CHAPTER 21

Conclusions: Outlook on Future Research

C. A. MIMS

A great variety of slow or persistent virus infections have been discussed, and each of these infections has its own fascination for us. We must, however, come to grips with the central problems that we face. It is in front of us on the landscape with its stern forbidding slopes, a mountain whose name is multiple sclerosis (MS). A few useful preliminary observations have been made, and some promising approaches have been seen, but the problem is still there. The great new hope that may help us scale this MS mountain is the possibility that it is caused by an infectious agent.

What can be learned from the known slow or persistent infections of the central nervous system (CNS) that will help us to understand MS? First, we are learning something in a very general sense about the immunology of these infections. However, it has been suspected that immunologic factors play only a secondary role in MS. It is very difficult to identify the earliest histologic lesion in MS, but should it consist of a decrease in oligodendroglial cells and some demyelination before there is a detectable cell infiltration, then this would suggest that immunologic factors were secondary. Whatever the case, we need to know about the immunology of the CNS in general if we are to understand MS. There are many important areas of ignorance. For instance, we know nothing about the small lymphoid cells present in normal cerebrospinal fluid (CSF)—how these cells get there, their turnover rates, and so on. We also need to find out more about neurons and glial cells. Are there any specific neuronal or glial cell membrane antigen markers that would assist immunologic studies and also help us to identify these cells? Antigens on neuronal and glial cells have been described, but some of these have been shown to be present in other types of cell in the normal embryo. In any case, most of the studies have been done with tumor cells from oligodendrogliomas or astrocytomas rather than with normal cells.

The second general area that deserves more study is genetics. The genetic studies point to a predisposition to MS, in that people with certain histocompatibility antigens on their cells are more likely to get MS. The parts of chromosome 6 that determine the histocompatibility types are closely associated with the immune response genes. Conceivably, genetically determined weak responses to a theoretic infectious agent causing

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MS make one more susceptible to the development of MS. On the other hand, it might be that this genotype confers susceptibility to the infection by coding for virus receptors on cell surfaces. But these are no more than possibilities, and it is really only our current bias that makes us think in terms of immune responses or receptors at all. It is possible that these genotypes are closely linked with factors that control quite separate things, such as the ability of a circulating microorganism to localize in the CNS or the susceptibility of certain enzymes to viral damage or even the presence of enzymes controlling cell susceptibility. The question of the basis of genetic predisposition is still open.

What about the infectious hypothesis of MS? What things should we be thinking of? What lessons should we have learned from the known slow virus infections? First, we cannot count on any infectious agent responsible for MS being a conventional virus. There is one human representative of the scrapie group, and we have to accept the possibility that there are other representatives in man of this same group of infectious agents. Moreover, additional agents of an unconventional type may be found that do not cause spongiform encephalopathies, and, of course, they might be very hard to detect if, like scrapie, they induce no trace of an immune response in the host.

Second, if we are thinking about a conventional virus, we will have to keep an open mind about it and not get bogged down, thinking only, for instance, about measles. In fact, we should perhaps remind ourselves that past cancer virus research has been described as a graveyard with the names of prominent virologists inscribed upon the tombstones. We have to recognize that we are in the same danger with MS. Nor can we expect that MS will necessarily operate just like any of the known slow virus infections of the CNS. The importance of these slow virus infections is that they have shown us the sort of things that *can* happen, and have suggested the sort of research approaches that might be useful. Indeed, the other slow virus infections, such as subacute sclerosing panencephalitis and progressive multifocal leukoencephalitis have given the whole field of MS research a fantastic new impetus. It was not long ago that one could define "virus" as a Latin term used by physicians to mean "your guess is as good as mine." Now we can be a little more precise, and at the same time the possibilities have expanded. If, for instance, I ask the visna experts whether there is a primate representative of visna virus or whether this virus is ridiculously unique to sheep and goats, they have to admit that they do not know. There are low titer visna virus neutralizing factors in the sera of many people, but these factors may be nonspecific inhibitors. Again, could the human oligodendrocyte, the cell especially involved in MS, be uniquely susceptible to a human C type virus? Whenever one grows mouse brain cells in bottles, C type particles seem to emerge. What about the human coronaviruses? They have always been regarded as respiratory viruses, but we really do not know very much about them. We never include them in our antibody studies for MS, and we must remember that many viruses reach unexpected parts of the body, especially in the imma-

ture host. After all, mouse hepatitis virus, a virus that causes one of the few primary demyelinating diseases, is a coronavirus.

One other way of looking at the slow and persistent infectious agents is to ask ourselves how they persist in the body and how they evade the normal host resistance factors that are designed to eliminate invading microorganisms. We have, in fact, identified some of the ways in which the immune defenses can be bypassed. But we must remember that many persistent infectious agents cause no lesions or illness. We are persistently infected with adenoviruses, herpes simplex, varicella-zoster, and cytomegalovirus; we carry the Epstein–Barr virus genome, and we probably all have the human C type virus. Nevertheless, with some exceptions, we have escaped any harmful consequences. Like all good parasites, these persisting viruses cause little trouble for the most part.

