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Oligoclonal IgG bands synthesized in the central nervous system are present in rats with experimental autoimmune encephalomyelitis

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Objective – Oligoclonal bands (OBs) in electrophoresis of cerebrospinal fluid (CSF) are present in multiple sclerosis and here is investigated whether these also occur in experimental autoimmune encephalomyelitis (EAE). **Material and methods** – Experimental autoimmune encephalomyelitis was induced in 42 DA rats after immunization with rat spinal chord homogenate and the occurrence of OBs were detected by electrophoresis of both sera and CSF. The relationship between disease symptoms, antibody response against myelin basic protein (MBP), myelin oligodendrocyte glycoprotein (MOG) and appearance of OBs was studied. **Results** – Development of CSF-specific OB was found to occur, 6 weeks after immunization, in seven of 42 rats. OB was detected in rats with an antibody response against MBP, whereas as a role no such bands were present in rats with an antibody response against MOG. Initially severe disease symptoms were correlated to a concomitant intense oligoclonal antibody response. **Conclusion** – Cerebrospinal fluid-specific OB occurs in EAE. It is present in rats with an anti-MBP, but not in rats with an anti-MOG antibody response. A severe disease results in an intense oligoclonal antibody response, which might have an anti-inflammatory effect.

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Key words: multiple sclerosis; experimental allergic encephalomyelitis; oligoclonal bands; B-cell stimulation; myelin basic protein; myelin oligodendrocyte glycoprotein

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Experimental autoimmune encephalomyelitis (EAE) has been claimed to be an experimental model for multiple sclerosis (MS). In EAE, there are inflammatory lesions in the central nervous system (CNS) at histological examination similar to those seen in MS.

The inflammatory process in the CNS of MS can in addition be visualized by magnetic resonance tomography of the brain, as multiple lesions in the white substance. The cerebrospinal fluid (CSF) contains immunoglobulin G (IgG) produced by a restricted number of B lymphocytes and migrates as oligoclonal bands (OBs), on electrophoresis of CSF. These OBs are likely to reflect intrathecal immunoglobulin synthesis when they are restricted only to CSF (1–7). Expectations were raised that determination of the specificity of the OB in MS

would provide information on the specificity of the immune response occurring in MS.

However, it has not been possible to show that the OBs uniformly contain antibodies specific for a certain CNS antigen. Instead a broader specificity is often found where the oligoclonal CSF bands contain antibodies reactive with various viral and bacteria antigens such as herpes simplex virus (HSV), measles, varicella and chlamydia pneumonia, with which the patient previously had been infected (2, 7). This antibody response is claimed to be caused by a polyclonal activation of B cells in the CNS. However, antibodies to various myelin components have also been found in CSF of some MS patients and some of the OB in MS has occasionally been found to contain antibodies against myelin basic protein (MBP) (8).

Serological tests with sensitive radioimmunoassay (RIA) have detected antibodies against MBP in CSF and brains from 90 to 95% of patients with MS whereas 2% had an antibody response to proteolipid protein (PLP) (9). Antibodies to myelin oligodendrocyte glycoprotein (MOG) have been detected in CSF from some patients with MS (10).

Presence of OB in the CSF does not exclusively occur in MS. In some patients with cerebrovascular disease the same unspecific oligoclonal IgG response in CSF as in MS has been observed (11, 12). This unspecific oligoclonal response is in patients with cerebrovascular disease transient in contrast to MS.

In CSF from patients with various infections, meningitis, because of mumps meningitis (13), herpes simplex meningoencephalitis (14), neurosyphilis (15), tuberculosis meningitis (16) and subacute sclerosing panencephalitis (SSPE) (17) a specific oligoclonal IgG response is seen. All OB have in these types of infections been found to contain antibodies against the aetiological agent in question. These OBs may disappear after recovery from the infection (18).

Further studies on the cause and pathogenic role of CSF-restricted OB in MS have been hampered by the lack of animal models showing the same type of CNS-derived OB.

Oligoclonal serum-restricted bands have been detected in rats (19), rabbits (20), guinea-pigs (21) and monkeys (22) with EAE, which is an experimental model for MS. The CSF OBs in these animal models have been interpreted as being derived from the serum compartment (23). CNS-specific bands have been observed in only one report where they were found in the CSF in one monkey of five with EAE (24).

In EAE most of the serum-restricted bands contain antibodies against MBP in addition to some weak activity to proteolipid apoprotein, while no activity against myelin-associated glycoprotein was found (25). A common finding in rats with EAE is production of oligoclonal antibodies against the adjuvant, mycobacterium tuberculosis, used for immunization (21).

