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The intrathecal, polyspecific and oligoclonal immune response in multiple sclerosis

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We report an extended set of neuroimmunological data detected in cerebrospinal fluid (CSF) from n=*267 patients with definite multiple sclerosis (MS). Known frequencies of oligoclonal IgG (98%), frequencies of intrathecal fractions of IgG, IgA and IgM (72%, 9% and 20%, respectively) were confirmed and quantitated as intrathecal fractions, Ig_{IF} or CSF concentrations, Ig_{Loc}. Eighty-nine per cent of the patients had a combined 'MRZ-reaction', i.e. intrathecal antibody synthesis (Antibody Index, AI > 1.4) against measles, rubella and/or varicella zoster virus. Frequencies of single antibodies decreased from measles (78%) to rubella (60%), VZV (55%) and HSV (28%). This MRZ-reaction, indicating a chronic autoimmune type disease already at time of first clinical symptoms, is less sensitive but more specific than detection of oligoclonal IgG. With increasing intrathecal IgG synthesis the number of different locally synthesized antibody species were increased as well as the amount per species (increased mean AI values). The concentration of MRZ antibodies in CSF represents together about 2% of intrathecally synthesized total IgG. But, as a very particular result the ratio of intrathecally synthesized specific antibody/intrathecally synthesized JgG was 5-fold higher (0.24–0.85%) compared to the corresponding ratio in blood (0.06–0.17%) of MS patients. This difference between brain ratio and blood ratio is discussed to be indicative for the anti-MRZ antibody forming B-lymphocyte subset in blood migrating into brain at earlier time of pathophysiological start of disease. These results give a concise explanation of neuroimmunological aspects in MS, not understood so far.*

Keywords: *measles-, rubella-, varicella zoster-, HSV-, toxoplasma-antibodies; intrathecal immune response; IgG; IgA; IgM; neuroimmunology*

Introduction

The intrathecal humoral immune response detectable in CSF is known as the most frequent and most stable pathological sign in the brain of MS patients. With the sensitive detection of oligoclonal IgG[1] a method for the laboratory-supported diagnosis of MS[2] was introduced. The observation of an intrathecal antibody synthesis against neurotropic viruses like measles (M), rubella (R) or varicella zoster (Z) virus with a 50- to 100-fold higher frequency in MS than in any other chronic or acute disease, led to the introduction of the 'MRZ-reaction'.[3,4] The detection of a combination of measles with rubella or measles with zoster virus antibody synthesis in brain has a very high plausibility not to be the consequence of two different acute diseases at the same time. With this empirical aspect the positive MRZ-reaction supports the diagnosis of a chronic disease like MS or autoimmune disease with involvement of the CNS[5], already at time of first clinical symptoms. The MRZ-reaction, preferentially seen in these chronic autoimmune-type diseases, is not understood at all, regarding its cause and pathophysiological meaning.

Obviously, there is no persistence of the antigens (e.g. of measles virus) observed in the brain.[6] In general, the polyspecific, oligoclonal immune response[1,17] in neurological diseases needs the understanding of the selforganized immunological network.[7,8] Besides this more general immunological concept, the particular meaning of the high frequencies of M, R, Z antibodies in CSF of MS patients led to several comparative investigations between these antibodies in acute diseases and MS regarding quantities[9] or affinities.[10]

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Our experience with the MRZ-reaction[3,4] since more than 10 years led to a collection of data, which, together with new quantitative evaluations might shed some light on the special meaning of this MRZ response in MS. We report these investigations together with the complete set of neuroimmunological CSF data collected for routine diagnosis in a large group of MS patients. All data are reevaluated by the more recent, improved parameters for the hyperbolic discrimination lines in the quotient graphs of Reiber.[11–13]

Materials and methods

Patients

The retrospectively evaluated data originate from patients with clinically definite MS, collected in the MS archive of Prof Dr Sigrid Poser, University Göttingen, in the time 1986–94.[18]

