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Multiple sclerosis patients lacking oligoclonal bands in the cerebrospinal fluid are less likely to develop neutralizing antibodies against interferon beta

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Abstract

Multiple sclerosis patients without cerebrospinal fluid oligoclonal IgG bands have been proposed to constitute an immunogenetically distinct subgroup of multiple sclerosis that may also differ in terms of prognosis. A proportion of patients with multiple sclerosis receiving IFNβ develop neutralizing antibodies, which interfere with treatment efficacy. Evidence suggests that the likelihood of developing neutralizing antibodies is partly genetically determined. Here, we hypothesized that absence of oligoclonal IgG bands reflects a property of B-cell responses in oligoclonal IgG band-negative patients characterized by a lessened propensity to develop neutralizing antibodies. We aimed to compare the development of neutralizing antibodies against IFNβ between oligoclonal IgG band-negative and oligoclonal IgG band-positive multiple sclerosis patients. Treatment, oligoclonal IgG band and neutralizing antibody information was obtained for 2219 patients from the Swedish multiple sclerosis registry and the Swedish neutralizing antibody registry. Additional data on genotype was available for 532 patients. A correlation was found between oligoclonal IgG band negativity and neutralizing antibody negativity (p = 0.02). This difference was confined to neutralizing antibodies against IFNβ-1a, since oligoclonal IgG band-negative patients were, to a lesser extent, neutralizing antibody positive compared with oligoclonal IgG band-positive patients if treated with IFNβ-1a (12% vs. 23%; p = 0.005). No difference was observed for IFNβ-1b-treated patients (44% vs. 46%). We propose that oligoclonal IgG band-negative patients differ immunologically from oligoclonal IgG band-positive patients, potentially influenced by distinct HLA-DRB1 alleles.

Keywords
cerebrospinal fluid, interferon beta, human, multiple sclerosis, neutralizing antibodies, oligoclonal bands

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Introduction

The presence of immunoglobulin G (IgG) oligoclonal bands (OCBs) or a general increase of IgG immunoglobulin in the cerebrospinal fluid (CSF) is part of the diagnostic criteria of multiple sclerosis (MS); however around 5% of patients with MS lack OCBs. How these two groups of patients differ in various aspects has been subject to numerous studies. Results from Japan and Sweden showed that OCB-positive and OCB-negative patients constitute two immunogenetically distinct subgroups of MS, as they are associated to different HLA-DRB1 alleles, HLA-DRB1*15 and HLA-DRB1*04, respectively. In a subsequent study by our group, OCB-negative patients were shown to have a better prognosis, i.e. higher median age at attainment of Expanded Disability Status Scale 6.0, if they at the same time were non-carriers for the HLA-alleles DRB1*15 and DRB1*04. Some studies looking at the clinical outcome and OCB status showed a favourable outcome for OCB-negative patients, whereas other authors have found no significant differences regarding clinical outcome between OCB-positive and OCB-negative patients. The effect of CSF-specific antibodies is currently unknown, as is the reason for why they are absent in some patients with MS. Whether the absence of oligoclonal IgG antibodies in CSF is due to a distinct immunogenetic background associated with the OCB-negative subgroup, or if the lack of OCBs could reflect
an underlying defect in B-cell mediated responses in general in these patients has not been studied.

Interferon beta (IFNβ) has been used as a first line disease-modifying treatment for MS for more than a decade. It is well established that a proportion of the IFNβ-treated MS patients eventually lose IFNβ efficacy due to formation of neutralizing antibodies (NAbs). It has recently been shown that the level of NAbs is influenced by genetic background, i.e. by certain HLA class II alleles, HLA-DRB1*0401 and HLA-DRB1*0408.9

Here we aimed to establish a possible relationship between the propensities for forming these two classes of IgG, i.e. OCBs and IFNβ NAbs, hypothesizing that a potential alteration in the humoral immune response of OCB-negative patients might be found in that case. Thus, we combined the information from the national Swedish MS (SMS) registry with data from the Swedish NAb registry to investigate whether patients with either OCBs or increased IgG immunoglobulin in the CSF are more likely to develop neutralizing antibodies (NAbs) against the different IFNβ preparations.

