GANGLIOSIDES AS MARKERS FOR MURINE LYMPHOCYTE SUBPOPULATIONS*

By KATHRYN E. STEIN-DOUGLAS,‡ GERALD A. SCHWARTING,§ MASAHARU NAIKI, AND DONALD M. MARCUS

(From the Departments of Microbiology and Immunology and Medicine, Albert Einstein College of Medicine, Bronx, N. Y. 10461)

Although theta antigens (Thy-1) have been widely used as a marker for thymocytes and peripheral T cells in the mouse, the chemical nature of these antigens has not been clearly established. On the basis of observations that theta antigen was nondialyzable and could not be recovered after lipid extraction, Reif and Allen (1) suggested that these antigens were lipoproteins. Vitetta et al. reported (2) that the antigenicity of Thy-1 was lost upon treatment with nonionic detergent and that cell surface Thy-1 could be labeled with radioactive galactose, but not with amino acids, and suggested that Thy-1 might be a glycolipid. Esselman and Miller (3) reported that the cytotoxicity of anti-Thy-1.2 serum was inhibited by G_{M1} ganglioside (Table I), and that G_{D1b} ganglioside inhibited the cytotoxicity of rabbit antibrain-associated theta antiserum (BAθ)[1] (4). They proposed that G_{M1} is θC3H (Thy-1.2) antigen, and that G_{D1b} is BAθ antigen.

We have previously reported (5) the properties of rabbit antibodies to G_{M1} ganglioside. In this report we describe the reactivity of purified antibodies to G_{M1} ganglioside with murine lymphoid cells. Anti-G_{M1} antibodies react with murine thymocytes and T cells, but this reactivity is independent of Thy-1 type, and it appears to be associated with cross-reacting antibodies rather than the antibody specific for G_{M1}.

Materials and Methods

Mice. AKR/J (*H-2*^{k}, Thy-1.1), C3H/HeJ and C3HeB/FeJ (*H-2*^{k}, Thy-1.2), C3H·SW (*H-2*^{b}, Thy-1.2), and BALB/c J (*H-2*^{d}, Thy-1.2) mice were purchased from The Jackson Laboratory, Bar Harbor, Maine. C3H/fAn (*H-2*^{k}, Thy-1.2) and A (*H-2*^{a},[2] Thy-1.2) mice were provided by Dr. Frank Lilly from colonies maintained at Albert Einstein College of Medicine. BALB/c NIH (*H-2*^{d}, Thy-1.2) mice, West Seneca Laboratory, West Seneca, New York, were provided by Dr. Matthew Scharff. Male nude, nu/nu, and heterozygous nude, +/nu, mice, which had been bred on BALB/c background, were provided by Dr. Seung-il Shin from colonies maintained at Albert Einstein. The nude mice used in these studies were routinely examined and found to lack thymuses.

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[1] *Abbreviations used in this paper:* BAθ, brain-associated theta; BSA, bovine serum albumin; CT, cholera toxin; FCS, fetal calf serum; IF, immunofluorescence; LPS, lipopolysaccharide; MEM, Eagle's minimum essential medium; MIg, mouse immunoglobulin; PBS, phosphate-buffered saline; RGG, rabbit gamma globulin; SIg, surface immunoglobulin.

[2] Recombinant strain between *H-2*^{k} and *H-2*^{d}.

TABLE I
*Glycosphingolipid Structures**

Asialo G_{M1}	Gal(β,1→3)GalNAc(β,1→4)Gal(β,1→4)Glc-Cer
G_{M1}	Gal(β,1→3)GalNAc(β,1→4)Gal(β,1→4)Glc-Cer 3 ↑ α 2 NeuNAc
G_{D1b}	Gal(β,1→3)GalNAc(β,1→4)Gal(β,1→4)Glc-Cer 3 ↑ α 2 NeuNac(α,8←2)NeuNAc

* Abbreviations: Glc, D-glucose; Gal, D-Galactose; GalNAc, *N*-acetyl-D-galactosamine; NeuNAc, *N*-acetylneuraminic acid; Cer, ceramide.

