Paediatric and adult multiple sclerosis: age-related differences and time course of the neuroimmunological response in cerebrospinal fluid*

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Abstract

We investigate common pathophysiology in paediatric and adult multiple sclerosis (MS) by comparison of cerebrospinal fluid (CSF) data. We compared cerebrospinal fluid (CSF) data from eight patient groups with onset of MS at 7 to 29 years ($n = 184$). A new statistics program allows sensitive detection, quantifies the mean amount of intrathecal Ig synthesis in groups based on the 96% reference range of 4100 non-inflammatory controls, corrects for age-related increase of blood-derived albumin and immunoglobulins in CSF, and presents graphical data interpretation in Reibergrams. Already at onset of MS before puberty (≤ 10 years) the frequency of intrathecal IgG synthesis (oligoclonal IgG) was 100% like in adults with 98%, but the amount of intrathecal IgG increases twofold during puberty. Intrathecal IgM synthesis is most frequent before and during puberty (in 57–67% of patients) compared with 41% in adults. The amount of intrathecal IgM synthesis before puberty is only 30% of that in adults. IgG and IgM Index are biased evaluations not suitable for characterizing age-related dynamics. A twofold age-related increase of the albumin quotient, Q_{Alb} , as a measure of the blood–CSF barrier function, represents normal physiological growth. Cell counts in CSF are low. The pre-puberty gender ratio is about 1:1. Intrathecal antibodies against measles, rubella and/or varicella zoster virus are detected in 73% of patients before puberty compared with 89% of adults. Individual paediatric patients ($n = 17$), with sequential punctures over 2–5 years, show constant quantities of intrathecal IgM and specific antibodies. In conclusion, paediatric MS already at first clinical manifestation shows the complete, neuroimmunological data pattern in CSF, i.e. inflammatory signs are not gradually evolving. Paediatric and adult MS differ quantitatively but not qualitatively in neuroimmunological patterns which does not allow for discrimination between ‘early’ and ‘late’ onset MS. CSF analysis may help to discriminate between acute and mono-symptomatic chronic inflammatory disease already at earliest clinical manifestation.

Keywords

paediatric multiple sclerosis, puberty, cerebrospinal fluid, IgG, IgA, IgM, intrathecal synthesis, statistics, measles, rubella, varicella zoster, antibody index, IgG index

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Introduction

For years the rediscovery^{1–3} of paediatric multiple sclerosis (MS) nourished the expectation of a better understanding of the pathophysiology of MS. Unfortunately much confusion entered this discussion due to inadequately performed or wrongly interpreted cerebrospinal fluid (CSF) analysis in both children and adults. However, there is an increasing consensus that paediatric MS with manifestations before the age of 16 years and adult MS^{3–5} are not different diseases.

The intrathecal humoral immune response is the prominent sign observed in CSF of MS patients.^{5–9}

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*This contribution is dedicated with high regard to the mentor of CSF analysis in Germany, Professor Dr Helmut Bauer (1914–2008), Göttingen, who motivated our research on paediatric MS as early as in the 1980s.

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The predominant IgG class reaction is detected by oligoclonal bands after isoelectric focusing¹⁰ in 98% of the adult MS patients. Owing to this high sensitivity the detection of oligoclonal IgG became an essential part of a qualified laboratory supported diagnosis of MS.¹¹ The less-frequent intrathecal IgM class response (20–50%) has been investigated⁵ mainly with respect to its predictive value for the course of the disease.^{12,13} The quantification of intrathecal IgG, IgA and IgM became possible due to the well-established and theoretically founded nonlinear reference range for blood-derived proteins in CSF^{14,15} avoiding incorrect interpretations due to linear IgG or IgM index with false-positive and false-negative interpretations in CSF routine diagnosis.⁶ Also the missing compensation for the physiological age-related change of the blood–CSF barrier function, particularly in children, led to a bias in the interpretation of earlier results. With the new statistics software⁸ we correct for this physiological increase of the blood-derived albumin, IgG and IgM fractions in CSF.

An extraordinarily frequent intrathecal antibody synthesis against measles, rubella and varicella zoster virus (VZV) was reported as a particular aspect of the humoral immune response in the central nervous system (CNS) of MS patients^{16–18} in both adults and children. In principle, a concomitant polyspecific immune response takes place in all immune reactions in the blood and brain.¹⁹ This is explained as a part of the immunological network response. The frequency of these particular antibodies against neurotropic viruses, also called MRZ-antibody reaction,¹⁶ is remarkably high in MS and also in autoimmune diseases with involvement of the CNS such as systemic lupus erythematosus or Sjögren's syndrome,²⁰ but not in other acute or chronic inflammatory diseases such as HSV encephalitis, neurotuberculosis or neurosyphilis.²¹ This makes the MRZ-antibody reaction more specific albeit less sensitive than detection of oligoclonal IgG, which in contrast can be found in any acute and chronic inflammatory process of the brain. This polyspecific immune response has a low intensity (smaller amount) compared with the antibody synthesis against a persisting causative antigen, which might be 20–60-fold stronger in HSV encephalitis, subacute sclerosing encephalitis or the Fuchs heterochromic cyclitis of the eye.¹⁹ Owing to its diagnostic potential for the detection of monosymptomatic MS at the time of its first manifestation, it seems reasonable to compare this polyspecific antibody response against neurotropic viruses in children and adult MS patients.

The new statistical approaches count the frequencies of intrathecal Ig fractions with higher sensitivities and calculate mean intensities of this synthesis with correction for the physiological variation of the age-related

blood–CSF barrier function. This should allow us to detect more reliably possible age-related differences between the weaker immune response in early-onset MS and the stronger response in late-onset MS.

As important complementary information to the statistical data of groups of patients at the time of disease onset we also report the time course of intrathecally synthesized amounts of immunoglobulins and antibodies in individual paediatric patients who underwent repeated lumbar punctures for diagnostic reasons.

With the sophisticated description of the neuroimmunological response in CSF of paediatric MS patients we aim to improve the differential diagnosis of acute and chronic inflammatory diseases of the CNS in childhood.^{3,22,23}

Methods and patients

Paediatric patients

All young patients were part of a larger, prospectively studied cohort with suspected MS, first admitted to the Department of Pediatric Neurology, Georg-August-University Göttingen, Germany, between 1987 and 2002. The study was approved by the Ethics committee of the Medical Faculty, University Göttingen, Germany.

