Myasthenia Gravis Under Monoclonal Scrutiny

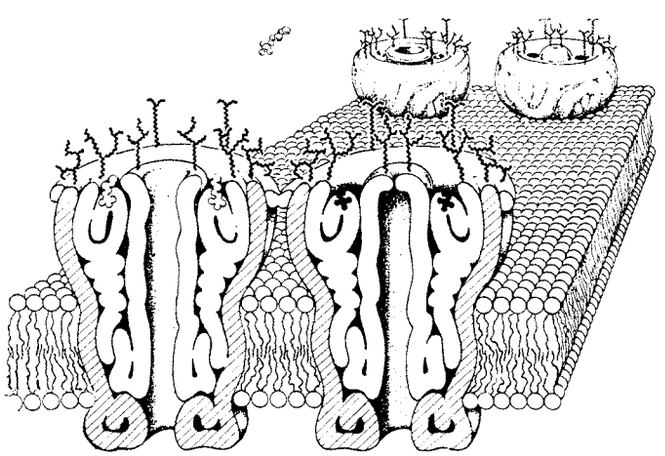
The recent application of the monoclonal antibody technique to myasthenia gravis is leading to a greater understanding of some fundamental aspects of the disease

A period of highly productive research during the 1970's has brought myasthenia gravis research to the position where, as Jon Lindstrom of the Salk Institute says, "The next breakthrough will be when someone declares 'I can cure it' or 'I've discovered the cause'." That point has yet to be reached, but the deployment by a rapidly growing number of research groups of the monoclonal antibody technique does at minimum seem likely to generate a greater understanding of the disease. "And," says Vanda Lennon of the Mayo Clinic, Rochester, "there has to be a reasonable chance that some kind of specific therapy might emerge from the work with monoclonal antibodies."

The subject of monoclonal antibodies created an interesting focus at a recent meeting sponsored jointly by the New York Academy of Sciences and the Myasthenia Gravis Foundation.

Myasthenia gravis is characterized by weakened and easily tired muscles. About 10 percent of patients die from the disease, but among those who survive the first 3 years there is a good chance that the condition will stabilize and there will be some degree of recovery. Paradoxically, myasthenia gravis is at once one of the most and yet least understood of diseases: the molecular machinery defective in the condition is being described in ever finer detail, while the cause of the disease remains a mystery.

Initiation of normal muscle contraction involves the transmission of an electrical signal along a nerve fiber, arriving at the muscle at a structure known as a motor end plate. Arrival of the signal at the nerve terminal triggers the release of a neurotransmitter, acetylcholine, which crosses the 600-angstrom synaptic gap in the end plate and interacts with an array of specific receptors located at the crests of the regularly folded postsynaptic membrane. This interaction opens channels in the receptors, allowing a sudden and massive flow of sodium and potassium ions through the membrane. This creates a full-blown end-plate potential that kicks the muscle into action. While the muscle is at rest a steady trickle of acetylcholine into the synaptic gap generates miniature end-plate potentials, but these are inadequate to initiate an action potential in the muscle fibers.



Acetylcholine receptors

The receptors on the left have acetylcholine molecules bound to them; the ion channel is therefore open. [*Courtesy of Jon Lindstrom,* Advances in Immunology, *vol. 27, page 1*]

An important feature of normal muscle action is the very large built-in "safety factor": about 10 times as many acetylcholine receptors are activated as are required to set up a full end-plate potential. This means that the end plate can sustain considerable damage before initiation of muscle contraction is affected. The damage incurred in myasthenia gravis must, therefore, be substantial.

What, then, is the source of the problem in the disease? "The synapse has been known to be the focus of the disease ever since the 1940's," explains Lindstrom. "Daniel Elmqvist and his colleagues made the important discovery in the mid-1960's that myasthenics have reduced miniature end-plate potentials, but they erroneously concluded that the defect lay with the release of acetylcholine from the nerve terminal." This led everyone astray for quite a long time. A sequence of important discoveries during the 1970's subsequently pinpointed the pathology to an immune-mediated disruption of the array of acetylcholine receptors in the postsynaptic membrane. An individual's immune system sets up what Lindstrom has called "an immunological civil war" against a range of chemical markers, or antigens, displayed on the surface of the acetylcholine receptor molecule.