The diagnosis of definite MS was clinically defined and laboratory supported according to the internationally accepted criteria.[2] The group of *n*=267 patients evaluated was recruited out of a total group of 817 patients, according to their set of immunoglobulin G, A, M detected, with a subgroup of *n*=177 patients with MRZ-reaction analysed, too. The ratio of female/male patients was in the total as well as in the subgroup 1.95/1, the mean age was 36 years. Only the first diagnostic puncture of each patient has been involved in this study. The quantitative analysis of antibody concentrations (mg/L) was performed with reference to actual data from a different group of 183 patients.[15]

Analytical procedures

The CSF and serum samples have been analysed on behalf of routine diagnosis. Albumin, IgG, IgA and IgM

have been quantitated by standard immunochemical nephelometry or alternatively by ELISA for IgA and IgM.[14] Virusspecific antibodies in CSF and serum samples were analysed by ELISA technique and evaluated as Antibody Index (AI) according to Reiber and Lange.[3] Intrathecal fractions (Ig_{IF}), intrathecal synthesis of IgG, IgA and IgM (Ig_{Loc}) and Antibody Index (AI) values for measles, rubella, VZV and HSV antibodies were calculated with the improved parameters (a/b, b^2, c according to Reiber,[11–13] also reported in, this Journal[19]). For detection of intrathecal antibody synthesis in a single patient we refer to Q_{IgG} or to the upper discrimination line, Q_{Lim}, of the reference range as zero synthesis in the Reiber graphs:[11,13,19]

$$Q_{Lim} = a/b\sqrt{Q_{Alb}{}^2 + b^2} - c$$

The intrathecal, locally synthesized contribution of Ig in CSF was calculated as Ig_{Loc} (in mg/L) or as relative intrathecal fraction Ig_{IF} (in %):[19]

$$Ig_{Loc} = \left[Q_{Ig} - Q_{Lim}(Ig)\right] \cdot Ig(serum)$$

and

$$Ig_{IF} = \left[Ig_{Loc}/Ig(CSF)\right] \cdot 100 = \left[1 - Q_{Lim}(Ig)/Q_{Ig}\right] \cdot 100$$

In case of statistical evaluations for comparison of means of groups, we refer to Q_{mean} with the different parameters (for a/b, b^2, c), given in[19] instead of Q_{Lim}. Q_{mean} represents the base for calculation of $Ig_{IF(mean)}$ or $Ig_{Loc(mean)}$.

Intrathecal synthesis of antibodies was calculated from Antibody Index, AI: $AI=Q_{spec}/Q_{IgG}$ or $AI=Q_{spec}/Q_{Lim}$ if ($Q_{Lim} < Q_{IgG}$), with Q_{spec}=AB(CSF)/AB (ser), the antibody (AB) concentrations in CSF and serum, usually determined as arbitrary units. The reference range is AI=0.7–1.3 and pathological values are AI>1.4.[3,4]

The quantitation of absolute measles-, rubella- and VZV-antibody concentrations (mg/L), instead of arbitrary units in CSF and serum, has been done by the modified ELISA method[15] according to Conrad *et al*.[9] Oligoclonal IgG was detected by IEF on macro-PA-gels with Coomassie- or silver-stain.[1]

Results

Immunoglobulin class response

In 98% of the MS patients (n=267) we observed an intrathecal IgG synthesis, either as oligoclonal IgG or direct by $Q_{IgG} > Q_{Alb}$. This is consistent with the many earlier reports.[1] Calculated as intrathecal fractions ($Ig_{IF} > 0$) in CSF, the other Ig-classes were much less frequent than IgG (72%) with 9% for IgA or 20% for IgM (Table 1). The absolute amounts of the mean immune response of IgG, IgA or IgM class in CSF are shown in Table 1 as Ig_{Loc} in mg/L. The comparison of intrathecal fractions (Ig_{IF}), which evaluate the intrathecal synthesis relative to the blood-derived Ig concentrations, shows more direct the relative intensity of IgM response than by absolute amounts with Ig_{Loc} in mg/L. The frequency or extent of IgM response was independent of the extent of IgG class response. The intrathecal IgA or IgM synthesis was always combined with an intrathecal IgG synthesis. There was no correlation between mean intrathecal IgG response ($IgG_{Loc(mean)}$) and the albumin quotient.