**Materials and methods**

**Subjects and inclusion criteria**

We obtained information on all patients listed in the SMS registry and NAb registry having undergone CSF testing and NAb testing up to February 2009. Informed consent was given by all registered patients. For inclusion into the study, patients were required to fulfill the following criteria: (1) a defined diagnosis of MS according to the McDonald criteria; (2) at least one serum sample examined for the presence of IgG and/or OCBs; (3) at least one serum sample tested for NAbs against IFNβ; and (4) available information about treatment with any of the IFNβ preparations Avonex, Rebif 22 µg (Rebif 22), Rebif 44 µg (Rebif 44) or Betaferon. A total of 2219 patients fulfilled all the required inclusion criteria. Additional data on HLA-DRB1 genotype was available for 532 patients.

Patients with more than one NAb sample were assigned the treatment information recorded for the first positive sample. If patients only had negative NAb samples the treatment information recorded for the first sample was considered. If treatment information was not stated for a selected sample, the treatment entered for the previous or, if this was not possible, the following NAb test was considered. Information on type of IFNβ treatment was obtained from the referral forms filled in at the time of sampling. Information on treatment onset was available for 2023 patients and for these, duration of IFNβ treatment was calculated as the time from IFNβ onset to the collection of the NAb sample described above.

The patients were grouped into two categories regarding treatment: either IFNβ-1a (Avonex, Rebif 22 and Rebif 44) treated or IFNβ-1b (Betaferon) treated. In the OCB-positive group, IFNβ-1a was prescribed to 1626 patients (Avonex, \(n=719\); Rebif 22, \(n=256\); Rebif 44, \(n=651\)) and IFNβ-1b to 444 patients, whereas in the OCB-negative group IFNβ-1a was prescribed to 117 patients (Avonex, \(n=66\); Rebif 22, \(n=13\); Rebif 44, \(n=38\)) and IFNβ-1b to 32 patients. The proportion of patients treated with each IFNβ preparation was similar in both groups, except for Avonex which was slightly more common in the OCB-negative group.

**CSF analysis and NAb testing**

CSF analysis was performed during the diagnostic workup for each patient, where paired CSF and plasma samples were analyzed using isoelectric focusing followed by IgG-specific immunolabelling. For this study, patients fulfilling the CSF criteria as stated in the revised McDonald criteria were declared OCB positive; all others were declared OCB negative. The presence of NAbs against IFNβ was determined by the myxovirus resistance protein A gene-expression assay as described by Bertolotto et al., and samples with NAb titers above 10 ten-fold reduction units (TRU)/ml were considered positive. Patients with at least one positive sample were declared NAb positive. Patients who only had tested negative were declared NAb negative. All test results were accessible through the SMS registry and the NAb registry.

**Statistics**

Comparisons between groups for categorical data were analyzed in 2 × 2 contingency tables using the Fischer’s exact test or the Chi-square test with Yates’ correction where appropriate. Differences in treatment duration between two groups were calculated using the two-sample (unpaired) t-test. All calculations were performed in GraphPad Prism version 4.0 for Windows (GraphPad Software, San Diego, CA, USA). To control for confounding effects and interaction between the two variables OCBs and IFNβ preparation on NAb outcome, logistic regression analyses were performed using R. All reported probability values are two-sided and considered significant if less than 0.05.

**Results**

Of all patients included in this study, 2070 (93%) were OCB positive and 149 (7%) were OCB negative. The overall NAb seroprevalence, i.e. frequency of NAb-positive patients, was significantly lower in the
OCB-negative group compared with the OCB-positive group (Figure 1).