Once antibodies to the receptor had been implicated in the disease, it was quickly assumed that an important target was the acetylcholine binding site on the receptor. "But this is not the case," explains Lennon. "Most of the antibodies bind at positions on the receptor other than the acetylcholine reaction site. The antibodies interfere with neuromuscular transmission by destroying the receptors." It turns out that some receptor antibodies interact with a component of the immune system known as complement; this results in the breakup or lysis of the postsynaptic membrane. Other antibodies cross-link between two receptors, causing them to be swallowed up into the membrane; this process is known as antigenic modulation. No one knows which of the two mechanisms is the more important in the disease, but in any case the end result is a severely disrupted membrane containing about one-third of the usual number of receptor molecules. Many of the remaining molecules have antibodies bound to them, significantly impairing their response to acetylcholine.

"One of the great puzzles of myasthenia gravis is the lack of correlation between the level of antibody in the sera and the clinical severity of the disease," said Daniel Drachman of Johns Hopkins University. "We must understand this phenomenon if we are to make significant progress." Although fluctuation of antibody levels within an individual is a good guide to his or her clinical condi-

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tion, the absolute amount of antibody in someone's blood is not a good predictor of the severity of the disease.

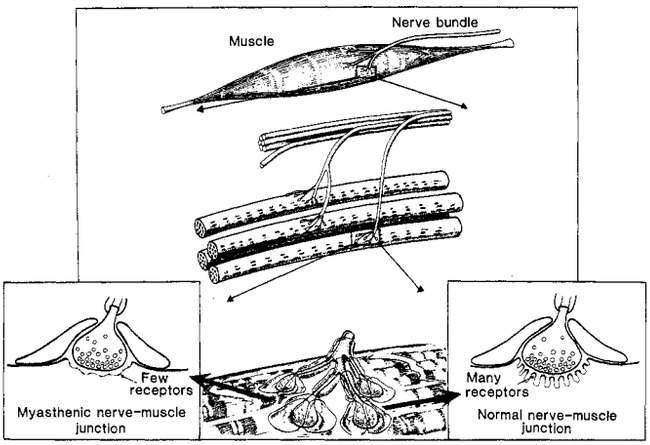
"The inference is that a spectrum of antibodies is produced in myasthenia gravis," suggests Lennon, "some of which are more important than others." In other words, one person may have a low titer of antibodies in the blood, but they happen to be of a particularly potent type, and so weakness is pronounced. Another individual may be generating high levels of relatively ineffective antibodies, and so will have a high titer but minimal clinical defects. It is important to know which antibodies are efficacious and which are not.

Myasthenia gravis researchers have so far benefitted from two gifts from nature: the electric organ from certain fish, which contains extremely high concentrations of acetylcholine receptor suitable for purification; and toxin from certain snakes, which binds specifically and tenaciously with the acetylcholine interaction site on the receptor molecule, facilitating detection and quantitation. Researchers now seem set to exploit a third gift: monoclonal antibodies.

The immune response against the acetylcholine receptor in myasthenics involves a range of antibodies, each specific to a different molecular conformation, or determinant, on the receptor. Each antibody, however, is the exclusive product of a single line, or clone, of lymphocytes. Separating antibodies into their discrete types from myasthenic serum is a horrendous task, and the monoclonal antibody technique circumvents this.

An animal immunized with acetylcholine receptor from, say, the electric organ of the torpedo fish responds by producing what has been termed experimental autoimmune myasthenia gravis, a condition that mimics many of the properties of the human disease. Cells from the immune system of such an animal can be mixed with myeloma cells (a certain type of tumor), and under favorable conditions pairs fuse to form antibody-producing hybridomas.

Unlike normal lymphocytes, hybridomas are virtually immortal and can readily be cultured in the laboratory. What the researcher has to do is, following the fusion stage, select out and separate a series of hybridoma clones giving, in Jon Lindstrom's words, "a library of monoclonal antibodies." This provides, he says, "a series of probes which have as tight a specificity and affinity for other parts of the receptor molecule as snake venom toxin has for the acetylcholine binding site."



