# Neuroblastomas of Infancy Exhibit a Characteristic Ganglioside Pattern

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**BACKGROUND.** Gangliosides are membrane-bound glycolipid molecules particularly prominent in neural tissue. Changes in ganglioside expression during embryologic development result from a shift in biosynthesis from the fetal *b* pathway to the adult *a* pathway. Tumor gangliosides may play a role in the clinical behavior of certain subtypes of neuroblastoma. Because neuroblastoma, which presents in infancy, has a different biologic and clinical phenotype than that which presents in older children, the authors determined whether differences in ganglioside biosynthesis exist between these two neuroblastoma subgroups.

**METHODS.** Sixty-eight tumor specimens (25 diagnosed by screening and 43 diagnosed clinically) were obtained from the Quebec Neuroblastoma Screening Project. Gangliosides were isolated and purified by solvent partitioning, separated by high performance thin-layer chromatography, and quantitated by scanning densitometry. The sum of a and *b* pathway gangliosides were determined for each tumor.

**RESULTS.** Gangliosides of the *b* (fetal) pathway predominated in both screened and clinically diagnosed tumors of patients younger than 1 year of age. Twenty-three of 25 screened patients (92%) and 21 of 23 patients with clinically diagnosed tumors at younger than 1 year of age (91%) had tumor *b* pathway ganglioside content greater than 60%. In contrast, tumors of only 8 of 20 patients 1 year or older (40%) had *b* pathway ganglioside predominance. Predominance of *b* pathway tumor gangliosides correlated with improved outcome. Event free survival was significantly higher among patients with *b* pathway ganglioside tumor content greater than 60% versus those with *b* pathway ganglioside tumor content less than 60% (118.1 $\pm$ 3.9 months vs. 69.2 $\pm$ 8.6 months, $P < 0.01$).

**CONCLUSIONS.** Fetal patterns of ganglioside biosynthesis predominate in neuroblastoma tumors from patients younger than 1 year of age and adult patterns of ganglioside biosynthesis predominate in tumors from older children, supporting the view that neuroblastoma consists of distinct but overlapping disorders, and that gangliosides may play a biologic role in the clinical differences among these patients. ***Cancer* 2001;91:785–93.** © *2001 American Cancer Society.*

**KEYWORDS: gangliosides, biosynthesis, patterns, neuroblastoma, screening.**

The emerging paradigm for neuroblastoma consists of two main disease categories. Favorable disease is characterized by an early age at diagnosis, low stage, a tendency toward adrenal, liver, and skin involvement, favorable biologic markers, and a good outcome with little or no therapy. Unfavorable disease generally presents later in childhood, usually with advanced disease, a tendency toward bone involvement, biologic markers associated with a poor prognosis, and a poor outcome despite aggressive therapy. Modest gains in response rates and survival have been made.[2] Overall, however, the prognosis for patients in the poorest risk group, those older than 1 year of

age who present with metastatic disease, remains guarded.[3,4] Therefore, the need to better understand the biology of this disease is compelling and fundamental to the development of more specific and effective therapy.

Tumor gangliosides, to which several important biologic activities have been attributed in recent years, may play a role in the clinical behavior of certain subtypes of neuroblastoma. Gangliosides are normal membrane-associated sialic acid–containing glycosphingolipid molecules especially prominent in neural tissue. Embryologically, the biosynthesis of gangliosides in human brain includes two prominent pathways designated *a* and *b* (see Fig. 1). In normal brain development, a shift from the *b* to the *a* pathway correlates with dendrite outgrowth, synaptogenesis, and other events signaling neuronal maturation.[5] Thus, the *a* pathway has been associated with more mature tissue, and the *b* pathway with developmentally more primitive brain.[6] In tumors, several lines of evidence suggest a functional role for gangliosides, such as in the biology of neuroblastoma. First, gangliosides are present in high concentrations in human neuroblastoma, as well as ganglioneuroblastoma and ganglioneuroma.[7] Second, tumor gangliosides are known to be shed from tumor cells,[8–14] and shed tumor gangliosides have been shown to alter host immune function[8,9,15,16] and permit enhanced tumor formation in mice.[8] Finally, alterations in ganglioside expression and shedding correlate with outcome.[17–22] We have demonstrated previously that the *b* pathway ganglioside $G_{D2}$ is a predominant ganglioside present in primary neuroblastoma, but that it is low or absent in the more differentiated (and generally less malignant) tumors, ganglioneuroblastoma and ganglioneuroma.[7] Furthermore, high plasma levels of tumor-derived $G_{D2}$ predict a poor outcome in children with advanced disease.[17]Schengrund et al. and Schengrund and Shochat observed absence of the *b* pathway ganglioside $G_{T1b}$ (a biosynthetic product of $G_{D2}$) and a predominance of the *a* pathway monosialogangliosides $G_{M1}$, $G_{M2}$, and $G_{M3}$ in clinically aggressive tumors.[18,19]

