scopy, using freeze-fracturing combined with immunolabeling, were reported by Dr. Monique Dubois-Dalcq of NIH. These methods have been applied to investigation of the distribution of membrane-viral antigens by means of the surface replication technique with SSPE measles virus. The techniques allow a better understanding of the maturation of slow-chronic viruses at the cell membrane.

The reports of what some have called "paramyxovirus-like" fuzzy tubules in multiple sclerosis brain tissues were discussed by Dr. Cedric S. Raine and by Dr. Dubois. Dr. Raine's report was in collaboration with Dr. Prineas. The structures have now been seen in tissues from patients with many different diseases, and thus are not specific for multiple sclerosis. Morphologically, they do not resemble paramyxoviruses and could not be labeled with specific measles or parainfluenza type 1 sera using the immunoperoxidase method. It appears that they may be related to degenerative changes of chromatin material. Dr. Dubois suggested that they should be called "dense strands," since they do not have a tubular structure.

The meeting closed with a presentation on new electron microscopic observations of labeling of IgG in multiple sclerosis brain tissue by Dr. Dubois. With immunoperoxidase-labeled anti-IgG, she used both light and electron microscopy to show intracellular localization of IgG in the plaque. Astrocytes and their gliofibrils stained for IgG throughout the cytoplasm, as did plasma cells. There was some IgG staining of the glial membrane of cells near capillaries and a slight stain of white matter. There was some IgG staining of the glial membrane of cells near capillaries and a slight stain of white matter. Detailed studies of these preliminary findings along with the necessary controls are now in progress.

NINDS plans to consider another possible meeting on "Perspectives in Multiple Sclerosis" in 1977.

Abstracts of the meeting

Genetic factors in multiple sclerosis: The major histocompatibility system (HL-A) and immunity

CASPER JERSILD and BO DUPONT, New York City, ARNE SVEJGAARD and PER J. PLATZ, Copenhagen, Denmark, KENNETH A. CIONGOLI, Philadelphia, and TORBEN FOG, Copenhagen

A survey of the studies of HL-A antigens in different diseases shows that several diseases are associated with the serological-detectable (SD) HL-A antigens. In some of these diseases a very strong association with specific HL-A antigens has been found, i.e., ankylosing spondylitis and W27. Other diseases have shown only a weak association to specific HL-A antigens, but in some of these diseases, i.e., multiple sclerosis and juvenile diabetes mellitus, a stronger association to specific mixed leukocyte culture, lymphocyte-defined (LD) determinants and the disease has been observed.

The studies of HL-A (SD) antigen frequencies in multiple sclerosis have shown a weak but consistent association to the following HL-A antigens: an increased frequency of HL-A3 and HL-A7, and a decreased frequency of HL-A2, HL-A12, and W15 antigens. These findings have been confirmed in several laboratories.

A detailed analysis of linkage disequilibrium between LA and FOUR Series antigens in normals as well as in multiple sclerosis patients shows linkage disequilibrium patterns in multiple sclerosis patients compatible with the one observed among normals. This indicates that the increased frequency of HL-A3 can be explained alone as being secondary to the increased frequency of HL-A7. Finally, the increased frequency of HL-A7 has been found to be secondary to the increased frequency of a specific HL-A7-linked MLC, LD determinant, LD-7a, which occurs in 60 to 70 percent of multiple sclerosis patients, as compared with 16 percent in normal, unrelated individuals.

An analysis of the influence of these genetic factors on the clinical course of the disease has shown a correlation to the disease activity, as measured by progression in neurologic disability scores in time. Patients with rapid progressing disease are only found among those patients who carry the HL-A7 antigen and/or the LD-7a determinant.

The finding of an association between the occurrence of high-titer, antimeasles antibodies and those multiple sclerosis patients who carry HL-A3, 7, and/or W18 indicates that genetic factors within this system contribute to the control of antibody production. The observation of similar high-titer, antimeasles antibodies among the family members of multiple sclerosis patients could point toward such a genetic factor in the regulation of antibody production, rather than suggesting the presence of an exogenic factor to which the family has been exposed.

Recent evidence shows that the serum levels of certain components of the complement system are controlled by genes located within or in close linkage with the HL-A chromosomal region. These findings may be of the utmost importance for the understanding of the influence of the HL-A system on disease susceptibility and resistance.

The lack of reactivity of multiple sclerosis patients'
leukocytes in the leukocyte migration agarose test (LMAT) after challenge with measles antigen has been found so far only among multiple sclerosis patients. A normal reactivity has been observed when multiple sclerosis patients' leukocytes are challenged with other bacterial and viral antigens. However, only some preparations of measles virus shows this reactivity pattern, and until now only little has been known about the component of the virus which antigens. However, only some preparations of measles sclerosis-causing agent, or whether it represents a with transfer factor, which normalizes this lack of reactivity. Secondary phenomenon related to other abnormal immune functions, is at present unknown. It was, however, the rationale behind treatment of multiple sclerosis patients with transfer factor, which normalizes this lack of reactivity.