Routes of transmission from nerve to muscle

The acetylcholine receptors of normal end plates are located at the crests of the folded postsynaptic membrane. An average end plate contains about 50 million receptors. An average nerve impulse releases 60 acetylcholine vesicles from the nerve terminal; each vesicle contains 10,000 neurotransmitter molecules. [Courtesy of Salk Institute]

Christopher Gomez, David Richman, and their colleagues at the University of Chicago reported at the New York meeting on their library of 15 characterized antibodies produced by rats immunized with torpedo receptor. Given that human myasthenia gravis is a polyclonal autoimmune response, with antibodies of presumed different potencies, one intriguing question is whether any single antibody could induce the disease. The Chicago group tried this by injecting rats with antibodies from single clones of hybridomas, and it worked: two out of six monoclonal antibodies initially tested produced passive experimental autoimmune myasthenia gravis. This was in line with results that Lennon and Lindstrom published early in 1980.

The corollary of this experiment is to see if the disease can be prevented by blocking a single antibody presumed to be important in the pathogenesis. The technique here is to pick out such an antibody; raise antibodies to this first antibody by carefully immunizing an experimental animal; and then challenge the animal with a dose of acetylcholine receptor. The antibody directed against the chosen receptor antibody (this is known as idiotype antibody) should block its production and thus perhaps remove the principal weapon in the autoimmune armory.

"Most researchers working with monoclonal antibodies have had it in mind to attempt this experiment," says Lindstrom, "and Vanda Lennon got there first." The result? "It didn't work," she reports, "and I'm not particularly surprised because we all knew that myasthenia gravis is a polyclonal response. But, although the animals producing the idiotype antibodies developed muscular weakness just like the other animals, we did notice a significant reduction of receptor antibody in their sera." Had a single idiotype antibody been able to block experimental autoimmune myasthenia gravis, specific immune therapy would immediately have been in the cards for human myasthenia. As it is, researchers will probably begin looking for a handful of idiotype antibodies that can lower receptor antibodies by 50 percent or more, this being the level of reduction typically observed in beneficial drug therapies.

"There's a faint element of hysteria surrounding monoclonal antibodies at the moment," comments Andrew Engel, also of the Mayo Clinic, "and the work won't become really important in terms of therapy until people start characterizing the human response with human monoclonal antibodies. That's the way to go." And this is precisely where Lennon is going. "We've shown the feasibility of the techniques with rats," she says, "and now that suitable human myeloma cells are available for fusion we'll begin with human monoclonal antibodies."

Meanwhile there is vast potential for the application of monoclonal antibodies in the more arcane but equally important business of dissecting the molecular structure of the acetylcholine receptor. "The acetylcholine receptor is already the most thoroughly characterized neurotransmitter receptor we have," comments Lindstrom. It seems set to become even more closely defined.

Most structural work has been done with the torpedo receptor, but it is becoming clear that all acetylcholine receptors follow a closely similar pattern. The receptor has a molecular weight of slightly more than 250,000 and is made up from four subunits, α, β, γ, and δ in the ratio 2:1:1:1; the molecular weights of the four are, respectively, 40,000,

50,000, 60,000, and 65,000. Using data available from other laboratories before the monoclonal antibody work got under way, Lindstrom constructed a hypothetical model of the receptor. It is a cylindrical molecule that protrudes through the postsynaptic membrane, having an ion channel running through the center, with two acetylcholine binding sites probably located on the α-subunit (see diagram).

Michael Raftery and his colleagues at the California Institute of Technology have analyzed part of the amino acid sequence of each of the four subunits and report a high degree of homology between them. "At 11 of the first 54 amino acid positions all four subunits have the same residue," says Raftery. "These data suggest that the genes encoding the four subunits descended from a single ancestral coding sequence. . . . We assume that the subunits evolved to perform discrete functions in the receptor complex."

"Apart from the obvious functions such as the site for binding with acetylcholine and the structures that make up the ion channel, there are other features on the receptor we need to know about," says Lindstrom. "There's the functional link between the binding site and the channel, for instance; and the molecule probably interacts specifically in some way with the basement membrane outside the postsynaptic membrane and with structural proteins inside it. We expect to be able to probe these functions using our library of monoclonal antibodies."

So far Lindstrom and his colleagues have isolated 70 monoclonal antibodies from rats, 17 of which came from immunization with torpedo receptor, 40 with electric eel, and 13 with fetal calf muscle. By testing with whole receptor, separate subunits, and individual subunits fragmented by proteolysis, the Salk group has begun the process of mapping the regions on the receptor to which the antibodies bind.