Cells. Mice were sacrificed by cervical dislocation, and thymuses and mesenteric lymph nodes were removed and minced in Eagle's minimum essential medium with glutamine (2 mM) (MEM) (Grand Island Biological Co., Grand Island, N. Y.) supplemented with 2% heat-inactivated fetal calf serum (FCS). Cell suspensions were prepared by passing the minced tissue several times through a double-hubbed 20-g needle attached to two 3-ml syringes. The suspensions were filtered through a funnel containing a loosely packed glass wool plug, prewashed in MEM plus 2% FCS, to remove debris, and the cells were centrifuged at 250 *g* for 10 min at 4°C and resuspended in 4 ml MEM plus 2% FCS. Lymphocytes were purified by Ficoll-Hypaque centrifugation (6), centrifuged at 300 *g* for 10 min at 4°C, washed three times at 250 *g* for 10 min at 4°C in pH 7.1–7.2 phosphate-buffered saline containing 2% bovine serum albumin and 4 mM NaN_3 (PBS-BSA), and resuspended for immunofluorescence (IF) in PBS-BSA at a concentration of 0.5–2.0 × 10^7 lymphocytes per ml. Splenic lymphocytes were prepared as above except that the spleens were processed in MEM containing 10% FCS, and cell suspensions were incubated in this medium with carbonyl iron for 60 min at 37°C before placing them on Ficoll-Hypaque. Splenic lymphocytes purified on Ficoll-Hypaque were processed as above for IF.

Absorption with Nude Spleen Cells. The spleens from six male nu/nu mice were minced, pooled, and a cell suspension was prepared, as described above, in MEM containing 5% FCS. The cells were washed three times in PBS-BSA and packed in a conical bottomed tube to which an equal volume of antibody was added. Absorption was carried out for 1 h in an ice bath, during which the tube was mixed frequently. The cells were centrifuged at 4°C, and the antibody was diluted appropriately for immunofluorescence in PBS-BSA and subjected to ultracentrifugation as described below.

Antisera. Rabbit antibodies to glycolipids (5) were purified on appropriate glycolipid-polyacrylamide gel immunoadsorbent columns (7). Rabbit antisera to mouse (CBA) brain, obtained from Dr. Edward Golub (Purdue University), was absorbed with CBA kidney, liver, and red cells and was used as a source of antibrain-associated theta (BAθ). Rhodamine-labeled sheep antirabbit gamma globulin (RGG) was prepared by the method of Preud'homme (8). Fluorescein-labeled goat antimouse IgG (MIg) was purchased from Meloy Laboratories, Inc., Springfield, Va. The specificity of the commercial anti-MIg was determined by cell surface staining of mouse myeloma cell lines, provided by Ms. Lucille Frank in the laboratory of Dr. Matthew Scharff (Albert Einstein College of Medicine). The antibody stained kappa, IgG2b (kappa), IgG1 (kappa), and IgM (lambda) producing lines, but not the nonproducing variant, NP2.

All antibodies to glycolipids were titered by a semiquantitative complement fixation assay, performed with a microtiter apparatus (5), with purified glycolipids. These antibodies were also titered by IF with thymocytes and lymph node lymphocytes. All experiments were performed with two preparations of immunoadsorbent-purified antibody from a pool of anti-G_{M1} serum made from several bleedings of a single rabbit (R525) (see Table II).

IF. With the exception of surface immunoglobulin, which was stained directly, an indirect IF