That there is no concomitant polyclonal B-cell activation in EAE was shown by the findings that monkeys with serologically detectable measles antibodies in serum before immunization did not develop measles-specific oligoclonal CSF IgG bands after immunization with MBP. The serum measles antibody titres did not change during the development of EAE and no measles antibodies were detected in CSF (22).

These investigations have revealed that there are two types of oligoclonal antibody responses: an unspecific, as in MS and cerebrovascular disease

and a specific oligoclonal antibody response as in infections and EAE.

The aim of the present work was to investigate the appearance of OB in CSF and/or serum of rats with EAE produced by immunization with spinal cord homogenate and incomplete Freund's adjuvant (IFA). As IFA does not contain mycobacterium tuberculosis the induction of immune responses to mycobacterium is avoided and the immune responses are directed to components within the spinal cord, i.e. the various proteins although the response to MBP seems to be the most significant (26). The relationship between disease symptoms, antibody responses against MBP and MOG and appearance of OBs was studied.

Material and methods

Animals, induction and evaluation of EAE

DA rats originating from the Zentralinstitut für Versuchstierzucht, Hannover, Germany, and bred in the animal house of Department of Medical Inflammation Research. At an age of 8–12 weeks they were immunized with rat spinal cord homogenate (10 mg/rat) emulsified in IFA (27).

Symptoms of neurological disease were evaluated by using a scoring system with nine points 0–8 and daily weighing (28).

Samples

Serum and CSF were taken during neurolept-anaesthesia (Midazolam and fentanyl/fluanisil) initially and within intervals of 5–7 days during 3 months. Blood was obtained by cutting the tip of the tail and CSF was taken by sub-occipital puncture. CSF was examined immediately for blood contamination, centrifuged and stored at -20°C .

Immunoassays

Determination of anti-MOG and anti-MBP IgG antibody response in serum and CSF was analysed by a europium streptavidin immunofluorescent method.

Microtitre plates (Thermo Labsystems, Chantilly, VA, USA) were coated with 1.0 $\mu\text{g}/\text{ml}$ MOG or 1.0 $\mu\text{g}/\text{ml}$ MBP in carbonate buffer pH 9.6 (MOG amino acid 1–125, a kind gift from C Linington) (29). The plates were blocked with 15% foetal calf serum (FCS) in phosphate-buffered saline (PBS). Rat sera and CSF were diluted 1:500 and 1:10, respectively, in 0.05% Tween-20/PBS.

The bound anti-MOG and anti-MBP rat IgG antibodies were after incubation with goat IgG anti-rat Ig antibodies (Biogoat alfa, southern Bio) diluted 1:5000 in Tween/PBS, detected by europium streptavidin (Wallac, Turku, Finland). The fluorescence was read on a fluorometer (Wallac 1420 Multilabel Counter, Wallac Oy, Turku, Finland).

The relative levels of anti-MBP- and anti-MOG-specific IgG antibody concentrations were calculated by comparison of the anti-MBP titres and anti-MOG titres with the titres from two reference serum from two rats with high antibody concentration of anti-MBP and anti-MOG antibodies, respectively.

The presence of oligoclonal IgG in rat serum and CSF was detected by isoelectric focusing (IEF) with a silverstaining method (30).

Twenty microlitres of serum diluted 1:50 in saline and 40 µl undiluted CSF were run in parallel on the polyacrylamide gel (PAG). The oligoclonal IgG migrate as bands in the gamma region on the PAG.

Statistics

We used Student's *t*-test and ANOVA for significant test and simple regression analyses for correlation of antibodies between sera and CSF. *P*-values < 0.05 were considered as significant. All analyses were performed using statview (SAS Institute, Cary, NC, USA) score and antibody titres were expressed as mean ± standard error of mean (SEM).

Results

Oligoclonal bands appear in the CNS after disease recovery

DA rats were immunized with rat spinal chord homogenate emulsified in IFA. They were followed daily for development of EAE and bled weekly for serum and sampled for CSF. Of 42 rats, 21 developed symptoms of neurological disease starting approximately day 10 with 1–2 weeks of duration.

When serum and CSF in some rats were investigated once a week OBs appeared in serum after 4–6 weeks and in CSF after 6 weeks. The OBs did not disappear during the observation period of 3 months.

The presence of OB was in all rats investigated by IEF 6 weeks after immunization, i.e. after the disease period.

An oligoclonal reaction restricted to the serum compartment was considered to be present when OB were detected on the IEF of serum without or with identical counterpart OB in CSF.

