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Quantitation of intrathecal antibodies in cerebrospinal fluid of subacute sclerosing panencephalitis, herpes simplex encephalitis and multiple sclerosis: Discrimination between microorganism-driven and polyspecific immune response

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Abstract

The detection of intrathecal antibody synthesis by qualitative methods or the Antibody-Index (AI) is a relevant tool for diagnosis of inflammatory neurological diseases. An increased AI can be observed for a causative antigen as well as part of a polyspecific immune response. The quantitation of the intrathecal antibody fraction in cerebrospinal fluid (CSF), F_S, helps to discriminate both cases: In contrast to AI, F_S needs an absolute antibody concentration detected in the ELISA in mg/L The intrathecally synthesized, "local" antibody concentration in CSF (AB_{Loc}) is expressed as the specific fraction of the intrathecally synthesized total IgG (IgG_{Loc}) in CSF with $F_S = AB_{Loc}/IgG_{Loc} \cdot 100$ in %. F_S for HSV or measles has about 20- to 60-fold higher values in virus-caused antibody synthesis in acute herpes simplex encephalitis (mean HSV — $F_S = 8.9\%$) or subacute sclerosing panencephalitis (mean measles-$F_S = 18.8\%$) compared to the polyspecific immune response against these antigens e.g., in multiple sclerosis (0.14% or 0.52%, correspondingly). F_S helps also to avoid misinterpretations of an increasing AI in cases of therapy control, and allows direct comparison of relative antibody concentrations (R_S) in blood and intrathecally synthesized fractions in CSF (F_S): In multiple sclerosis patients F_S:R_S has a mean ratio of about 3 for the measles, rubella and VZV antibodies. Together with the large variability we find by ranking that about two third of MS patients have no direct correlation of the relative concentrations in serum and intrathecal synthesis. So this concept gains increasingly relevance for analysis of the polyspecific immune response in brain.

Keywords: Cerebrospinal fluid; Measles-, rubella-, varicella zoster-, herpes simplex-antibodies; Multiple sclerosis; Herpes simplex encephalitis; Subacute sclerosing panencephalitis; Intrathecal synthesis

1. Introduction

The detection of an intrathecal antibody synthesis against a causative antigen represents an important support of the suggested diagnosis of a neurological disease. In addition to the qualitative detection by western blots and antigen-driven immunoblots (Moyle et al., 1984; Dorries and ter Meulen, 1984; Sindic et al., 1994a,b; Monteyne et al., 1997) the quantitative analysis by ELISA with calculation of the Antibody-Index (AI) are of actual diagnostic relevance (Reiber and Lange, 1991; Reiber and Peter, 2001). But, the accompanying polyspecific immune response with intrathecal antibodies in multiple sclerosis (MS) against a variety of non-causative antigens can lead to misinterpretations or to a wrong diagnosis in the worst case. The intrathecal synthesis of chlamydia antibodies (Rostasy et al., 2003) in MS is an example.

It was a long way to understand that antibodies in MS, which are synthesized in brain and in blood, are not only directed against a causative antigen but also against other antigens, which are not involved in the cause of the disease. The first detection of intrathecal measles antibody synthesis (Adams and Imagawa, 1962) in MS led to the misleading hypothesis of a virus aetiology of MS. Later observations showed a polyspecific antibody

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synthesis against rubella, varicella zoster, herpes simplex, mumps viruses (Vandvik et al., 1979) in the single MS-patient. Meanwhile there are also reports about the intrathecal synthesis of antibodies against chlamydia pneumoniae (Rostasy et al., 2003), or of autoantibodies against ds-DNA in a fraction of MS patients (Graef et al., 1994).