technique was used in all experiments. To avoid possible staining by aggregated IgG, all antisera were ultracentrifuged (140,000 *g* for 40 min) once a week. Cell surface staining was performed on viable lymphocytes by the method of Preud'homme and Seligmann (9). Briefly, 0.025 ml of cell suspension in PBS-BSA were incubated with 0.025 ml of antibody for 30 min at 4°C. The cells were washed three times with cold PBS-BSA and resuspended in the last drop of the third wash (after draining the tubes well in an inverted position). The cells were then treated with 0.025 ml of fluorescent antibody, incubated, washed, and resuspended as before. (In experiments including direct staining, those cells were also carried through a two-stage procedure to treat all tubes identically; PBS-BSA was used during the first incubation and the fluorescein-labeled anti-MIg during the second.) In double-label experiments, cells were first incubated with rabbit anti-G_{M1} and then with a mixture of rhodamine-labeled sheep anti-RGG and fluorescein-labeled goat anti-MIg. In addition, the percent of G_{M1} and surface immunoglobulin (SIg)-positive cells was determined separately in tubes of cells stained for a single specificity. Another control tube was set up in which cells were stained for SIg with fluorescein-labeled anti-MIg in the presence of rhodamine-labeled anti-RGG, but in the absence of anti-G_{M1}.

Smears of the stained cells were made on glass slides, and the cells were air dried, fixed in absolute ethanol for 5 min, and rehydrated by three brief rinses in cold PBS. The cells were mounted in a small drop of PBS-Glycerol (1:9, vol/vol), covered with a No. 1 coverslip, and sealed with nail polish. The slides were stored at −20°C until they were counted (generally within 2 days of preparation). Slides were viewed using a 100 X oil immersion objective lens in a Zeiss Universal Microscope equipped with an HBO 200 W mercury lamp and a vertical illuminator. The following filters were used: 58 barrier, 580 reflector and F1546 and KP600 exciter filters for rhodamine; 50 barrier, FL (green) reflector and FITC and BG 12 exciter filters for fluorescein. The percent of positive cells was determined by counting 500 cells per slide. In double-label experiments each field was viewed alternately with rhodamine and fluorescein filters, and all staining combinations were recorded independently. Only 100 cells were counted on the double-label slides since the percent of rhodamine- or fluorescein-stained cells was found to be the same, or differed by no more than 5%, whether cells were stained in the presence of both fluorescent antibodies or with only one (determined on the basis of counting 500 cells).

Results

General Properties of G_{M1} Staining. Immunoadsorbent purified anti-G_{M1} was titered by IF with AKR and C3H thymocytes and was used at the highest dilution which gave maximal percent staining. Data on the titer and specificity of the antibody are shown in Table II. As reported previously (5), antibodies made against purified G_{M1} ganglioside cross-react with asialo G_{M1} and G_{D1b}. After absorption by passage through an asialo G_{M1} affinity column, the antibody could be concentrated to the original titer against G_{M1}, but with significantly reduced titers against the cross-reacting antigens.

Both male and female AKR and BALB/c mice were studied with anti-G_{M1} and anti-MIg, and no sex differences were found in the staining properties with either antibody. G_{M1} and SIg staining were found to be the same in comparisons of BALB/cJ and BALB/c NIH and C3H/fAn and C3H/HeJ mice, therefore, cells from both lines of each strain were used interchangeably. No consistent differences in the percent of G_{M1} or SIg-positive cells was seen in mice ranging in age from 3–9 wk. Animals were not selected by age except that thymuses were generally taken from mice aged 3–5 wk.

Staining of Thymocytes and Lymphocytes. When cells were stained with anti-G_{M1} and rhodamine-labeled anti-RGG, a uniform distribution of fluorescent spots was seen, although on some cells the high density of spots gave a ring-like appearance (see below). Fig. 1 shows the pattern of staining of C3H and AKR thymocytes. Approximately 75% of thymocytes of both C3H and AKR mice were

TABLE II
Specificity of Anti-G_{M1} Antibodies

Antigen*	Titer of complement-fixing antibodies	
	Anti-G_{M1}‡	Absorbed§ Anti-G_{M1}
G_{M1}	1/160	1/160
Asialo G_{M1}	1/80	1/5
G_{D1b}	1/40	1/5

* The antigen preparations consisted of 20 ng glycolipid, 70 ng lecithin, and 200 ng cholesterol.

‡ Column 134 antibody was obtained by passage of pooled R525 anti-G_{M1} serum through a G_{M1} immunoadsorbent column and elution of the bound antibody with 2 M potassium thiocyanate in 0.2% BSA.

