fect of KCl on ventricular muscle. Two possibilities have been suggested: One invokes the stimulation of Na^+ pump, reducing the intracellular sodium ion concentration, $[Na^+]_i$, thus suppressing Ca^{2+} influx through the Ca-Na counter exchanger, which in turn leads to reduction of tension (*14, 15*); the other suggests that K^+ competes more directly for the Ca^{2+} transport site on the surface membrane in a manner similar to that already postulated for Na^+(*5, 12*). The rapidity with which K^+ suppresses tension in depolarized ventricular strips (Fig. 2) makes it improbable that $[Na^+]_i$ has sufficient time to change significantly in order to alter tension. Thus the data are more compatible with a direct effect of K^+ on the Ca^{2+} transport site.

Regardless of the mechanism by which the negative inotropic effect of K^+ is mediated, it is clear that in frog ventricular muscle, KCl solutions should not be used solely as depolarizing agents to quantify development of tension. It is, in fact, misleading to assume that K does not interfere at all with the Ca^{2+} transport mechanism, an assumption based on studies in skeletal muscle, in which activator Ca^{2+} is released primarily from an intracellular compartment.

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Therapy of Mouse Lymphoma with Monoclonal Antibodies to Glycolipid: Selection of Low Antigenic Variants in vivo

Abstract. *Growth of mouse lymphoma L5178Y, which contains large quantities of the gangliotriosylceramide ($GgOs_3Cer$), in DBA/2 mice was suppressed by passive immunization with monoclonal immunoglobulin G3 antibodies to $GgOs_3Cer$, but not by immunoglobulin M antibodies with or without added complement. Most groups of mice treated with monoclonal immunoglobulin G3 antibodies did not develop tumors, but the tumor that appeared in a treated animal had a much lower amount of the $GgOs_3Cer$ than the cells used for inoculation. Thus, passive immunization either prevented growth of the lymphoma or caused selection of a variant with a lower quantity of the antigen $GgOs_3Cer$.*

Two general types of changes of glycolipid patterns resulting from malignant transformation are (i) synthesis of new glycolipid determinants as a result activation of normally unexpressed glycosyltransferases and (ii) deletion of more complex glycolipids because of a block in synthesis that frequently leads to accumulation of precursor structures [for a review, see (*1*)]. Although both of these changes can produce tumor-distinctive glycolipids, no studies have demonstrated that such chemically well-characterized glycolipid markers can be used for tumor diagnosis or therapy. We now report that passive immunization of DBA/2 mice with monoclonal immunoglobulin G3 (IgG3) antibodies specific for the glycolipid ganglio-*N*-triosylceramide ($GgOs_3Cer$; GalNAcβ1→4Galβ1→4Glcβ1→1ceramide) (*2*) dramatically suppressed the growth of tumor cells bearing that glycolipid marker. In addition, the tumor cells that eventually appeared in one treated mouse contained decreased amounts of $GgOs_3Cer$.

We used variants of the DBA/2 lymphoma cell line L5178Y that differed in their display of the neutral glycolipid $GgOs_3Cer$ (*3*). One of these variants, designated 1A1, was chosen as an optimal target cell for immunotherapy because it contained a large amount of $GgOs_3Cer$ and was sensitive to lysis in vitro by monoclonal immunoglobulin M (IgM) antibody to $GgOs_3Cer$ plus complement (*3*). Another variant, clone 27AV, lacked detectable $GgOs_3Cer$ and thus provided an appropriate negative control cell line.

For immunotherapy we used two Balb/c monoclonal antibodies that are specific for distinct portions of the nonreducing terminus of $GgOs_3Cer$ (*4*). Pooled ascites fluids from mice bearing each of these hybridomas contained, per milliliter, 15 mg of the IgM antibody produced by clone 2D4 (*4*) and 4 mg per milliliter, of the IgG3 antibody produced by clone D11G10 (*4*). A nonspecific control ascites fluid contained, per milliliter, 10 mg of the monoclonal IgG3 antibody produced by clone 3. Because mice of the DBA/2 strain are known to have low concentrations of complement component C5 (*5*), certain groups of mice received injections of native guinea pig serum as a complement source.

