defect associated with the *Lps* gene. A fundamental alteration in membrane structure would be consistent with all of the data on LPS hyporesponsiveness in C3H/HeJ mice. Further investigation of glycolipid and sialic acid metabolism in this strain is needed to determine whether the altered ganglioside accessibility is directly related to the LPS hyporesponsiveness or whether it is an additional consequence of the *Lps* gene defect.

ACKNOWLEDGMENTS

We acknowledge helpful discussions with John W. Mellors and appreciate the assistance of Karen A. Marino in preparing the manuscript.

This research was supported by the Veterans Administration and by Public Health Service grant AI 18099 from the National Institutes of Health (to J.L.R.).

LITERATURE CITED

1. **Barton, N. W., and A. Rosenberg.** 1973. Action of *Vibrio cholerae* neuraminidase (sialidase) upon the surface of intact cells and their isolated sialolipid components. J. Biol. Chem. **248**:7353–7358.
2. **Berenson, C. S., H. C. Yohe, and J. L. Ryan.** 1989. Factors mediating lipopolysaccharide-induced ganglioside expression in murine peritoneal macrophages. J. Leukocyte Biol. **45**:221–230.
3. **Besanson, F., H. Ankel, and S. Basu.** 1976. Specificity and reversibility of interferon-ganglioside interaction. Nature (London) **259**:576–578.
4. **Chaby, R., M.-J. Morelec, D. Enserqueix, and R. Girad.** 1986. Membrane glycolipid and phospholipid composition of lipopolysaccharide-responsive and -nonresponsive murine B lymphocytes. Infect. Immun. **52**:777–785.
5. **Correa, M., H. C. Miller, and W. J. Esselman.** 1980. Antigen induced modulation by shed lymphocyte membrane gangliosides. Immunol. Commun. **9**:543–557.
6. **Coutinho, A., L. Forni, and T. Watanabe.** 1978. Genetic and functional characterization of an antiserum to the lipid A-specific triggering receptor on murine B lymphocytes. Eur. J. Immunol. **8**:63–67.
7. **Cuny, C. L., H. C. Yohe, M. Saito, and J. L. Ryan.** 1989. Gangliosides of elicited murine macrophages: presence of sialidase-sensitive G_{M1}. FASEB J. **3**:A1104.
8. **Dijkstra, J., J. W. Larrick, J. L. Ryan, and F. C. Szoka.** 1988. Incorporation of LPS in liposomes diminishes its ability to induce tumoricidal activity and tumor necrosis factor secretion in murine macrophages. J. Leukocyte Biol. **43**:436–444.
9. **Dijkstra, J., J. W. Mellors, J. L. Ryan, and F. C. Szoka.** 1987. Modulation of the biological activity of bacterial endotoxin by incorporation into liposomes. J. Immunol. **138**:2663–2670.
10. **Fishman, P. H.** 1982. Role of membrane gangliosides in the binding and action of bacterial toxins. J. Membr. Biol. **69**:85–97.
11. **Flebbe, L., S. W. Vukajlovich, and D. C. Morrison.** 1989. Immunostimulation of C3H/HeJ lymphoid cells by R-chemotype lipopolysaccharide preparations. J. Immunol. **142**:642–652.
12. **Gamberg, C. G., K. Itaya, and S.-I. Hakomori.** 1976. External labeling of cell surface carbohydrates, p. 179–210. *In* E. D. Kore (ed.), Methods in membrane biology, vol. 7. Plenum Publishing Corp., New York.
13. **Hampton, R. Y., D. T. Golenbock, and C. R. H. Raetz.** 1988. Lipid A binding sites in membranes of macrophage tumor cells. J. Biol. Chem. **263**:14802–14807.
14. **Holmgren, J., L. Svennerholm, H. Elwing, P. Fredman, and O. Strannegard.** 1980. Sendai virus receptor: proposed recognition structure based on binding to plastic-adsorbed gangliosides. Proc. Natl. Acad. Sci. USA **77**:1947–1950.
15. **Jakobovits, A., N. Sharon, and I. Zan-Bar.** 1982. Acquisition of mitogenic responsiveness by nonresponding lymphocytes upon insertion of appropriate membrane components. J. Exp. Med. **156**:1274–1279.
16. **Kohn, L. D., S. M. Aloj, F. Beguinot, P. Vitti, E. Yanin, Z. Yavin, P. Laccetti, E. F. Grollman, and W. A. Valente.** 1982. Molecular interactions at the cell surface: role of glycoconjugates and membrane lipids in receptor recognition processes. Prog. Clin. Biol. Res. **97**:55–83.
17. **Kuus-Reichel, K., and R. J. Ulevitch.** 1986. Partial restoration of the lipopolysaccharide-induced proliferative response in splenic B cells from C3H/HeJ mice. J. Immunol. **137**:472–477.
18. **Lei, M.-G., and D. C. Morrison.** 1988. Specific endotoxic lipopolysaccharide-binding proteins on murine splenocytes. I. Detection of lipopolysaccharide-binding sites on splenocytes and splenocyte populations. J. Immunol. **141**:996–1005.
19. **Lei, M.-G., and D. C. Morrison.** 1988. Specific endotoxic lipopolysaccharide-binding proteins on murine splenocytes. II. Membrane localization and binding characteristics. J. Immunol. **141**:1006–1011.
20. **Miller, H. C., W. G. Chaney, N. R. Klinman, and W. J. Esselman.** 1982. Regulation of B cell tolerance by murine gangliosides. Cell. Immunol. **67**:390–395.
21. **Parker, J., G. Caldini, C. Krishnamurti, P. B. Ahrens, and H. Ankel.** 1984. Binding of interleukin 2 to gangliosides. FEBS Lett. **170**:391–395.
22. **Rosenstreich, D. L.** 1985. Genetic control of endotoxin response: C3H/HeJ mice, p. 82–122. *In* L. J. Berry (ed.), Handbook of endotoxin, vol. 3. Cellular biology of endotoxin. Elsevier Science Publishers, Amsterdam.
23. **Ryan, J. L., R. D. Arbeit, H. B. Dickler, and P. A. Henkart.** 1975. Inhibition of lymphocyte mitogenesis by immobilized antigen-antibody complexes. J. Exp. Med. **142**:814–826.
24. **Scibienski, R. J.** 1981. Defects in murine responsiveness to bacterial lipopolysaccharide. The C3H/HeJ and C57BL/10ScCr strains, p. 241–258. *In* M. E. Gershwin and B. Merchant (ed.), Immunologic defects in laboratory animals. Plenum Publishing Corp., New York.
25. **Smith, A. G., A. Phillips, and E. J. Hahn.** 1985. X-ray films; suppression of reciprocity failure by astronomical techniques. J. Imaging Technol. **11**:27–32.
26. **Spiegel, S., and P. H. Fishman.** 1987. Gangliosides as bimodal regulators of cell growth. Proc. Natl. Acad. Sci. USA **84**:141–145.
27. **Stein, K. E., G. A. Schwarting, and D. M. Marcus.** 1978. Glycolipid markers of murine lymphocyte subpopulations. J. Immunol. **120**:676–679.
28. **Sultzer, B. M.** 1968. Genetic control of leukocyte responses to endotoxin. Nature (London) **219**:1253–1254.
29. **Suzuki, Y., M. Matsunaga, and M. Matsumoto.** 1985. N-acetylneuraminyllactosylceramide, G_{M3}-NeuAc, a new influenza A virus receptor which mediates the adsorption-fusion process of viral infection. J. Biol. Chem. **260**:1362–1365.
30. **Svennerholm, L.** 1963. Chromatographic separation of human brain gangliosides. J. Neurochem. **10**:613–623.
31. **Symington, F. W., and S.-I. Hakomori.** 1985. Glycosphingolipids of immune cells. Lymphokines **12**:201–238.
32. **Tobias, P. S., and T. N. Kirkland.** 1988. Binding of LPS to a specific membrane protein of 70Z/3 cells is required for cell activation. FASEB J. **2**:A991.
33. **Vengris, V. E., R. H. Reynolds, Jr., M. D. Hollenberg, and P. J. Pitha.** 1976. Interferon action: role of membrane gangliosides. Virology **72**:486–493.