§ Column 145 antibody was obtained by passage of Col. 134 antibody through an asialo G_{M1} immunoadsorbent to remove the antibodies cross-reactive with asialo G_{M1} and G_{D1b}.

stained by anti-G_{M1} (Table III). Another preparation[3] of purified anti-G_{M1}, used in earlier studies, stained 90–95% of thymocytes and approximately the same percent of lymphocytes as reported here. Anti-BAθ stained greater than 98% of thymocytes in all experiments.

Fewer lymph node and splenic lymphocytes than thymocytes reacted with anti-G_{M1}, and an inverse relationship was seen between the number of G_{M1}-positive and surface immunoglobulin-positive cells in both AKR and C3H lymphoid tissues. There was a striking difference in percent of G_{M1}-positive lymphocytes between AKR and C3H mice, although both strains had the same percent of G_{M1}-positive thymocytes.

Double-Label IF. Double-label IF experiments were performed to determine the relationship between G_{M1} and SIg-positive cells. Lymphocytes from mesenteric lymph nodes were stained with both anti-G_{M1} and anti-MIg, and they were scored as G_{M1}+SIg−, G_{M1}−SIg+, G_{M1}+SIg+, or G_{M1}−SIg− (Fig. 2). In AKR, A, and BALB/c mice, SIg and G_{M1} were found on separate cells, with only 2–7% of cells exhibiting both markers. C3H, +/nu, and nu/nu mice, however, all had 25–30% of cells that were labeled with both anti-G_{M1} and anti-MIg. This is reflected in an elevated total G_{M1} percent in C3H mice and an elevated total SIg percent in +/nu and nu/nu mice. The inverse relationship between G_{M1} and SIg-positive cells is clearly seen in these mice and suggests that G_{M1}+SIg− cells are T cells. The studies in nude mice were consistent with this interpretation in that nu/nu mice have only a few percent of cells that are G_{M1}+SIg−.

To ascertain whether the differences between C3H and the other inbred mice were demonstrable in another lymphoid tissue, double-label experiments were performed with AKR and C3H splenic lymphocytes (Table IV). The percent of double-labeled cells was even higher in C3H splenic lymphocytes than in mesenteric lymph nodes and differed markedly from that in AKR cells.

Specificity of Anti-G_{M1} Staining. Anti-G_{M1} was absorbed with asialo G_{M1} to remove the cross-reactive antibodies to asialo G_{M1} and G_{D1b} (Table II). The

[3] This antibody was from a different rabbit than the one used in the present studies.

