Cellular and Humoral Components of the Cerebrospinal Fluid in Multiple Sclerosis

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This presentation is mainly a synopsis of the workshop on "Cellular and Humoral Components of the Cerebrospinal Fluid in Multiple Sclerosis" held under the auspices of the International Federation of Multiple Sclerosis Societies as a NATO workshop at Hengelhoef, Belgium, April 20-24, 1986. Two reports have already been written but not yet published about this meeting: one by Brosnan and Wekerle (1) and the other for World Neurology (7). In addition, the abstracts were published in Acta Neurologica Belgica (8) and the complete text of all the presentations will be published by Plenum Press (11). Whe shall add to this synopsis some observations reported in recent publications or taken from personal studies.

The purpose of the meeting was to update our knowledge on cerebrospinal fluid (CSF) of patients with multiple sclerosis (MS). We classified the presentations in four categories: (1) experimental or spontaneous diseases in animals which could serve as a model for MS; (2) modifications of the CSF in MS due to humoral immune reactions including modifications of specific antigens and antibodies; (3) changes in the CSF revealed by studies of cellular immunology; (4) factors controlling or modulating immune reactions.

A. Models

1. Acute experimental allergic encephalomyelitis (EAE) was mentioned more often than its chronic relapsing form. Little information was given about the antibodies raised in this disease. Several speakers spoke about cellular immune reactions.

We wish to stress two points:

1. The distribution of T-cell subsets does not run parallel in the blood, the nervous tissue and the CSF,

2. The crucial role of the macrophages marked with the I-region-associated antigen (Ia), a surface antigen, has been emphasized. These macrophages would be good markers for the disease. Ia antigen is also found to be associated with astrocytes and may play a role in immunoregulation.

The hypothesis was put forward that the action of T-cells would induce the formation of plaques, whereas macrophages would cause demyelination.

2. A second disease, murine meningoencephalitis due to JHM hepatitis virus, has been presented as a model. It is not fatal. The clinical evolution of this disease recalls that of MS. The changes in the CSF

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and the central nervous system do not run parallel, nor do the modifications of cellular and humoral immunity. For example, the antibodies appear before the activated B-cells can be found in the periphery, so that one must conclude that these antibodies can be synthesized in the central nervous system.

3. Rat encephalomyelitis due to corona virus was another model discussed. As was mentioned during the meeting, this disease induces astrocytes to present viral antigens and thus the astrocytes can be considered to play a role in the immune reaction. The hypothesis that the antibodies are produced in the central nervous system and the CSF has been developed.

The important suggestion was made that blocking a stage of the immune reaction might make it possible to contain the disease by blocking pathological immune reactions. This is worth considering with respect to the treatment of MS. This has also been suggested in other diseases, wich do not involve the nervous system, in which attempts have been made to block the development of the disease at a well-defined level by reacting with the agent at one of the steps of its cycle and thereby completely stopping the evolution of the disease. This has been suggested among others for the treatment of acquired immune deficiency syndrome (AIDS). Confirmation of this approach for MS has recently been reported by Hafler (4), who has used monoclonal anti-T_{12} antibodies directed against a determinant expressed by most T-cells in clinical therapeutical trials in MS.

4. It is obvious that the encephalomyelitis due to rabies vaccination is not a model for MS, but the study of the lymphocytic reaction is interesting and should not be overlooked.

In general the question as to which is the optimal model for MS remains open. EAE is still considered to be the best. The confusion between different demyelinating diseases persists as well for models as between human diseases such as slow sclerosing panencephalitis (SSPE) and MS. We believe that the study of the model diseases described can permit understanding of well-circumscribed immunological phenomena, but not the whole of the immunological reaction seen in MS. Some findings should be emphasized: the appearance of the Ia antigens on the membrane of macrophages and astrocytes, the role of astrocytes in the immune reaction, the transmission of viral neurological diseases through the spleen, and the possibility, indicated by clinical trials, of blocking the chain of immune reactions at a particular locus.

B. Humoral Immunity

1. The antigens triggering the disease are still being sought. Numerous antigens were discussed.

Myelin basic protein (MBP) is still of major interest. Its study in the CSF is relatively difficult because of the presence of proteases and their inhibitors. It is still agreed that MBP increases during exacerbations of MS. Warren and Catz (13) showed that antibodies against MBP appear at the same times. Bound and free MBP and MBP antibodies increase during bouts, progression and exacerbations. The most striking presentation in this field dealt with MBP polypeptides. Using monoclonal antibodies, it has been shown that these polypeptides can also appear in the urine but that the polypeptides in the urine and in the CSF are not identical. This is probably the first time that urine has been found to be of value in diagnosing MS.