Cellular immune response

The known relations of a dominantly normal cell count ($\leqslant 4/\mu$L) in 42% of the cases and only 2% of patients with values $>40/\mu$L and no case larger than $96/\mu$L are reported in detail in Table 2, confirming earlier reports. Activated B-lymphocytes[16] in CSF were found in 59.5% of the patients with a clinical sensitivity below that of plasma cells. The frequency, but not the mean cell count of activated B-cells increased somewhat with increasing intrathecal IgG fractions in CSF.

Intrathecal, polyspecific immune response (MRZ-reaction)

The intrathecal synthesis of measles (M), rubella (R), varicella zoster (Z) and herpes simplex (H) virus antibodies was detected by calculation of the Antibody

Table 2 Cellular immune response in CSF of MS patients (n=267)

	Intervals					
Cell count/µl	0–4	5–9	10–14	15–24	25–34	>34
Frequency (%)	42.6	26.2	13.1	11.2	3.8	4.1

Table 1 Frequency and intensity of the IgG, IgA, IgM class response in multiple sclerosis (n=267) with median and maximum

Ig-class	*Frequency*[a]	*Intrathecal fract. Ig_{IF} (%)*[c] *median (max)*	*Local Ig-synthesis Ig_{Loc} (mg/L) median (max)*	*Blood conc. Ig(g/L) median*
IgG	72%[b]	43 (86)	26.2 (172)	10.7
IgA	9%	24 (80)	1.3 (31)	1.7
IgM	20%	55 (92)	1.2 (12)	1.4

[a] Relative number of cases with $IgG_{IF} \geqslant 10\%$, calculated from Reiber's hyperbolic discrimination function.[11] [b]98% of the patients had an intrathecal IgG synthesis as detected by oligoclonal bands in CSF. [c]Intrathecal fraction $Ig_{IF}=Ig_{Loc}/Ig\ (CSF) \bullet 100$ [%] with $Ig_{Loc}=(Q_{Ig}-Q_{Lim}) \bullet Ig(ser)$ and Ig(CSF) as total Ig concentration in CSF

Index (AI). The frequencies of intrathecal synthesis of each single species in a subgroup of *n*=177 (*n*=94 for HSV, respectively) patients are reported in Table 3 with median and maximal AI values.

Eighty-nine per cent of the MS patients had a combined intrathecal antibody synthesis against one, two or three of the M, R, Z viruses, and negligibly more (90%) if H is additionally combined with MRZ (Table 3).

The largest frequency of a single species in the oligoclonal immune response was observed for measles antibodies, seen in 78% of all patients with definite MS (Table 3). An isolated measles antibody response was observed in 15% only, in 14% as a combination with increased rubella-AI (M+R), or 11% as combination M+Z and 4% as R+Z. The combination with all three AI values increased (M+R+Z) was seen with a mean frequency of 38%. The detection of isolated intrathecal HSV antibody response was very rare in only 2% of the MRZH. Combinations of M+R, M+Z, or R+Z are a plausible indication for a chronic autoimmune type disease, not seen in any other acute, subacute or chronic inflammatory neurological disease, only with frequencies below 0.1%. In contrast, in acute infections, Z, H and the combination of Z+H (together 5% frequency in MS) is of diagnostic relevance, e.g. as cause of facial nerve palsy an increased Z-AI is seen in 11/11 cases[13,14] at time of first diagnostic puncture.