In the initial experiment mice received on day 0 intraperitoneal inoculations of 10^6 subclone 1A1 cells (a dose at least 2 logs greater than the 100 percent lethal dose). Therapy began on day 1 and consisted of intraperitoneal injections of hybridoma ascites fluids with or without guinea pig serum (Fig. 1A). Whereas the median survival time of untreated mice was 34 days, treatment with guinea pig serum alone or with IgM antibody to $GgOs_3Cer$ alone did not significantly improve survival. Only one of three mice treated with the IgM antibody to $GgOs_3Cer$ plus complement was a long-term survivor. In contrast, the mice treated with the IgG3 antibody to $GgOs_3Cer$, either with or without complement, showed prolonged survival. In fact, five of these seven mice were apparently cured as judged by their survival 120 days after they were inoculated with the tumor cells.

In the second experiment this protective effect of the IgG3 antibody to $GgOs_3Cer$ was reproduced (Fig. 1B). To rule out nonspecific effects of IgG3 antibodies, we treated one group of mice with ascites fluid containing the nonspecific monoclonal IgG3 antibodies produced by clone 3, which did not show reactivity with the subclone 1A1 lymphoma cells by immunofluorescence (data not shown). This antibody failed to protect mice against challenge with the subclone 1A1 cells; the median survival for this group was 31 days compared to 30 days for the untreated group. In this experiment the IgM antibody to $GgOs_3Cer$ had a slight protective effect. However, therapy with the ascites fluid containing IgG3 antibody to $GgOs_3Cer$, either with or without complement, was most effective. Of the 12 mice treated with intraperitoneal injections of the IgG3 antibody, nine survived at least 120 days after they were injected with tumor cells. In addition, intravenous therapy with IgG3 antibody to $GgOs_3Cer$ pro-

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Table 1. Concentrations of $GgOs_3Cer$ in L5178Y lymphoma cells. The 1A1-tu cells were harvested from the peritoneal cavity of an untreated mouse 71 days after it was challenged with 10^5 1A1 cells. The 1A1-G3 cells were harvested from a mouse 76 days after it was challenged with 10^6 1A1 cells; this mouse was treated with ascites fluid containing IgG3 antibody to $GgOs_3Cer$ (Fig. 1A, group 5). The 1A1-G3 cells were cloned by limiting dilution at a density of one cell in every three wells, and the resultant clones were screened for binding to ^{125}I-labeled antibody to $GgOs_3Cer$ as previously described (*3*). Three clones (AF7, BC11, and AG10), which showed minimal binding (data not shown), were expanded for chemical analysis. Neutral glycolipid fractions were obtained from cultured cells as previously described (*3*) and analyzed by thin-layer chromatography in a solvent containing chloroform, methanol, and water (65:25:4, by volume). Neutral glycolipids were visualized with orcinol spray reagent and quantitated by the comparative dilution method (*17*). The $GgOs_3Cer$ was present in these lymphoma cells as a triplet of orcinol-positive bands (*3*). The concentrations of $GgOs_3Cer$ are shown separately for the three bands.

Cell type	$GgOs_3Cer$ (micrograms per 10^7 cells)			
	Fast band	Intermediate band	Slow band	Total
Subclone 1A1	4.0	1.5	1.2	6.7
Subclone 1A1-tu	1.8	1.3	0.5	3.6
Subclone 1A1-G3	0.4	0.2	0.0	0.6
Clone AF7	0.15	0.05	0.0	0.2
Clone BC11	0.1	0.05	0.05	0.2
Clone AG10	0.15	0.1	0.05	0.3