Myelin-associated glycoprotein (MAG) is still actively studied in some laboratories. The presentations of studies with this antigen were derived from rather few cases. The anti-MAG antibody is certainly heterogeneous. It probably plays a role in MS.

The studies on MAG do not exclude those devoted to specific *proteins of the nervous tissue*. Studies have been concerned with the levels in CSF of GFAP, S100, and MBP (9), among others. During clinical exacerbations of MS, a large percentage of patients have increased MBP in their CSF. The changes in GFAP are less clear, and those of S100 even less. Determinations of enolase or neurofilament proteins have not yet given unequivocal results. It has to be pointed out that the antibody levels against neurofilaments are very low in the serum of MS patients (6). One should also mention a study of neural-cell adhesion molecules (NCAM) whose concentrations are increased in the CSF of MS patients during relapses.

Numerous other autoantigens, originating from the central nervous system, have been shown to play a possible role in MS.

Interesting studies refer to *immune complexes*. It is known at present that immune complexes are increased in the CSF and perhaps the blood of MS patients. Could the study of the immune complexes lead to the identification of specific antigens? It was demonstrated that some of the antigens detected in the immune complexes have epitopes in common with measles virus.

We may summarize these studies by saying that although nothing specific has been found up to now, nevertheless, they appear promising.

It is interesting to note that among proteins, very few enzymes have been dealt with during the symposium, except for NCAM and enolase.

2. *The Antibodies*. Although it is generally agreed that the IgGs are increased in the CSF, opinions are divided about IgM, IgA and particularly IgE. Henriksson et al. (5) in a recent contribution, showed that stimulated lymphocytes from the CSF produce mainly IgG.

It is widely agreed that the oligoclonal reaction is the most valuable biological reaction to confirm the diagnosis of MS. Technical improvements are still expected to display this immune reaction better. The significance of the reaction remains mysterious, and the hypothesis that this is a non sense reaction has been discussed. The origin of these bands was once more discussed.

A very large number of studies were concerned with better definition of the IgGs. The presence in the CSF of light free kappa, and to a lesser degree lambda chains during MS bouts has been mentioned (12) and was regarded as a test for confirming the clinical diagnosis by these authors. The kappa chains are present as dimers. Bidimensional electrophoresis provides more information (2, 14). An oligoclonal pattern in the light-chain region is then seen. This pattern differs from one disease to the other, with oligoclonal IgGs in the CSF. It was noted with interest that in the population Gm allotypes of the IgGs have a distribution similar to the epidemiological distribution of MS. This challenges some of the conclusions drawn from epidemiological studies. However a close relationship between these allotypes and MS has not been demonstrated. Studies have been devoted to the investigation of IgG subclasses and idiotypic IgGs. Certainly an effort is needed to investigate the IgGs more intensively and, consequently, the humoral immune reaction in MS. Final conclusions can not yet be drawn.

We can summarize these studies concerned with the antibodies, by saying that there are still technical problems to be solved and that the significance of the oligoclonal reaction and even the name of this reaction may have to be reconsidered. For many authors, the hypothesis that MS is an autoimmune reaction is challenging. However, the question is whether this immune reaction is a *cause*, or a *consequence* of the demyelination. The antigen causing MS and the antigen(s) responsible for the oligoclonal reaction are still unknown.

C. Cellular Immunity

A lot of work has been dedicated to this topic and it is therefore not an easy task to write a synopsis. Only the most important observations will be stressed.

A first series of experiments was devoted to the study of T-cell (subset and activation) antigens. As reported earlier, a decrease in the proportion of the $CD8^+$ cytotoxic/suppressor T-cell subset (and concomitantly, an increase in the CD4/CD8 ratio) in peripheral blood and CSF of some active MS patients (with chronic progression or relapse) was found. This observation is however hardly correlated with the clinical course of the disease. In addition, an increased percentage of cells expressing certain activation antigens, such as IL2 receptor (Tac antigen), major histocompatibility complex class II (HLA-DR) and Ta_1 antigen, has been observed in MS CSF. Data have been presented at the workshop, indicating that the latter antigen is a marker of memory T-cells. Differences in the expression of IL2 receptor (and responsiveness to IL2) by T-cell lines from MS patients and normal individuals have been demonstrated. MS T-cell lines were aberrant in that they showed maximal IL2-receptor expression within 2 days of mitogen or antigen stimulation (earlier than normal T-cells) and did not down-regulate this IL2 receptor expression until after 22 days in the presence of recombinant T-cell growth factor (much later than normal T-cells).