When a possible infectious agent for MS was discussed, I was reminded very strongly of chronic glomerulonephritis in man. Most of this glomerulonephritis is thought to be caused by immune complexes, and in this particular disease we have superb animal models that have told us how immune complexes cause glomerulonephritis. Although the pathogenesis has been worked out, we still know almost nothing of the (possibly microbial) antigens that are present in the complexes. In MS, however, we know very little of the pathogenesis and even less of the possible virus. Norrby told us of the oligoclonal antibodies in the CSF in MS, but no one knows what antigen provoked them. This problem should be investigated because it may be possible to study the antigen specificities of the oligoclonal antibodies. Such a study might be more promising than a search for viral antigens in the glomerular deposit from a kidney biopsy in glomerulonephritis. We should also look at nucleic acid sequences in brain cells, just as tumor virologists have had to do in tumor cells. One of the difficulties with our modern approach to MS is that we need live cells from the human brain for cocultivation, fusion, and so on. In the old days of virology, the great standby in the isolation of viruses was the availability of deep frozen tissues that could be used for reisolation. If we must grow the cells from a brain specimen, this type of reisolation becomes impossible.

Other difficulties abound. It is possible that the infectious agent gets into the body, initiates the changes in the CNS that lead to MS, and then totally disappears leaving nothing of itself behind. The changes that slowly evolve are no longer dependent on the presence of the virus. We have good precedent for this in Richard Johnson's studies of hamster neonatal infections with mumps and influenza viruses. It is necessary, therefore, to look at infected animals for long periods of time, as we have learned from slow virus research in general.

I have always been astounded at people's prejudiced, or perhaps weighted, attitudes to experimental animals. If I wrote a paper describing the results of my experiments on three mice, I would be laughed at. But if I use a large and expensive animal, small numbers do not seem to matter. Benedict based his entire book, *The Physiology of the Elephant*, on a single circus elephant called something like Clarabella. It is said that in the early

days of immunology there was an entire text on immunochemistry that was literally a one-horse textbook. Yet each little mouse is as complete an individual and as complete a host for an infectious disease as a horse, elephant, or chimpanzee. This is a serious problem if we cannot help using large expensive animals, as Gajdusek discovered in his classic pioneering studies with primates, but wherever possible we must use a convenient, small, and inexpensive laboratory animal. Our progress is then more rapid. Research on scrapie leaped ahead when Chandler discovered that mice were susceptible. The mouse is the ideal animal. We know so much about its genetics, its immunology, and so on, that one can go so far as to say that we are frankly foolish if we do not pursue our studies in a mouse, if it is at all possible and not too irrelevant a host.

It has been crippling for research on equine infectious anemia that one must have horses. Experiments, therefore, have to be done with just a few animals. If only some magician–geneticist could have devised a minihorse so that we could keep six of them in a mouse cage, hearing the thunder of little hoofs as we lifted the lid, then I am sure we'd know as much about equine infectious anemia as we do about lymphocytic choriomeningitis.

If MS is an infection, the disease evolves over a long period of time. Millar did a study of 700 MS patients over many years and showed that the average time from when MS was first diagnosed until death was 21.5 years. That is the median time, and it is not the picture of any progressive infectious process that we know about. To understand the highly irregular course of the disease process in MS, we have to think, for instance, of a disease such as tuberculosis where the defense system is waxing and waning over the years. In other words, there is a long drawn out, sometimes life long, battle between infectious agent and host defenses. We might also ask whether in the normal aging process there are changes in the ability of cells in the CNS to regenerate damaged cell membrane, so that a steady, low grade, and initially reversible pathologic process would progress with age. If we are pessimists, we might suggest that it is more complicated, and there are infections or associated immunologic events that also wax and wane and contribute to this very long drawn out type of infection.

At the fundamental level we need to learn a great deal more about neurons and glial cells and their responses to injury and to infection with all varieties of viruses. Even if the virus etiology of MS should prove to be incorrect, it will, nevertheless, have been of immense importance, simply because the virologic interest has generated so much work relevant to MS. We have learned how to grow brain cells, and we can now study them in bottles; we are investigating immune events in the CNS. Until recently MS research was a neglected field, which did not attract the young and interested people. The focusing of modern virology and immunology on to the problem of MS has, in fact, been a transfusion of life and hope.

I must counter, therefore, Porter's sober but very depressing survey of the virologic approach to MS. It is not that we are necessarily going to discover that this or that virus causes MS, but that we have generated a great ground swell of research effort and active interest on the part of young

people. It is this interest that will enable us to assault and conquer the MS mountain. We need the general biologic studies on glial and tumor cells. The oligodendrocyte must be our central focus, but unfortunately 90% of this cell in the intact brain is wrapped spirally around the axon, producing myelin. If we study in the bottle only the little cell that was sitting beside the neuron, we will miss out a lot of what, in fact, the oligodendrocyte is in the body. But studies are progressing, and Ponten in Uppsala can now clone glial cells. He studies their replication in little tissue culture gardens on the surface of agar, and he can make studies of the offspring of single cells. There is a great future in the general biologic approach to MS.

Finally, we must keep our options open about the virus being considered, but advance against the MS mountain on all fronts. The infectious hypothesis is the most important one for the moment, and as we study the infection and immunology of the CNS, the pathophysiology of neurons and glial cells, the prospects of success will be high. As good scientists, we have to temper our enthusiasm and hope with self-criticism and skepticism.