We report only those patients with a complete set of analytical data: 82 MS patients with a disease manifestation before the age of 16 (54 girls, 28 boys); 17 of these patients had a repeated lumbar puncture.

Basic data collection included gender, age at disease onset, clinical presentation, neuroradiological and CSF findings. Detailed clinical data are summarized elsewhere.²⁴ Mean age at MS onset was 12.5 years (range: 4–16 years). The statistically evaluated data represent the analysis of the first 'diagnostic' puncture. In this cohort of paediatric MS patients ($n = 135$) the initial spinal tap was performed during the first attack in 106 patients (78%), during the second attack in 26 (19%) and during the third attack in 3 (2%) patients. Haemolytic, blood-contaminated or turbid CSF samples were excluded. The age-related gender distribution is reported in Table 2. Cases with onset between 7 and 10 years are interpreted separately as a pre-puberty group.

Serial analytical data

A subgroup ($n = 17$) of these young patients was punctured two to five times for diagnostic purposes over a period of up to 6 years after manifestation of the disease. The patients had 2–10 relapses during this time. The serial analysis was performed for intrathecal IgG and IgM.

The MRZ antibody analysis (Table 2) was performed in 78 children. A serial antibody analysis was also performed in all 17 children who underwent repeated lumbar punctures.

Adult patients

The data of adult MS groups originate from a cohort of 267 patients with clinically definite MS²⁵ with a mean age of 36 years and a female-to-male ratio of 1.95:1, published earlier.⁵ We report the statistical data of a subgroup with 103 MS patients between 17 and 29 years of age (76 women and 27 men). The data of the age-related groups (Table 2) are recalculated with the new statistics software.⁸

Control group

The control group for CSF data with absence of an intrathecal humoral immune response is constituted from 4100 patients.¹⁴ These data were obtained from CSF routine analysis in the same laboratory (Neurochemistry Laboratory, Department of Neurology, Georg-August-University, Göttingen). Main exclusion criteria were CSF oligoclonal IgG and an atypical relation in the QIgG/QIgA/QIgM pattern. These data constituted the reference range of the CSF/serum quotient diagrams.¹⁴

Protein analysis

Albumin, IgG, IgA and IgM in CSF and serum were analysed with immunochemical nephelometry, particle-amplified for IgA and IgM (Dade Behring Nephelometer Prospec, Marburg, Germany). CSF and diluted serum samples were analysed paired in the same analytical run. Data were recorded online and interpreted numerically and graphically in the integrated CSF report including CSF/serum quotient diagrams (Reibergrams).⁶

Blood-CSF barrier function

The albumin quotient, $Q_{Alb} = \text{Alb}(\text{CSF})/\text{Alb}(\text{ser})$, as a measure of the individual blood CSF barrier function was interpreted with the age-related reference range,^{6,7} $Q_{Alb} = (4 + \text{age}(\text{years})/15) \times 10^{-3}$. The significance of a barrier dysfunction is calculated on a 10% and 20% level above the age-related border of the reference range.⁸

CSF statistics tool

It is important to understand the basic difference between the diagnostic approach for the single patient

and the statistical approach for groups: for diagnostic purposes in routine analysis, the intrathecal synthesis is quantified as the relative intrathecal fraction, Ig_{IF} , as a percentage of total Ig in CSF, with reference to Q_{lim} (Figure 1), to allow a comparison of different Ig classes response and disease related immunoglobulin patterns⁶ in the single patient.

The absolute amount of intrathecal Ig synthesis, Ig_{loc} , in milligrams per litre CSF is preferred for a quantitative description of the time course of intrathecal synthesis of a single immunoglobulin. In the case of diagnostic interpretations the reference for detection of the intrathecally synthesized amounts (Ig_{IF} or Ig_{loc}) is the upper borderline of the reference range, Q_{lim} ($=Q_{mean} + 3SD$ (standard deviations)) or 99% range for highest specificity.

For a comparison of groups we usually compare means with their SD. Therefore, the calculation of intrathecal amounts (preferentially quantity of locally synthesized Ig fraction, Ig_{loc} , in milligrams per litre CSF) refers to the mean of the reference range, Q_{mean} , expressed as $Ig_{loc}(\text{mean})$ or $Ig_{loc}(m)$ in Tables 1–3.

Table 1 presents two representative calculation examples for diagnostic and statistical approaches with Q_{lim} and Q_{mean} related values: for a 9-year-old MS patient with $Q_{Alb} = 2.8 \times 10^{-3}$ and a 30-year-old MS patient with $Q_{Alb} = 5.3 \times 10^{-3}$. Both patients have different amounts of intrathecally synthesized IgG ($Ig_{G_{loc}}$), but due to their different blood-derived IgG fractions ($IgG(\text{CSF}) - Ig_{G_{loc}}$), they have similar relative intrathecal fractions (Ig_{IF}). This demonstrates why for comparison of groups the $Ig_{loc}(m)$ values must be preferred.

Subsequently the frequency of intrathecal synthesis in a group of patients counts the numbers of cases with $QIg > Q_{mean} + 2SD$ (96% range) instead of $QIg > Q_{lim} = Q_{mean} + 3SD$ (99% range). The different frequencies of intrathecal synthesis for both decision borders of the reference range are calculated and reported for MS groups in Table 2.