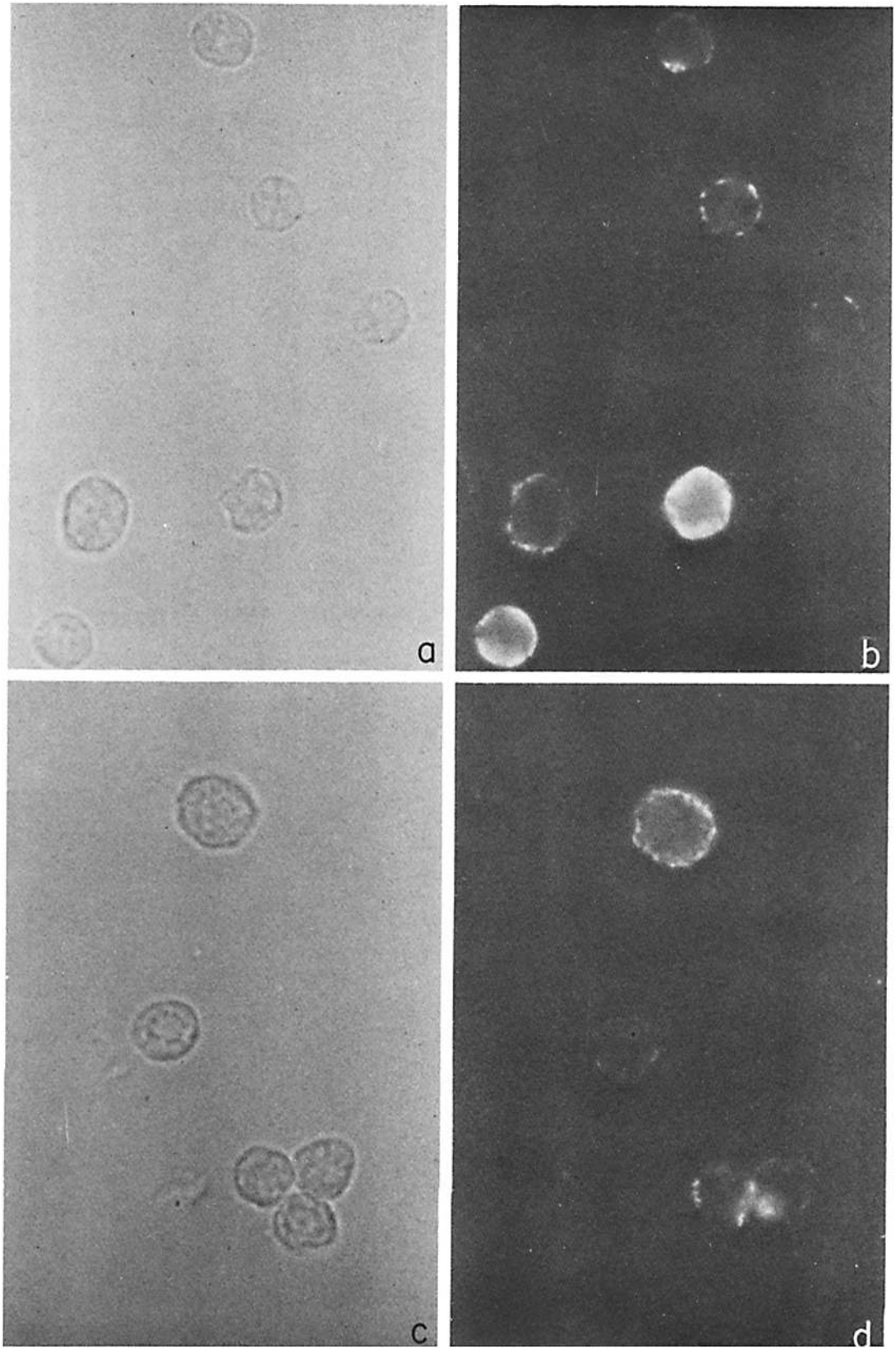
a
b
c
d

TABLE III
Immunofluorescent Staining of Murine Lymphoid Cells by Anti-G_{M1}, Absorbed Anti-G_{M1}, and Antimouse Immunoglobulin

	Percent positive cells*		
	Anti-G_{M1}‡	Anti-MIg	Absorbed anti-G_{M1}‡
Thymus			
AKR/J	75.33 ± 2.16 (6)§	1.00 ± 1.00 (3)	<1 (3)
C3H/HeJ	73.5 ± 7.41 (4)	0.33 ± 0.58 (3)	<1 (3)
Lymph node			
AKR/J	45.50 ± 11.54 (10)	52.70 ± 9.84 (10)	3.5, 2.0‖
C3H/HeJ	69.37 ± 7.74 (8)	41.2 ± 4.73 (8)	<1, 3.0
Splenic Lymphocytes			
AKR/J	51.75 ± 8.77 (4)	43.0 ± 6.2 (4)	4.33 ± 1.52 (3)
C3H/HeJ	67.0 ± 2.16 (4)	41.5 ± 9.84 (4)	2.50 ± 1.73 (4)

* Mean ± SD.
‡ See table II.
§ Parenthesis, number of mice.
‖ Results of individual experiments.

absorbed anti-G_{M1}, concentrated to the same anti-G_{M1} titer as the unabsorbed, failed to react with a significant number of thymocytes or peripheral lymphocytes in either C3H or AKR mice (Table III).

To determine the chemical nature of the reactive site on lymphocytes, the cells were prefixed in either 4% formaldehyde or absolute ethanol and stained with anti-G_{M1}. Cell surface staining was present after aldehyde fixation, but was abolished by alcohol fixation, suggesting that anti-G_{M1} is indeed reacting with glycolipids on the cell.