The suggestion that ganglioside metabolism may differ among neuroblastomas with different malignant potential is supported by 1) the shift in ganglioside metabolism during normal neuronal cell differentiation[23,24] and 2) the characteristic ganglioside patterns associated with aggressive neuroblastoma.[7,18,19] In addition, substantial experimental evidence associates ganglioside changes with alterations in tumor formation and metastasis in vivo.[25] Because recent evidence suggests that neuroblastoma that presents before 1 year of age, whether diagnosed clinically or by screening, has a different biologic and clinical phenotype than that which presents in older children,[3,26–28] we hypothesized that differences in ganglioside biosynthesis may exist between these two neuroblastoma subgroups. To test this hypothesis, we compared patterns of ganglioside biosynthesis among patients with tumors diagnosed both before and after 1 year of age. In addition, we utilized of a uniquely available group of tumors, those diagnosed by infant screening. Population-based screening programs using urinary vanillylmandelic acid (VMA) and homovanillic acid (HVA) have been underway in Japan, and more recently in North America. Most tumors detected by screening have favorable histology[29] by the Shimada classification and biologic markers that are associated with a favorable prognosis (i.e., hyperdiploid or triploid DNA and lack of MYCN amplification or structural chromosomal abnormalities, such as 1p deletions, homogeneous staining regions, and double minutes).[30–33] Furthermore, these tumors are not clinically aggressive, suggesting that neuroblastoma diagnosed by screening closely resembles that diagnosed clinically in patients younger than 1 year of age.[27,30] The purpose of our study was to determine the pattern of ganglioside biosynthesis among screened tumors, and to compare that pattern with those of clinically diagnosed tumors. Here, we report that ganglioside biosynthesis in tumors from patients diagnosed before 1 year of age (whether diagnosed clinically or by screening) differs substantially from that in tumors diagnosed in older patients.

## MATERIALS AND METHODS

### Tumor Samples

Sixty-eight confirmed pretreatment neuroblastoma samples obtained through the Quebec Neuroblastoma Screening Project[34] during a 5-year accrual period were studied. Samples were obtained both from infants with tumors diagnosed by urinary HVA and VMA screening at 3 weeks or 6 months of age (25 samples), and from patients who presented clinically during the same period of study (43 samples). Children with disease diagnosed clinically either presented before the onset of the screening program, underwent screening but disease was not detected, or were never screened. Tumor biopsies were obtained at the time of diagnosis and were stored at −70 °C until analyzed for their ganglioside content. Appropriate informed consent was obtained for all patients. All staging and outcome data were obtained from the Quebec Neuroblastoma Project database.

### Ganglioside Isolation and Purification

Neuroblastoma specimens (0.1–0.5 g) were thawed, homogenized, and lyophilized. Gangliosides were purified as previously described.[35] Briefly, total lipids were extracted twice with chloroform:methanol (1:1). The extracts were reduced in volume, cooled to −20 °C to free insoluble material, and dried under $N_2$. The dried total lipid extract then was partitioned in diisopropyl ether:1-butanol (60:40) and 0.3% NaCl (10 mL/g tissue), organic:aqueous (2:1). After the first extraction, the ganglioside-containing aqueous phase was repartitioned with fresh organic phase, and the final aqueous phase was lyophilized and purified by Sephadex G-50 gel filtration. The ganglioside-containing void volume then was collected and lyophilized before separation and quantitation by thin-layer chromatography (TLC).

### Quantitation of Ganglioside Content

Gangliosides were separated by high performance thin-layer chromatography (HPTLC) and analyzed densitometrically as previously described.[7] The total sample of dried tumor gangliosides was resuspended in C:M, centrifuged to remove residual protein, and 6–10 nmol lipid-bound sialic acid (LBSA; 200 nmol LBSA/g neuroblastoma tissue, estimated) were spotted as 1-cm lanes onto 10 × 10– or 10 × 20–cm precoated silica gel HPTLC plates (E. Merck, Darmstadt, Germany). The plates were preactivated by heating to 110 °C for 1 hour. A known quantity of human brain gangliosides (HBG) was spotted on each plate as an internal standard. The TLC plates were dried overnight in a desiccator, then developed in tanks containing chloroform:methanol: 0.25% $CaCl_2 \cdot 2H_20$ (60:40:9). The TLC plates then were dried, placed in an oven at 110 °C for 20 seconds, sprayed with resorcinol-HC1 reagent, and heated again at 110 °C for 20 minutes. Gangliosides then were directly visible as purple bands.

Final ganglioside quantitation was accomplished by scanning each lane with a photo densitometer (Shimadzu CS 9000, Kyoto, Japan). The area under each peak generated by each ganglioside band was integrated, and the relative percentage of each ganglioside band was calculated.