**Immunogenetic aspects of demyelinating disease**

BARRY G. W. ARNASON and EWA CHELMICKA-SZORC, M.D. Boston

There is agreement that the serologically determined histocompatibility antigens HLA-3 and HLA-7 are over-represented in multiple sclerosis as compared with control populations, and that the MLR determinant LD-7a is strikingly over-represented in multiple sclerosis. In optic neuritis patients who do not go on to develop multiple sclerosis, HLA-3 and HLA-7 are not increased. It was, however, the suggests that a given "insult" may not progress to frank multiple sclerosis in the absence of certain genetically determined immune response genes.

Measles antibodies tend to be higher in multiple sclerosis patients than in controls, but persons who bear HLA-3 have higher measles antibody titers than those who do not, whether they have multiple sclerosis or not. Thus, increased measles antibodies in multiple sclerosis may be an epiphrenomenon.

B-cells, as determined by EAC rosetting techniques, are increased in peripheral blood in a significant proportion of multiple sclerosis patients, but this increase does not correlate with disease activity, disease severity, or CSF IgG levels. T-cell percentage is not significantly depressed in multiple sclerosis, but "avid" T-cells (i.e., T-cells that attach 10 or more sheep red blood cells during rosetting) are significantly decreased. A subset of T-cells may be abnormal in multiple sclerosis. Response of peripheral blood lymphocytes to PWM (primarily a B-cell mitogen) is brisk in multiple sclerosis. Possibly this reflects the increase in circulating B-cell percentage seen in this disease. Response to PHA (a T-cell mitogen) is often not depressed in multiple sclerosis, but is often increased. In peripheral blood, the abnormality in T-cells detected by rosetting techniques.

Experimental allergic encephalomyelitis (EAE) in experimental animals has been proposed as a model for multiple sclerosis in man. In inbred strains of rats, susceptibility to EAE is linked to the major histocompatibility locus of the species, a situation which parallels that observed in multiple sclerosis in man. Strain 13 guinea pigs are susceptible to EAE but lack the immune response gene that determines T-cell responsiveness to poly-L-lysine (PLL). Strain 2 guinea pigs respond to PLL but are relatively refractory to EAE. PLL, given subcutaneously either early or late during disease induction in nontoxic doses, will protect outbred Hartley guinea pigs against both clinical and histologic EAE, but PLL administration will not protect them against experimental allergic orchitis. Thus, immunosuppression by PLL in guinea pigs is disease-specific. Administration of protamine, another basic polypeptide, will not protect guinea pigs against EAE. Rather, disease severity — both clinical and histologic — is potentiated by protamine treatment in guinea pigs, even though immune responsiveness to protamine and to PLL are determined by the same Ir gene.

**Multiple sclerosis susceptibility and histocompatibility determinants**

MILTON ALTER, MARY HARSHE, and EDMOND J. YUNIS, Minneapolis.

The association between multiple sclerosis and HL-A tissue types was analyzed in nine families in which at least two individuals had multiple sclerosis. In all families, the individuals with multiple sclerosis inherited a haplotype (i.e., a set of two HL-A tissue types) that the nonaffected individuals, with one exception, did not inherit. Thus, multiple sclerosis segregated with a single haplotype within the family. Because the specific HL-A types differed among the families, another determinant, closely linked to the HL-A genes, was implicated, i.e., a multiple sclerosis susceptibility gene, perhaps associated with or the same as an immune response gene. Multiple sclerosis susceptibility may be mediated through immune mechanisms controlled by this gene. Differences in exposure to an environmental trigger or modifying genes may explain why not all individuals with the genetic susceptibility show the disease.

**Transfer factor treatment in multiple sclerosis**

TORBEN FOG, C. JERSILD, B. DUPONT, P. J. PLATZ, A. SVEJGAARD, M. THOMSEN, S. MIDHOLM, and N. E. RAUN, Copenhagen, Denmark, and P. GROB, Zurich, Switzerland

Transfer factor treatment in multiple sclerosis is difficult to evaluate for several reasons. The practical problem of getting enough substance for a double-blind trial limits the possibility of drawing definite conclusions. Instead, a pilot study has been started in which a group of patients (10 in all) with a known progressive course before treatment is being treated to see if this progress continues. If demonstrable progress occurs during treatment lasting 1 to
2 years, we may conclude that there is reasonable doubt about continuing this study. If not, the need of enough substance for a double-blind trial is imperative.