The early approach to quantitate the intrathecally synthesized fraction of measles-specific IgG in subacute sclerosing panencephalitis (SSPE) (Mehta et al., 1977) has been improved by a better method and evaluation technique which showed that in SSPE only about 20% of the intrathecally synthesized IgG is measles-specific (Conrad et al., 1994), i.e., the remaining 80% of the intrathecal IgG, the largest fraction of the antibody response, involves antibodies against non-causative antigens. This polyspecific immune response has been observed also as part of the immune reactions in blood (Terryberry et al., 1995), i.e., it is not a particular property of the immune reactions in brain. With these and other observations it became clear that the polyspecific immune response against non-causative antigens represents a general phenomenon of all inflammatory processes in acute, subacute as well as in chronic diseases. This "polyspecific immune response" does not depend on the persistence of a corresponding antigen (Godec et al., 1992). It is the recognition of an immunological network (Varela and Coutinho, 1991), which gives the clue to understand the polyspecific immune response.

So, what we observe as oligoclonal bands in cerebrospinal fluid (CSF) (Andersson et al., 1994) of many patients with an inflammatory neurological disease is the consequence of a humoral immune response which is oligoclonal and polyspecific. In the actual meaning the term "oligoclonal" refers to a restricted number of B-cell clones. The detection of oligoclonal IgG in CSF is a basic part of a laboratory supported diagnosis of MS (Andersson et al., 1994; McDonald et al., 2001; Poser et al., 1983). An extension of this approach comes from the detection of a polyspecific immune response in MS, in particular the measles-, rubella- and VZV antibodies (M–R–Z-antibody reaction) (Reiber et al., 1998). The unexplained high frequency of this M–R–Z antibody reaction, in up to 94% of the MS patients is not specific, as also detected in autoimmune diseases with involvement of the CNS (Graef et al., 1994). But, as the MRZ combination is not seen in any other acute or subacute inflammatory disease (< 0.1%). Only the single antibodies are detected more frequently e.g. measles specific antibodies in varicella zoster-virus (VZV)-meningitis (Sindic et al., 1994a). So the presence of this MRZ antibody reaction allows the recognition of a chronic inflammatory process (autoimmune type) already at the time of the first clinical manifestation (Reiber and Peter, 2001; Reiber et al., 1998). This is particularly important for diagnosis of cases with a monosymptomatic start of the disease like an optical neuritis, an uveitis intermedia or periphlebitis retinae (Quentin and Reiber, 2004).

The detection of intrathecal antibody synthesis in CSF has a long tradition. The earliest approach, the Goldmann–Witmer-Index (Goldmann and Witmer, 1954) is still frequently used in ophthalmology (GW-I=Q_{spec}/Q_{IgG}). Felgenhauer et al. (1982) introduced an approach with comparison of optical densities in CSF and serum samples matched for IgG concentrations. Qualitative approaches have been used since 1984 and have been compared with quantitative methods (Dorries and ter Meulen, 1984; Monteyne et al., 1997; Moyle et al., 1984). Limitations due to non-specific or low affinity binding have been described (Chapman et al., 2006; O'Connor et al., 2003; Sindic and Laterre, 1991). Reiber and Lange (1991) improved the sensitivity of the AI by a correction to avoid a false negative interpretation in cases with a strong intrathecal IgG synthesis. The AI presents a very sensitive value for the quantity of intrathecally synthesized specific antibodies. But the value of the AI does not correspond with the intensity of the intrathecal synthesis, as the value depends also on the amount of the blood derived fraction in CSF. With the invention of the measurement of absolute antibody concentrations (Conrad et al., 1994) and with an improved calculation of the specific antibody fraction in CSF, F_S, as reported in this paper, a virus-driven antibody synthesis can now be discriminated from a polyspecific, network-related immune response. These methodological improvements are based on the evaluation of immunoglobulin quotients (Q_{IgG}, Q_{IgA}, Q_{IgM}) with a non-linear, hyperbolic discrimination function, Q_{Lim} (Reiber, 1994), which allows the sensitive discrimination between blood- and brain-derived immunoglobulin fractions (i.e., intrathecal synthesis of IgG, IgA and IgM) in CSF. This replaces the earlier linear approaches, like IgG-Index, which leads to false interpretations in cases of a blood/CSF barrier dysfunction as demonstrated in detail (Ref. in Reiber and Peter, 2001). With this improved quantitation of the specific fraction, F_S, in CSF we gain also a new tool to study pathophysiological aspects of the MRZ reaction in multiple sclerosis.