### Statistical Analysis

The sum of the *a* pathway gangliosides ($G_{D1a}$, $G_{M1}$, $G_{M2}$, and $G_{M3}$) and the sum of the *b* pathway gangliosides ($G_{Q1b}$, $G_{T1b}$, $G_{D1b}$, $G_{D2}$, and $G_{D3}$) were determined for each tumor. Differences in the relative percentage of the *a* and *b* pathway content were analyzed by the Wilcoxon rank-sum test. The differences in content of *b* pathway gangliosides among patient groups were analyzed using the Fisher exact test. Event free survival was estimated using the Kaplan–Meier method. Event free survival was measured as the time from study entry to progressive disease, recurrence, or death. Survivor functions were compared using the Wilcoxon rank-sum test.

## RESULTS

The two main pathways for ganglioside biosynthesis are depicted in Figure 1. Pathway *a* is predominant in normal differentiated neuronal tissue. Pathway *b* is predominant in fetal neuronal tissue (< 20 weeks gestation), and a single specific *b* pathway ganglioside, $G_{D2}$, can be isolated from most primary neuroblastoma tumors.

We studied the ganglioside content of 68 neuroblastoma samples (25 from screened patients, 43 from patients with clinically diagnosed disease). The clinical stages (International Neuroblastoma Staging System) of all 68 patients are summarized in Table 1. Tumor ganglioside content was analyzed by TLC as described above. We anticipated that the TLCs from the samples studied would reveal a predominance of gangliosides from one or the other metabolic pathways, or alternatively, a relatively even distribution of gangliosides from both pathways, suggesting no particular biosynthetic pathway predominance. Figure 2 shows the TLC patterns of five of the tumor samples analyzed for this study, three samples from screened patients (lanes 3–5), and two samples from patients with clinically diagnosed disease (lanes 1 and 2). Qualitatively, the ganglioside patterns of samples 1, 3, 4, and 5 have a high degree of similarity, with a predominance of $G_{Q1b}$, $G_{T1b}$, $G_{D1b}$, $G_{D2}$, and $G_{D3}$ (*b* pathway gangliosides). Lane 2 differs from the other 4 samples in that the gangliosides $G_{D1a}$ and $G_{M1}$ (*a* pathway gangliosides), in addition to $G_{M3}$, are present in increased concentration. Scanning densitometry confirmed these observations. Samples represented in lanes 1, 3, 4, and 5 contain greater than or equal to 90% *b* pathway gangliosides (i.e., less than or equal to 10% *a* pathway gangliosides). The sample represented in lane 2, which has a higher content of $G_{D1a}$ and $G_{M1}$ relative to the other 4 samples, contains 68% *b* pathway gangliosides (i.e., 32% *a* pathway gangliosides).

To determine whether differences in ganglioside biosynthesis characterize clinically distinct types of neuroblastoma, we analyzed all 68 tumor samples for their relative content of *a* and *b* pathway gangliosides by TLC and scanning densitometry. Figure 3 summarizes our data. Overall the screened tumors had a high percentage of *b* pathway gangliosides (median, 85%). The *b* ganglioside content of tumors from patients

