surface ligand recognize an identical or closely related receptor binding site.

Thrombospondin (TSP), an adhesive glycoprotein, has also been proposed as a receptor for cytoadherence of infected erythrocytes (*18*). Our purified CD36 was not contaminated with TSP, as determined by Coomassie blue and silver staining of protein separated by SDS-PAGE and by enzyme immunoassay with antiserum to TSP (*17*). In addition, TSP is synthesized by many cells that do not have the capacity to bind *P. falciparum*–infected cells (*9*). This lack of correlation between the presence of TSP and binding argues against the specificity of TSP as a cytoadherence receptor. Surface expression of a receptor recognized by MAb OKM5 is correlated with the ability of various monocyte and melanoma cell lines to bind infected erythrocytes (*9, 10*). Furthermore, much higher concentrations of TSP than of CD36 are required for significant inhibition of cytoadherence to C32 melanoma cells (200 μg/ml versus <10 μg/ml), and neither TSP nor antibodies to TSP have been reported to reverse cytoadherence. It is possible that CD36 and TSP have sequence similarities that account for the binding of infected erythrocytes to either of these purified proteins when immobilized on plastic. Alternatively, CD36 and TSP may bind to independent ligands on the surface of K+-infected erythrocytes.

Although these results strongly suggest that CD36 is a malaria sequestration receptor, the identity of the complementary ligand on the infected erythrocyte surface is still unknown. It has been suggested that the cytoadherence ligand is a poorly immunogenic conserved domain that is masked by a more immunogenic, strain-specific moiety on the surface of *P. falciparum*–infected erythrocytes (*19*). The availability of purified CD36 should allow identification and characterization of epitopes on the cytoadherence ligand that may be useful for a malaria vaccine. Soluble CD36, or a fragment thereof that can reverse cytoadherence in vitro, may also be useful therapeutically for rapid reversal of sequestration in cerebral malaria or other complicated forms of this disease.

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The Mutations in Ashkenazi Jews with Adult G_{M2} Gangliosidosis, the Adult Form of Tay-Sachs Disease

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The adult form of Tay-Sachs disease, adult G_{M2} gangliosidosis, is an autosomal recessive disorder that results from mutations in the α chain of β-hexosaminidase A. This disorder, like infantile Tay-Sachs disease, is more frequent in the Ashkenazi Jewish population. A point mutation in the α-chain gene was identified that results in the substitution of Gly^{269} with Ser in eight Ashkenazi adult G_{M2} gangliosidosis patients from five different families. This amino acid substitution was shown to depress drastically the catalytic activity of the α chain after expression in COS-1 cells. All of these patients proved to be compound heterozygotes of the allele with the Gly to Ser change and one of the two Ashkenazi infantile Tay-Sachs alleles. These findings will aid in the diagnosis and understanding of β-hexosaminidase A deficiency disorders.

BOTH ADULT G_{M2} gangliosidosis and the infantile form of Tay-Sachs disease are autosomal recessive diseases caused by a deficiency of β-hexosaminidase A, a lysosomal enzyme composed of α and β chains (*1*). The enzyme deficiency is a consequence of mutations in the α-chain gene. As a result, there is a progressive accumulation of G_{M2} ganglioside, the natural substrate of β-hexosaminidase A, leading to degenerative changes in the nervous system. However, with respect to onset and severity, the two disorders are very different. In infantile Tay-Sachs disease, the age of onset is within the first year of life with death ensuing in early childhood after progressive motor and mental deterioration. In the adult disease (*2, 3*), onset usually occurs in the second or third decade with lower motor neuron, pyramidal tract, and cerebellar deterioration. In many cases psychosis seems to be an integral part of the disease and precedes the other neurological manifestations (*3*). Both the adult and infantile diseases are more prevalent in Ashkenazi Jews than in the general population, with the infantile form predominating.

Recently it has been shown that two different mutations in the α-chain gene un-

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derlie Ashkenazi infantile Tay-Sachs disease: a splice junction mutation (*4–6*) at the 5′ end of intron 12 accounts for ~30% of the mutant alleles (*4, 5*) and a 4-base-pair insertion in exon 11 accounts for the remaining ~70% (*7*). Both of the infantile Tay-Sachs mutations result in nearly undetectable levels of mRNA (*6, 8–10*), as well as a complete absence of α-chain protein (*11*) and of β-hexosaminidase A activity (*1*). In contrast, α-chain mRNA (*12*) and a precursor α-chain polypeptide are readily detectable in fibroblasts from adult patients (*13, 14*). Apparently this mutant α chain is able to form a small amount of active enzyme because these adult patients demonstrate a low level of activity if G_{M2} ganglioside is used as a substrate (*15*). No significant activity is detected with synthetic substrates (*2*).