The difference in NAb seroprevalence between the two subgroups was further investigated by comparing the humoral immune response towards the two types of recombinant IFNβ; the eukaryotic recombinant IFNβ-1a (Avonex, Rebif 22 and Rebif 44), and the prokaryotic recombinant IFNβ-1b (Betaferon). The NAb seroprevalence for IFNβ-1a in the OCB-positive group was 23% (Avonex, n = 71; Rebif 22, n = 79; Rebif 44, n = 218) and 12% in the OCB-negative group (Avonex, n = 4; Rebif 22, n = 3; Rebif 44, n = 7), while IFNβ-1b had a seroprevalence of 46% (n = 205) and 44% (n = 14) in the respective groups (Table 1). Thus, OCB-negative patients treated with IFNβ-1a were significantly less likely to develop NABs compared with OCB-positive patients, whereas no difference was detected between OCB-negative and OCB-positive patients treated with IFNβ-1b. In both subgroups a significantly larger proportion of the IFNβ-1b treated patients had a NAb-positive status compared with the IFNβ-1a-treated patients (p < 0.0005).

Although treatment with Avonex was slightly more common among the OCB-negative compared with the OCB-positive patients (44% vs. 35%; p = 0.02) this did not explain the difference in NAb outcome between these two groups, since the effect of OCBs on NAb outcome was independent of IFNβ preparation (p = 0.02). No interaction between the variables OCBs and treatment was detected (p = 0.15).

To ascertain that the difference in NAb seroprevalence between OCB-positive and OCB-negative patients was not influenced by a difference in duration of IFNβ treatment, the time from treatment onset to collection of the NAb samples was compared. No variation in treatment duration was detected between the OCB-positive (n = 1886, mean ± SEM: 103.9 months ±1.7; 95% CI 100.4–107.3) and the OCB-negative group (n = 137, mean ± SEM: 98.9 months ±6.2; 95% CI: 86.7–111.1; p = 0.46), nor was a difference detected between the NAb-positive (n = 549, mean ± SEM: 103.2 months ±3.0; 95% CI: 97.2–109.2) and NAb-negative group (n = 1474, mean ± SEM: 103.6 months ±2.0; 95% CI: 99.7–107.6; p = 0.91).

Information on HLA-DRB1 genotype was available for 26 of the OCB-negative (17%) and 506 of the OCB-positive patients (24%). A significant difference in carriage frequencies between the groups was observed for HLA-DRB1*04 and HLA-DRB1*15 (Table 2). Of the genotyped patients, two OCB-negative patients (8%) and 134 OCB-positive patients (26%) were NAb positive.

### Discussion

The examination of CSF for the presence of two or more OCBs, which are absent in serum, is used as a paraclinical test to support the diagnosis of MS. These bands are believed to reflect an elevated intrathecal synthesis of antibodies of the IgG subclass produced by specific B-cell clones, which indicates an ongoing inflammatory response within the central nervous system. Increased IgG levels and OCBs are found in the majority of patients with MS, although a subgroup...
of patients does not display these MS typical features. To date there are few clues to why some MS patients lack OCBs.

In this study we have for the first time characterized MS patients who lack the typical increase in IgG levels, both in respect of their immunological response towards therapeutic IFNβ and the presence of OCBs in CSF. We describe a correlation between the production of IgG in CSF and the risk of developing antibodies with neutralizing capacity against therapeutic IFNβ. OCB-negative MS patients are less likely to develop NAbs against IFNβ compared with OCB-positive MS patients, supporting the suggestion that an immunological difference between these two MS subgroups might exist.

One hypothesis explaining the absence of OCBs in the CSF in a minority of MS patients could be a general defect in the B-cell response in these patients. However, since the proportion of NAb-positive patients treated with IFNβ-1b does not differ between OCB-negative and OCB-positive patients, our results do not support that OCB-negative patients suffer from a general defective B-cell response. Instead we observed that only the OCB-negative patients treated with IFNβ-1a develop NAbs to a significantly lesser extent than OCB-positive patients, as well as compared with the expected frequency of NAb-positive patients based on historical data reported for the Swedish population.13

Since Avonex is known to be less immunogenic than Rebif 22, Rebif 44 and Betaferon, and treatment with Avonex was more common in the OCB-negative compared with the OCB-positive group, we performed a logistic regression analysis to control whether the observed difference in NAb seroprevalence between OCB-negative and OCB-positive individuals was a result of IFNβ preparation instead of OCB status. Treatment was not identified to confound the effect of OCBs on NAb outcome, neither was any interaction between treatment and OCB status observed. The proportion of NAb-positive patients among the IFNβ-1b-treated patients was larger in both the OCB-positive and OCB-negative group, compared with the IFNβ-1a treated. However, this is not unexpected since IFNβ-1b is known to have the highest seroprevalence among the different preparations.13,14