The occurrence of oligoclonal IgG restricted to the CNS compartment was considered to occur

when OB were present on IEF of CSF without any counterpart in serum.

The rats were classified into four groups:

Group A: In 15 rats we found no evidence of OB in CSF or in serum.

Group B: In 14 rats we detected OB only in the serum compartment.

Group C: In seven rats we found OB only in the CSF (Fig. 1).

Group D: In six rats we found OB selectively derived from the CSF as well as from the serum compartment (Fig. 2).

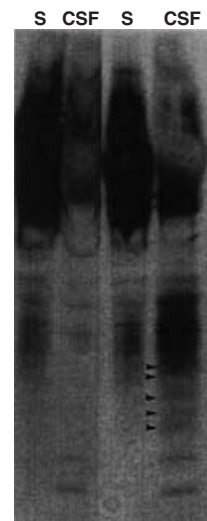


Figure 1. PAG IEF of serum and cerebrospinal fluid (CSF) from a rat day 4 and day 89 after immunization with E3 SCH and IFA. The CSF-specific OBs present on day 89 are indicated.



Figure 2. PAG IEF of serum and CSF from a rat 26 and 64 days after immunization with E3 SCH and IFA. The CSF- and serum-specific OBs present on day 64 are indicated.

No evidence for formation of OBs because of high levels of serum anti-MBP or anti-MOG antibodies

There was no correlation between the anti-MBP antibody levels in serum, measured by the maximal anti-MBP serum titre, and the occurrence of OBs in serum or CSF (group A vs B + C + D, $P = 0.48$, $n = 42$) (Fig. 3A).

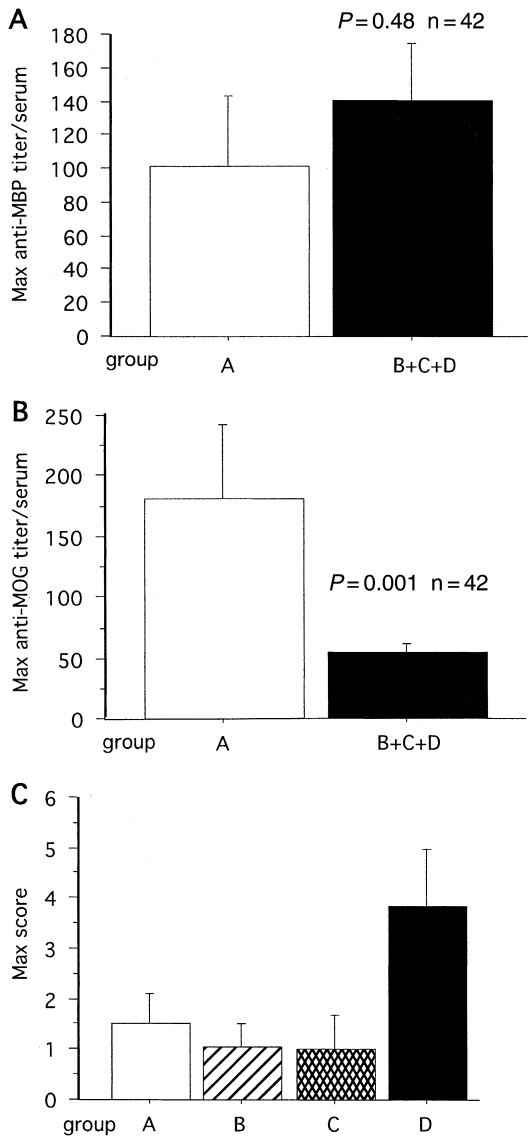


Figure 3. Relationship between the presence of oligoclonal bands (OBs), anti-MBP and anti-MOG titres and disease activity. The rats were classified into the following categories: group A ($n = 15$), no OBs; group B ($n = 14$), OBs in serum; group C ($n = 7$), OBs only in cerebrospinal fluid (CSF); and group D ($n = 6$), OBs produced in serum and in CSF. (A) Maximal anti-MBP titre serum in the absence (group A) and the presence of oligoclonal bands (group B + C + D). (B) Maximal anti-MOG titre serum in the absence (group A) and the presence of oligoclonal bands (group B + C + D). (C) Maximal disease activity, score, in rats with different oligoclonal patterns vs type of 'oligoclonal reaction'. Group D: vs A, $P = 0.03$; vs B, $P = 0.01$; vs C, $P = 0.02$; vs A + B + C, $P = 0.007$, $n = 42$.

The occurrence of OBs are negatively correlated with the presence of an antibody response against MOG in serum

The occurrence of OB in serum and/or CSF was negatively correlated to the anti-MOG titre in serum (group A vs B + C + D, $P = 0.001$, $n = 42$) (Fig. 3B).