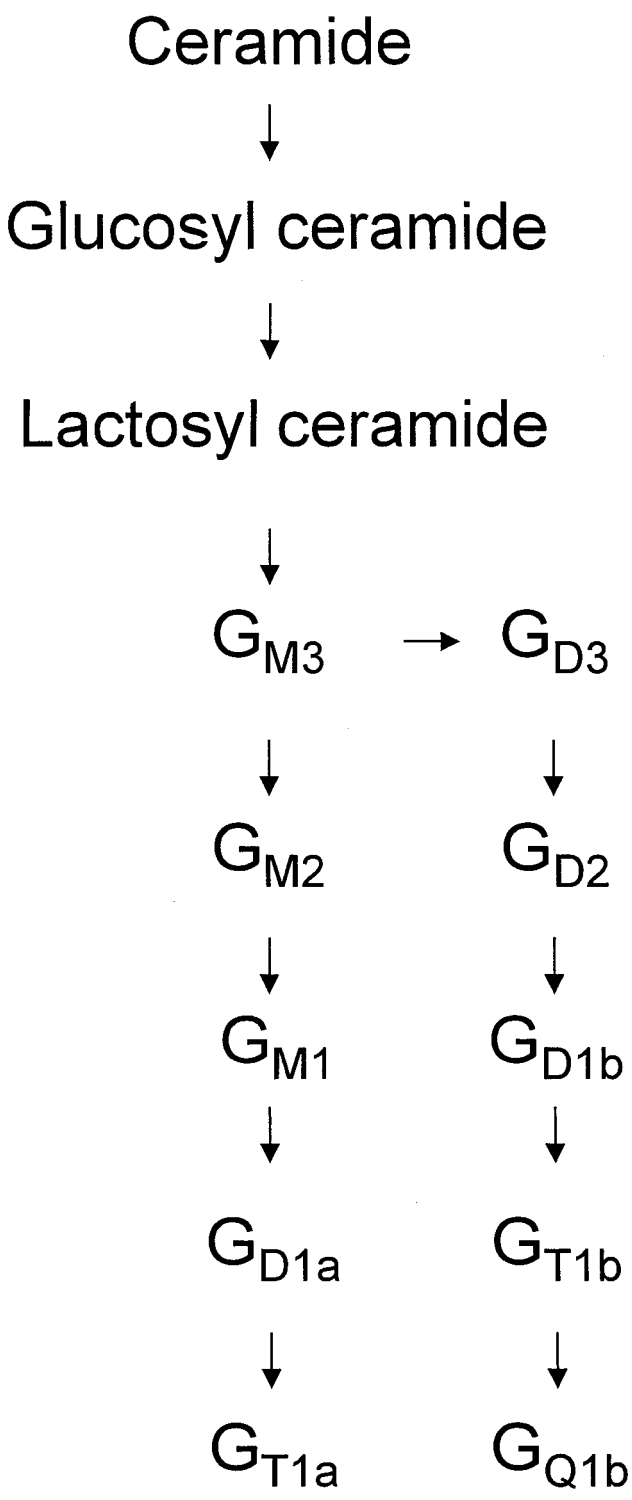


**FIGURE 1.** Schematic representation of the major pathways of ganglioside biosynthesis. *a* pathway $F_{M3} \rightarrow \rightarrow \rightarrow G_{T1a}$; *b* pathway $G_{D3} \rightarrow \rightarrow \rightarrow G_{Q1b}$.

younger than 1 year of age with clinically diagnosed disease was found to be similar to that of the screened group (median, 80%, $P < 0.29$). However, the ganglioside distribution of clinically diagnosed tumors from children 1 year of age or older (n = 20) were highly variable. In this group, the median percentage of *b* pathway gangliosides was 55%, substantially lower than the other two groups. This difference was highly statistically significant ($P < 0.0002$). Similarly, the relative number of patients in each of the 3 groups with *b* pathway ganglioside content greater than 60% was 92% (23 of 25) for the screened group and 91% (21 of 23) for the patients younger than 1 year of age with clinically diagnosed disease, but only 40% (8 of 20) for the patients 1 year of age or older with clinically diagnosed disease. The differences between the patients 1 year of age or older with clinically diagnosed disease and both the screened group and patients younger than 1 year of age with clinically diagnosed disease were highly significant ($P < 0.0002$ and $P < 0.0007$, respectively). In Figure 2, the heterogeneity observed in the ganglioside patterns of the clinical specimens is exemplified by lanes 1 and 2, which reveal a predominance of *b* and *a* pathway gangliosides, respectively. In contrast, the screened specimens depicted in lanes 3–5 reflect greater homogeneity of *b* pathway predominance.

Finally, to determine whether differences in tumor cell ganglioside biosynthesis correlate with the clinical behavior of neuroblastoma tumors, we segregated tumors on the basis of the patients' International Neuroblastoma Staging System (INSS) clinical stage (Fig. 4) and outcome (Fig. 5), rather than age. We observed that greater than 85% of patients with low stage disease (Stages 1 and 4S) had tumors with a *b* pathway ganglioside predominance. Tumors from patients with disseminated disease at diagnosis (Stage 4) were almost equally divided between those with and without *b* pathway predominance (Fig. 4). These data suggest that *b* pathway ganglioside content greater than 60% is associated with lower stage; however, because of the small sample size there was not adequate power to detect differences among these groups.

Further analysis of patient outcome revealed that only 3 of 7 tumors (43%) from Stage 4 patients who progressed, experienced recurrence, or died had a *b* pathway ganglioside predominance. When all patients who progressed, experienced recurrence , or died are considered (1 Stage 2 patient, 1 Stage 3 patient, and 7 Stage 4 patients) only 3 of 9 tumors (33%) had a *b* pathway predominance. Kaplan–Meier analysis of all 68 patients segregated on the basis of tumor *b* pathway ganglioside content (greater than or < 60%)

**TABLE 1**
**Clinical Stages of 68 Patients Whose Tumors Were Analyzed for Ganglioside Content Categorized by Age and Mode of Diagnosis**

| | | Stage | | | | |
|---|---|---|---|---|---|---|
| **Age of patients** | **Total** | **4S** | **1** | **2** | **3** | **4** |
| Younger than 1 yr | 48 | 5 | 20 | 11 | 4 | 8 |
| Screened | 25 | 4 | 7 | 9 | 3 | 2 |
| Clinical | 23 | 1 | 13 | 2 | 1 | 6 |
| 1 yr or older | 20 | 2[a] | 5 | 6 | 1 | 6 |

[a] Both patients were 12 mos of age at diagnosis.