To identify the molecular defect in adult G_{M2} gangliosidosis, a cDNA library was constructed (*16*) with polyadenylated RNA prepared from the skin fibroblasts of a well studied 45-year-old Ashkenazi patient (S.R.) (*17*). This patient is an obligate carrier of an infantile Tay-Sachs allele, as one of his children was affected with this disorder. Since the Tay-Sachs infantile alleles give rise to little detectable mRNA, any cDNA clone isolated from this library was likely to be derived from the mRNA-positive adult G_{M2} gangliosidosis allele. The cDNA library was screened for α-chain recombinants and the longest insert obtained, 1.2 kb, was sequenced. This analysis revealed that the cDNA was truncated at the 5′ end and contained 1033 of the 1587 base pairs of the α-chain protein coding region (*9*). The only difference between the normal and the mutant cDNA sequence was a single nucleotide substitution, G to A, changing Gly^{269} (GGT) to Ser (AGT) (Fig. 1). The cDNA showed no evidence of either of the Ashkenazi infantile mutations.

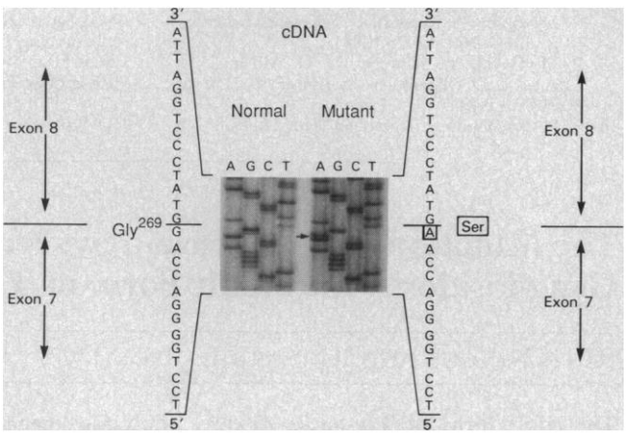

Fig. 1. Nucleotide sequence of α-chain cDNA from a patient with G_{M2} gangliosidosis. An α-chain cDNA isolated from patient S.R. was subcloned into the Eco RI site of pUC13. The normal α-chain cDNA has been described (*9*). The cDNAs were sequenced by the dideoxy-chain termination method (*30*) with an α chain–specific primer, ^{35}S-labeled deoxyadenylate triphosphate (dATP), and Sequenase (U.S. Biochemicals). A portion of autoradiograph is shown for the sequencing gel of the normal cDNA (normal) and of the patient's cDNA (mutant). The nucleotide change (G to A) in the patient's cDNA is boxed. The sequence is labeled 5′ and 3′ in reference to the orientation of the α-chain gene (*18*).