The theoretic base for this study is discussed. HL-A studies have shown that a dominance of the MLC-type 7a is demonstrable in a multiple sclerosis population, and this type correlates with a rather fast progress of the disease. The MLC-focus has a possible relation to the immune response in the animal, and also in man. The possibility of a genetic susceptibility in the multiple sclerosis patient toward the unknown exogenous factor or factors therefore exists. In multiple sclerosis, a pathologic reaction is demonstrable in vitro in the so-called migration inhibition test in almost all cases. By using transfer factor this reaction is reversed towards normal values, even if it seems as if this reversal is temporary or perhaps dose-dependent. However, transfer factor may have other capacities than those demonstrable in this in vitro test, and may possibly change the susceptibility toward a better resistance.

The problem of a statistically satisfying method for evaluating any treatment in the multiple sclerosis patient is discussed. A new method, based upon a collection of up to several hundred data at each examination of the patient at repeated 1-month intervals during treatment, is demonstrated. By this method, a semiquantitative analysis is possible.

Ten patients have been treated, beginning in February 1974, with the last patient beginning treatment in June. The score of single systems — motor system, sensory system, coordinative system, brainstem system, and visual system — and the total score during treatment is correlated with the course of the MIF test toward measles, parainfluenza-virus, and PPD. It is concluded that no side effects have been demonstrable. The MIF reaction seems only temporarily reversed during treatment.

The study will continue during the following months. Definite conclusions may not be drawn at this moment.

Immunologic studies with transfer factor in multiple sclerosis patients

J. B. ZABRISKIE, V. UTERMOHLEN, L. R. ESPINOZA, C. R. PLANK, and R. C. COLLINS, New York City

Using the in vitro technique of direct leukocyte migration inhibition as our index of cellular recoginition to a given antigen, previous studies in this laboratory have demonstrated a selective cellular suppression to measles antigen in leukocytes obtained from multiple sclerosis patients. This selective suppression prompted us to investigate the effect of transfer factor, both in vitro and in vivo, on cellular recognition to measles virus in these patients.

Dialysable transfer factor was prepared as described by Lawrence (In Vitro Methods in Cell-Mediated Immunity, Academic Press, 1971) with some minor modifications. Following isolation of theuffy coat layer, an aliquot of the total leukocyte pellet was removed, adjusted to 20 million lymphocytes per cc and migrated against measles antigen. The remaining cell pellet was adjusted to 80 million lymphocytes per cc and frozen at -70°C until use. Only those individuals who exhibited a high degree of cellular reactivity to measles antigen were selected for production of transfer factor. Using these selected transfer factor units, the following immunologic studies were carried out:

1. In vitro migration inhibition studies to measles antigen with and without the addition of transfer factor were carried out in 13 patients with multiple sclerosis. Eleven of the patients had significantly higher values to measles antigen after the addition of transfer factor to the medium. Transfer factor alone had a negligible effect on the migration values.

2. Sixteen patients received subcutaneous injections of transfer factor approximately 1 week apart. Migration values were determined before and after injections of transfer factor. The majority of patients exhibited an increase in their cellular response to measles antigen. The increased migration values were maintained for at least 6 weeks following injection of transfer factor.

The clinical and immunologic parameters of these patients will be discussed.

Immunosuppressive treatment of multiple sclerosis with ALG and/or thoracic duct drainage

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Twenty patients, seven with the chronic progressive form of multiple sclerosis and 13 with the intermittent relapsing form, have been treated with intravenous antilymphocyte globulin (seven), thoracic-duct-drainage (five), or a combination of both (eight), together with azathioprine and steroids, which had been given before treatment without success. Five different batches of horse antihuman lymphocyte globulin were used; the animals had been immunized three times with thoracic duct cells, once with thymocytes, and once with cultured lymphoblasts. The IgG preparations were made by Behringwerke AG. During thoracic duct drainage, the lymph was removed over a period of 6 to 23 days, with an average loss of 66 x 10⁹ lymphocytes per week.

In 13 ALG-treated patients, induction of immunologic unresponsiveness against IgG was attempted, and this was successful in eight. The clinical effect was evaluated using the Bronx and Fog scales, with follow-up for 1 to 5 years. Eleven patients showed significant clinical improvement, with the best results achieved by combined therapy. Part of the results have been published (Brendel W, et al: Proc R Soc Med 65:531, 1972, and Behring Inst Res Comm 51:176, 1972; and Ringe J, et al: Lancet 2:1093, 1974).

Lymphopenia seemed to be some index of the therapeutic effect: In patients in whom gross lymphopenia was never achieved during treatment, clinical changes were only slight. By the induction of immunologic unresponsiveness against horse IgG, the clinical efficacy of ALG was significantly improved: All eight patients who
were regarded as "unresponsive," according to the criteria of immunodiffusion, direct and indirect hemagglutination, skin test, and immune elimination, showed immediate improvement of an average of 18.3 points on the Fog scale and 2.8 points on the Bronx scale; long-term observations showed that in six of the eight patients, there was still an improvement of 13.5 points on the Fog scale and 2.8 points on the Bronx scale. In the "not unresponsive" patients, only two showed very slight improvements of only 3.3 points on the Fog scale during or immediately after therapy.