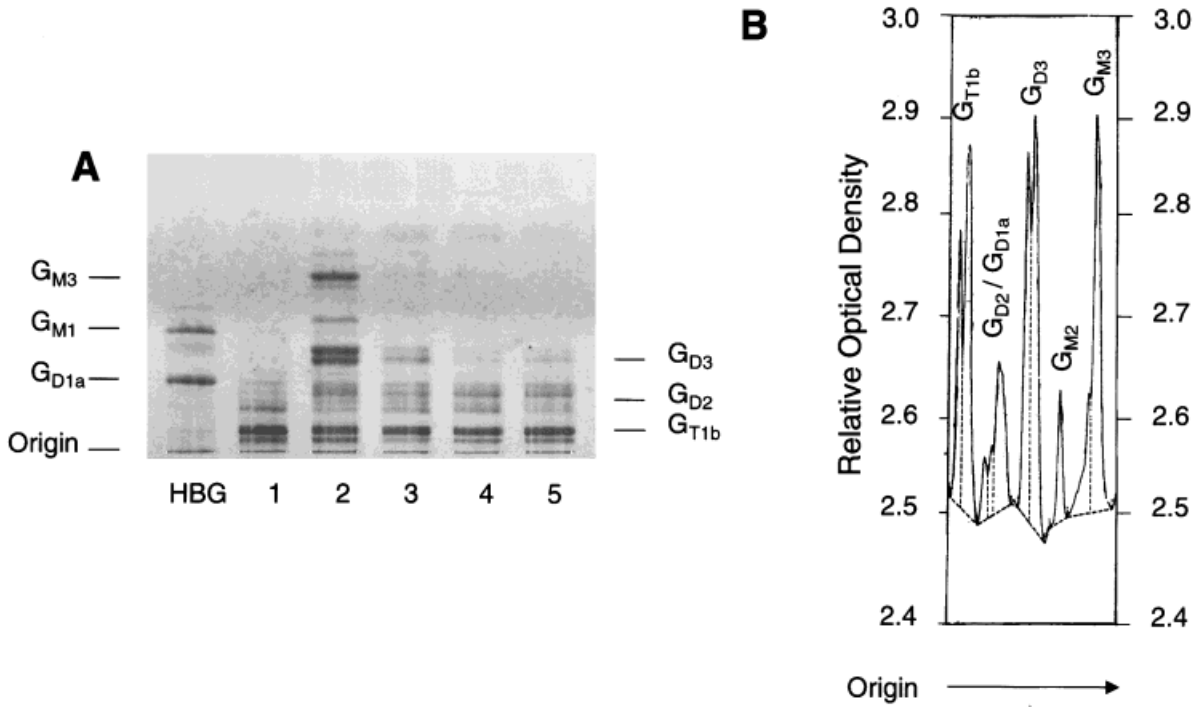


**FIGURE 2.** (A) HPTLC of highly purified human neuroblastoma gangliosides from five tumor samples, three from screened patients (lanes 3–5) and two from patients with clinically diagnosed disease (lanes 1 and 2). Neuroblastoma tissue (200 nmol LBSA/g) was spotted, and the HPTLC developed in chloroform:methanol: 0.25% $CaCl_2 \cdot 2H_2O$ (60:40:9) and stained with resorcinol:HCl. (B) Representative scanning densitometry of the sample shown in lane 2 of the HPTLC. The area under each peak generated by its corresponding ganglioside band was integrated and the percentage of each ganglioside was calculated.

showed a significant difference in event free survival among the two groups with mean intervals of progression free survival of 118.1 ± 3.9 months in patients with greater than 60% *b* pathway tumor gangliosides, and 69.2 ± 8.6 months in patients with less than 60% *b* pathway tumor gangliosides ($P < 0.01$; Fig. 5).

We conclude from our data that most neuroblastoma tumors diagnosed in infants, either through screening or clinically, contain a predominance of *b* series gangliosides, and in that sense, tumors diagnosed by screening and those diagnosed clinically in infants are indistinguishable. In contrast, the *b* pathway ganglioside content of tumors in children older than 1 year of age is highly variable, with some tumors having a high content of *b* pathway gangliosides, but many having a predominance of *a* pathway gangliosides. Furthermore, from studying all patients together, independent of their age or mode of diagnosis, we conclude that *b* pathway ganglioside predominance is associated with improved clinical outcome.