The level of pokeweed-mitogen-induced IgG secretion was increased while that of concanavalin A-induced suppressor activity was decreased in lymphocytes of progressive MS patients as compared to age-matched controls. No correlation could be found between results in one of these assays and number of $CD8^+$ or $CD4^+$ lymphocytes.

Functional studies in MS CSF have been hampered by the small number of cells that can be obtained through lumbar puncture. By applying modern tissue-culture techniques (limiting dilution in the presence of irradiated feeder cells and polyclonal activators such as mitogenic lectin or anti-CD3 monoclonal antibodies with subsequent addition of IL2), many authors have succeeded in propagating T-cell lines and clones big enough to perform phenotypic and functional analysis. As concerns the specificity of these cell populations, no clear picture has yet emerged. On sporadic occasions only, a rather weak reactivity against viral (mumps) antigens and auto-antigens (myelin basic protein, cerebrosides, gangliosides) was noted.

A preliminary study was presented at the workshop, which investigated the possible oligoclonality of CSF T-cells. This was done by looking for gene rearrangement patterns by means of a T cell receptor β-chain-specific cDNA probe. No evidence for oligoclonality of T-cells was obtained.

In summary, the studies on cellular immunity are to be considered as very important; for one, due to the fact that new techniques are deve-

loped: cell cultures, particularly culture of lymphocytes, cloning of these lymphocytes and use of monoclonal antibodies. Besides lymphocytes, astrocytes may play a role. The cause of the anomalies observed in the serum, CSF or nervous tissue lymphocytes, and the mechanism which triggers activation of lymphocytes remains unknown. Passage from one compartment to the other remains under discussion and even questionable. However it seems established that the lymphocytes do not evolve in a completely independent manner in the three above-mentioned compartments.

D. Immunological Modulation

Nearly everyone agrees that in MS there exists a significant immunological anomaly that can be shown in the CSF. The oligoclonal reaction, even if considered a non-sense reaction as well as numerous changes observed in lymphocytes, monocytes, and finally astrocytes, are evidence for this anomaly. Interferon, IL1 and 2 (which has been found in the CSF for the first time, receptors to the latter, glial growth factor, autoimmune factors, and prostaglandins have been incriminated. The prostaglandins are apparently increased in the CSF of MS. Endocrinologically, a somatostatin might play a role. It should be noted that epitopes shared by immunological and endocrinological cell types were discussed, and results agree with recent psychiatric studies on the role of the CNS in immunological reactions. Among the other factors mentioned, IAP, neopterine (a marker for carcinomas), and the Fc gamma receptors were discussed. Quite suprising results with respect to the latter were presented. The Fc gamma receptors are not increased in the CSF of MS, as they do in SSPE patients. The humoral immune reaction seen in SSPE is often compared to that of MS, but it differs in that its antigen has been identified and that most antibodies in the serum and in the CSF are directed against the antigen, measles virus. The difference between the immune reaction in the CSF observed in MS and SSPE, as shown by the presence of free Fc receptors, has been discussed. Modifications in the C9 component of the complement cascade have also been demonstrated.

Personal Assessment of the Symposium

We wish to make the following comments:

1. *New techniques* have been applied to the study of modifications of the cellular and the humoral immunity in the CSF of MS. These new techniques, which often use monoclonal antibodies, involve a number of their own problems. Monoclonal antibodies present specificities different to those of polyclonal antibodies. They often share their specificity with quite unexpected antigens. Whereas, in the past, CSF-protein studies have relied techniques examining the chemical properties of the different proteins, immunology now has monoclonal antibodies as a new tool for investigation. However numerous and laborious studies must be carried out before results can be interpreted objectively.