**Intensive immunosuppression in multiple sclerosis**

EUGENE M. LANCE, Honolulu, and J. ABBOSH, M. KREMER, V. JONES, S. KNIGHT, and SIR PETER MEDAWAR, Harrow, England.

We undertook a small trial (14 patients) of the remedial effects of intensive immunosuppression: the combined administration of corticosteroid, azathioprine (Imuran®), and antilymphocyte globulin (ALG). Of the 14 patients, 10 had the disease for 3 years or less and 12 had active disease at the outset. Taking day zero as the first day of treatment, azathioprine, 3 mg/kg, was started on day zero and continued at this level throughout the first year. Prednisone was initiated at a dosage of 200 mg per day and tapered rapidly to 20 mg per day by day 7. All patients received an intravenous infusion of aggregate-free normal horse IgG on days 1 and 4 at dosages of 60 mg/kg and 30 mg/kg respectively. Intravenous ALG, 500 mg, was given on day 7 and on the weekdays of the following 3 weeks. Following discharge from hospital, patients were maintained on a daily dose of prednisone, 20 mg, and azathioprine 3 mg/kg. At the end of the year, immunosuppressive drugs were tapered in preparation for complete withdrawal.

A number of undesirable side effects were identified. Most of these were minor and related to steroid administration. One patient developed signs of serum sickness requiring cessation of ALG treatment and another patient developed mild symptoms of anaphylaxis requiring reduction in ALG dosage.

The clinical appraisal was based on a four-point scalar evaluation of sensory and motor modalities, balance, speech, and vision. The final ratings were agreed on by three assessors. Every patient reported improvement during the first 2 to 3 weeks of treatment, especially during the first few days of ALG therapy. The degree and nature of improvement varied greatly, and in a few cases symptoms of many years standing improved. As most patients had multiple sclerosis of the intermittently active type, special attention was paid to relapse rates before, during, and after treatment. Using patients as their own controls, there was found to be a significant reduction in relapse rate compared with the number predicted on the basis of their experience before treatment. Many patients underwent relapse a few weeks or months after significant drug reduction; for this reason, some patients required continued immunosuppressive treatment, though all drugs have been withdrawn in most cases.

**Lymphocyte-mediated cytotoxicity to viruses in patients with multiple sclerosis: Presence of a blocking factor**

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Lymphocyte-mediated cytotoxicity to virus-infected target cells was investigated in 14 patients with multiple sclerosis using a $^{51}$Cr-release lymphocytotoxicity micro assay. Lymphocytes from patients or normal controls were incubated with $^{51}$Cr-labeled target cells persistently infected with either measles, SSPE, or rubella virus, and the culture supernatants were assayed for released $^{51}$Cr, in the presence or absence of serum from normal measles-immune individuals or from patients with multiple sclerosis.

In the absence of serum, specific immune release (SIR) was similar in both groups studied with all three virus strains. Serum from patients with multiple sclerosis, however, inhibited the SIR effected by normal or patient lymphocytes from measles or SSPE-infected cells, but not from rubella-infected cells; normal immune sera of comparable titer did not significantly inhibit the SIR from any of the target cell lines with any of the lymphocytes tested. Partial characterization of this blocking factor by gel filtration with Sephadex G-200 revealed it to be associated with the 7S fraction.

These data suggest a normal lymphocyte "killer" function in patients with multiple sclerosis, but present evidence for a serum blocking factor with specificity for measles and SSPE viruses.

**The visna provirus and slow virus disease**

ASHLEY T. HAASE, San Francisco

Visna virus is the cause of a chronic inflammatory and demyelinating disease of the central nervous system of sheep which first appeared in Iceland in the 1930s. The virus is a prototype of agents designated slow viruses by Sigurdsson, because disease follows a prolonged subclinical phase and runs a protracted course.

There are a number of striking parallels between visna virus and RNA-containing viruses responsible for tumors in animals, although visna virus itself does not cause tumors in its natural host. Visna virus resembles RNA tumor
viruses in morphology, maturation by budding from the membrane of infected cells, and biochemical properties. These include construction from a similar number and size of polypeptide, a segmented 60-70 genome, and the presence of an RNA-dependent DNA polymerase or reverse transcriptase in the virions.

This enzyme functions in viral replication to transfer the genetic information of the virus from RNA to a DNA intermediate, the provirus. The existence of a provirus in the replicative cycle of a slow virus was first shown by hybridization of virus-specific nucleic acid to infected cell DNA. Final proof that replication proceeds through a DNA provirus rests on the demonstration that DNA extracted from the infected cell prior to synthesis of new viral progeny contains all of the genetic information of the virus. Experiments to be described will show that this is the case, since DNA from infected cells confers on uninfected cells the capability to synthesize new virus. In both these DNA infectivity experiments and the nucleic acid hybridization work, evidence also was obtained that the proviral DNA is physically integrated into the genome of the infected host cell.