No evidence for formation or absence of selectively CSF-derived OB because of high levels of serum anti-MBP and anti-MOG antibodies

No correlation was found when anti-MBP and anti-MOG antibody levels in serum were compared between rats without and with CSF-specific OB (group A + B vs C + D $P = 0.86$, $n = 42$ and $P = 0.14$, $n = 42$, respectively).

Anti-MBP antibodies are easily detected in CSF in rats with a strong serum antibody response against MBP

A significant correlation was found between the serum titres and CSF anti-MBP titres (maximal anti-MBP serum titre vs maximal anti-MBP CSF titre, $P < 0.0001$, $n = 23$) (Fig. 4).

The CSF MOG antibody titre was nearly constant and not related to the serum MOG antibody titre

In contrast, no correlation was found between the anti-MOG serum titres and anti-MOG CSF titres (maximal anti-MOG serum titres vs maximal anti-MOG CSF titres, $P = 0.53$, $n = 23$) (Fig. 5).

Occurrence of OB, but not high serum titre, correlates with severe disease

No significant correlation was found between the severity of neurological symptoms (maximal score)

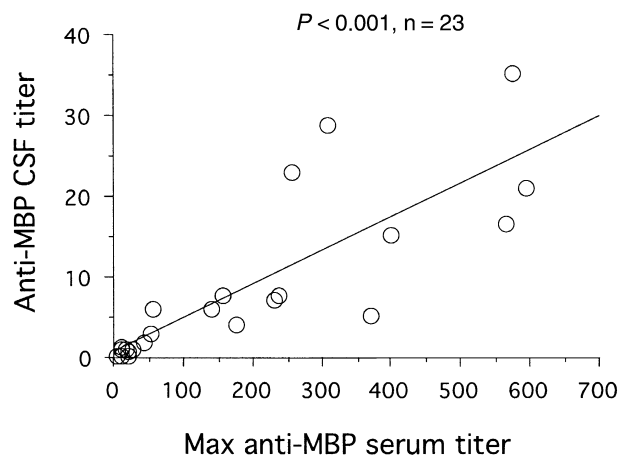


Figure 4. Correlation between anti-MBP CSF titre and maximal anti-MBP serum titre.

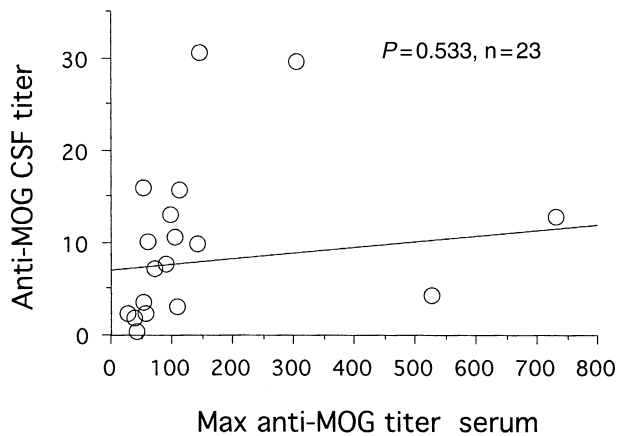


Figure 5. Correlation between anti-MOG CSF titre and maximal anti-MOG serum titre.

and maximal anti-MBP serum titre ($P = 0.34$, $n = 42$) or maximal anti-MOG serum titre ($P = 0.16$, $n = 42$).

Interestingly, the presence of serum- and CSF-derived OB concomitantly was connected with the most severe symptoms (Fig. 3C).

Discussion

In similarity with MS we have observed that CNS-specific OBs are present in the spinal fluid in EAE. The proportion of rats with CSF-specific OB were less (seven of 42) than in humans with MS (90%). The formation of OBs in the CNS of the rats is most likely related to intrathecal IgG synthesis as has also been found in MS. The oligoclonal reaction is in EAE a late phenomenon occurring 6 weeks after immunization. In MS this is already a persistent phenomenon when the patients develop the first symptom of neurological disease. However, in the human disease a subclinical disease process might have preceded the first symptoms for a longer time mimicking the observations in the animal model. In patients with such symptoms suspected to be a first attack of MS the presence of an oligoclonal reaction seems to predict those patients who are going to develop a chronic demyelinating disease, MS (31). However in MS 10% of the patients does not have any OB.

The disease incidence (50%) in this EAE study is less than expected. This together with the absence of relapsing symptoms may be the result of stress or/and depletion frequently of CSF and serum.