The new CSF statistics approach is based on two observations:¹⁴

- (1) In the control group the distribution (variation) of the immunoglobulins (QIgG, QIgA, QIgM) at a certain albumin CSF/serum quotient, Q_{Alb} , is symmetrical (Gaussian) around Q_{mean} .
- (2) The mean coefficient of variation in the control group with $CV(QIgG) = 15\%$, $CV(QIgA) = 23\%$ and $CV(QIgM) = 60\%$ is constant, i.e. valid for all Q_{Alb} values, or independent of CSF flow rate. The $Q_{mean} + 2SD$ border is calculated with $[(Q_{lim} - Q_{mean}) = 3SD]$ for the individual Q_{Alb} value of the single patient, reported in the knowledge base of the statistics tool.⁸

Figure 1. Comparison of CSF data from MS patients in the age groups of 7–11 years (squares) and 28–29 years (open circles) in CSF/serum quotient diagrams (Reibergrams).^{6,7} These diagrams are double logarithmic graphs for the reference range of blood-derived immunoglobulins in CSF, constructed from 4100 non-inflammatory control patients.¹⁴ This is the range between the thick upper hyperbolic border line (Q_{lim}) and the lower hyperbolic borderline (Q_{low}), including 99% of the controls or $Q_{mean} \pm 3SD$. The range with vertical lines is the range of increased albumin quotients, Q_{Alb} , due to a blood–CSF barrier dysfunction. Values above the upper limit of the reference range, Q_{lim} , are regarded as an intrathecal synthesis, the extent of which is read from the dashed hyperbolic lines presenting the intrathecal fractions, Ig_{IF} with 20%, 40%, 60% or 80% of total CSF Ig concentration. The intrathecally synthesized amount of IgG, IgA or IgM is calculated for diagnostic purposes with reference to Q_{lim} and for statistics of the whole group with reference to Q_{mean} , the mean hyperbolic function between Q_{lim} and Q_{low} , not shown in the diagram (see the methods section). The CSF/serum quotient diagrams are explained in detail with applications in the literature.^{6,7,26} Corresponding statistics of the both MS groups are shown in Table 2. The diagram is created with the new CSF statistics tool.⁸

Table 1. Calculation examples in diagnostic and statistical CSF data interpretation with Q_{lim} or Q_{mean} related evaluation. Two MS patients with Q_{Alb} values representative for the mean of their age group (e.g. in Figure 1)

Analytical data				Diagnostic interpretation				Statistical evaluation		
Age (years)	$Q_{Alb} \times 10^3$	IgG_{CSF} (mg/l)	$QIgG \times 10^3$	$Q_{lim} \times 10^3$	IgG_{loc} (mg/l)	IgG_{IF} (%)	IgG index	$Q_{mean} \times 10^3$	$IgG_{loc(m)}$ (mg/l)	$IgG_{IF(m)}$ (%)
9	2.8	28	3.0	1.76	11.8	42.2	1.1	1.19	17.1	61.0
30	5.3	70	6.4	3.73	29.3	41.9	1.2	2.5	42.7	61.0

IgG_{CSF} , concentration of IgG in CSF; Q_{lim} , upper border of the reference range; Q_{mean} , mean of the reference range; IgG_{loc} , amount of locally synthesized, intrathecal IgG in CSF; IgG_{IF} , relative amount of intrathecal IgG in CSF with reference to Q_{lim} , zero synthesis; $IgG_{loc(m)}$, amount of intrathecal IgG in CSF; $IgG_{IF(m)}$, relative amount of intrathecal IgG in CSF with reference to Q_{mean} , zero synthesis in statistics approach; IgG index, $QIgG/Q_{Alb}$.

Table 2. Age-related CSF data in MS at the time of the first 'diagnostic' puncture of a patient

Age-related data (means of groups)									
Parameter									
Age-Range	units	7–	12–	14	15–	17–	22–	25–	28–
		11	13		16	21	24	27	29
Total <i>n</i>	years	18	29	17	18	25	23	26	29
Q_{Alb}	$\times 10^3$	3.2	3.8	4.0	3.9	4.8	5.0	4.7	5.3
Dysf. ($\geq 10\%$)		1/18	3/29	1/17	2/18	8/25	4/23	3/26	5/29
OCB	%	89	86	100	100	95	100	92	100
$IgG_{loc(m)}$	mg/l	13.6	22.5	24.7	29.3	27.3	24.2	34.4	40.1
IgG -Fr (2SD)	%	67	71	82	83	76	70	81	97
IgG -Fr (3SD)	%	61	68	71	78	68	65	77	79
IgG index	Med	0.74	0.74	0.81	0.76	0.96	0.70	1.0	1.0
$IgA_{loc(m)}$	mg/l	0.31	0.36	0.39	0.18	0.45	0.3	0.5	0.5
IgA -Fr (2SD)	%	29	15	19	12	16	9	12	17
$IgM_{loc(m)}$	mg/l	0.48	1.25	1.16	0.97	0.88	1.22	0.8	0.8
IgM -Fr (2SD)	%	56	56	50	59	48	44	46	41
IgM -Fr (3SD)	%	31	48	38	47	24	39	31	21
Gender	m:f	8:10	10:19	6:11	4:14	5:20	6:17	10:16	6:23
Cell count median	c/ μ l	3	10	10	15	20	12	12	12
Cell count range	c/ μ l	0–8	1–78	1–38	0–42	1–96	1–32	0–30	3–44
M-AI ≥ 1.5	%	75	56	50	62	63	71	64	86
R-AI ≥ 1.5	%	36	41	21	47	55	41	67	67
Z-AI ≥ 1.5	%	54	61	36	33	38	55	60	63
MRZ pos.		12/17	19/27	8/16	11/18	17/25	20/23	22/26	28/29

Statistics are calculated with a new CSF statistics tool.⁸ The distribution of patients in diagrams is compared in Figure 1 for the 7–11 years and the 28–29 years groups. $Ig_{loc(m)}$, Q_{mean} related intrathecal Ig synthesis. IgG – fraction (2SD) frequency of cases with $QIg > Q_{mean} + 2SD$. IgG – fraction (3SD) frequency of cases with $QIg > Q_{mean} + 3SD (=Q_{lim})$ (see Table 1). Frequency of increased antibody index for measles (M-AI), rubella (R-AI) and Varicella zoster (Z-AI) antibodies in the fraction of patients with detectable values. MRZ reaction is taken as positive for any patient with one to three of the intrathecally synthesized MRZ antibodies. The borders of the age groups were selected to get somehow groups of similar size. In addition to the age group 7–11 years we report the data of a definitely prepuberty subgroup (≤ 10 years) in Table 3.