34. **Watson, J., K. Kelly, and C. Whitlock.** 1980. Genetic control of endotoxin sensitivity, p. 4–10. *In* D. Schlessinger (ed.), Microbiology—1980. American Society for Microbiology, Washington, D.C.
35. **Wollenweber, H.-W., and D. C. Morrison.** 1985. Synthesis and biochemical characterization of a photoactivatable, iodinatable, cleavable bacterial lipopolysaccharide derivative. J. Biol. Chem. **260**:15068–15074.
36. **Wysocki, L. J., and V. I. Sato.** 1978. "Panning" for lymphocytes: a method for cell selection. Proc. Natl. Acad. Sci. USA **75**:2844–2848.
37. **Yohe, H. C., D. L. Coleman, and J. L. Ryan.** 1985. Ganglioside alterations in stimulated murine macrophages. Biochim. Biophys. Acta **818**:81–86.
38. **Yohe, H. C., C. L. Cuny, C. S. Berenson, and J. L. Ryan.** 1988. Comparison of thymocyte and T lymphocyte gangliosides from C3H/HeN and C3H/HeJ mice. J. Leukocyte Biol. **44**:521–528.
39. **Yohe, H. C., and J. L. Ryan.** 1986. Ganglioside expression in macrophages from endotoxin responder and nonresponder mice. J. Immunol. **137**:3921–3927.
40. **Yu, R. K., and R. Ledeen.** 1970. Gas-liquid chromatographic assay of lipid-bound sialic acids: measurement of gangliosides in brain of several species. J. Lipid Res. **11**:506–516.