2. Materials and methods

2.1. Patients and samples

The patients were admitted to the Neurology and Pediatric Neurology Department, University of Göttingen. CSF and serum samples of these patients were collected for routine. Residual sample volumes were stored at − 70 °C.

2.1.1. Patient groups

2.1.1.1. Multiple sclerosis. From a larger group, $n=71$ patients were selected, which all had a complete set of data analysed (including oligoclonal IgG in CSF and a polyspecific intrathecal antibody synthesis against measles-, rubella-, varicella zoster- and in 16 cases additionally against herpes simplex-virus). To make sure that the patients fulfill the criteria of a chronic inflammatory disease of the autoimmune type, we included only those patients with antibodies against at least two neurotropic antigens (possible combinations: measles–varicella zoster, measles–rubella, rubella–varicella zoster or measles–rubella–varicella zoster). Frequencies are summarized in Table 1.

2.1.1.2. SSPE. $n=5$ patients with clinically defined SSPE, some with several punctures are involved. Diagnosis was supported by increased measles AI values.

Table 1
Frequency of intrathecal antibodies (AI ≥ 1.5) against measles (M)-, rubella (R)-, varicella zoster (V)- and herpes simplex (H)-virus in patient groups investigated

	Antibodies				
	M	R	Z	H	M–R–Z [a]
MS [b]	60/71	62/71	52/71	3/16	32/71
SSPE [c]	5/5	0/5	0/5	0/5	0/5
HSV-E [d]	0/4	0/4	4/4	4/4	0/4
CONTROL	0/28	0/28	0/28	0/28	0/28

[a] Patients with intrathecal synthesis of antibodies against all three antigens M, R, Z.
[b] MS — Multiple sclerosis.
[c] SSPE — Subacute sclerosing panencephalitis.
[d] HSV — Herpes simplex encephalitis.

2.1.1.3. Herpes simplex-virus encephalitis. $n=4$ patients with a clinically defined HSV-E, supported by specific antibody response, are involved in this group.

2.1.1.4. Controls. $n=28$ patients with other non-inflammatory neurological disorders (OND) were involved, including patients with tension headache ($n=10$), stroke ($n=6$), psychiatric disorders ($n=4$), seizures ($n=3$), cranial nerve palsy ($n=2$), neurodegenerative disorders ($n=2$), pseudotumor cerebri ($n=1$). The controls had no oligoclonal IgG in CSF and no intrathecal synthesis of the examined antibodies. The frequency of M–R–Z–H-antibody synthesis in the patient groups are shown in Table 1.

2.2. Protein analysis in CSF and serum

Albumin and IgG in CSF and serum were analysed with immunochemical nephelometry (Dade Behring Nephelometer). CSF and (correspondingly diluted) serum samples are analysed in the same analytical run to avoid imprecisions due to different standardizations in different assays (Reiber et al., 2003). Data are evaluated in the CSF/serum quotient diagrams (Reiber and Peter, 2001), to detect an intrathecal immune reaction by reference to the established hyperbolic discrimination line (Q_{Lim}), upper border line of the reference range for blood-derived immunoglobulins in CSF. The intrathecal fraction IgG_{IF} in % or the locally synthesized contribution to CSF concentration IgG_{Loc} in mg/L are calculated (Reiber, 1994) with reference to Q_{Lim}. Q_{Lim} (IgG) $=(0.93(Q_{Alb}^2-6)^{0.5}-1.7)\cdot 10^{-3}$ with $Q_{Alb}=$ Alb(CSF)/Alb(ser) with the empirical albumin (Alb) concentrations in CSF and serum (Q_{Alb} value multiplied with 10^3 for insertion in the above version of Q_{Lim}). $IgG_{Loc}=(Q_{IgG}-Q_{Lim})\cdot$ IgG(ser) in mg/L with $Q_{IgG}=$ IgG(CSF)/IgG(ser) the empirical concentrations in CSF and serum. The intrathecal fraction, $IgG_{IF}=(IgG_{Loc}/IgG_{CSF})\cdot 100$, or $IgG_{IF}=(1-Q_{Lim}/Q_{IgG})\cdot 100$ in %.