In this way the CSF Statistics tool provides access to a large non-inflammatory control group for everybody who analyses CSF/serum quotients according to the state of the art as method-independent values.²⁶

All calculations of means and SD for Q_{Alb} and $Ig_{loc(m)}$ in groups of patients were performed with this tool.⁸ It provides all data needed to perform a *t*-test to show that there is not a significant difference

Table 3. Prepuberty and postpuberty differences in CSF of MS patients

Age range	years	7–10	15–16
Q _{Alb}	× 10 ³	2.8	3.9
OCB	%	100	100
IgG _{loc} (m)	mg/l	14.1	29.3
IgG-Fr (>2SD)	%	70	83
IgM _{loc} (m)	mg/l	0.3	1.0
IgM-Fr (>2SD)	%	67	59
IgM-Fr (>3SD)	%	33	47
Gender	m:f	1: 0.7	1:3.5
Cell count median	c/μl	3	15
M-AI		80%	61%
R-AI		25%	45%
Z-AI		50%	40%
MRZ positive		75%	66%

For abbreviations, see the legend of Table 2. Mean of group data. The prepuberty age group is restricted to an age ≤10 years to avoid an overlap and bias possible in the group ≤11 years in Table 2.

between a patient group and the control group (null hypothesis), possible where both groups have a Gaussian distribution.²⁷ Groups with intrathecal synthesis which usually have no Gaussian distribution of data need another usual program to calculate the significance of differences. The software also closes the gap in CSF diagnostics enabling the researcher to process data from Excel tables of patients' data into pdf files with Reibergrams (such as Figures 1–4).

The formulae are as follows (all functions can be calculated by the CSF Statistics Tool [8] from patients CSF and serum concentrations):

$IgG_{Loc} = (Q_{IgG} - Q_{Lim}) \cdot IgG(ser)$ and $Ig_{loc} (m) = (Q_{Ig} - Q_{mean} (Ig)) \cdot Ig \text{ serum (mg/l)}$ $IgG_{IF} = (IgG_{Loc}/IgG_{CSF}) \cdot 100[\%]$ or $IgG_{IF} = (1 - Q_{Lim}/Q_{IgG}) \cdot 100 [\%]$. Q_{Lim} and $Q_{mean} (= a/b(Q_{Alb}^2 + b^2)^{0.5} - c)$ are calculated with the following values of the variables a/b , b^2 , c : $Q_{Lim} (IgG) = (0.93[Q_{Alb}^2 + 6]^{0.5} - 1.7) \times 10^{-3}$ and $Q_{mean} (IgG) = (0.65 [Q_{Alb}^2 + 8]^{0.5} - 1.4) \times 10^{-3}$ or for IgM class: $Q_{Lim} (IgM) = (0.67[Q_{Alb}^2 + 120]^{0.5} - 7.1) \times 10^{-3}$ and $Q_{mean} (IgM) = (0.33 [Q_{Alb}^2 + 306]^{0.5} - 5.7) \times 10^{-3}$ [14].

Oligoclonal IgG

Detection of oligoclonal IgG was performed with 10 μl unconcentrated CSF and serum (prediluted to a similar IgG concentration) by immunoblot (NCA foil ECL RPN303D, GE healthcare, Freiburg, Germany) after isoelectric focusing on in-house agarose gels (pH 3–10). The quality is assessed by participation in the CSF survey of INSTAND, Dusseldorf, Germany. The results were expressed as five different types as

recommended by a European and an international expert group.^{10,11} A typical performance from our laboratory is shown in a figure of Freedman et al.¹¹

Antibody index

The intrathecal synthesis of IgG class antibodies of measles (M), varicella zoster (Z), rubella (R) and herpes simplex (H) was analysed using an enzyme-linked immunosorbent assay (ELISA) technique (Enzygnost, Siemens (Behring), Marburg Germany) and interpreted as antibody index, AI [18], calculated by $AI = [Q_{spec}/Q_{IgG}]$ or with correction (in the case of $Q_{IgG} > Q_{lim}$) by $AI = [Q_{spec}/Q_{lim}]$ with reference to the Q_{lim} . Diluted CSF (1 : 15) and serum (1 : 3000) samples are analysed paired in the same analytical run with a standard curve, to calculate from arbitrary concentration units the specific CSF/serum antibody quotient, Q_{spec} .

The reference range of normal $AI = 1.0 \pm 0.3$ and the clinically defined cut-off for pathologically increased AI values $AI \geq 1.5$ were detected earlier.¹⁸

For diagnosis of a 'chronic' inflammatory process in CNS, combinations of MR, MZ or RZ have the highest plausibility.⁵ For statistics the MRZ antibody reaction was counted as positive if one, two or three of the MRZ antibodies were synthesized intrathecally.

For detection of a change in the individual intrathecal antibody reactions in the course of the disease in a single patient, instead of AI values we used the relative concentrations of intrathecal antibody (AB) in CSF, $AB_{loc}(m)$, a value calculated with analogy to the $IgG_{loc}(m)$: Q_{spec} was calculated retrospectively from AI

Figure 2. Dynamics of intrathecal IgG, IgA and IgM synthesis in individual patients. The first 'diagnostic' puncture is indicated by a square; the last puncture by a triangle in the diagrams created with the CSF statistics tool.⁸ The left diagram is from a patient with first exacerbation at age of 12 years followed by five relapses in 5 years (CSF punctures at 12, 13.5 and 17.5 years). The middle diagram is from a patient with first clinical symptoms and CSF puncture at age of 13.5 years followed by seven relapses in the next 5 years (punctures at 13.5, 17 and 18.5 years of age). The right diagram is from a patient with first clinical symptoms and CSF puncture at age of 16.5 years. The clinical course in this patient was of the rare chronic progressive type. Samples were obtained at 2, 3 and 3.3 years after the first puncture.