2. The *oligoclonal reaction* in routine clinical use remains the most reliable biological reaction for confirmation of the diagnosis of MS. Some points, however, need to be clarified: one should not mistake the oligoclonal reaction seen in MS with that observed by some authors in other neurological diseases, e.g., in vascular diseases, with respect to the intensity of the reaction and the rate of mobility of the gamma globulins (Fig. 1). The oligoclonal reaction is said to be due

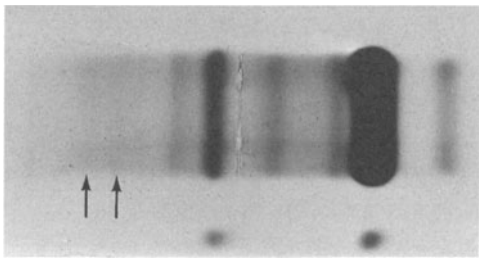

Fig. 1. Cerebrospinal fluid agar-gel-protein electrophoresis in a case of vascular cerebral disease. Faint fractions are seen

to the intrathecal synthesis of the IgGs. This synthesis probably takes place in cells coming from the peripheral blood, which have infiltrated the central nervous system and multiplied there. Nevertheless, one may ask whether astrocytes play a role in this intrathecal synthesis. What is the origin of those astrocytes? Are they modified blood cells or solely nervous system cells? Nearly everyone agrees that the oligoclonal reaction does not parallel the clinical symptoms. The electrophoretic pattern of this reaction is very stable. Its morphology is not specific to MS. Diseases other than MS have been invoked to justify the hypothesis that the intrathecal synthesis of the IgG is specific. It should be noted that there is so far no proof for the existence of clones giving rise to oligoclonal IgGs and therefore logically we should preserve the concept of the restricted heterogeneity of the IgGs.

3. The specificity of the *antibodies* involved in the oligoclonal reaction remains practically unknown. They are relatively more concentrated in the CSF than in the serum, but their absolute concentration is much less in the CSF than in the serum. These antibodies are probably partially the result of a nonspecific reaction, but that does not preclude their being also a specific reaction. The hypothesis that it is a nonsense reaction has not received much support.

4. The *antigen* responsible for MS remains unknown. Are there one or more antigens? Are we dealing here with autoantigens or with factors of viral origin? It is impossible to answer these questions. The possibility of a virus closely related to HLTV I is far from proven.

5. The interpretation of the results based on *cellular immunology* in the CSF is tied to the properties of an inducing factor which may be present in the disease. It seems that in the cellular immune reaction there is a relative increase of the helper T-cells and a relative decrease of the killer T-cells. Correlations between these modifications and the clinical conditions of the patient are difficult to make, as quoted further. The results on cultures and cloning of lymphocytes and their stimulation have been stressed; these allow the study of surface antigens which could play a more or less decisive role as receptors in MS. We wish to add that lesions undetected clinically may be revealed by means of evoked potentials and in particular by MRI. The disclosure of plaques in persons presenting no clinical signs raises considerable difficulties when classifying the patients. This has been confirmed in a recent paper by Duquette and Charest (3). Correlations between biological findings and neurological observations, including evoked potentials and MRI examinations, must be reappraised.

6. One of the most debated problems, for many years, has been the *location of the immunological reaction:* it is only a reaction in the nervous tissue? Is it a generalised reaction? Does it start in the nervous tissue or must it pass the blood-brain barrier? Is MS only a neurological disease or it is a generalized disease?

7. Part of the research presented at the workshop takes its inspiration from a whole new *methodology*. It concerns mainly a considerable contribution to the study of MS but two methodological developments should be precited from papers presented or recently published:

a) The comparison between the modifications seen in the CSF and in the blood with the lesions in the tissue is very difficult to make. The morphological study of the tissue lesions is often inadequate. The neuropathology is not always investigated fully. One may ask what is the point of counting the lymphocytes in tissue, and how much the results of cell typing in the tissues can be compared to similar lymphocyte typing in the CSF.

b) The clinical material is not always well defined or well studied. In general there are too few cases in the groups studied, and the statistical interpretation is unreliable. The problem, mentioned already, concerning the clinical classification of the patient remains unsolved.

In general, there is no doubt that the Hengelhoef meeting showed new ways for the immunological study of CSF in MS. This new lead in research was indispensable. It is just starting. Numerous research studies will be discarded. In general research work should start from better-defined clinical material. It is clearly premature to draw clear conclusions. A recent paper by Oger (10) confirms this viewpoint and proves that critical work is needed.

Acknowledgements

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