The intensity of the oligoclonal immunoglobulin reaction in EAE might reflect the disease activity as the most severely sick rats had in addition to intrathecal oligoclonal IgG production an oligoclonal reaction in the serum compartment. This

indicates that these rats had a generally activated immune response in addition to a specific CNS immune response resulting in severe neurological symptoms. There are no reports that the disease activity is influenced by the presence or absence of OB in MS.

The sources of the oligoclonal immunoglobulins and of the immunoglobulin producing cells in the CSF from patients with MS are unknown. The observation in our rat model that unique CNS OB are formed argues that these antibodies might be produced by plasma cells within the CNS. The finding that the anti-MBP, but not anti-MOG, antibody concentration in serum was positively correlated to the anti-MBP antibody concentration in CSF is most readily explained if the inflammatory lesions predominantly contain anti-MBP secreting plasma cells. Alternatively, anti-MBP antibodies may have a higher capacity to pass the blood-brain barrier than anti-MOG antibodies. Another explanation for a low anti-MOG IgG antibody concentration in CSF, despite raised serum concentration, may be binding of most of these antibodies to CNS tissue resulting in low antibody concentration in CSF. In MS anti-MOG antibodies have been shown to bind to myelin and the statement above may thus be applicable to MS as well as EAE (32).

The antibody response against MBP and MOG in our study was analysed in an effort to find a correlation between these titres and the presence of OB in CSF or serum. The OB in serum from rats with EAE has previously been claimed to contain anti-MBP antibodies (25). A correlation between the anti-MBP antibody response and the presence of OB in serum or CSF was therefore anticipated. However, no statistically significant correlation was found.

In MS some of the OBs have been reported to occasionally consist of anti-MBP antibodies. The major part of the oligoclonal immune response is however in MS unspecific with a variety of antigen specificities, which is claimed to be caused by a concomitant polyclonal B-cell activation. No proof of such activation has been found in EAE (8, 22, 23). The oligoclonal anti-MBP antibody response in EAE is probably a specific anti-MBP immune response in contrast to the situation in MS where the oligoclonal anti-MBP antibody response might be part of an unspecific immune response with a huge variety of antibody specificities.

No studies have shown the presence of anti-MOG antibodies in serum or CSF OB in rats with EAE and therefore we did not expect to find a positive correlation between the anti-MOG antibody response, serum titre, and the occurrence of

OB from serum and/or CSF in EAE. Unexpectedly we found that the occurrence of OB in serum and/or CSF was negatively correlated to the anti-MOG antibody titre in serum (group A vs B + C + D $P = 0.001$, $n = 42$) (Fig. 3B).

The polyclonal B-cell stimulator LPS gives, when used as an adjuvant together with an antigen a specific antibody response. When LPS is given without any other antigen a natural preexisting antibody response will be stimulated (33). If brain tissue components released during brain tissue damage in EAE and MS act as an adjuvant like LPS the suggested specific oligoclonal MBP antibody response in EAE is similar to the effect of LPS with a specific antigen and the unspecific oligoclonal antibody response in MS is similar to the effect of LPS without specific antigen. Another possibility is that MBP gives rise to a specific oligoclonal antibody response without requirement of a B-cell stimulator. This may be similar to mycobacterium tuberculosis which might *per se* give rise to an oligoclonal antibody response in pleuritis (34). Our rats with a high antibody titre against MOG does not have OB. The absence of an oligoclonal response could be caused by inactivation of the B-cell stimulator by the MOG antibodies. Another explanation is that MOG does not *per se* give rise to a specific oligoclonal IgG response. In conclusion, our study demonstrated that seven of 42 rats with EAE developed CSF-specific OB 4–6 weeks after the immunization, whereas EAE symptoms were present in 21 rats from day 10 with 1–2 weeks of duration. This is in contrast with the situation in MS, where 90% of the patients have OBs simultaneously with disease symptoms. Moreover, whereas OBs were detected in rats with an antibody response against MBP, as a role no such bands were present in rats with a strong antibody response against MOG. A possible interpretation of these data, together with previous observations, is that the oligoclonal antibody response in spinal chord-induced EAE is directed towards the immunodominant protein, i.e. MBP, in the spinal chord. In contrast, the OB in MS might signal a secondary phenomenon to the disease process. It is possible that this prominent oligoclonal IgG response in the CSF in MS has an anti-inflammatory effect. As it has been shown that B cells play a key role in the recovery from EAE via the production of an anti-inflammatory cytokine, interleukin (IL)-10 (35).

In addition, immunoglobulins given intravenously have an anti-inflammatory effect in the treatment of inflammatory diseases of the nervous system, i.e. MS (36).

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