postsynaptic events into bursts of impulses; the mechanism involves the closing of special potassium channels. We have demonstrated that the number of M1 receptors remains unchanged in patients with SDAT and after experimental cortical cholinergic denervation in rats; these results suggest a postsynaptic location for these sites. Taken together, these data indicate that M1 and M2 receptors are differently disposed at cholinergic synapses in the cerebral cortex and have different mechanisms and functions.

The selective reduction in the number of M2 receptors in patients with SDAT has therapeutic implications. When cholinergic nerve terminals are gone, there is a need to replace the action of acetylcholine on postsynaptic M1 receptors. Replacement therapy is being attempted directly with acetylcholine analogs (*26*) and indirectly with esterase inhibitors (*27*). It is also necessary to avoid M1 antagonists, such as atropine, some drugs used to treat Parkinson's disease, certain antihistamines and neuroleptics, and possibly quinidine. We hypothesize that early in the course of Alzheimer's disease, an M2-selective antagonist might be useful in addition to an esterase inhibitor, to prevent acetylcholine from inhibiting its own release. Part of the reason why physostigmine has not been very useful by itself in early SDAT (*27*) may be that as it maintains the presence of acetylcholine, which is highly M2-selective, further transmitter release is decreased. Thus both M1 agonists and M2 antagonists deserve clinical trials.

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Circulating Autoantibodies to the 200,000-Dalton Protein of Neurofilaments in the Serum of Healthy Individuals

Abstract. *There is substantial evidence that human serum contains antibodies to many autoantigens. For example, all healthy people have autoantibodies (immunoglobulin M) to some undefined brain antigens. In this study immunoblots and immunohistochemical staining were used to detect antibodies to neural tissues in serum samples from 200 healthy people and 200 patients with various neurological diseases. Ninety-nine percent of the 400 subjects had serum immunoglobulin M and 95 percent had immunoglobulin G that bound to a 200-kilodalton protein in homogenates of neural tissues. In most cases there were no antibodies to anything else in the homogenates. The 200-kilodalton protein was the heaviest of the neurofilament triplet proteins. These observations do not support a role for antibodies to the 200-kilodalton protein of neurofilaments in the pathogenesis of neurological diseases.*

In his clonal selection theory, Burnet (*1*) proposed that lymphocytes that respond to antigenic stimulation during development are killed. This would mean that lymphocytes that respond to autoantigens present during development are also extinguished. Therefore, autoantibodies should only appear in pathologic conditions, either from emergence of new clones of lymphocytes or from exposure of the immune system to autoantigens that either were not expressed or were sequestered during development. According to the clonal selection theory, the presence of autoantibodies signifies disease.

There has been considerable recent evidence against the clonal selection theory (*2–6*). It is, for example, probable that most people have serum antibodies to certain autoantigens (*3*). It is even possible that most people have in their serum antibodies to virtually all autoantigens. However, most of the self-reactive antibodies in the serum of healthy people are present at low titers, and they may be less specific than induced antibodies (*5*). The existence of these autoantibodies in the serum of healthy people makes unclear the implications of finding autoantibodies against components of the affected organs in the serum of patients.

Doar and Fabre (*3*) reported that healthy people have in their serum organ-specific immunoglobulin M (IgM) autoantibodies to liver, heart, and brain. We attempted to determine whether the serum IgM antibodies to brain tissue in healthy people bind to specific antigens and, if so, to which. Using immunoblots containing electrophoresed proteins from homogenates of various neural tissues and isolated neurofilaments, we found that most healthy people have in their serum both IgM and immunoglobulin G (IgG) antibodies that are directed against the 200-kD neurofilament protein (NFP) and that only a few people have a high enough titer of serum antibodies to other proteins (including other NFP's) in the neural homogenates to be detected by this method.

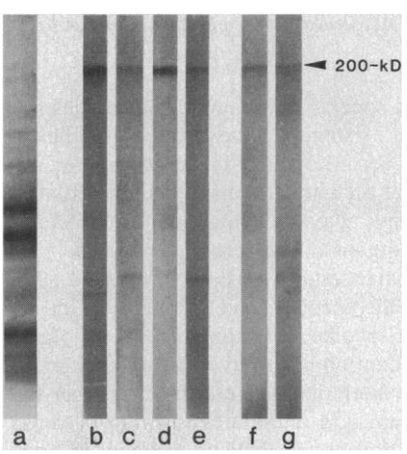
Table 1. Diagnoses of the subjects with neurological diseases.

Diagnosis	Number of patients
Myasthenia	24
Amyotrophic lateral sclerosis	36
Guillain-Barré syndrome	10
Neuropathies of unknown pathogenesis	30
Alzheimer's disease	10
Central nervous system lupus	10
Parkinson's disease	10
Multiple sclerosis	25
Astrocytomas	10
Stroke	20
Subacute sclerosing panencephalitis	5
Total	200

Serum from 200 healthy men and women 20 to 80 years of age and from 200 patients with various neurological diseases (Table 1) were obtained from a serum collection at the Department of Neurology, University of Chicago. IgG was isolated from the serum of two individuals that had high titers of IgG against the 200-kD NFP (*7*). Subsequently, $F(ab')_2$ fragments were prepared by treating the isolated IgG with pepsin (*8*). The fragments were separated from the intact IgG by cationic exchange chromatography (MonoS, Pharmacia), and the $F(ab')_2$ fragments were separated from the Fc portions by anionic exchange chromatography (MonoQ, Pharmacia) with a fast protein, peptide, and polynucleotide liquid chromatographic instrument (Pharmacia).