The existence of a provirus in replication suggests one solution to the fundamental problem in understanding slow diseases, the problem of how virus is able to persist in its host. In this view, the provirus is the subviral repository of virus genetic information, allowing the virus to reside indefinitely in its host cell secure from immunologic and other defensive measures mounted by the host in response to infection.

The logical consequence of the provirus explanation of viral persistence is that cells in involved organ systems, such as the central nervous system and the reticuloendothelial system, will harbor the provirus at a time when complete infectious virus is not being produced. An animal model system is now available which appears favorable to test this prediction experimentally. Tissues obtained from the central nervous system of animals infected with visna virus in utero do not produce virus, as assessed by direct infectivity of homogenates or electron micrographic examination of the tissue. However, when the tissues are cultivated in vitro for some time, virus synthesis is readily detected. This situation is compatible with the notion that virus resides in the cell as a provirus which is later activated, in the conditions of growth in vitro, to express itself in the synthesis of new progeny. Appropriate experimental strategies to test for the presence of provirus in latently infected tissue include transfection and nucleic acid hybridization. These methods, recent findings in the visna system, and potential applications in multiple sclerosis will be discussed.

Defective interfering particles

DAVID BALTIMORE, Cambridge, MA, and ALICE HUANG, Boston

Most animal viruses spawn defective interfering (DI) particles during their growth. These particles have three fundamental characteristics: (1) they are lacking some fraction of the genome of the standard virus, (2) they interfere with the growth of standard virus in co-infected cells, and (3) they are able to enrich themselves such that the yield from co-infected cells contains a higher proportion of the DI particle than does the inoculum which infected the cell. While the first characteristic is indicative of a deletion mutant, not all deletion mutants will have the properties of interference and enrichment.

Detailed study of DI particles has been carried out in two viral systems: poliovirus and vesicular stomatitis virus (VSV). In the poliovirus system the deletion in DI particles involves less than 20 percent of the genome. Poliovirus DI particles interfere with standard virus because the infected cell can only produce a fixed amount of viral RNA, and this synthetic capacity is partitioned by the infecting viruses in proportion to their percentage in the viral inoculum. The mechanism by which enrichment occurs has not been elucidated except that we know enrichment occurs as an early step during the viral growth cycle.

The VSV DI particles are deleted to various extents. The best-characterized of these, the DI-T particle, has only one-third of the RNA of standard VSV. VSV carries out two types of RNA synthesis: transcription and replication. Different mechanisms are involved in the two processes, although at least part of the enzymatic machinery for the two processes is shared. DI-T particles interfere with replication but do not interfere with transcription. In addition, they do not appear to interfere with translation. Their interference with replication leads, ipso facto, to enrichment.

These two systems provide two examples of how DI particles act. The VSV particles are much more effective biologically because they have a much greater interfering and enriching effect. VSV DI particles are able to ameliorate the lethal effects of standard VSV for mice. In smaller amounts, VSV DI particles can change an acute infection to a chronic infection. Cyclic production of VSV and its DI particles occurs in long-term cell cultures.

We have postulated that DI particles may play a critical role in the self-limiting nature of viral diseases, in persistent viral diseases, and in latent infection. In a disease such as multiple sclerosis, DI particles of an otherwise innocuous virus could allow the virus to linger on for very long periods of time, causing slow degenerative effects or occasional exacerbations. In such a situation, infectious virus may not be evident and thus would have to be detected by either nucleic acid hybridization or radioimmunoassay for virus-specific antigens.

A non-cytopathic infectious agent associated with multiple sclerosis material

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In our initial studies, total cell yields in cultures of a mouse cell line termed "PAM" were reduced markedly after exposure to multiple sclerosis homogenates. Reductions were noted as early as the second subcultivation. The effect was produced by eight of eight multiple sclerosis brain homogenates and three of three multiple sclerosis spleen homogenates; the samples were from 10 multiple sclerosis cases. The reduction in total cell
The relationship between measles virus-specific antibodies and oligoclonal IgG in the cerebrospinal fluid in patients with multiple sclerosis

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A comparison of measles virus antibody titers in sera and cerebrospinal fluid (CSF) samples has demonstrated that antibodies appearing in the latter type of samples are locally produced in the central nervous system in about 60 percent of patients with multiple sclerosis. The relationship between these locally produced measles virus antibodies and the oligoclonal IgG in selected cases was studied by the use of two different techniques. One technique was to absorb concentrated CSF with purified measles virus particles to analyze their capacity to remove oligoclonal IgG; antigen-antibody complexes formed were isolated by sedimentation, after which IgG was eluted at pH levels of 4.0, 3.0, and 2.0. The second technique was to make a preparatory electrophoresis of concentrated CSF and then to analyze the distribution of antibodies against different measles virus antigens in the fractions collected. For comparison, material from patients with SSPE was studied by use of the same techniques.