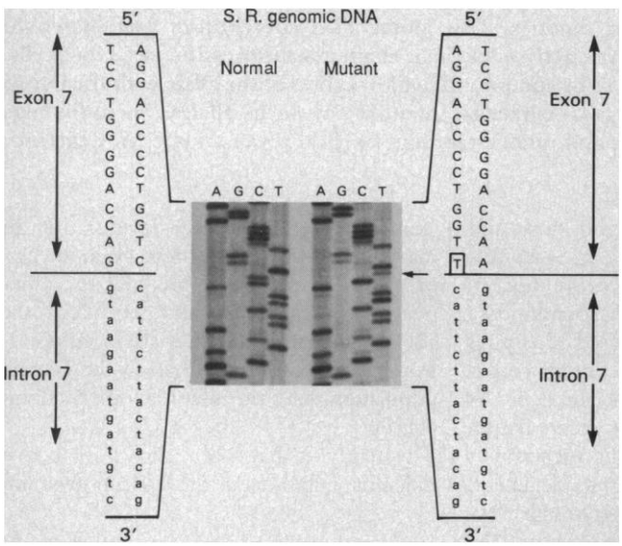

Fig. 2. Nucleotide sequence of the α-chain gene from a patient with G_{M2} gangliosidosis. Genomic DNA from patient S.R. was amplified by the PCR technique with Taq DNA polymerase (Perkin-Elmer Cetus) and oligonucleotides primers, 5′-GGGTCCTACAACCCTGTCACCCAC-3′ and 5′-AAGCTTCACTCTGAGCATAACAAG-3′, specific for exon 7 and intron 7, respectively, of the α-chain gene (*18, 31*). The DNA was subjected to amplification on a Perkin-Elmer Cetus thermocycler for 39 cycles. Each cycle was 2 min at 94°C for denaturation, 2.5 min at 60°C for annealing, and 3 min at 72°C for primer extension. The amplified 190-base-pair segment was cleaved with Hind III and Hpa II. The resulting 117-base-pair fragment was isolated from a 4% NuSieve agarose gel (FMC BioProducts) and ligated into the Hind III and Acc I sites of the M13mp18 vector. From the recombinant clones, single-stranded DNA was isolated and sequenced by the dideoxy-chain termination method with the universal primer and Sequenase. Shown are representatives of the two types of clones isolated; Normal indicates a sequence identical with the normal α-chain gene sequence, and mutant indicates a sequence with the G to A change described in the text. The sequence is labeled 5′ and 3′ in reference to the orientation of the α-chain gene (*18*).

Since the nucleotide change in the cDNA occurs at the 3′ nucleotide of exon 7 in the α-chain gene (*18*), we investigated the possibility that the mutation might extend into the intron. Using the polymerase chain reaction (PCR) (*19*), we amplified 190 base pairs of this patient's genomic DNA surrounding the G to A base change. A restriction fragment of the amplified DNA was cloned and sequenced. Two types of clones were found, one containing a normal sequence and the other with the same G to A change as in the cDNA with no alteration of the intron sequence (Fig. 2). The two sequences demonstrate, as was anticipated (*17*), that this patient is a heterozygote for the allele containing the G to A point mutation.

We next developed an assay to screen for this point mutation in other adult patients. The 190-base-pair region surrounding the mutation was amplified from genomic DNA by the PCR technique, and the product was blotted onto a hybridization membrane. The samples were hybridized with allele-specific oligonucleotide probes to detect either the sequence containing the G to A change or the corresponding normal sequence. Amplified genomic DNA of eight Ashkenazi adult patients from five unrelated families were positive for the point mutation (Fig. 3). A normal Ashkenazi control and two Ashkenazi infantile Tay-Sachs carriers, each with a different mutation, were negative for the G to A change. To exclude the possibility that the G to A change is a neutral polymorphism, we screened genomic DNA from an additional ten normal Ashkenazi Jews and ten heterozygote carriers of one of the Ashkenazi infantile Tay-Sachs alleles. None of these DNA samples were positive for the G to A mutation. Both patients and controls were positive with the probe carrying the normal sequence (Fig. 3) and could not be distinguished at any of the washing temperatures tested, consistent with compound heterozygosity in all of the patients.

We determined if the presumed compound heterozygosity was a result of the allele bearing the G to A change in combination with either of the infantile Tay-Sachs alleles. The regions surrounding the infantile splice junction mutation in intron 12

Table 1. Expression of α chain–associated β-hexosaminidase activity in transfected COS-1 cells. COS-1 cells were transfected with pSVLα, pSVLα Gly^{269}→Ser, or were mock-transfected (*21*). After 48 hours the cell extracts were assayed with 4-methylumbelliferyl-6-sulfo-2-acetamido-2-deoxy-D-glucopyranoside (Toronto Research Chemicals) (*22*). One unit of enzyme activity is defined as the activity that releases 1 nmol of 4-methylumbelliferone per hour.

DNA	β-Hexosaminidase (activity units per milligram of protein)
pSVLα	8089
pSVLα Gly^{269}→Ser	328
Mock transfection	352

and the 4-base-pair insertion in exon 11 were amplified by the PCR technique. The amplified DNA samples were blotted on a filter and hybridized to allele-specific oligonucleotide probes under conditions that detect the infantile mutations (*4, 7*). The autoradiograms (Fig. 3) clearly indicate that each adult patient carries an Ashkenazi infantile Tay-Sachs mutant allele; two patients carry the splice junction mutation in intron 12 and the remaining six carry the more common insertion in exon 11. These results demonstrate that all the patients are compound heterozygotes with one allele containing the G to A change in exon 7 and the other harboring one of the two Ashkenazi infantile Tay-Sachs mutations.