## DISCUSSION

Several studies have addressed the potential role of cell surface gangliosides in the biology of human neuroblastoma.[7,17–19,36] However, there has been no reported analysis of alterations in ganglioside biosyn-

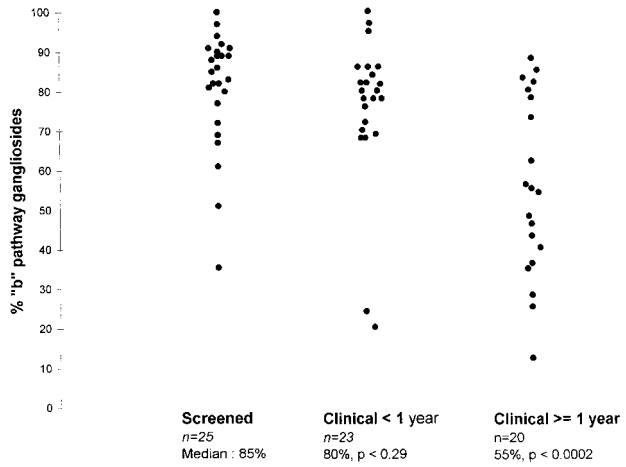


**FIGURE 3.** Content of *b* pathway gangliosides in neuroblastoma tumor samples from screened patients, patients younger than 1 year of age with clinically diagnosed disease, and patients 1 year of age or older with clinically diagnosed disease. Sample number, median percentage *b* pathway ganglioside content, and statistical significance of median percentage *b* pathway ganglioside content compared with screened samples is summarized for each group.

thesis associated with clinically distinct subgroups of neuroblastoma. In addition, although studies of ganglioside expression and its relation to disease stage and/or outcome have been valuable in establishing that alterations in ganglioside expression exist in neuroblastoma, a complete picture of the changes in ganglioside metabolism associated with neuroblastoma is lacking. Gangliosides are of interest in the study of the biology of neuroblastoma for several reasons. Experimentally, shed tumor gangliosides have been shown to enhance tumor formation,[8] and appear to be locally immunosuppressive,[15,16] and their presence has been demonstrated in the plasma of patients with neuroblastoma but not in healthy controls.[37]

This study was undertaken initially as part of the biologic study arm of the Quebec Neuroblastoma Screening Project, which had as its goal the delineation of biologic differences between screened tumors and those detected clinically. A body of data subsequently has emerged strongly suggesting that neuroblastoma diagnosed by screening is biologically and clinically similar to that diagnosed clinically in patients younger than 1 year of age, i.e., the screened tumors had excellent clinical outcomes and lacked unfavorable markers such as MYCN amplification, chromosome 1p deletions, and others.[30–33] The clinical and biologic similarities among tumors diagnosed by screening and those diagnosed clinically in patients younger than 1 year of age led us to stratify the patients with clinically diagnosed disease based on age, to compare ganglioside biosynthesis among these 2 groups and the screened patients.

One major finding of this work is that neuroblastomas diagnosed through screening have a characteristic pattern consisting of gangliosides derived from the *b* metabolic pathway. Furthermore, the tumors diagnosed by screening have essentially the same ganglioside biosynthesis phenotype as those diagnosed clinically in patients younger than 1 year of age. Both of these clinical groups tend to have tumors with favorable biologic phenotypes (i.e., favorable histology, single copy MYCN, chromosome 1p deletion, high trk-A expression) and outcomes. In contrast, the ganglioside patterns from tumors diagnosed in older children are more variable, with significantly fewer tumors exhibiting a predominance of *b* pathway gangliosides. These results lend support to the paradigm for neuroblastoma in which tumors diagnosed in patients younger than 1 year of age (either clinically or by screening) are clinically and biologically distinct from those that present in older children.

The second major finding of this work is that tumor *b* pathway ganglioside predominance is associated with improved clinical outcome in neuroblastoma. Furthermore, our work suggests a correlation between *b* pathway ganglioside predominance and localized disease (low stage) at diagnosis. Published work in other tumors supports our findings here. Specifically, studies of ganglioside metabolic profiles in human astrocytoma demonstrate progressive loss of *b* pathway gangliosides associated with histologically more malignant phenotypes.[21] Similar work with renal carcinoma reveals a predominance of *a* pathway gangliosides in tumor specimens, compared with normal kidney.[38] These studies support the concept that malignant transformation is associated with alterations in ganglioside biosynthesis. The combined conclusion from our findings and those mentioned above is that the malignant potential of tumor cells may be associated with either loss of *b* series gangliosides or accumulation of *a* series gangliosides in the tumor cell.