In the case of SSPE, repeated absorptions with measles virus antigens caused an almost complete removal of all oligoclonal IgG. After preparatory electrophoresis, peaks of antibody activities correlated in their electrophoretic position to the occurrence of bands of oligoclonal IgG. Different bands were interpreted to carry different antibody activities.

Results of studies of materials from multiple sclerosis patients differed somewhat from those obtained in characterization of materials from SSPE patients. Only in exceptional cases could absorption with purified virus particles modify the band pattern of oligoclonal IgG, and generally absorption with measles virus antigens in the selected cases did not influence the level of IgG in the samples. However, measles virus antibody activities and a few bands of oligoclonal IgG were recovered in low pH eluates. Preparatory electrophoresis of concentrated CSF material from multiple sclerosis patients resulted in a varying distribution of antibodies against different measles virus antigens, but a distinct correlation between accumulation of antibody activity and bands present in the electropherogram could not be discerned.

It is concluded that in the case of SSPE, the oligoclonal IgG represents an oligoclonal IgG response to an intense hyperimmunization with measles virus antigens. In contrast, a local immunization with measles virus antigens might account only for a few bands of oligoclonal IgG in cases of multiple sclerosis, whereas the potential antibody activity of the major part of oligoclonal IgG remains to be explained. The possibility that oligoclonal IgG in multiple sclerosis patients may derive from activation of selected antibody-producing cells in the absence of specific antigen should be considered. (Supported by grants from the Swedish Medical Research Council — Project No. 16X-116.)
Paramyxoviruses are prominent among those agents that have been considered as candidates for an etiologic role in multiple sclerosis and other chronic diseases. Immunologic findings and virus isolation studies have implicated measles and parainfluenza viruses, and several features of the biology of paramyxoviruses are compatible with their involvement in chronic diseases.

A general property of this virus group is the ability to produce persistent infection in cultured cells, and in some cases in animals, often with the production of little or no infectious virus. Events at the cell membrane play an important role in such infections. The virion is assembled by budding from the cell surface and contains an envelope that consists of a bilayer of lipids derived from the host cell, two glycoproteins (HN and F) that form the spike-like projections on the virus surface, and a non-glycosylated protein (M) that is associated with the inner surface of the membrane.

In virus assembly, a specific recognition occurs between the internal helical nucleocapsid of the virus and the M protein, which is associated with regions of the cell membrane containing virus proteins. Failure of virus assembly to proceed, which may involve both host and viral factors, results in the accumulation of nucleocapsids within the cell, and such accumulations are characteristic of persistent infections with paramyxoviruses. Possible factors in defective virus assembly include failure of recognition between the nucleocapsid and the M protein, which might result from cleavage of the nucleocapsid protein subunit, and failure of the synthesis, proper processing, or association with the cell membrane of the viral envelope proteins.

Envelope proteins of several paramyxoviruses, SV5, NDV, and Sendai virus, have been isolated and their biochemical and biological properties studied. The larger viral glycoprotein (HN) possesses both receptor binding and neuraminidase activities. The smaller glycoprotein (F) has been shown to be derived from a biologically inactive precursor (Fo), which, with Sendai virus, has been shown to be activated by host-dependent proteolytic cleavage. This cleavage is accompanied by activation of cell fusing and hemolyzing activities and the acquisition of infectivity. Thus the ability of the virus to initiate infection and to spread from cell to cell depends on the cleavage of the Fo protein, and this cleavage, in turn, is dependent on the presence of the appropriate protease in the host. Whereas wild type Sendai virus can multiply in the chick embryo due to the presence of trypsin-like activity, it cannot undergo multiple cycles of growth in cells that lack this enzymatic activity, unless trypsin is added to the medium. In contrast, mutants have been isolated that cannot be activated by trypsin, but require other proteases for activation, e.g., chymotrypsin, and such mutants do not undergo multiple cycle replication unless the activating enzyme is added.

These results provide a possible mechanism for the dependence of host range and the spread of paramyxovirus infections on the availability of the appropriate protease. They also suggest a factor that may be involved in the persistent infection of cells without the elaboration of infective virus, i.e., lack of proteolytic cleavage by a host enzyme of a viral protein can prevent the production of infective virus and cell death due to cell fusion. The possibility of the emergence, under natural conditions, of mutants resistant to cleavage by the enzymes present in the host tissue also might play a role in chronic infection.