Figure 3. Two antibody quotients in the course of the disease between 14.5 and 15.5 years of age. Triangle, VZV Q_{spec} ; open circles, rubella Q_{spec} ; square, Q_{IgG} . Graph created with CSF statistics tool.⁸

Figure 4. Biased Interpretations with IgG index. Changing IgG index (3.1 to 1.5 to 1.2) and relative intrathecal fractions ($\text{IgG}_{\text{IF}} = 75\%$ to 45% to 25%) with increasing Q_{Alb} (3.4 to 12.6 to 30×10^{-3}) but constant amount of intrathecal IgG synthesis ($\text{IgG}_{\text{loc}} = 75 \text{ mg/l}$) at all three times of investigation. See also Tables 1 and 2. Graph created with CSF statistics tool.⁸

with Q_{IgG} or $Q_{\text{lim}}(\text{IgG})$ and Q_{spec} inserted into the calculation formula for $\text{IgG}_{\text{loc}}(\text{m})$ instead of Q_{IgG} . The result is a concentration for the intrathecally synthesized antibody in CSF, e.g. $\text{VZV}_{\text{loc}}(\text{m})$ in Table 5, in arbitrary units (U/ml) exceeding the mean of the reference range $[(Q_{\text{spec}} - Q_{\text{mean}}) \times \text{IgGser}]$. This retrospective calculation is relevant only for comparison of data from a single antibody species in the same patient. For detection of absolute values of AB_{loc} we need antibody serum concentrations, Abser , instead of IgGser .^{5,28}

Results

Blood–CSF barrier function

The mean albumin quotient, as a measure of the individual blood–CSF barrier function, increases with increasing age in MS patients from $Q_{\text{Alb}} = 3.2 \times 10^{-3}$ (7–11 years) to $Q_{\text{Alb}} = 5.3 \times 10^{-3}$ (28–29 years; see Table 2 and Figure 1) corresponding to 3.1 – 4.6×10^{-3} in age-matched normal controls. The frequency of blood–CSF barrier dysfunction was similar in all age groups (Table 2) with a slight tendency to higher frequencies in older age.

Intrathecal IgG synthesis

Frequencies of intrathecal IgG synthesis, detected as CSF restricted oligoclonal bands (OCBs), were similar in all age groups studied (Table 2). Already in the youngest pre-puberty age group (≤ 10 years), all 10 cases investigated (100%) had oligoclonal IgG in CSF (Table 3). The variation between the groups (Table 2) is mainly due to the small numbers. To see that the low frequency of OCBs in the 7–11 years group is not due to an age-related trend, we evaluated the subgroup 7–10 years, a definitely pre-puberty age group, separately in Figure 3. In the interpretation by quantitative intrathecal fractions ($\text{IgG-Fr} > 2\text{SD}$) in Table 2) the frequencies for $Q_{\text{IgG}} > Q_{\text{mean}} + 2\text{SD}$ (67–97%) matches this result of OCBs better than for the diagnostically relevant decision border $Q_{\text{IgG}} > Q_{\text{lim}} (=Q_{\text{mean}} + 3\text{SD})$ ($\text{IgG-Fr} > 3\text{SD}$) in Table 2): instead of a mean frequency of 72% of the adults we now obtain a mean frequency of 82% and instead of the mean frequency of 69% of the total group of paediatric patients we obtain 75% (Table 4).

The mean amount of IgG in CSF (mg/l), i.e. the mean intensity of intrathecal IgG synthesis is larger at older age. The increase of mean $\text{IgG}_{\text{loc}}(\text{m})$ compared with the mean of the control group is significant in all groups ($p < 0.01$) as well as the difference between the pre-puberty and postpuberty groups with ages 7–10 and 15–16 (Table 3).

Table 4. Comparison of frequencies (percentage of the group) of intrathecal IgG, IgA and IgM synthesis in MS detected with reference to Q_{lim} (=3SD) (99% reference range) and $Q_{mean} + 2SD$ (=2SD) (96% reference range)

	Adult MS patients		All paediatric MS patients (≤ 16 years)	
	3SD ¹	2SD ²	3SD ³	2SD
IgG	72	82	69	75
IgA	9	14	6	17
IgM	20	41	36	55

¹ $n = 267$ (Reiber et al.⁵); ²17–29 years ($n = 103$, this study); ³ $n = 72$ (Pohl et al.²⁶).

Table 5. Dynamics of intrathecal IgG and IgM with duration of the disease in individual patients

	Individual time course of intrathecal IgG, IgM synthesis		
	Decreasing	Constant	Increasing
IgG _{loc} (m)	5/17	6/17	6/17
IgM _{loc} (m)	2/16	10/16	4/16

The data for the intrathecally synthesized IgG and IgM (as Ig_{loc}(m) in mg/l) come from 17 children with MS and repeated punctures over a period up to 6 years (examples in Figure 2). The data were interpreted according to the tendency between the first diagnostic puncture and the last puncture after 2–6 years.

IgG index: The more than twofold increase of intrathecal IgG synthesis in the age between 7 and 29 years is not mirrored by the median of the linear IgG Index (Table 2). This systematic bias explains why an IgG Index was the source of many wrong interpretations in MS research.

Intrathecal IgM synthesis

The frequency of intrathecal IgM synthesis (IgM-Fr in Table 2) at the time of first clinical investigation of the MS patients is slightly higher at younger age, observed in 67% (Table 3) compared with 41% (Tables 2 and 4) of the older patients. These frequency data from 2SD discrimination line are higher than those reported earlier with reference to Q_{lim} such as the data in Table 2 with reference to Q_{lim} . In particular, in young children these frequencies are much higher than those referring to the 3SD borderline (IgM-Fr = 33% in Table 3). These discrepant frequencies between the 2SD and 3SD evaluation are mainly in the very young children (Tables 2–4) due to the more sensitive method which is obviously necessary to detect the intrathecal IgM synthesis due to a smaller amount of intrathecal IgM synthesis in young children. The decrease of frequencies of IgM synthesis at the time of first available lumbar puncture from pre-puberty (67%) or from total paediatric group (55%) to the adult group (41%) is significant (Table 4).

The mean amount of intrathecal IgM synthesis in prepuberty children (IgM_{loc}(m) = 0.3 mg/l CSF in Table 3) is much smaller than in the postpuberty MS patients with rather invariant values not depending on age (IgM_{loc}(m) = 0.8–1.2 mg/l CSF in Table 2). From Figure 1 we recognize the much larger variation of the quantity of intrathecal IgM synthesis between different patients than in IgG or IgA synthesis.

Intrathecal IgA synthesis

The IgA class reaction in paediatric MS (7–16 years) is found more frequently (Table 4) with reference to the 2SD border (about 17%) instead of 6% with reference to Q_{lim} , and in adult patients with 14% versus 9% reported earlier.²⁹ The prepuberty frequencies are higher than the frequencies in the adult patients (Table 2). In the paediatric MS group all of the few patients with an intrathecal IgA synthesis also had an intrathecal IgM synthesis.