2.3. Antibody-Index, AI

Antibody analysis (measles, rubella, herpes simplex, varicella zoster,) is performed on commercial microtiter plates with antigen and control antigen (Enzygnost OWLNG14C02; OWBFG14C01; OWLTG14C01; OWMXG14C01, Dade Behring, Marburg, Germany). Dilution buffer, washing solution, antibody solution, substrate solution and stopping solution were used as described earlier (Reiber and Lange, 1991). The calibrations curves were obtained by serial dilution of a pooled serum (6 serial dilutions of 1:3). The predilution of the highest standard concentration was chosen to get an optical density about 2.0, defined as 100 arbitrary concentration units. CSF and serum samples are analysed paired in the same analytical run with reference to the standard curve. Standard sample dilutions, CSF (1:15) and serum (1:3000) are chosen to get OD values in the reliable range of the standard curve In cases of large antibody synthesis CSF needs a higher dilution. The measured absorbancy (OD) is transferred into arbitrary concentration units (AU), by reference to the standard curve (Reiber and Lange, 1991). After multiplication of AU with the dilution factor to get the sample antibody concentration AB (still arbitrary units) we calculate the specific antibody quotient, $Q_{spec}=$ AB(CSF)/AB(ser).

The Antibody-Index, AI, is calculated either with $AI=Q_{spec}/Q_{IgG}$ if $Q_{IgG}<Q_{Lim}$ or $AI=Q_{spec}/Q_{Lim}$ if $Q_{IgG}>Q_{Lim}$. Q_{Lim} is calculated with the improved parameters (Reiber, 1994), different from the data base in Reiber and Lange (1991). The reference range is AI = 0.7–1.3. Pathological values are AI = 1.5.

2.4. Quantitative evaluation of antibody data from AI assay

The absolute concentrations of measles-, rubella-, varicella zoster- and herpes simplex antibodies in CSF and serum samples were detected by quantitation (in mg/L) of the measles-, rubella-, varicella zoster- and herpes simplex antibody concentration in the standard solution, used for the detection of the Antibody-Index. The ELISA established was modified from a previously described method (Conrad et al., 1994) by using Fab specific IgG for coating and, Fc specific AB for detection, omitting the blocking with rabbit antiserum, PBS buffer was replaced by our particular mixture described in Reiber and Lange (1991). The microtiter plate was constructed as a combination of five stripes: one coated with anti-Human IgG and four stripes with measles antigen, rubella antigen, varicella zoster antigen and herpes simplex antigen (Behring microtiter plates, see above), respectively.

For the total IgG analysis (calibration curve) on the first stripe, ELISA microtiter plates (Maxi Sorp, Nunc, Roskilde, Denmark) were coated with 100 µl of gamma-chain-specific–anti-Human IgG (Sigma, St. Louis, USA), diluted to 5 µg/ml in a coating solution prepared with Na-Carbonat 0.1M/L (pH 9.6) and incubated on a shaker (Titertek, Hunstville, USA) at 4 °C overnight.