Characterization of DNA polymerase from visna virus

FU HAI LIN and HALLDOR THORMAR, Staten Island, NY

Visna virus contains DNA polymerase which can be directed by endogenous RNA and exogenous DNA. The soluble DNA polymerase can be resolved into multiple activities by DEAE-cellulose column. Glycerin gradient centrifugation separates the DEAE fractions into 3.4, 5.5, 7.0, and 11S components. The 5.5S component, equivalent to a molecular weight of 95,000 daltons, highly prefers visna virus RNA to activated calf thymus DNA as a template. In contrast, the 7S component responds equally well to these two natural nucleic acids. The 3.4S component represents a small fraction of the visna enzyme. Preliminary experiments indicate that 3.4 and 7S components are capable of incorporating TNP by using oligo T6-10 as initiator, in the absence of template suggesting a terminal deoxynucleotidyl transferase activity.

The template activity of 60-70S, 3S, and 4S visna RNA for DEAE-cellulose fraction was studied in an enzyme reaction mixture (0.2 ml) containing 1 μg of RNA. Under these conditions, the 60-70S molecule alone was three times as active as the 3S or 4S molecule alone. The addition of 0.5 μg of oligo T6-10 to the RNA template increased the template activity 20 times for 60-70S, 80 times for 3S, and three times for 4S RNA. A mixture of equal A260 units of 60-70S and 4S RNA or of 3S and 4S RNA did not stimulate the template activity, as compared with 60-70S or 3S alone. In the presence of the oligo T primer, the template activity of the 3S RNA was higher than that of 60-70S. These results indicate (1) that when the 60-70S RNA is dissociated, a primer is lost, (2) that the 3S and 4S components retain the PolYA tract which hybridizes with the oligo T and forms an active template-primer and (3) that the 4S RNA of visna virus is not a primer.
**Structural changes in the membrane of Vero cells infected with subacute sclerosing panencephalitis virus**

MONIQUE DUBOIS-DALCQ and T. S. REESE, Bethesda

Structural changes in the cell membrane of Vero cells infected with SSPE virus were studied by means of surface replication and freeze-fracturing combined with immunolabeling techniques. These observations were integrated with data obtained from thin sections of similarly infected cells. Surface replicas were used to reveal structures lying on the outer surface of the cell membrane. Early in infection, the surface of Vero cells was embossed by narrow strands of granular material apparently coextensive with the viral nucleocapsids present under the membrane. Incubation with peroxidase-labeled SSPE antibody was used in order to visualize antigenic sites in the surface replicas. Viral antigen was distributed diffusely over regions of the membrane marked by strands. At later stages, when more nucleocapsids contacted the plasmalemma, the strands progressively increased in number and width, while most of the viral antigens on the membrane surface became organized into stripes lying over these strands. Finally, regions of the membrane displaying strands protruded to form ridges over the underlying nucleocapsids. The spacing between ridges decreased during viral budding so that the true outer surface of buds and freshly detached virus was covered with a continuous layer of antigens.

Viral maturation also was studied with the freeze-fracture technique, which splits biological membranes to reveal details of their internal structure. Particles on these split membranes are thought to be membrane proteins. In infected cells, fractured ridges in the cell membrane were covered with small particles. Similar small particles were diffusely spread over the inside of the membrane of viral buds. In mature viruses where the free nucleocapsids have lost their intimate relationship with the viral membrane, there were unusually large particles inside the fractured membrane. Deep etching experiments were used to reveal regions of the outer surface next to fractures splitting the membrane in order to determine the precise correlation between structures on the outer surface with structures inside the membrane.

These techniques have revealed fine structural details in cell membranes and viral envelopes and have allowed the relationship of these structures to viral antigens to be followed during SSPE viral maturation. Our data suggest that: (1) organization of surface antigens, which are continuously added to the plasmalemma, occurs by means of lateral movement of viral proteins into strands and ridges. (2) the small particles present inside the membranes of ridges and buds reflect the presence of proteins which are the attachment sites for nucleocapsids during the process of viral assembly at the cell membrane, and (3) budding and late viral maturation involve further changes in the organization of these intramembranous proteins and surface antigens.

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**“Paramyxovirus-like” fuzzy tubules and multiple sclerosis**

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The significance and specificity of “paramyxovirus-like” inclusion material (fuzzy tubules) to multiple sclerosis is currently under debate in view of the existence of a number of recent reports on their occurrence in other conditions unrelated to multiple sclerosis. This presentation will review our current knowledge on fuzzy tubules in terms of their incidence, location, and nature in multiple sclerosis lesions.

Fuzzy tubules were first reported in 1972 in unidentified cells in acute lesions from a case of chronic, relapsing multiple sclerosis. Their morphologic similarity to paramyxovirus nucleocapsids was noted. Occasionally, they presented a striated appearance in longitudinal plane and were tubular in cross section. They occurred both in nuclei and in cytoplasmic fragments and were absent or difficult to locate in normal areas of white matter or old lesions.