"Many of the antibodies are species-specific: they bind only to receptors against which they were produced," reports Lindstrom. "But about half of them cross-react with receptors of other species. It is interesting that many of these cross-reacting antibodies bind with the α-subunit. There's an area on the α-subunit that provokes a powerful immune response: many antibodies react with determinants here. We call it the Main Immunogenic Region, and it is not the acetylcholine binding site." Similar regions are found in torpedo, eel, and

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Biology and Culture Meet in Milk

William Durham, an anthropologist from Stanford University, is concerned about a gap he sees between biological and cultural anthropology. "A major goal of anthropology has been to explain and interpret the diversity of human attributes around the world, past and present," says Durham. And yet, he complains, "there have been relatively few attempts to interrelate or integrate the dichotomous schools of thought." Speaking at one of the few sessions on sociobiology at this year's meeting, Durham offered to bridge that gap with a case study on the biological and cultural aspects of dairying.

"The interaction of biology and culture proceeds through the influence that culture can have on the fitness of individuals and genotypes, and through the influence biology can have on the fitness of cultural practices," he stated. Calling on the data of cultural geographer Frederick J. Simoons, Durham claimed that the distribution of certain dairying practices and the physiological ability to metabolize lactose "proves in this instance that cultural changes directly affected human survival and reproduction."

Some populations rely heavily on milk products as part of their diet; others do not. This is the cultural diversity on the equation. In addition, some of the "dairying populations" consume their dairy products as milk, others as cheese, yoghurt, and similar derivatives, thus extending the cultural diversity. Durham adduced archeological data for an indication of the importance of dairying in different cultures' history.

The biological variable in the equation is the possession, or not, of lactase, the enzyme required for the absorption of lactose from the intestine. Although all infants have lactase in their gut juices, for obvious reasons, levels of the enzyme plummet to near zero in many populations of adults. This state of zero lactase in adults was the norm in humans until animal domestication became important after the Agricultural Revolution some 15,000 years ago, Durham hypothesized. The recently evolved high levels of lactase in some adult populations provides the biological diversity.

Durham analyzed biological and cultural data on 32 populations living between 60°W and 60°E (that is, roughly between Greenland and Saudi Arabia) and classified people into dairying and nondairying populations. Imposed on this cultural split was the finding, not surprisingly, that the lactase levels in nondairying people were low. The dairying people, however, were divided into low-lactase and high-lactase groups. This biological difference was explained by the discovery that these low lactase absorbers, who had a long history of dairying, consumed their milk produce mainly as cheese and other derivatives (the most extreme example is Jordan, where 86 percent of its milk is converted into cheese).

The question to be answered, says Durham, is "why in the milk-consuming populations there was a biological rather than a cultural solution to the problem of lactose absorption." The answer, he suggests, has to do with the diminishing amount of natural synthesis of vitamin D in the skin with increasing distance from the equator. "Clinical studies have shown that lactose behaves biochemically like vitamin D, facilitating the absorption of calcium from the small intestine, but only for absorbers," explains Durham. "The frequency of lactose absorption in dairying populations would therefore be expected to increase directly with increasing distance from the equator. . . . This prediction is precisely what one finds."

This pattern of lactose absorption among dairying people is mirrored directly by the cultural pattern of milk consumption and inversely by cheese consumption. "Dairying is thus a good case of what I have called coevolution—the idea that through both biological and cultural evolution attributes tend to facilitate human survival and reproduction," claims Durham, making the assumption that the ability to assimilate dairy products significantly widened people's base of food resources. Coevolution—or coincidence? Some biologists are certain to offer the latter as an equally valid interpretation.

The American Anthropological Association held its 79th annual meeting in Washington, D.C., 3 to 7 December.

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calf muscle receptors, and there's preliminary evidence of an equivalent region on human receptor too. "These similarities imply that this region is highly conserved between species," observes Lindstrom. "It might be functionally very important and it might also be a prime target in the autoimmune response of myasthenia gravis."

In addition to using monoclonal antibodies to probe the structure of the acetylcholine receptor, the Salk group is planning to determine their pathogenicity, as are other groups. "For instance," Lindstrom suggests, "if we can isolate antibodies than can fix complement and those that can't, and ones that can cause antigenic modulation and those that can't, then we might be able to determine which of these two processes are most important in destruction of receptors in the disease." A systematic approach of this sort might eventually build up a picture of pathologically vulnerable regions of the receptor molecule, and might even expose an immunological Achilles' heel.