To prepare antigens, we homogenized pieces of normal human cerebral white and gray matter, cerebellum, and spinal cord (obtained at autopsy) separately in a solution containing 6.3*M* urea, 1.0 percent (weight to volume) sodium dodecyl sulfate (SDS), and 1 percent (by volume) 2-mercaptoethanol. The solution was then boiled for 3 minutes. Neurofilaments were isolated from bovine spinal cord (*9*) and solubilized in the same solution as the homogenates or used to absorb serum.

Proteins in the homogenates and the neurofilament preparations were separated on 5.6 percent SDS–polyacrylamide slab gels (*10*), and the gels were stained with Coomassie blue or the proteins were electrically transferred onto nitrocellulose by Trans-blot cell (Bio-Rad) at 60 V for 3 hours in 20 m*M* tris-HCl buffer (*p*H 7.5) with 20 percent methanol. The nitrocellulose sheets were cut into strips, which were placed individually into capped test tubes and stained with antibodies from the serum (*11*). All the human serum was used at a dilution of 1:50, and some samples were also used at greater dilutions (up to 1:1000). As second antibodies, peroxidase-labeled rabbit antibodies to the human μ or γ chain were used (DAKO).



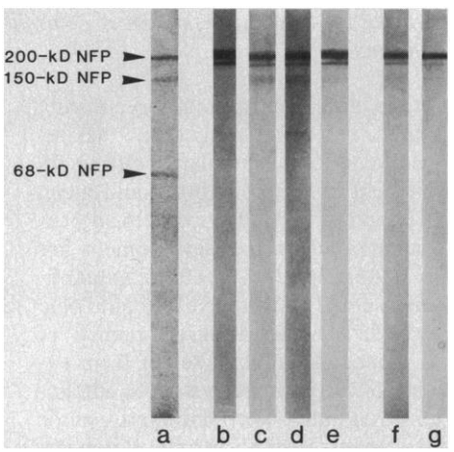


Fig. 1 (left). Antibodies in serum from healthy people and patients with neurological diseases against the 200-kD protein in a homogenate of human white matter. Lane a shows Coomassie blue–stained SDS–polyacrylamide gel (*10*) containing electrophoresed proteins from the homogenate. Lanes b to g show nitrocellulose strips to which proteins from a gel equivalent to that in lane a were electrically transferred. Each of the nitrocellulose strips was incubated separately with human serum diluted 1:50. Binding of IgM (lanes b to e) or IgG (lanes g and h) from the serum to proteins on the nitrocellulose was revealed by an indirect peroxidase method (*11*). The arrow points to a ~200-kD protein that was identified by antibodies in all the samples represented here. Fig. 2 (right). Antibodies in serum from healthy people and patients with neurological diseases against the 200-kD protein in bovine neurofilaments. Lanes a to g show nitrocellulose strips containing electrophoresed proteins from isolated neurofilaments. The strip in lane a was stained with amido black, which shows the total proteins (*23*). The strips in lanes b to g were each incubated separately with human serum diluted 1:50. Binding of IgM (lanes b to e) or IgG (lanes f and g) from the serum to proteins on the nitrocellulose was revealed by an indirect peroxidase method (*11*). All the serum samples represented here had antibodies to the 200-kD NFP.

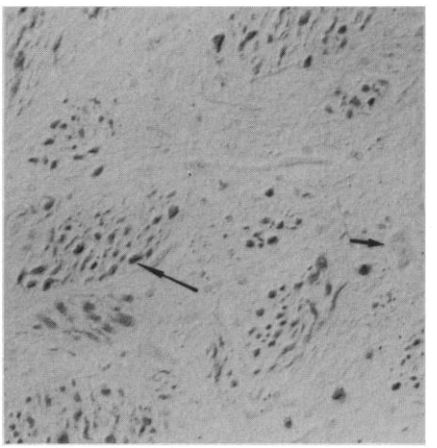


Fig. 3. Binding of IgM from the serum of a healthy individual to axons in the human central nervous system. The photomicrograph (×300) shows a cross section of human spinal cord after it was overlaid with the serum from a healthy individual diluted 1:50 and after binding of IgM from the serum to the tissue section had been revealed by a biotin-avidin-peroxidase method (*12*). The tissue section was also counterstained lightly with hematoxylin. The long arrow points to an axon stained darkly with IgM from the serum; the short arrow to a neuron stained faintly with hematoxylin.

Sections of human spinal cord, fixed in Formalin and embedded in paraffin, were stained immunohistochemically with human serum at the dilution 1:50 by a biotin-avidin-peroxidase method (*12*).