Subsequent reports from a number of laboratories have confirmed these observations, but the possible viral nature of the particles has remained unclear. These studies have shown that, unlike paramyxovirus nucleocapsids, fuzzy tubules are mainly intranuclear and have an extremely electron-dense, granular structure, whereas intranuclear paramyxovirus nucleocapsids are less dense and are clearly defined. Furthermore, using immunoperoxidase techniques, Dubois-Dalcq and associates were unable to show binding of measles antibodies in these areas. Recently, similar structures have been seen in a number of unrelated conditions, suggesting that they are not specific for multiple sclerosis.

If the particles are not viral, what are they? Firstly, they may be the result of autolysis. This seems unlikely, since they have been described both in autopsy and biopsy tissue. Also, in multiple sclerosis they seem to be restricted to active lesions. Studies in autolysed nuclei have not shown these structures. Their occurrence in degenerating cells in biopsy tissue and in early inflammatory lesions might support an autolytic origin. In some cases, however, apparently healthy cells contain fuzzy tubules. That they are caused by an unusual coiling of chromatin fibers, as suggested by one recent report, remains to be proven.

Preliminary results will be presented from recent experiments on (1) the further characterization of fuzzy tubules by negative staining and nucleic acid staining techniques, (2) attempts to produce this material in macrophages in vitro, and (3) ongoing work on measles variants.
Immunoperoxidase studies on multiple sclerosis brain

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Several investigators have recently described the presence of what some have called "paramyxovirus-like" structures in brain lesions of multiple sclerosis patients. However, these structures do not appear to meet the criteria for the identification of intracellular paramyxovirus nucleocapsid under electron microscopy (EM). Indeed, if these were paramyxoviruses, certain key findings would be expected:

1. Helically arranged nucleoprotein would always appear hollow in cross section. There are, however, very few EM pictures in the literature illustrating hollow inner cores in these multiple sclerosis-related structures. Thus the term "dense strands" would be more appropriate.

2. They should have a rather constant diameter (approximately 18 nm). The diameter of the "dense strands," however, varies rather widely from one illustration to another, a fact which has led some authors to divide them into type 1 and type 2. The latter (up to 26 nm), however, doesn't correspond to the measles fuzzy nucleocapsids usually observed in the cytoplasm.

3. They should display periodic striations. However, these are rarely if ever present in the described multiple sclerosis cases. Thus the criteria for paramyxovirus nucleocapsids are not fulfilled, and the controversy about their nature will probably not be solved by conventional EM studies.

One approach to the detection of viral antigens is ultrastructural immunohistology. The aim of our studies was to determine if measles virus antigen was present in the active multiple sclerosis plaques; more precisely, in cells containing dense nuclear strands, which were seen in two of our three multiple sclerosis cases. Peroxidase-labeled SSPE globulin, with a high measles antibody titer, has been shown earlier to stain with specificity SSPE viral antigen in the hamster brain (Dubois-Dalcq and associates, in press in Lab Invest 1975).

The advantage of the immunoperoxidase (IP) technique as compared with the fluorescent antibody (FA) technique is that it allows precise correlation between the specific label and the neuropathologic features on plastic-embedded sections under the light microscope and further correlations with the ultrastructural localization of the label.

Plaque areas from three multiple sclerosis brains taken 3 to 6 hours after death were fixed in formaldehyde, chopped in 50 μ sections, and incubated with the SSPE conjugate, using the direct technique. The conjugate did not stain dense strands or any other structures in the multiple sclerosis plaques. These results correlate with the lack of complement-fixing measles antibodies in the cerebrospinal fluids of these two patients. The possibility of inactivation of viral antigen after death or of another viral antigen being present in these brains is being explored.

We also have studied the intracellular localization of IgG in the multiple sclerosis plaques, using goat antihuman peroxidase-labeled antibody. IgG is known to be present in large amounts in plaques, as shown by fluorescent antibody technique (Simpson and associates, Arch Neurol 20:373, 1969). Similarly, with the IP technique, the stain was very intense in the plaques of the three cases and absent in control white matter. However, the labeling was present mostly inside the cells, in contrast to what was described in unfixed frozen brain stained by the FA techniques. Indeed, plasma cells and hypertrophied astrocytes were stained, whereas the oligodendrocytes and myelin present in shadow plaques were spared. Under EM, the extensive parallel endoplasmic reticulum of cisternae of plasma cells were labeled, as well as the ribosomes and gliofibrilles within the astrocytes. The specialized perivascular astrocytic membrane also was strongly labeled.

The results confirm that plasma cells are producing IgG in the plaques and raises the question of the possible role of hypertrophied astrocytes in the immunopathogenesis of the disease. Appropriate controlled studies are now in progress.