Absorption with Nude Spleen. Since nude mice lack mature T cells, it was important to determine whether the antigen on nude cells that reacts with anti-G_{M1} was the same as that on thymocytes of normal mice. Anti-G_{M1} absorbed with spleen cells from nude mice reacted with 5% of nude lymphocytes compared to 30% with the unabsorbed anti-G_{M1}. The antibody was tested without further absorption with the thymuses of three AKR and four C3H mice. The nude-absorbed anti-G_{M1} reacted with an average of 20% of thymocytes, compared to 75% with the unabsorbed antibody, and the staining in all experiments was weak. No differences were noted between C3H and AKR cells in the percent of stained cells or in the intensity of staining. It is, therefore, clear that significant cross-reactivity exists between nude lymphocytes and normal thymocytes as measured by anti-G_{M1}.

Staining Intensity and Pattern. Under the conditions used in this study, the intensity of cell surface staining with anti-G_{M1} varied widely. Cells could be

FIG. 1. Thymocytes were stained with rabbit anti-G_{M1}, followed by rhodamine-conjugated sheep anti-RGG: (*a*) AKR thymocytes, bright field; (*b*) same field as in (*a*), UV; (*c*) C3H thymocytes, bright field; (*d*) same field as in (*c*) UV.

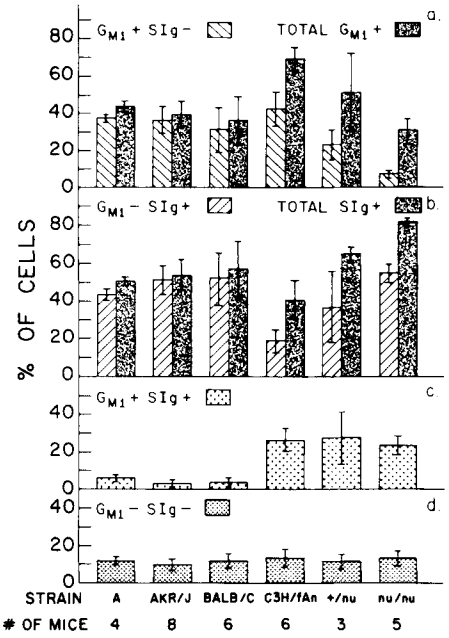

FIG. 2. Double-label immunofluorescence studies of lymph node lymphocytes. Mesenteric lymph node lymphocytes were stained for both G_{M1} and SIg.

TABLE IV
Double-Label Immunofluorescence Studies of Splenic Lymphocytes

Strain	Total G_{M1}	Total SIg	G_{M1}+ SIg−	G_{M1}− SIg+	G_{M1}+ SIg+	G_{M1}− SIg−
	%	%	%	%	%	%
AKR/J						
(Exp. 1)	36	45	34	43	2	21
(Exp. 2)	42	54	33	45	9	13
C3H/HeJ						
(Exp. 1)	70	46	33	9	37	21
(Exp. 2)	70	46	27	3	43	27

grouped into three categories: weak stippled staining, bright stippled staining, and very bright ring staining. Examples are shown in Fig. 1. In AKR mice an average of 25% of G_{M1}-positive thymocytes and 50% of G_{M1}-positive lymph node and splenic lymphocytes exhibited very bright ring fluorescence. In a limited number of studies quantitated in this manner, the percent of very brightly staining G_{M1}-positive cells in C3H thymus and spleen was consistently lower than in AKR tissues. The distribution of very brightly staining cells in +/nu mice was similar to C3H mice. nu/nu lymph nodes, however, contained only rare very brightly staining cells, consistent with the few percent of G_{M1}+SIg− cells present in the nodes (spleens were not examined). In all mice G_{M1}+SIg+ cells exhibited either weak or bright stippled G_{M1} staining, but not very bright ring staining.

When cells were stained with anti-G_{M1} at 4°C, followed by anti-RGG at 37°C,[4] 85% of the stained cells had caps, and the remaining positive cells had patches of various sizes.