Immunoglobulin dynamics in individual patients

The time course of CSF protein concentrations in some individual patients is shown representatively in Figure 2. In the course of the disease the amounts of intrathecally synthesized immunoglobulins in the

three examples are either decreasing, constant or increasing. As summarized in Table 5 there is no uniform tendency for the time course of the IgG or IgM class response in individual patients, i.e. there is neither an isotype switch from IgM to IgG class nor a general correlation of the quantity of humoral immune response with the duration of the disease.

Polyspecific antibody response

In prepuberty MS patients the frequency of intrathecal synthesis ($AI \geq 1.5$) of measles (75%) and VZV (50–54%) antibodies (Tables 2 and 3) is similar to the results in adult MS patients (78% and 55%, respectively).⁵ The rubella antibody synthesis was less frequent before puberty (Table 3) with 25% versus 65% in adults.⁵ The frequency of an antibody synthesis against one to three of the MRZ antibodies with 71–75% in the prepuberty and about 50–70% in the postpuberty age (12–16 years) (Tables 2 and 3) is somewhat lower than frequencies detected in adults with 68–97% (17–29 years in Table 2) or 89% in the large group ($n = 177$) of adult patients (mean age 36 years).⁵ A combination of two or three antibodies (MR, MZ, RZ or MRZ), which allows the early diagnosis of a chronic inflammatory disease was observed in 40% of the paediatric MS patients compared with 67% in the adult MS group.⁵

Dynamics of specific antibody synthesis in the CNS of a single patient: We report the time course of the concentration of intrathecal antibodies in CSF during the course of the disease in an individual patient (see the example in Figure 3). All 17 patients investigated had oligoclonal IgG in CSF. On the basis of the quantitative values for the intrathecal antibody synthesis ($s.VZV_{loc(m)}$) in Table 6) we evaluated 15 patients which had at least two CSF punctures in a time range up to 6 years analysed for MRZ antibodies. Five of 15 patients had no MRZ antibody reaction, in 10 of 15

patients with paediatric MS one to three of the MRZ antibodies were synthesized intrathecally (Figure 3). In these 10 patients a total of 21 antibody time courses could be followed. Fourteen of 21 remained constant over a time range of 2–5 years, 6 of 21 had an increase and only one antibody concentration (VZV) decreased over time. However, this was a patient who had at the same time between the first (at 16 years) and fourth lumbar puncture (at 19 years) a fourfold increase of measles antibodies and a constant intrathecal rubella antibody concentration in CSF. In three cases of antibody species the initially normal AI was detected as pathologically increased about 3–4 years after first diagnostic puncture (two cases of rubella and one case of VZV antibodies). All three cases could be interpreted as a current immunization (rubella) or fresh systemic infection (VZV). As a consequence we learn that the amount of the intrathecal polyspecific antibody synthesis remains constant in the single patient and that the antibody pattern did not change with only few exceptions.

The polyspecific intrathecal antibody synthesis in MS shows that, at the earliest studied time of the disease, the complete neuroimmunological pattern already exists and the amount is not decreasing, as would be expected in case of an acute inflammatory disease.

Prepuberty and postpuberty differences

In Table 3 we report data of the subgroup (≤ 10 years) of the 7–11-year-old patients to avoid any bias by an eventually early onset of puberty in some paediatric patients. The main differences between patients ≤ 10 years and the group with patients aged 15–16 years are summarized in Table 3:

- **Gender ratio.** The prepuberty ratio of male and female patients is 1:1 changing after puberty to about 1:2, supporting earlier reports.

Table 6. Quantification of intrathecal antibody concentration for retrospective comparison in the course of the disease in the same patient with several punctures

	Q_{Alb} $\times 10^3$	Q_{IgG} $\times 10^3$	IgG Ser (g/l)	Q_{lim} $\times 10^3$	VZV-AI	Q_{spec} $\times 10^3$	VZV _{loc(m)} U/L	IgG _{loc(m)} (mg/l)
1. P	3.0	2.1	8.1	1.90	3	5.7	36	6.7
2. P	5.7	3.8	10.8	4.07	1.7	6.5	40	11.1

The method works on the precondition of only a small change in the antibody/total IgG relation in serum between different punctures. The arbitrary concentrations are calculated like Ig_{loc(m)}. The change of the albumin quotient (blood–CSF barrier function) between first and second puncture changes the AI in spite of a rather constant amount of intrathecal VZV synthesis. VZV, varicella zoster virus; AI, antibody index; Q_{spec} , AB(CSF)/AB(Ser); VZV_{loc(m)}, intrathecally synthesized VZV antibodies in CSF (arbitrary concentration units per millilitre), calculated with reference to Q_{mean} ; IgG_{loc(m)}, intrathecally synthesized total IgG in CSF, calculated with reference to Q_{mean} .

- *Cellular immune response.* The median lymphocyte/monocyte cell count in CSF (Table 3) was lowest in the age group of 7–10 years ($3/\mu\text{l}$), but still in the combined, larger group ($n=26$) of patients in the age range 7–12 years, the median cell count was lower ($6/\mu\text{l}$) than in the adult age group, with medians $>10\text{--}20/\mu\text{l}$.
- *IgG.* The frequency of intrathecal IgG synthesis (OCBs or IgG-Fr (2s)) was consistently high at all ages but the amount of intrathecal IgG in CSF increases strongly during puberty (Table 3) and increases thereafter only slightly with age (IgGloc(m) in Table 2).
- *IgM.* The frequency of intrathecal IgM synthesis is highest at disease onset before and during puberty (57–65%) and slowly decreases with age afterwards to 41% of the patients. Most noticeably the amount of intrathecal IgM in CSF at onset in prepuberty is only 30% (Table 3) of the amount at onset after puberty, but remains at the same level of frequency (Table 2) in spite of this threefold higher mean amount.
- *MRZ antibodies.* The frequency of intrathecal MRZ antibodies is lower at earlier age (12/17 versus 28/29 in Table 2). This has to be considered as an expression of the less intense intrathecal IgG class response (IgGloc(m) in Table 2) with a lesser detectability of the MRZ response.⁵

Discussion

Blood–CSF barrier function: albumin quotients

There are two explanations for the age-related increase of mean albumin concentration in CSF of children. It is a normal physiological function depending on the age-related decrease of CSF production rate, i.e. reduced CSF flow rate.¹⁴ In addition, in the growing child and adolescent, the increasing distance between ventricles and lumbar location of CSF puncture contributes to the increase of protein concentrations in lumbar CSF, as the flow distance along the subarachnoid space becomes longer with body growth, which allows more blood-derived proteins to diffuse into CSF along the prolonged flow path. This is the reason why with the increase of albumin concentration, Q_{Alb} , the blood-derived fractions of IgG and IgM also increase in CSF (Table 1 and Figure 1). An advantage of the applied new software⁸ is the ability to compensate for this possible bias in the calculation of intrathecally synthesized fractions of IgG and IgM in CSF (see, for example, in Table 1).