So far less than half a dozen papers on monoclonal antibodies relating to myasthenia gravis have appeared in the scientific literature, but a flood of them is on its way.—ROGER LEWIN

Consensus on Bypass Surgery

In most cases, the operation has not been shown to save lives, but patients do say they feel better after surgery

Since its inception, coronary artery bypass surgery has been controversial. A few years ago, a number of physicians at major surgical centers claimed that it saved lives, although good data from clinical trials were not available (*Science*, 17 December 1976). Now the emphasis has shifted to its role in improving the quality of life. Although quality of life can be hard to measure, patients say they feel better after the operation and the surgery is popular—110,000 operations were performed in the United States last year at an average cost of $15,000 per patient.

On 3 to 5 December 1980, a consensus conference, sponsored by the National Heart, Lung, and Blood Institute and the National Center for Health Care Technology, considered the scientific and clinical aspects of bypass surgery. The consensus panel, whose chairman was Robert Frye of the Mayo Clinic, said at the outset that it would not make recommendations. Instead, it would determine what is now known about the procedure, which meant considering data on the diagnosis of coronary artery disease, the survival rates of surgical patients, and the quality of life following surgery.

The consensus, reached after 2 days of animated discussions among the meeting participants, was conservative. Although the panel declared bypass surgery a "major advance," it did not claim miracles for the treatment and took an extremely cautious view of the epidemiological data presented. It concluded that the operation can improve blood flow to the heart, can improve the quality of life, and can, in some patients, prolong life. But it stopped short of concluding that the great number of bypass operations performed each year is fully justified.

By grafting sections of vein or internal mammary artery to blocked coronary arteries, a surgeon can bypass occlusions that slow blood flow to the heart. The operation can be somewhat risky, however. In the best of circumstances and among patients with the best prognoses, the consensus panel concluded, 1 to 4 percent of patients die as a result of the procedure and there is a small chance—about 1 percent—that a patient who survives will have neurological damage. In the worst cases—patients with congestive heart failure whose hearts cannot pump blood efficiently—the lowest operative mortality rates attainable are between 10 and 15 percent, the panel said. But the operation today is far safer than it was even a few years ago. According to James Ware, a meeting participant and biostatistician at the Harvard School of Public Health, the mortality rates in the best cases 5 years ago ranged from 6 to 10 percent. "I think there's a lot to be impressed about here," he says.

Most patients who undergo bypass surgery have angina pectoris, which is a tightening or heaviness in the chest that occurs during exertion or, in some people, even at rest, because portions of the heart do not receive enough blood. It can be a debilitating and frightening symptom of heart disease.

The consensus panel concluded that in one, or possibly two, subgroups of patients, the surgery does seem to prolong life. The best evidence is in patients with blocked left main coronary arteries. (Three arterial vessels directly feed the heart; two are forks of the left main coronary artery.) A randomized, controlled trial conducted by the Veterans Administration in which nearly 700 men were studied for 6 years showed that the expected mortality rate of 10 percent per year in this subgroup of patients was cut in half by the surgery. These results were confirmed by the European Collaborative Study, a randomized, controlled trial in which nearly 900 patients were followed for 6 years.

The VA and European trials also provided evidence that patients with three-vessel disease, in which the three arterial vessels that reach the heart are obstructed but the left main artery is not, may also live longer as a result of bypass surgery. However, the data here are less conclusive and the consensus panel expressed some hesitation about accepting the results on three-vessel disease without further confirmation.

Only a minority of patients with angina have three-vessel disease or blocked left main coronary arteries, however. For example, Michael Mock of the Heart, Lung, and Blood Institute says that 9 percent of the 18,143 men in a registry for the Institute's Coronary Artery Surgery Study had left main disease and 34 percent had three-vessel disease. But a major rationale for doing bypass surgery is to relieve angina—not necesssarily to prolong life. There was some feeling at the consensus meeting that a significant number of patients are deciding too quickly that surgery is the only acceptable way to improve the quality of their lives. T. Joseph Reeves, director of the Cardiovascular Laboratory at St. Eliza-

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