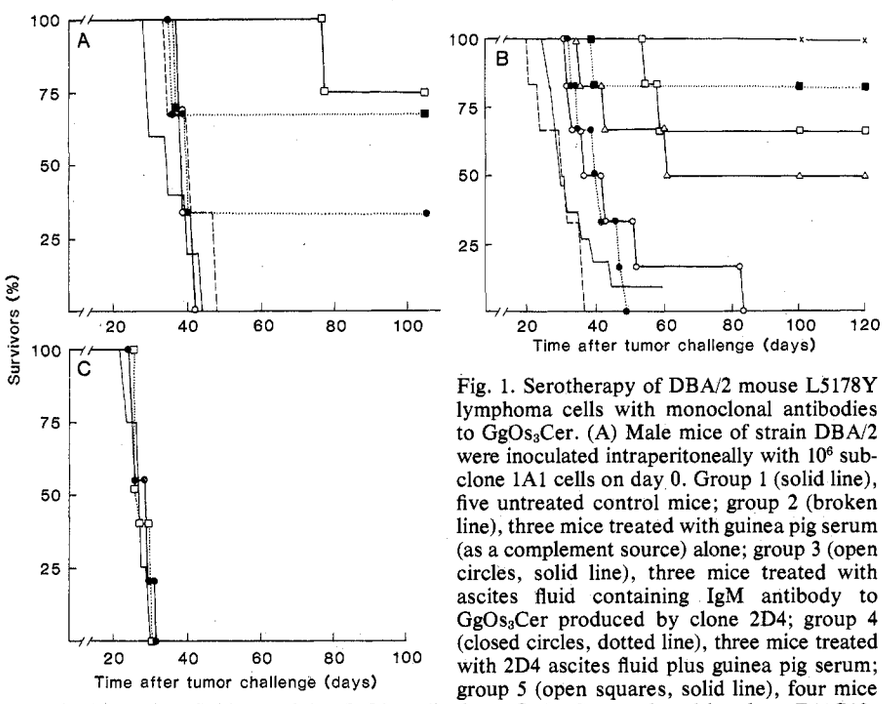

Fig. 1. Serotherapy of DBA/2 mouse L5178Y lymphoma cells with monoclonal antibodies to $GgOs_3Cer$. (A) Male mice of strain DBA/2 were inoculated intraperitoneally with 10^6 subclone 1A1 cells on day 0. Group 1 (solid line), five untreated control mice; group 2 (broken line), three mice treated with guinea pig serum (as a complement source) alone; group 3 (open circles, solid line), three mice treated with ascites fluid containing IgM antibody to $GgOs_3Cer$ produced by clone 2D4; group 4 (closed circles, dotted line), three mice treated with 2D4 ascites fluid plus guinea pig serum; group 5 (open squares, solid line), four mice treated with ascites fluid containing IgG3 antibody to $GgOs_3Cer$ produced by clone D11G10; and group 6 (closed squares, dotted line), three mice treated with ascites fluid from clone D11G10 plus guinea pig serum. Treatment consisted of intraperitoneal injections of 100 μl of ascites fluid or guinea pig serum, or both, on days 1, 3, 7, and 10. (B) Mice were inoculated intraperitoneally with 10^6 subclone 1A1 cells on day 0. Group 1 (solid line), 11 untreated control mice; group 2 (broken line), six mice treated with nonspecific ascites fluid containing monoclonal IgG3 produced by clone 3; group 3 (open circles, solid lines), six mice treated with ascites fluid containing IgM antibody produced by clone 2D4; group 4 (closed circles, dotted line), six mice treated with ascites fluid from clone 2D4 plus guinea pig serum; group 5 (open squares, solid line), six mice treated with ascites fluid from clone D11G10; group 6 (closed squares, dotted line), six mice treated with D11G10 ascites fluid plus guinea pig serum; group 7 (open triangles), six mice treated with D11G10 ascites fluid injected intravenously; and group 8 (crosses, solid line), five mice treated with purified IgG3 antibody to $GgOs_3Cer$. The treatment protocol was similar to that in (A) except for group 7 which received intravenous instead of intraperitoneal injections of ascites fluid. (C) Mice were inoculated intraperitoneally with 10^5 clone 27AV cells (a variant that had no $GgOs_3Cer$) on day 0. Group 1 (solid line), four untreated control mice; group 2 (closed circles), five mice treated with 2D4 ascites fluid plus guinea pig serum; and group 3 (open squares, dotted line), five mice treated with D11G10 ascites fluid. The treatment protocol was the same as that in (A).

vided significant protection in three of six mice, indicating that the IgG3 antibody was effective not only after local injection in the peritoneal cavity but also systemically.