The slightly higher mean albumin quotients in MS compared to the age-matched controls (5.3×10^{-3} versus 4.6×10^{-3}) is a consequence of some cases with

a moderate blood–CSF barrier dysfunction in the MS group. By exclusion of these cases with barrier dysfunction we obtain in the MS group the same mean as the controls (4.6×10^{-3}). The almost twofold increase of Q_{Alb} in MS patients between 7 and 29 years is therefore predominantly a consequence of the normal physiological age-related increase of Q_{Alb} . In paediatric MS the number of cases with a blood–CSF barrier dysfunction (Table 2) was slightly lower compared with adults (8–18%).

Humoral immune response in CSF

The qualitative analysis of OCBs allows for the detection of a small fraction as low as 0.5–1% of oligoclonal IgG within the total blood-derived IgG fraction in CSF. In comparison, up to 100% increase is necessary in the quantitative evaluation of the intrathecal fraction to obtain a value which is significantly above the statistically defined reference range [26]. Correspondingly the clinical sensitivity for detection of an inflammatory process in adult MS is 98% for oligoclonal IgG compared to 72% ($Q_{\text{IgG}} > Q_{\text{lim}}$) or now 82% ($Q_{\text{IgG}} > Q_{\text{mean}} + 2\text{SD}$) with the quantitative method (Table 4). So OCB, as the most sensitive method, allows an analysis unbiased by calculation with empirical reference values. Nevertheless the quantitative analysis cannot be omitted in CSF analysis for detection of disease-related immunoglobulin patterns^{6,7} and for calculation of the AI for specific antibodies.

In the sequential analysis of individual patients, recorded up to 5 years, we did not find any changes in the OCB pattern, as expected from many earlier reports. The search for the pathophysiological meaning of this rather stable immune response gained new relevance since we recognized its local variability in the individual patient by comparison of CSF and aqueous humour of the eye of the same MS patient.³⁰

For the detection of intrathecal IgM and IgA class response, there is no such direct method available with the equivalent quality and sensitivity of oligoclonal IgG. The detection of ‘oligoclonal IgM’ as reported by a research group¹³ is biased by the disassociation of the IgM pentamer into monomers before isoelectrofocusing, which destroys any individual pattern and does not allow a sufficiently qualitative discrimination of blood- and brain-derived IgM. Therefore, we still have to refer to the quantitative method with the statistically defined upper limit of the reference range for the blood-derived fractions in CSF.

For the most sensitive detection and statistical interpretation of intrathecal IgG, IgA and IgM synthesis in groups of patients, we refer now to $Q_{\text{mean}} + 2\text{SD}$ instead of Q_{lim} ($=Q_{\text{mean}} + 3\text{SD}$).²⁶ Instead of the

diagnostically more specific but less sensitive reference range, including 99% of the non-inflammatory controls ($n = 4100$), we introduce the reference for the more sensitive 96% range (Table 2) which approaches, but does not reach, the clinical sensitivity of the oligoclonal bands in MS. This improved sensitivity for IgG_{loc} is not a replacement of oligoclonal IgG analysis, but demonstrates the improved reliability for IgA and IgM group analysis (Table 2).

This higher sensitivity is needed as demonstrated in particular for the frequency of intrathecal IgM synthesis in MS: former reports⁵ found a frequency of IgM synthesis in 20% of adults which is now about 41% (Table 4). In particular, the difference in the prepuberty children is striking (Table 3): instead of a frequency of 33% with reference to Q_{lim} we now detect a frequency of intrathecal IgM in 67% of the paediatric patients. The higher sensitivity of this method explains why we report results that appear controversial in comparison with former calculations from the same group of patients.²⁹ Consequently, instead of an age-related higher frequency we now report an age-related lower frequency of intrathecal IgM at later disease onset (Table 2). In contrast to the frequencies of IgM synthesis at time of disease onset, we find from the examples in Figure 2 and the summary in Table 5 that there is no uniform increase or decrease of the immunoglobulin class response during the individual course of the disease: the intrathecal immune response in a single patient remains rather constant at the initial level of intrathecal IgM synthesis, as reported earlier for the IgG response too.

The presence or absence of an intrathecal IgM response in juvenile MS seems to be of prognostic relevance²⁴ albeit different from that obtained by the reports based on a qualitative method.¹³

Bias of IgG and IgM Index evaluations

There is particular controversy in the field of MS research which comes from earlier reports comparing IgM synthesis in patient groups with the linear IgM index.¹² This evaluation has an inherent bias by not compensating for the age-related increase or change of the serum protein fractions in CSF. This is demonstrated for the median IgG Index values in Table 2 and the corresponding Figure 4. In Figure 4 with a constant amount of intrathecal IgG synthesis ($\text{IgG}_{\text{loc}}(\text{m}) = 75 \text{ mg/l}$) at different times of CSF punctures the IgG index is decreasing from 3.1 to 1.5 and 1.2. This is simply due to the physiologically increasing Q_{Alb} . Also, in case of the threefold higher amount of intrathecal IgG ($\text{IgG}_{\text{loc}}(\text{m})$ in Table 1), the IgG index values remain about the same.