Discussion

Using antibodies prepared against a chemically characterized antigen, G_{M1} ganglioside, we have demonstrated that the expression of the reactive site(s) on the surface of murine lymphoid cells is a property of thymocytes and peripheral T cells. The data that support this conclusion are that anti-G_{M1} and anti-MIg label different cell populations, and together they account for 90% of lymph node and splenic lymphocytes. Nude mice, which lack T cells and have at best only a few percent of theta-positive lymphocytes, have only 7% G_{M1}+SIg− cells in their lymph nodes. The percent of cells labeled by anti-G_{M1} is similar to that reported for theta-positive peripheral lymphocytes (10), but the presence of this marker is unrelated to Thy-1 type as it has the same distribution in Thy-1.1 and Thy-1.2 mice.

In A, AKR, and BALB/c mice only 2–7% of lymph node lymphocytes exhibit both G_{M1} and SIg staining. This is consistent with the finding of Roelants et al., who used BAθ and SIg as markers (11). Surprisingly, we have found that approximately 28% of lymph node cells of C3H mice exhibit both markers. Since the percent of G_{M1}+SIg− cells is the same as in A, AKR, and BALB/c mice, the total percent of G_{M1} positive cells in C3H mice is increased. The B cells (G_{M1}−SIg+) in C3H mice are reduced in number in the lymph nodes and spleen.

Lymphocytes of C3H mice exhibit a weak response to lipopolysaccharide (LPS) of *Escherichia coli* and other bacteria (12, 13). The defect is specific for mitogen and antigen-induced B-cell responses to LPS, it is controlled by a single locus that is not linked to *H-2*, and it is not found in other C3H lines. Since we found the pattern of G_{M1} staining to be the same in C3H/HeJ and C3H/fAn mice we tested two lines of C3H which were reported to have a normal LPS response. In two experiments, C3H·SW and C3HeB/FeJ mice each had the same percent of G_{M1}+ and G_{M1}+SIg+ cells as the C3H/HeJ and C3H/fAn mice. This indicates that the decreased percent of G_{M1}−SIg+ cells is not directly related to the failure to respond to LPS. Heterozygous nude mice also have 25–30% G_{M1}+SIg+ cells, but they have a normal number of B cells and a somewhat reduced level of G_{M1}+SIg− cells. This pattern is magnified in homozygous nude mice which have only 7% G_{M1}+SIg− cells in their lymph nodes.

Since we have not yet performed functional studies on isolated G_{M1}+SIg+ cells we cannot comment directly on the nature of these cells. To determine whether the immunoglobulin was synthesized by these cells or was passively acquired by binding to an Fc receptor, C3H lymph node lymphocytes were trypsinized (9) to remove SIg, maintained in culture for 18 h, and tested for G_{M1} and SIg by double-label IF. The results indicate that the SIg present on G_{M1}+SIg+ cells in C3H mice is synthesized by these cells. This finding leads us to speculate that G_{M1}+SIg+ might be precursors of both T and B cells. Since it is known that T precursor cells lack theta (14) it would be of interest to know if anti-G_{M1} reacts with these cells. Others (15, 16) have suggested that B and T

[4] Cells were stained for 30 min in azide-free PBS-BSA.

cells may have a common precursor which has surface immunoglobulin. In this regard, Greenberg and Zatz (17) have recently reported malignant lymphomas which express both theta and surface immunoglobulin which may have arisen from a normal population of cells that express both markers.

An important aspect of the specificity of anti-G_{M1} staining relates to the cross-reacting antibodies present in anti-G_{M1} sera. Since no staining was observed with anti-G_{M1} from which the cross-reacting antibodies had been removed, it is possible that unabsorbed anti-G_{M1} reacts with asialo G_{M1}, G_{D1b}, or both glycolipids on lymphocytes. Experiments performed with antibodies prepared against asialo G_{M1} indicate that both specificities may be detectable (Stein-Douglas, Schwarting, and Marcus. Unpublished Observations.). Since we are dealing with anticarbohydrate antibodies of relatively low affinity which must be used at 4°C to demonstrate good staining, we cannot exclude the possibility that the absorbed anti-G_{M1} has been depleted of the highest affinity antibodies and is unreactive for this reason.