The detection of the MRZ-reaction is of different quality compared to oligoclonal IgG. The absence of oligoclonal IgG speaks against a suggested diagnosis 'MS' and its presence is not specific at all. The MRZ-reaction gives a much more specific information, which allows to state the presence of a chronic disease, already at time of first clinical symptoms. The MRZ-reaction is clinically less sensitive (89%) than the detection of oligoclonal IgG (98%), in particular there was no case with MRZ-reaction in absence of oligoclonal IgG. So the detection of a MRZ-reaction could be restricted to cases with oligoclonal IgG.

Table 3 Frequency and intensity of intrathecal, polyspecific antibody response in MS, shown for the single virus antibodies M, R, Z, or H and the combinations of M, R, Z (and/or i.e. one to three AI values increased) and the combination of MRZH (and/or i.e. one to four AI values increased)

	Frequency[a]	*Antibody Index (AI)*[b]	
Antibody Species	*[%]*	*median*	*maxima*
Measles (M)	78	3.3	46.3
Rubella (R)	60	3.9	33.5
Varizella Zoster (Z)	55	3.6	28.9
Herpes simplex (H)	28	1.8	9.5
Total M, R, Z (and/or)	89	–	–
Total M, R, Z H (and/or)	90	–	–

[a]Calculated as % of all MS patients investigated for MRZ (*n*=177). The MRZH-group included only *n*=94 cases. [b]Evaluated for subgroups of patients with AI>1.4 for the single antibody species

MRZ and other intrathecal antibodies in neurological diseases

The lower frequency of intrathecal HSV-antibodies (28%) in MS patients is still larger than that of other antibody species, like intrathecal toxoplasma-antibodies (10% amongst *n*=84 patients) or intrathecal autoantibody synthesis against dsDNA (10% of *n*=60 patients). These results emphasize the extraordinary role of the MRZ-reaction in MS. Other neurological diseases like neuroborreliosis, neurosyphilis, neurotuberculosis had a frequency of MRZ-reactions below 1% for the single species and below 0.1% for M+R+Z, restricted to exceptional cases of a chronic course of disease (e.g. neuroborreliosis[3]).

Table 4 Frequency of antibody combinations in the MRZ reaction with none (n_0), one (n_1), two (n_2), or three (n_3) different antibody species in correlation with increasing intrathecal total IgG synthesis, $IgG_{Loc\ (mean)}$

$IgG_{Loc\ (mean)}$[a]		*Mean number of*	*Fractions*[c]			
Intervals (mg/L)	*n/Interval*	*AB-species*[b]	$n_0/n \cdot 100$	$n_1/n \cdot 100$	$n_2/n \cdot 100$	$n_3/n \cdot 100$
0–10	32	0.9	31.3%	53.1%	12.5%	3.1%
>10–20	34	1.6	14.7%	26.5%	38.2%	20.6%
>20–30	23	2.1	4.3%	21.7%	30.4%	43.5%
>30–40	25	2.2	4.0%	20.0%	32.0%	44.0%
>40–50	20	2.2	10.0%	5.0%	40.0%	45.0%
>50–60	4	2.8	0.0%	0.0%	25.0%	75.0%
>60–70	5	2.8	0.0%	0.0%	20.0%	80.0%
>70–80	5	2.6	0.0%	0.0%	40.0%	60.0%
>80	25	2.7	0.0%	4.0%	24.0%	72.0%

[a]IgG_{Loc} (mean), represents the intrathecal IgG synthesis with reference to Q_{mean}, the mean of the reference range for blood-derived IgG fractions in CSF as zero intrathecal synthesis. [b]Intrathecally synthesized AB-species with AI>1.4, regarding the number of one species (n_1), two species (n_2) or three species (n_3) with pathological values. The mean is calculated as $(n_1+2n_2+3n_3)/n$ with ''n'' as the total number of pathological AB-species in an interval. [c]Fractions: number of combined antibody species/total n in a single interval with zero (n_0), one (n_1), two (n_2), or three (n_3) antibody species