In addition to the earlier report⁶ where we pointed to false positive and false negative interpretations of IgG concentrations in CSF with the IgG Index, we now demonstrate also false interpretations with the IgG Index of the dynamics of intrathecal synthesis due to changing Q_{Alb} . Owing to the stronger hyperbolic curvature the fault is still more prominent with the IgM Index than that shown for the IgG index.²⁶

These data again support the statement that the linear IgG, IgA or IgM index is obsolete for a reliable detection of intrathecal IgG, IgA or IgM synthesis²⁶ in contradiction to the proposal in the McDonald criteria for diagnosis of MS.²⁵

Specific antibody synthesis in CNS (MRZ antibodies)

For more than 40 years the pathophysiological relevance of the intrathecal antibody synthesis in MS has remained part of ongoing discussions about microorganisms causing MS or initiating new exacerbations.^{31–36} These speculations are caused by the frequent misinterpretation of intrathecal antibodies in CSF. The polyspecific antibody synthesis is part of all acute and chronic immune reactions in blood and CNS²⁶ and does not need the persistence of the corresponding antigen.^{37,38}

It was our hope that the understanding of the extraordinarily high frequency of the intrathecal MRZ antibody response in MS and autoimmune diseases with involvement of the brain,²⁰ could contribute to a better understanding of the neuroimmunological reaction in the development of this type of chronic inflammatory processes.

As a first result in this study we found that there are only minor differences in the frequencies for measles or VZV antibodies at times of early or late onset of MS (Table 2). The lower frequency of rubella antibodies before adolescence might be a consequence of the immunization program varying in different populations.³⁹ As a second result we recognized that the intrathecal antibody synthesis remains constant or might increase in intensity during the course of the disease, observed over several years.

To obtain this second result we had to calculate retrospectively the amount of intrathecal antibodies in CSF. This was needed as the AI is a relative value.²⁶ This is shown in Table 6 and Figure 3: if with decreasing CSF flow rate Q_{Alb} and Q_{IgG} is increased, the AI will decrease even if the same amount of intrathecal VZV antibody, $Z_{\text{loc}}(\text{m})$ (U/L), is synthesized.

These data of the polyspecific immune response contribute two aspects to the field: to the diagnosis of chronic inflammatory diseases and to the understanding of the pathophysiological process.

Diagnostic relevance of MRZ antibodies and oligoclonal IgG

The presence of a humoral immune response with oligoclonal IgG (100%), intrathecal IgM (67%) and/or a combined MRZ-antibody response (40%) offers a strong argument for the differential diagnosis of paediatric MS and low probability for a suggested diagnosis, e.g. of an acute disseminated encephalomyelitis,^{22,23,40} which rarely (<20%) presents oligoclonal IgG.

Since the first report about its diagnostic relevance,¹⁶ it is now confirmed that a simultaneous presence of two or three antibodies (MR, MZ, RZ or MRZ) has a high probability of being an expression of a chronic inflammatory disease of the autoimmune type.^{20,26} This was observed in 40% of the paediatric MS patients compared with 67% in the adult MS group.⁵ In spite of its lower frequency in paediatric MS this is a helpful diagnostic tool for detecting the chronic process already at the time of a first clinical manifestation and also in the case of a monosymptomatic appearance. This aspect is underestimated in the international discussion, which focuses primarily on imaging techniques²⁵ as a diagnostic tool. The absence of oligoclonal IgG in CSF in an acute neurological childhood disease does not permit the diagnosis of definite or evolving MS, rather the absence points to another disease and needs further diagnostic efforts. CSF analysis therefore represents another strong and probably more powerful tool for differential diagnosis in childhood compared to imaging findings.²⁵

Pathophysiological relevance of MRZ antibodies

The particularly high frequency of the MRZ antibodies among the antibodies against neurotropic viruses is not understood at all. Nevertheless the study of this polyspecific immune response contributes to the understanding of the pathophysiology of MS.

The MRZ antibody reaction in MS does not need the persistence of the microorganism as in acute or chronic inflammatory diseases with a persisting causative antigen (SSPE,⁴¹ HSV encephalitis,²⁸ Fuchs heterochromic cyclitis,¹⁹ etc.). As the pattern of polyspecific antibodies in CSF is rather stable with the duration of disease, occasionally increasing in amount, and rarely does a new antibody synthesis appear, we can conclude that there is not a permanent invasion of B-cells into the CSF. This is supported by the earlier observation that there is no direct correlation of blood antibody patterns²⁸ and CSF antibody patterns.²⁹ However, the constancy of the intrathecal response also indicates that the disease is not starting as an acute form showing decline of the intrathecal

antibody synthesis as usually observed in acute inflammatory diseases of the brain.²⁶ This view is also supported by the other data reported for IgM synthesis and cell count in CSF: an intrathecal IgM fraction in CSF is principally not a sign of acuity. The acute inflammatory disease is recognized by an increased CSF cell count and increased Q_{Alb}. Both observations are not prominent in young children with MS, whereas the cell count in the group with the earliest onset is low or at least not higher than in later stages (Table 3) as could be expected for acute diseases.

This intrathecal synthesis of specific MRZ antibodies is also seen in aqueous humour of MS patients with an uveitis intermedia or periphlebitis retinae.¹⁹ With the recent observation that the MRZ antibody response pattern is different in aqueous humour and CSF of the same MS patient³⁰ we obtain an important argument for the local variability of the B-cell invasion into the CNS in MS patients. At least it is a further argument against the often-repeated suggestion of a causative role for microorganisms against which this polyspecific immune response is directed. Clinical immunology would benefit from a better understanding of the regulation in the immunological networks as the source of a chronification instead of relying solely on the clonal selection concept.

The finding that the frequency of an antibody synthesis against one to three of the MRZ antibodies with 71–75% in the prepuberty and 50–70% in the postpuberty age (Tables 2 and 3) is lower than frequencies detected in adults⁵ with 89% is, in fact, a consequence of the higher amount of intrathecally synthesized total IgG (IgGloc(m) in Table 2). This correlation was extensively studied in adult MS patients.⁵ Again the prepuberty differences present only with a quantitative but not a qualitative difference of the local immune response.

Pathophysiological conclusion

Manifestation of the neuroimmunological pattern in MS is not a slowly developing process. Already at the earliest clinical manifestation of MS in childhood, the complete neuroimmunological response pattern is unfolded. This aspect has diagnostic relevance and suggests a rethinking of the earliest pathophysiological processes of MS. Paediatric and adult MS differ only quantitatively but not qualitatively in neuroimmunological patterns in CSF which does not allow for discrimination between an 'early' and 'late' onset MS.

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