We studied the segregation of the alleles bearing the G to A mutation and the infantile insertion mutation in three generations of the family of the two related patients, R.O. and A.O. (Fig. 4, III-1 and III-2). The allele bearing the G to A mutation was inherited from the patients' father (II-1) and is also carried by their grandmother (I-1) (Fig. 4). The infantile Tay-Sachs allele segregated from the maternal side; the patients' mother (II-2), aunt (II-3), and cousin (III-4) harbor this insertion defect. The unaffected sibling (III-3) carries the adult but not the infantile mutation.

We investigated the consequence of the G to A point mutation on the catalytic activity associated with the α chain. The G to A change was introduced into the normal α-chain cDNA by oligonucleotide mediated site-directed mutagenesis (*20*). The normal and the mutated α-chain cDNAs were inserted into the eukaryotic expression vector, pSVL, and the plasmid DNAs were transfected into COS-1 cells (*21*). The cell extracts were assayed with a synthetic substrate (*22*) specific for the catalytic activity of the α chain (*23*). Transfection with the normal α chain (pSVLα) resulted in a large increase of catalytic activity. Most of this activity is likely to represent β-hexosaminidase S (αα) because greater than 90% of the activity was not precipitable with antibodies reactive with the β chain. In contrast to the normal cDNA, no increase over background activity was observed with the cDNA containing the G to A change (pSVLα Gly^{269} → Ser) (Table 1). The cells transfected with the mutant cDNA were found, however, to synthesize an α-chain polypeptide (*12*).

We have shown that all adult Tay-Sachs patients tested thus far carry a mutated α-chain allele with a Gly^{269} to Ser change. In all of the patients, the allele bearing this point mutation was found in combination with one of the two Ashkenazi infantile Tay-Sachs alleles. Although the frequency of the adult mutation is not yet established, it is thought to be rare (*24*). Thus, the appearance of the adult disorder in Ashkenazi Jews is, in large part, due to the high carrier frequency (1/30) of the infantile Tay-Sachs alleles.

The Gly^{269} to Ser change is functionally significant because it eliminated the catalytic activity associated with the α chain. A previous study had demonstrated that the α chain expressed in fibroblasts from adult G_{M2} gangliosidosis patients is defective in subunit assembly (*14*). Thus, the lack of activity after expression in COS-1 cells may be the inability of the α chain with the Gly^{269} to Ser change to dimerize and form catalytically active β-hexosaminidase S (αα) (*1*). The importance of this Gly at position 269 is also suggested by its conservation in both the homologous human β chain (*10, 25*) and in β-hexosaminidase from the slime mold *Dictyostylium discoideum* (*26*).

Although the cDNA that we isolated appeared to be derived from a correctly spliced mRNA, it is possible that the G to A mutation, because of its position at the 3′ end of exon 7, might disrupt the splicing of a fraction of the α-chain mRNA. The spf^{ash} mutation in the mouse ornithine transcarbamylase gene, also a G to A change occurring in the last nucleotide of an exon, has been shown to result in some misspliced mRNA (*27*).

The identification of the mutation in adult G_{M2} gangliosidosis, together with the two previously identified infantile Tay-Sachs mutations, will facilitate the study and diagnosis of β-hexosaminidase deficiencies in the Ashkenazi population. With the exception of an unidentified allele in a single patient