Relations between polyspecific, oligoclonal MRZ antibodies and intrathecal IgG fraction in CSF

For the evaluation of means of different groups of parameters we refer to the mean blood-derived IgG fraction, Q_{mean}, in CSF instead of the upper limit of the reference range, Q_{Lim}, used for the diagnosis-relevant identification of an intrathecal fraction in a single patient. In Table 4 the AI-data from patients with a MRZ-reaction are grouped in intervals with an increasing amount of intrathecally synthesized total IgG (IgG_{Loc}(mean) in mg/L. In the interval with 0–10 mg/L intrathecal IgG synthesis (with reference to Q_{mean} as zero intrathecal synthesis), 31% of the patients had no M, R or Z reaction, i.e. all M, R, Z Antibody Index values were AI<1.4, and only 3.1% had a pathological MRZ-reaction with all three antibody species synthesized intrathecally (M-AI>1.4, R-AI>1.4 and Z-AI>1.4). With increasing intrathecal IgG concentration an increasing number between zero and three species had pathological AI values in a single patient, e.g. in the interval with IgG_{Loc} (mean)>80 mg/L all patients had at least one increased AI value (0% n_0 in Table 4) and the number of cases, where all three species had pathological AI values (M+R+Z) increased to 72% (n_3 in Table 4). The mean number of simultaneously intrathecally synthesized MRZ antibody species increased from 0.9–2.7, as indicated in a separate column in Table 4.

With increasing frequency of intrathecal MRZ antibodies also the amount of the antibodies, produced intrathecally, is increased. This is shown in Table 5 regarding the increasing mean Antibody Index values (median) with increasing intrathecal fraction $Ig_{IF(mean)}$. Both values AI and $IgG_{IF(mean)}$ are a relative measure of intrathecal IgG synthesis, calculating the excess over the blood-derived fraction. The AI values increase fivefold (median) to 10-fold (maxima), corresponding to a fivefold increase of the intrathecal total IgG fraction in CSF.

From both results, regarding frequency (Table 4 and intensity (Table 5) of MRZ intrathecal antibody response, we learn that a larger polyspecific, oligoclonal total IgG response in the brain is associated with an increasing number of different antibody species detectable, in particular with or due to an increasing number of B-cells per clone or an increasing number of cell clones, synthesizing locally antibodies of a single species. The evaluation of AI-values referring the intrathecal synthesis to the blood-derived amounts did not allow to understand the reasons why measles, rubella or zoster antibody producing B-cells are more frequent than other antibody producing B-cells of MS patients, and why these MRZ antibody producing B-cells are in general more frequent in MS than in other polyspecific, oligoclonal immune reactions of acute infectious diseases of the brain. As an approach to answer these questions, we made a quantitative analysis of M, R, Z antibody concentrations in CSF and blood.

Quantitation of MRZ antibody/total IgG concentration ratio in CSF and serum

The quantitative absolute amount of measles, rubella and VZV antibodies (AB) in CSF and serum were analysed by a particular ELISA technique, introduced by Conrad *et al*.[9] and modified by us.[15]

This quantitation allows to calculate the AB/total IgG ratio in serum, in CSF and, in particular, in the intrathecally synthesized IgG fraction. We evaluated these AB/IgG ratios for the AB species against measles, rubella and VZV from cases with AI>1.4 and n=100 cases in each group. The total intrathecally synthesized M+R+Z antibody concentration in CSF represents less than 2% of total intrathecally synthesized (oligoclonal) IgG. The data for the single species are given in Table 6. The group of patients with measles-AI>1.4 had a measles-AB/total IgG ratio in CSF of 0.0027 or R_{CSF}=0.27% (median) compared to the ratio in serum R_S of MS patients with 0.13% and a somewhat lower value of R_S in blood of normal controls (0.1%). If the intrathecal fractions of AB and IgG (AB_{Loc} and IgG_{Loc}) are calculated separately we get the ratio $R_{Loc}=AB_{Loc}/IgG_{Loc}.100=0.54\%$.