One possible mechanism for the observed difference in NAb seroprevalence is the way these slightly different products may elicit humoral immunity. Recombinant IFNβ-1a, which is identical to natural IFNβ, is thought to trigger an antibody response mainly by the T-cell-dependent activation of B cells through presentation of peptides bound to HLA class II molecules to activated T cells. Recombinant IFNβ-1b differs from natural IFNβ and IFNβ-1a by a few modifications. The lack of glycosylation makes IFNβ-1b molecules less soluble in water compared with IFNβ-1a molecules, and this also makes IFNβ-1b molecules more likely to form aggregates (reviewed by Buttmann and Rieckmann 15). We hypothesize that, in addition to the T-cell-dependent B-cell activation pathway, aggregated IFNβ-1b molecules might also be able to cross-link B-cell receptors and thereby stimulate B cells to produce antibodies through a T-cell-independent pathway.13

The lower NAb seroprevalence observed in OCB-negative patients treated with the IFNβ-1a preparations could thus reflect a difference in their antigen presenting ability of B cells to T cells compared with OCB-positive patients. The efficiency of antigen presentation could be influenced by several genes encoding for essential structures of the antigen presenting pathway, or by environmental factors influencing the efficiency of the presentation. It could also potentially be influenced by the peptide-binding capacity of distinct HLA-DRB1 alleles between these subgroups.

To further assess whether the lower NAb seroprevalence observed in OCB-negative patients treated with the IFNβ-1a preparations might reflect a difference in their antigen presenting ability, compared with the OCB-positive patients, the potential influence of HLA-DRB1 was investigated. Unfortunately, as genotype data was available for 26 of the 149 OCB-negative patients, and only two of these patients were NAb positive, no reliable results could be achieved regarding the influence of HLA-DRB1 on NAb status between the groups. Thus, this connection to HLA needs to be elucidated further.

Although the number of genotyped patients was small, a significant difference in HLA-DRB1*04 and HLA-DRB1*15 carriage between OCB-negative and OCB-positive individuals could be detected, with a significantly larger proportion of the OCB-negative patients carrying HLA-DRB1*04 and carriage of HLA-DRB1*15 being significantly more frequent
among OCB-positive patients. This confirms previously published results on the immunogenetic difference between these two subgroups.\(^{4,5}\) Since genotype data for a larger number of the OCB-negative patients would be needed to have enough power to detect differences between the groups, we cannot exclude the possibility that there might also be differences in carriage frequencies for other HLA-DRB1 alleles between the groups.

In the study, we included patients who had at least one CSF sample tested for the presence of OCBs, regardless of their disease duration. Although 7% of the patients were OCB negative, we do not consider this frequency to be atypical and that it could have been introduced by a biased selection of OCB-negative subjects. However, this is not excluded and could therefore be a potential weakness of our study. Since patients who become NAb positive have been suggested to generally develop NAbs between 9 and 18 months after treatment onset,\(^{16}\) treatment duration of IFN\(\beta\) was compared between OCB-positive and OCB-negative patients, as well as between NAb-positive and negative patients. The mean treatment duration was similar for the compared groups; therefore the observed difference in NAb development between OCB-positive and OCB-negative patients does not seem to be affected by how long they have been treated with IFN\(\beta\).

If B-cell-mediated responses are affected in OCB-negative MS patients, their reaction to other autoimmune diseases, and possibly also to vaccinations, would be expected to differ in comparison with OCB-positive MS patients. So far it is unknown whether this is the case as studies on this have, to our knowledge, not yet been performed.

In conclusion, we show that the immunological response towards therapeutic IFN\(\beta\) is less frequent in OCB-negative MS patients than in OCB-positive MS patients, possibly due to the lower immunogenic potential of the IFN\(\beta\)-1a preparations in these patients.

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