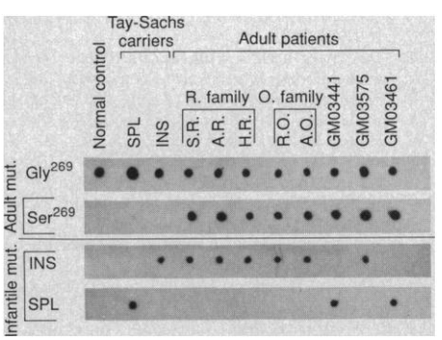

Fig. 3. The α-chain mutations in Ashkenazi adult G_{M2} gangliosidosis patients. Genomic DNA (indicated at the top of the figure) was isolated from the blood samples of a normal Ashkenazi individual (normal control), and from two infantile Tay-Sachs carriers; one harboring the splice junction mutation (SPL) and the other carrying the 4-base-pair insertion mutation (INS). The other DNAs were from adult G_{M2} gangliosidosis patients; from the cultured fibroblasts from three sibling patients (S.R., A.R., and H.R.), from blood samples of two affected sisters (R.O. and A.O.), and from three lymphoblast cultures (GM03441, GM03575, and GM03461) obtained from the Human Genetic Mutant Cell Repository (Camden, New Jersey). The regions surrounding the G to A point mutation (described in Fig. 2) and each of the two infantile Tay-Sachs mutations (*4, 7*) were amplified by the PCR technique. The amplified DNA was blotted on Gene Screen Plus filters (Du Pont, Biotechnology Systems), denatured, neutralized, and dried. Prehybridization was for 1 hour at 37°C in a solution containing 1*M* NaCl, 0.005*M* EDTA, 1% SDS, and denatured salmon sperm DNA (100 μg/ml). Hybridization was performed overnight at 37°C after addition of the appropriate ^{32}P-labeled allele-specific oligonucleotide probe (~2.5 ng/ml). The oligonucleotide probes, indicated on the left of the figure, were as follows: Gly^{269}, 5′-TGGGGACCAGGTAAGAATG-3′, corresponding to the normal sequence in the region of the G to A change; Ser^{269}, 5′-TGGGGACCAAGTAAGAATG-3′, corresponding to the mutant sequence containing the G to A change; SPL, 5′-CAGGCTCTGTGCTAAGGGTT-3′, corresponding to the splice junction mutation found in infantile Tay-Sachs disease (*4*); and INS, 5′-GAACCGTATATCTATCCTA-3′, corresponding to the 4-base-pair insertion found in infantile Tay-Sachs disease (*7*). The blots were washed twice for 30 min in 2× saline sodium citrate (SSC) containing 0.5% SDS at room temperature and then for 5 min at 55°C with 2× SSC.

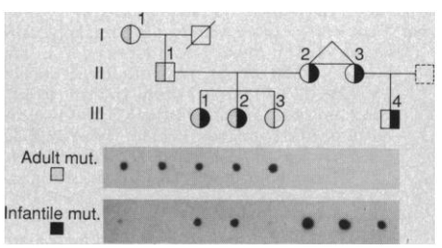

Fig. 4. Genotype analysis of an Ashkenazi family with two affected siblings with adult G_{M2} gangliosidosis. Details of the assay to detect the G to A point mutation (adult mut.) and the infantile Tay-Sachs 4-base-pair insertion (infantile mut.) are described in the legends to Figs. 2 and 3. The family pedigree is shown at the top of the figure. Patients R.O. and A.O. (Fig. 3) are represented by III-1 and III-2. All other members of the family are clinically normal.

(7), these three α-chain mutations are responsible for the β-hexosaminidase A deficiency in all Ashkenazi adult G_{M2} gangliosidosis patients and infantile Tay-Sachs patients tested thus far. Diagnosis based on these mutations will be helpful for carrier detection and genetic counseling when the enzymatic assay is inconclusive. Furthermore, it is now possible, with allele-specific probes, to distinguish carriers of the adult mutation from carriers of the infantile mutations, which is not achievable with the conventional enzymatic assay. Finally, it will be of interest to determine if the mutation described here is present in the various clinical forms (juvenile, chronic, and adult) associated with β-hexosaminidase A deficiency in Ashkenazi Jews and in other ethnic groups (*28*) and to assess the clinical consequences of this mutation in a homozygous form.

Note added in proof: We have learned that Paw *et al.* (*29*) have also identified The Gly^{269} to Ser charge in other patients with adult-onset G_{M2} gangliosidosis.

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Quisqualate Activates a Rapidly Inactivating High Conductance Ionic Channel in Hippocampal Neurons

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Glutamate activates a number of different receptor-channel complexes, each of which may contribute to generation of excitatory postsynaptic potentials in the mammalian central nervous system. The rapid application of the selective glutamate agonist, quisqualate, activates a large rapidly inactivating current (3 to 8 milliseconds), which is mediated by a neuronal ionic channel with high unitary conductance (35 picosiemens). The current through this channel shows pharmacologic characteristics similar to those observed for the fast excitatory postsynaptic current (EPSC); it reverses near 0 millivolts and shows no significant voltage dependence. The amplitude of the current through this channel is many times larger than that through the other non-NMDA (*N*-methyl-D-aspartate) channels. These results suggest that this high-conductance quisqualate-activated channel may mediate the fast EPSC in the mammalian central nervous system.

GLUTAMATE IS BELIEVED TO BE AN important excitatory neurotransmitter in the mammalian central nervous system (*1*). The glutamate-evoked current is the result of the behavior of more than one type of glutamate channel coexisting on the same neuron (*2, 3*). These different receptor-channel complexes are postulated to play a variety of physiological roles (*4–7*). A rapidly desensitizing component of the glutamate-evoked current is seen when the agonist is applied rapidly (*8–11*). Here, we identify and characterize the properties

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