As a new interpretation, the relation between the serum ratio R_S and the CSF ratio R_{CSF} represents the Antibody Index $AI=R_{CSF}/R_S$. This is easily shown by

Table 5 Intrathecal antibody synthesis (M, R, Z-species) in relation to total IgG intrathecally synthesized (as IgG_{IF} (mean))

	Distribution of pathological Antibody-Index values (AI>1.4)								
$IgG_{IF\ (mean)}$	*Measles-AI*			*Rubella-AI*			*Zoster-AI*		
Intervals (%)	*n/Interval*	*Median*	*range*	*n/Interval*	*Median*	*range*	*n/Interval*	*Median*	*range*
10–19	15	1.8	1.6–4.0	1	–	6	0	–	–
20–29	14	2.65	1.95–4.6	7	2.1	1.5–3.9	5	1.7	1.5–2.4
30–39	18	2.1	1.5–5.7	6	2.7	1.8–3.1	5	3.6	1.5–5.2
40–49	28	2.75	1.7–12.2	17	2.9	1.5–7.3	17	1.7	1.5–7.3
50–59	32	2.8	1.5–19.2	22	2.85	1.5–9.0	17	3.0	1.6–9.3
60–69	25	3.9	1.6–45.0	20	4.4	1.0–10.0	19	3.2	1.9–13.9
70–79	26	4.95	1.6–46.3	19	8.9	1.7–28.0	22	3.75	1.5–28.9
>79%	14	8.7	3.4–33.1	13	13.0	4.8–33.5	12	9.1	1.8–26.3

IgG_{IF} (mean) represents the intrathecal fraction (IgGLoc/IgGSF · 100) with reference to Q_{mean}, the mean of the reference range for blood-derived IgG fractions in CSF as zero intrathecal synthesis. The group of n=176 patients was recruited according to the detection of at least one increased AI value of M, R, Z

Table 6 Quantitation of ratios (R) between specific antibody (AB) to total IgG class concentrations in CSF and blood of MS patients (median of $n=100$) and control (median of $n=32$)

	Multiple Sclerosis				*Control*
Antibody Species	R_{Loc}[a] *[%]*	R_{CSF}[b] *[%]*	R_S[c] *[%]*	*AI*[d]	R_S[c] *[%]*
Measles-AB	0.54	0.4	0.13	2.8 (1.5 – 16.9)	0.1 (0.014 – 0.37)
Rubella-AB	0.54	0.31	0.16	2.5 (1.5 – 14.4)	0.11 (0.007 – 0.5)
VZV-AB	0.24	0.15	0.06	2.7 (1.5 – 21.0)	0.06 (0.014 – 0.41)
HSV-AB	0.85	0.27	0.17	1.9 (1.6 – 3.3)	0.3 (0.15 – 0.47)

[a]$R_{Loc} = AB_{Loc}$ (mean)/$IgG_{Loc\ (mean)}$; · 100 in [%]. [b]$R_{CSF} = AB_{CSF}/IgG_{CSF}$; · 100 in [%]. [c]$R_S = AB_S/IgG_S \cdot 100$ in [%]. Mean of total IgG in blood was 10.3 g/L in the MS group and 10.3 g/L in the control group. [d]Antibody-Index, $AI = R_{CSF}/R_S$ got a new interpretation compared to original definition $AI = Q_{spec}/Q_{IgG}$ or $AI = Q_{spec}/Q_{Lim}$ (if $Q_{IgG} > Q_{lim}$). [e]HSV-AB data refer to $n=16$ patients

rearrangement of $AI=Q_{spec}/Q_{IgG}=Q_{AB}/Q_{IgG}=AB_{CSF}/AB_S \cdot IgG_S/IgG_{CSF}$ with $R_{CSF}=AB_{CSF}/IgG_{CSF}$ and $R_S=AB_S/IgG_S$. According to the shown correlations of AI values with increasing IgG_{IF} (Table 5) IgG_{Loc} we find increasing differences betwen R_{CSF} and R_S with increasing IgG_{Loc}.[15] So, the local synthesis of measles antibodies in brain of MS patients in relation to total intrathecal IgG synthesis is about fivefold exceeding the ratio of measles AB/total IgG in blood of these MS patients. Similar results are obtained for rubella and VZV antibodies (Table 6).