The IgG calibration curve was obtained by a serial dilution of a defined IgG-standard (Beckmann calibrator, Germany) with 20 µg/l, 10 µg/l, 5 µg/l, 2.5 µg/l, 1.25 µg/l, 1.25 µg/l, 0.625 µg/l, 0.3125 µg/l. With these dilutions we got optical densities on the first stripe from 1.6 to 0.04. Each dilution of the standard was run in duplicate. As positive control we used a serum with a defined IgG-concentration (Beckmann control, Germany). As negative control the wells of the control antigen were filled with 100 µl of the dilution buffer.

The MRZH standards were diluted to be measured in the steep area of the standard curve for IgG. Each sample (100 µl/

Table 2
Example of transfer parameters for calculation of absolute concentrations of M-, R-, Z-, H-antibodies from arbitrary units (routine AI-analysis) with a homemade standard (pooled serum samples)

	Antibodies			
	M	R	Z	H
Conversion factor [a]	0.097 µg/L	0.149 µg/L	0.095 µg/L	0.097 µg/L
AU (CSF) [b]	467	817	168	228
CSF AB-concentration [c]	0.045 mg/l	0.122 mg/l	0.016 mg/l	0.022 mg/l

[a] Valid only for this single homemade standard.
[b] Determined arbitrary concentration units from a MS patient.
[c] Corresponding CSF concentration.

well) was measured in four serial dilution steps (first dilution step of our actual MRZH standard was 1:350).

The microtiter plates with the five stripes were incubated 1.5 h at room temperature on a shaker, washed three times with the washing solution (SLT-ELISA Washer EAW II plus). 100 µl of the antibody solution (peroxidase conjugated gamma-chain specific anti-human IgG) were incubated for 1.5 h at room temperature on a shaker and washed four times with the washing solution. 100 µl substrate solution were added and the reaction stopped after 15 min with the stopping solution. Optical density (OD) was measured on a microtiter plate reader (SLT-ELISA–Reader EAR 400 AT) at 450 nm and 620 nm. The concentration of MRZH antibodies in the standard samples were calculated under the definition that 1 mg specific antibodies has the same OD as 1 mg total IgG. From this quantitation of MRZH concentrations we get calculation factors (shown for our actual standard sample in Table 3) to transfer the AU-values measured for AI into absolute concentrations in the samples analysed with reference to this particular standard.

2.5. Fraction of specific intrathecal antibodies in CSF, F_S

The specific fraction, F_S, in % is the ratio of the intrathecally synthesized concentration of specific antibodies (AB_{Loc}), and the intrathecally synthesized concentration of total IgG (IgG_{Loc}). This calculation of F_S, for comparison of means in different groups, refers to Q_{mean}, the mean function (Reiber, 1994) of the reference range instead of the upper limit Q_{Lim} used for AI: $F_S = AB_{Loc}(mean)/IgG_{Loc}(mean) \cdot 100$ in %. With $Q_{mean}(IgG) = (0.65\ (Q_{Alb}^2 - 8)^{0.5} - 1.4) \cdot 10^{-3}$, we calculate $IgG_{Loc}(mean) = (Q_{IgG} - Q_{mean}) \cdot IgG(ser)$ in mg/L or $AB_{Loc}(mean) = (Q_{spec} - Q_{mean}) \cdot AB(ser)$ in mg/L. Q_{mean} in this function is Q_{mean} (IgG) for IgG-class antibodies.

F_S can not be calculated for $Q_{IgG} < Q_{mean}$ and should not be calculated for cases were not definitely an intrathecal antibody synthesis is found by AI = 1.5, to avoid false positive interpretations from F_S.

Once the conversion factors of arbitrary units into absolute concentrations for the antibodies in the actually used standard in the AI assay (Table 2) are performed, F_S, can be calculated with the AI, also retrospectively, as far as the arbitrary units in the antibody assay have been analysed with reference to the same standard.