The negative reaction of monospecific anti-G_{M1} with murine thymocytes and lymphocytes is in apparent conflict with recent studies of the interaction of cholera toxin (CT) with lymphocytes (18, 19) in which G_{M1} was assumed to be the cell receptor. Inasmuch as CT, like antibodies to G_{M1}, also binds to G_{D1b} and asialo G_{M1} (20), G_{M1} is not necessarily the primary receptor for CT on all cells. Any of these glycolipids, or a glycoprotein with a similar carbohydrate moiety, could serve as a receptor for CT. CT has a high association constant for murine thymocytes, 7×10^8 liters/mol at 37°C (18), and the binding is essentially complete within 5 min. Even though G_{D1b} and asialo G_{M1} are approximately 1% as effective as G_{M1} in inhibiting CT (20), because of its high association constant CT may bind effectively to cells containing G_{D1b} or asialo G_{M1} but no G_{M1}.

Revesz and Greaves (19) found that, at high concentrations, CT binds equally to thymocytes and T and B lymphocytes. The differences between their data and ours are probably a consequence of the greater sensitivity of their system and of the lower molecule weight of CT (84,000 daltons) compared to IgG antibodies (160,000 daltons). Gahmberg and Hakomori (21) have reported that some cell surface glycolipids are labeled by galactose oxidase (mol wt of 70,000 daltons), but not by larger antibody molecules. We cannot rule out the possibilities that G_{M1} is present on the lymphocyte membrane or that it is present on thymocytes and T and B cells in different amounts. Nevertheless, anti-G_{M1} functions as a marker for T cells and apparently reacts with asialo G_{M1}, G_{D1b}, or both determinants on the cell.

Esselman and Miller have suggested that G_{M1} is C3H theta (Thy-1.2) (3) and that G_{D1b} is BAθ (22), but they have not supported their thesis that G_{M1} is Thy-1.2 with inhibition studies of anti-Thy-1.1. In view of the cross-reactivity observed with our antibodies to G_{M1}, it is surprising that they observe very little cross-reactivity between G_{M1} and G_{D1b} in their system. In our experiments, anti-G_{M1} reacts equally well with Thy-1.1 and Thy-1.2 cells, and monospecific anti-G_{M1} fails to react with either cell type. These findings, and the data of others demonstrating the glycoprotein nature of Thy-1.2 (23) and Thy-1.1 (24), do not support their conclusion that G_{M1} is Thy-1.2. Our findings are compatible with the suggestion of Esselman and Miller (22) that antibodies to G_{D1b} are among the specificities present in anti-BAθ sera.

Our findings demonstrate that anti-G_{M1} can be used to detect T cells in many strains of mice. Moreover, using this reagent we have detected a subpopulation of lymphocytes that has both G_{M1} and surface immunoglobulin. The existence of this subpopulation reveals a previously unrecognized heterogeneity of the surface immunoglobulin-bearing cells in C3H and nude mice and offers a new means of approaching the analysis of functional subpopulations of lymphocytes.

Summary

Antibodies to G_{M1} ganglioside were used to study murine lymphocyte populations. In A, AKR, and BALB/c mice, anti-G_{M1} reacts with thymocytes and peripheral T cells. This reactivity of anti-G_{M1}, studied by immunofluorescence, is independent of Thy-1 type and appears to be related to the reactivity of cross-reacting antibodies to asialo G_{M1} and G_{D1b}, rather than G_{M1} itself. In addition, a subpopulation of lymphocytes reacting with anti-G_{M1} and anti-immunoglobulin has been found in approximately 26% of the peripheral lymphocytes of C3H mice, nude mice, and nude heterozygotes. This subpopulation is found in small numbers in A, AKR, and BALB/c mice. These studies demonstrate that antibodies to a chemically defined antigen can be used to identify T cells in many strains of mice and may delineate previously unrecognized lymphocyte subpopulations.

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