These results represent an important new aspect for the understanding of the pathophysiology of MS. The species distribution of AB-producing B-cells in brain is different from that in blood, i.e. there is no permanent equilibration/invasion of B-cells into brain, with a representative distribution for the blood B-cell populations at time of lumbar puncture.

This raises a set of basic questions: Is there a different preference for homing of B-lymphocyte in brain tissue from other tissues, or does the brain B-cell ratio represent the blood B-cell ratio at an earlier time of lymphocyte migration into brain, different from actual B-cell ratio in blood? Does the MRZ-reaction represent a scar, dating from time of first pathophysiological events? Do active B-cells (plasma cells) migrate more easily into CNS than resting B-cells, late after infection or immunization?

With this knowledge we open a new window to look at B-cell-related pathophysiological events in the early immune reaction of MS. This view is supported by our observations that also the ratio of intrathecal IgG to IgM-class response does not mirror the actual ratio in blood nor the clinical state of disease. Our first results[27] in a group of n=12 young MS patients (5 – 12 years) show that in this time a 4-fold increase in R_S values for measles but not for rubella or VSV occured. But, the intrathecal synthesis of the specific antibodies was not age-related, i.e. not different from the groups of older MS patients. Corresponding with intrathecal fractions IgG_{IF} between 0 to 73%, measles-AI values were increased in 6/9 patients with measles-AI from 1.6 to 42. Also the relative frequencies for intrathecal measles-AB > rubella-AB and VZV-AB were similar to the results reported for adult MS patients.

Discussion

The many earlier reports about IgG, IgA and IgM class response in the brain of MS patients could be confirmed by our larger set of data emphasizing that the evaluated group of MS patients was representative also for earlier reports.[1] Discrepancies regarding the frequency of intrathecal IgM response in MS depend on the analytical and evaluation methods. The qualitative analysis by electrophoretic methods give higher frequencies than the evaluation by reference to the statistically defined upper limit of the reference range. The approved diagnostic relevance of the MRZ-reaction[4] to detect a chronic inflammatory disease at time of first clinical symptoms of MS finds increasing acceptance.[23] Our data support the conclusion that it is sufficient to investigate the IgG class response and, in particular, only for the three most frequent antibodies M, R, Z. The reported frequencies of MRZ-reaction, which vary between 84 – 94%[4] depend obviously on the arbitrary collection of patients with more or less strong intrathecal immune response. For practical reasons, the MRZ-reaction, which is clinically less sensitive than the detection of oligoclonal IgG should be analysed only in case of positive oligoclonal IgG.

With the introduction of Q_{mean}, the mean of the reference range of blood-derived concentrations in CSF, as reference for calculation of intrathecal synthesis, we report a base for improved statistical evaluations. These evaluations avoid the discontinuity of AB_{Loc} and IgG_{Loc} values in the range of Q_{Lim}. As an application of this evaluation concept we describe the correlation of increasing frequency and increasing intensity of specific antibody response (MRZ) with the total intrathecal IgG synthesis. This result is understood as a consequence of a generally larger set of B-cell clones for different species, which have been migrating into brain. In addition, a larger proliferation of single clones or a larger number of clones per species should count for increasing AI-values with larger intrathecal synthesis of total IgG (IgG_{Loc} or IgG_{IF}).