During the second trimester of fetal development *b* pathway gangliosides predominate, with a shift at approximately 20 weeks to *a* pathway gangliosides. Thus, *b* pathway gangliosides are associated, at least in the brain, with a relatively less differentiated cellular state. Although our study was limited to patients stratified on the basis of age and mode of diagnosis, not clinical stage, our current data suggest that in patients younger than 1 year of age, most of whom have tumors with favorable histology, ganglioside biosynthesis mimics that of relatively undifferentiated neuronal cells. In contrast, the disease in older children appears to result from malignant transformation and is char-

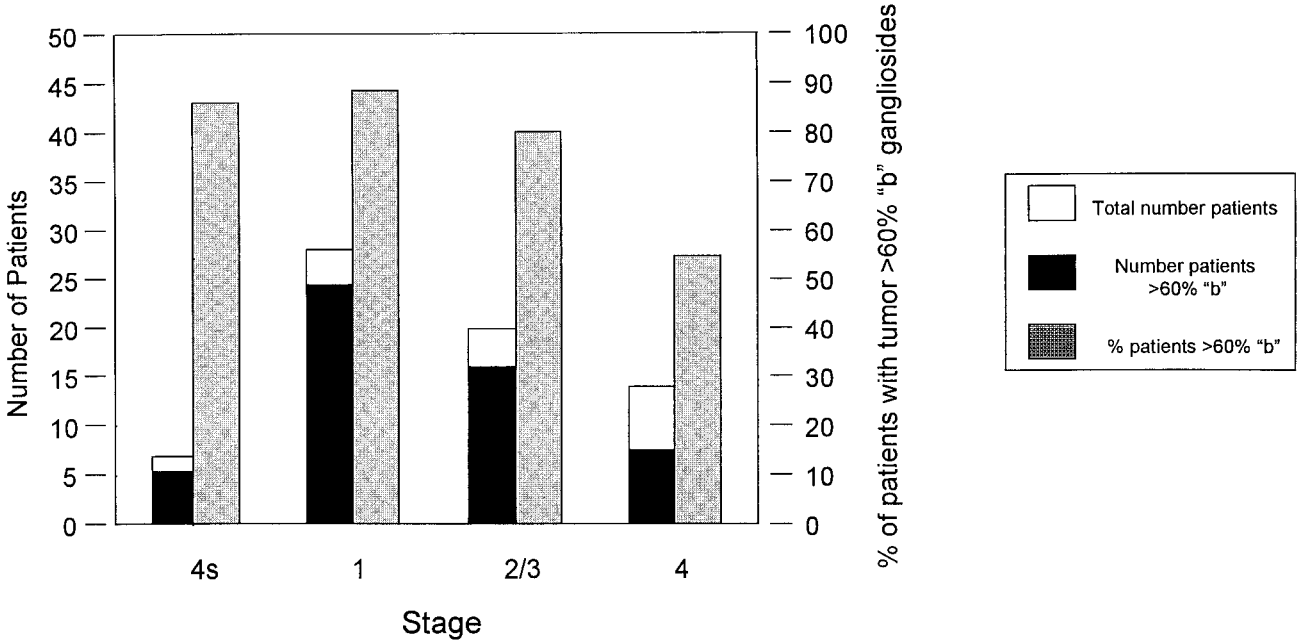


**FIGURE 4.** The proportion of tumors with *b* pathway ganglioside predominance (> 60% *b* pathway ganglioside content) from 68 patients categorized by INSS clinical stage. The stacked bar represents the number of patients in each group with tumors containing greater than 60% *b* pathway gangliosides (black area) as a subset of the total number of patients in each group. The shaded bar represents the percentage of patients in each group with tumors containing greater than 60% *b* pathway gangliosides.