In two control experiments we tested whether this protection was due to a specific effect of the antibody to $GgOs_3Cer$ or to a nonspecific antitumor activity present in the hybridoma ascites fluid. First, IgG3 antibody to $GgOs_3Cer$ was purified from ascites fluid on a Protein A–Sepharose column (Pharmacia) (*6*). Treatment with doses (0.31 mg of protein) of the purified antibody equivalent to the concentration in the ascites fluid effectively protected mice against growth of subclone 1A1 tumor cells (Fig. 1B). Second, Fig. 1C shows that the antibody to $GgOs_3Cer$ was ineffective in protecting mice against challenge with the lymphoma cell variant clone 27AV that did not display $GgOs_3Cer$. Neither the IgM antibody plus complement nor the IgG3 antibody altered the survival of mice innoculated intraperitoneally with 10^5 clone 27AV cells.

A crucial problem for specific immunotherapy is the potential escape of antigen-deficient variants from immune control. To determine if the tumor cells that survived treatment with the IgG3 antibody to $GgOs_3Cer$ contained altered amounts of $GgOs_3Cer$, we compared ascites cells from a treated mouse that survived 76 days with those from an untreated mouse (Table 1). The cells were cultured in vitro for 1 month prior to glycolipid analysis to eliminate host cells. In addition, to detect any possible variation in the amounts of $GgOs_3Cer$ among these cells resistant to IgG3 immunotherapy, we determined the glycolipid pattern of a few clones derived from 1A1G3 (Table 1). The amount of $GgOs_3Cer$ in cells from the untreated mouse was approximately half that of the 1A1 cells used for challenge. In contrast, the cells from the treated mouse (1A1G3) contained only 10 percent of the amount of $GgOs_3Cer$ present in the 1A1 cells. Furthermore, clones derived from the surviving cells contained 20 to 30 times less $GgOs_3Cer$ than the 1A1 cells (Table 1). These results suggest that treatment with the antibodies to glycolipid resulted in the selection of glycolipid antigen-deficient tumor cells. It will be of interest to determine the enzymatic basis for the altered amount of $GgOs_3Cer$.

These studies indicate that antibodies specific for tumor cell glycolipid can protect host animals against inoculated tumor cells. Unlike most so-called "tumor-associated antigens" that are defined serologically, glycolipids offer the ad-

vantage of chemical structures that can be completely defined. In fact $GgOs_3Cer$ has been synthesized chemically (*7*). Many glycolipids are present in various organs of different species, thus providing a source of glycolipid antigen for immunization; for example, the $GgOs_3Cer$ used to prepare the monoclonal antibodies described in this report was obtained from guinea pig erythrocytes (*4*). Recently, the neutral glycolipid ganglio-*N*-tetraosylceramide ($GgOs_4Cer$) was defined as a marker for human acute lymphocytic leukemia cells (*8*). The present study in mice may serve as a model for future therapeutic attempts to use this human tumor glycolipid marker.

Most attempts to use specific antibodies for tumor immunotherapy have met with limited success [for a review, see (*9*)]. However, using the hybridoma technique (*10*) to generate large amounts of specific antibodies, several investigators have demonstrated suppression of tumor growth by serotherapy (*11, 12*). In another study (*13*) serotherapy was only effective with IgG antibodies to Thy-1; monoclonal IgM antibodies were not effective. Similarly, in the present study, IgM antibody to $GgOs_3Cer$ plus complement caused only a marginal increase in the survival time of mice (Fig. 1, A and B), even though this antibody mediated complement-dependent lysis of L51781A1 in vitro. Thus, the effect of the IgG3 antibody to $GgOs_3Cer$ in our system may be due to antibody-dependent cellular cytolytic (ADCC) mechanisms, because IgG in vitro mediates ADCC but IgM does not (*13*). In fact, Tracey and Silberman (*14*) recently found host ADCC effector cells present among the ascites cells of mice bearing the L5178Y lymphoma. Alternatively, IgM may not readily diffuse to tumor cells in vivo.