Of the 400 serum samples, 396 contained IgM against the 200-kD protein in the neural tissue homogenates and 380 contained IgG against this protein (Fig. 1). Only ten samples from healthy individuals had antibodies to anything else in the homogenates, and of these only two had antibodies to the same additional protein. Forty-five of the samples from the patients with neurological diseases contained antibodies to proteins in the homogenates other than the 200-kD protein. The other proteins against which these 45 patients had antibodies were, to a certain extent, dictated by the diagnosis they carried.

Of the 400 samples, 396 contained IgM against the 200-kD NFP and 380 contained IgG against this NFP (Fig. 2). The binding of IgG and IgM to the 200-kD protein in the homogenates of the neural tissues was absorbed with isolated neurofilaments. Isolated $F(ab')_2$ fragments of IgG also bound specifically to the same protein. Thirty-one percent of the samples in both groups had serum IgM and 12 percent had serum IgG that immuno-

histochemically stained axons in the human spinal cord (Fig. 3), which is consistent with binding of antibodies to neurofilaments. None of the disease categories was associated with higher titers of antibodies to the 200-kD NFP or to axons than those found among the healthy controls.

In summary, a large sample of healthy people and patients with neurological diseases had in their serum antibodies against the 200-kD NFP, and this NFP was the only protein in homogenates of neural tissues against which most of the healthy subjects had serum antibodies. The neurofilaments are the intermediate filaments of neurons and are composed of three proteins of 200, 150, and 68 kD. The 68-kD protein forms the backbone of the neurofilaments and the 200- and 150-kD proteins appear to be more peripherally located (*13, 14*). It is also possible that the 200-kD NFP gives rise to smaller protease-resistant fragments that retain immunoreactivity, as seen in transsected nerves (*15*). Antibodies to cytoskeletal components, including the intermediate filaments, are commonly found in the serum of healthy people (*16*), and so are antibodies to many other autoantigens (*5*). These autoantibodies may even serve a physiological role in the normal catabolism of tissues or as vehicles for transporting blood-borne substances (*17*).

This does not, however, explain the relatively high titer of antibodies to the 200-kD NFP compared with other autoantigens in the homogenates of neural tissues used in our work. In the rat the 200-kD NFP appears late in development (*18*); according to the clonal selection theory (*3*), the immune system could therefore have less tolerance for it than proteins that appear at an earlier stage. We did not find increased titers of antibodies to the 200-kD NFP associated with any of the diseases (Table 1). This is in contrast to the results of Sotelo *et al.* (*19*) and Bahmanyar *et al.* (*20*), who found a higher incidence of antibodies to "neurofilament antigens" in serum from patients with various neurological diseases than in serum from healthy individuals, but agrees with the results of Elizan *et al.* (*21*), who did not find any such difference. However, Elizan *et al.* did observe a greater incidence of serum antibodies to neurofilaments in people over 70 years of age. We found the same incidence of antibodies against the 200-kD NFP in the serum in all age groups.

It is difficult to compare our data with those of the aforementioned investigators because they used immunohistochemical staining of axons to detect antibodies to neurofilaments or neurofilament antigens, whereas we used immunoblots to detect antibodies to the 200-kD NFP, and the methods differ substantially in their sensitivity and specificity. Recently, Bahmanyar *et al.* (*22*) showed that a chimpanzee that was inoculated intracerebrally with brain tissue from a patient with Creutzfeldt-Jakob disease (CJD) and that developed CJD 14 months later had serum antibodies to the 200-kD NFP after onset of the disease but not before the inoculation. However, our results underscore the need for caution when interpreting the finding of antibodies to neurofilaments in the serum of patients with neurological diseases.

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Male Reproductive Parasitism: A Factor in the Africanization of European Honey-Bee Populations

Abstract. *Africanized drone honey bees* (Apis mellifera) *migrate into European honey-bee colonies in large numbers, but Africanized colonies only rarely host drones from other colonies. This migration leads to a strong mating advantage for Africanized bees since it both inhibits European drone production and enhances Africanized drone production.*

Colonization by Africanized honey bees in the continental United States is a possibility within 5 years (*1*). This honey bee (*Apis mellifera scutellata*; formerly *A. m. adansonii*) (*2*) was introduced into Brazil in 1956 with the escape of 26 queens (*3*) and the possibly 250 drones with which they had mated in Africa. In less than 30 years the genetic materials from this bee have overwhelmed the vastly larger and more diverse gene pool of previously imported European honey bees (mainly *A. m. mellifera* and *A. m. ligustica*) (*4*) throughout most of South America and lately much of Central America. Taxonomic (*5*) and behavioral (*6*) descriptions of the Africanized population are quite similar to descriptions of the parental African bee population (*2, 3, 7, 8*) in spite of interbreeding with European bee stocks. After a few years the European bee is largely replaced by the Africanized bee (*9*), which retains fully its several objectionable characteristics including severe stinging (*6*) and poor honey production (*10*).

The rapid change of European bee colonies into Africanized colonies strongly suggests a mating advantage. An earlier study (*11*) used equal numbers