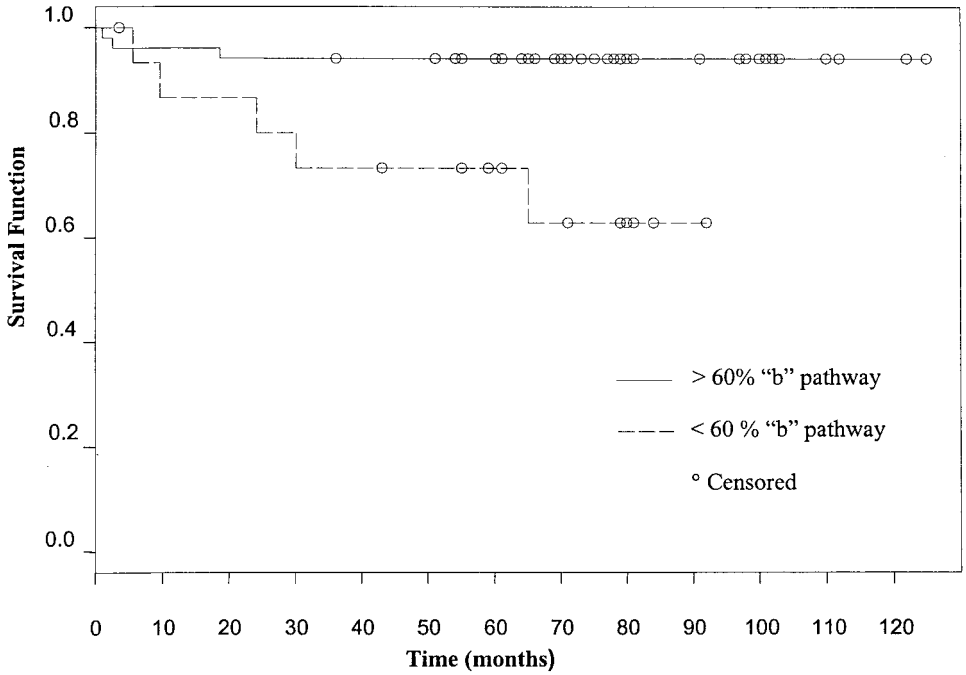


**FIGURE 5.** Kaplan–Meier analysis of 68 neuroblastoma segregated on the basis of tumor cell ganglioside content (greater than or < 60% *b* pathway ganglioside content). The difference in the mean interval of event free survival between the two groups is significant ($P < 0.01$).

acterized by chromosomal derangements and a greater tendency toward the biosynthesis of *a* pathway gangliosides. Because 1) *b* pathway gangliosides normally are associated with early events in normal brain development, and 2) the *b* pathway ganglioside $G_{D2}$ is associated with more malignant forms of neuroblastoma, this finding that gangliosides of the *b* pathway predominate in clinically favorable tumors is somewhat unexpected. One possible explanation for our results is the observation that tumors of favorable

histology contain undifferentiated neuroblastic elements, which may be the source of *b* pathway gangliosides. Using this model, it is clear that the apparent biologic differences in neuroblastomas diagnosed in patients younger than 1 year of age versus those diagnosed at 1 year of age or older suggest that the infant disease represents a congenital "holdover" of fetal tissue characterized by remnants of earlier biologic phenotypes (i.e., *b* pathway ganglioside predominance). This explanation is supported by the well described finding of nodules of neuroblasts in the adrenal glands of fetuses (of 17–20 weeks gestation) and infants (younger than 3 months of age).

The relationship of *b* pathway gangliosides to outcome in our study is supported by findings in other tumor systems (astrocytomas, medulloblastoma).[21,22] As has been suggested for astrocytomas, it may be that deletion of a specific galactosyl transferase underlies the observed patterns of ganglioside biosynthesis in clinically aggressive neuroblastomas. Published studies, which demonstrate elevated levels of $G_{D2}$[7] and absence of $G_{T1b}$[18,19] (both *b* pathway gangliosides) in clinically aggressive neuroblastomas, make it interesting to speculate that absence or inhibition of the enzyme $G_{D1b}$ synthase may account for the ganglioside profiles of these tumors. If this enzyme were nonfunctional, conversion of $G_{D2}$ to $G_{D1b}$ could be prevented, resulting in increased levels of $G_{D2}$. Because $G_{D1b}$ is the substrate for $G_{T1b}$ synthesis, $G_{T1b}$ (as well as $G_{Q1b}$) levels would be decreased or absent (Fig. 1).

Previously published studies, including our own previous work, demonstrate both expression and shedding of the *b* pathway ganglioside $G_{D2}$ in human neuroblastoma tumors, but not in ganglioneuroma or ganglioneuroblastoma. However, in these previous studies, other *b* pathway gangliosides were not measured, making it impossible to ascertain whether $G_{D2}$ expression represented a more generalized *b* pathway ganglioside predominance or an *a* pathway predominance with a biosynthetic block downstream from $G_{D2}$. The lack of detectable $G_{D2}$ in ganglioneuroma or ganglioneuroblastoma does not contradict our current findings, because these tumors are histologically and biologically distinct from neuroblastoma, despite their common embryologic derivation.

In summary, the current findings of homogeneity in ganglioside biosynthesis among neuroblastoma tumors diagnosed in infancy (whether by screening or clinically), and the variability in patterns of ganglioside biosynthesis in tumors from children older than 1 year of age, support the view that neuroblastoma consists of at least two distinct but overlapping disorders characterized by different biologic and clinical phenotypes. The predominance of fetal patterns of ganglioside biosynthesis in infant tumors may be indicative of the fetal derivation of these tumors and may account in part for some of their clinical manifestations (i.e., patterns of metastatic spread, tumor–host immune response). In addition, these findings raise the possibility that the role of gangliosides in the biology of neuroblastoma may be best approached by studying alterations in overall ganglioside biosynthesis, in addition to the behavior of individual ganglioside species, and relating these to other established biologic prognostic factors. Finally, it will be necessary to address the prognostic significance of altered ganglioside metabolism in neuroblastoma, especially when compared with other prognostic variables, in a larger series of patients.

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