The successful suppression of L51781A1 cells with IgG_3 antibodies may be partially due to the greater susceptibility of lymphoma cells to destruction by antibody compared to solid tumors, and use of a similar approach to the suppression of solid tumors (fibrosarcoma) bearing the same glycolipid marker (*15*) would be interesting. We and others have described the presence of a trace of $GgOs_3Cer$ in mouse spleen (*15, 16*). Thus, injection of antibody to $GgOs_3Cer$ may modify host lymphoid components which then could retard tumor growth.

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Water Intake in Hypovolemic Sheep: Effects of Crushing the Left Atrial Appendage

Abstract. *Sheep increased their water intake in proportion to the amount of protein-free, isosmotic fluid that was removed from their blood by ultrafiltration. This behavioral response to hypovolemia was eliminated by crushing the left atrial appendage of the heart. The surgical maneuver had no effect on basal water intake or on the drinking response to a salt load. These findings suggest that left atrial stretch receptors, which influence secretion of antidiuretic hormone when stimulated, may also play an important role in mediating thirst during hypovolemia.*

Loss of plasma volume (hypovolemia) provokes compensatory increases in the secretion of antidiuretic hormone (*1*) and the consumption of water (*2, 3*). The nature of the thirst stimulus remains uncertain; increased concentrations of angiotensin II (*4*) and altered neural activity in vascular baroreceptors (*3, 4*) have been proposed as important factors. The secretion of antidiuretic hormone in response to plasma volume deficits is believed to be stimulated in large part by the activation of stretch receptors in the area of the left atrium of the heart (*5*). This report presents evidence that the receptors may also mediate thirst during hypovolemia.

We performed the experiments on female mixed-breed sheep (*6*). Acute intravascular dehydration was produced in conscious animals by ultrafiltration of the blood with a parallel flow dialyzer (*7*). During a 5-hour period in which the sheep were deprived of food and water, their blood was continuously pumped from a saphenous vein catheter through the filter and back into the animal via the contralateral catheter. Protein-free, isosmotic fluid was separated under negative pressure and collected. Simultaneous measures of arterial blood pressure (ABP), glomerular filtration rate (GFR), plasma sodium (PNa) concentration, plasma protein (PP) concentration, he-

Table 1. Effects of hypovolemia on ABP, GFR, PNa, and PP concentrations, Hct and PRA in unoperated sheep, sheep with damaged left atrial appendages (operated), and unoperated sheep treated with furosemide. Values are means ± standard errors.

Treatment group	ABP (mm-Hg)	GFR (ml/min)	PNA (mEq/liter)	PP (g%)	Hct (%)	PRA (ng AI/ml-hour)
Unoperated (N = 8)						
Before filtration	88 ± 1	67 ± 4	147 ± 1	6.3 ± 0.2	29 ± 1	1.0 ± 0.2
After filtration	71 ± 7*	22 ± 7†	147 ± 1	7.7 ± 0.2†	39 ± 2†	19.9 ± 5.8†
Operated (N = 11)						
Before filtration	84 ± 1	70 ± 4	145 ± 1	6.7 ± 0.2	25 ± 1	1.7 ± 0.4
After filtration	58 ± 3†	6 ± 4†	144 ± 2	9.1 ± 0.7†	35 ± 3†	28.4 ± 5.7†
Furosemide (N = 4)						
Before filtration	84 ± 2	72 ± 4	145 ± 1	6.2 ± 0.1	30 ± 1	0.7 ± 0.1
After filtration	61 ± 6*	13 ± 8†	145 ± 2	8.3 ± 0.3†	38 ± 1†	25.8 ± 7.2†

*Significantly different from the control value at $P < .05$. †Significantly different at $P < .01$.

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