The Antibody Index as a ratio of two quotients can be expressed in two different relations either as $AI=Q_{spec}/Q_{IgG}$ with Q_{spec} as the CSF/serum ratio of

specific antibodies (e.g. measles-AB) or as $AI=R_{CSF}/R_S$. Q_{spec} can be determined as relation of arbitrary units[3] and does not need absolute AB concentrations. This is an advantage for the practical approaches. The second relation of the Antibody Index, $AI=R_{CSF}/R_S$ uses the same parameters as in the first relation but needs for the single ratios $R_{CSF}=AB_{CSF}/IgG_{CSF}\cdot 100$ and $R_S=AB_S/IgG_S\cdot 100$ absolute values for the antibody concentrations in CSF and serum, AB_{CSF} and AB_S, respectively. With the ratios R_{CSF} and R_S we get a new interpretation of the Antibody Index as the relation between specific antibody to total IgG in blood and in CSF.

The AB concentration in CSF (AB_{CSF}) represents a mixture of blood-derived and brain-derived fractions. This is true also for the ratio R_{CSF}, respectively. To approach the real ratio of specific antibody to total IgG in the intrathecally synthesized fraction($R_{Loc}=AB_{Loc}/IgG_{Loc}\cdot 100$) we need to calculate $AB_{Loc}=(Q_{AB}-Q_{mean})\ AB_S$ in analogy to $IgG_{Loc}=(Q_{IgG}-Q_{mean})\cdot IgG_S$. As shown in Table 6, reasonably we find for R_{Loc} larger values than for R_{CSF}. This means that the empirical AI value does not represent the pure ratio of brain to blood-derived fraction and would need a correction for that theoretical purpose in the sense of $AI_{Loc}=R_{Loc}/R_S$.

This evaluation makes sense only for those cases with $IgG_{Loc}>0$ (75% of the MS cases). In case of statistical evaluations and reference to Q_{mean}, $IgG_{Loc(mean)}>0$ would be the case in almost 100% of MS cases. This is the evaluation used for theoretical aspects in Table 6. The clinically relevant evaluations for diagnosis of MS in a single patient have to refer to the empirical AI value as the best choice. But, if the brain-derived R_{Loc} would not be different from R_S we would not detect an intrathecal synthesis of AB in cases with $Q_{IgG}<Q_{Lim}$ for $Q_{spec}=Q_{IgG}$ in this case would give an AI=1, different from $Q_{spec}=Q_{IgG}$ if $Q_{IgG}>Q_{Lim}$ and $AI=Q_{spec}/Q_{Lim}>1$.

From a detailed analysis of the ratio R_S in children and adults we learn that the blood ratio R_S changes in early life time. But in contrast to the steadily changing absolute titer values[20], the serum ratio R_S remains constant after the age of 12–15 years. As the most important aspect of the age-related evaluation of the MRZ reaction we find that already in the young MS patients with the earliest start of clinical symptoms there are the same patterns as in the adult patients. This could be interpreted in the sense that the brain B-cell repertoire in MS patients represents the blood repertoire at time of first B-cell invasion into brain at time of early pathological events in MS. The locally proliferating and maturating B-cell clones then would not be influenced by later migration of blood-derived B-cell clones into brain. If that is true, we get an answer on the question – why the intrathecal IgG synthesis is very different from patient to patient but remains constant over decades independent of clinical course of disease.

With this aspect of B-cell migration[25,26] we emphasize the necessity to improve the understanding of the function of B-cells in pathophysiology of MS.[24] The early observation of a measles antibody synthesis in MS brains induced virological investigations over the past fifty years, but the viral etiology of the disease remains a hypothesis without grounds for confirmation or rejection.[23] The studies of migration of activated T-lymphocytes into brain taught us details about possible pathomechanism in experimental animals,[24] but with very restricted analogy to the cause and clinical course of multiple sclerosis.[21,22] It could be reasonable to take our results on B-lymphocyte function as an impulse to rethink the concepts for interpretation of the huge collection of immunological data in MS regarding the immune network[7,8] and its selforganization capacity.

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