Table 3
Method of ranking for AB-concentration of M, R, Z in intrathecal synthesis (AB_{Loc}) and blood (AB_S) and AB_{Loc}/AB_S (calculated in %)

Measles			Rubella			Varicella zoster		
AB_S	AB_{Loc}	AB_{Loc}/AB_S	AB_S	AB_{Loc}	AB_{Loc}/AB_S	AB_S	AB_{Loc}	AB_{Loc}/AB_S
17.2	0.58	3.4%	22.8	0.1	0.4%	7.4	0.09	1.2%
2.		1.	1.		3.	3.		2.

2.6. Method of ranking for AB concentrations of M,R,Z in intrathecal synthesis and blood

In a subgroup of MS patients we made a ranking for the antibody concentrations in blood (AB_S), and the ratio of intrathecally synthesized antibodies (AB_{Loc}) and AB_S (AB_{Loc}/AB_S). The measles-, rubella- and zoster antibody concentrations in blood (AB_S) were ranked according to the size of concentration values (1.,2.,3.). Correspondingly, the ratio AB_{Loc}/AB_S was ranked according to their size (calculated in %). An example from a single patient in Table 3 shows the sequence in blood (R, M,Z) different from the relative intrathecal synthesis AB_{Loc}/AB_S (R,Z,M).

2.7. Oligoclonal IgG

The detection of oligoclonal bands in cerebrospinal fluid and serum is performed by immunoblot after isoelectric focussing and interpretation according to the international consensus (Andersson et al., 1994).

3. Results

3.1. Quantitations of intrathecal humoral immune response

In Table 4 we report the data of 10 MS patients with a representative variation of data. All patients had oligoclonal IgG in CSF and an intrathecal measles antibody synthesis. The Table contains all data necessary for the reader, to recalculate all relevant data, intrathecal fraction (IgG_{IF}), Antibody-Index (AI),

Table 4
Examples of basic CSF data and quantitation of measles antibody response in blood and brain of adult MS-patients, assorted for increasing intrathecal IgG synthesis, IgG_{Loc} (mean)

Q_{Alb} · 10^3	Q_{IgG} · 10^3	IgG_s [g/L]	AB_S [mg/L]	IgG_{Loc} (mean) [mg/L]	AB_{Loc} (mean) [mg/L]	$Q_{spec\text{-}M}$ · 10^3	AI [a]	F_S [b] [%]
5.2	2.72	7.5	16.9	1.9	0.04	4.6	1.7	2.10
3.8	2.1	11.8	8.4	5.0	0.03	5.3	2.5	0.60
3.3	2.2	13.2	13.7	10.2	0.04	4.7	2.2	0.39
2.5	1.89	12.3	5.4	10.4	0.02	4.8	3.1	0.19
12.3	8.3	10.2	31.1	15.3	0.24	14.9	1.8	1.57
2.1	2.52	11.0	36.5	17.7	0.46	13.7	10.5	2.60
4.2	3.82	14.3	89.6	27.3	0.46	7.1	2.5	1.68
9.3	8.2	14.2	33.7	46.6	2.06	66.6	9.2	4.42
4.6	6.6	11.2	2.4	50.3	0.07	29.6	9.4	0.14
8.1	18.65	12.6	20.3	181.7	0.91	49.4	8.0	0.50

[a] Antibody-Index, $AI = Q_{spec}/Q_{IgG}$ or $AI = Q_{spec}/Q_{Lim}$ (if $Q_{Lim} < Q_{IgG}$).
[b] Specific fraction, $F_S = AB_{Loc}(mean)/IgG_{Loc}(mean) \cdot 100$ [%]. F_S is the mean of all separately calculated single F_S values, not identical with the quotient of the means in this table.

or specific of antibodies in CSF, F_S. The Antibody-Index, the diagnostically most sensitive parameter, is in this set of data between AI=1.7 and 10.5. In contrast to AI the specific intrathecal fraction, F_S, represents a quantitative measure of intrathecally synthesized measles antibodies. This quantitative evaluation refers to the mean of the reference range and varies between 0.14% and 4.42%, i.e., only 0.14% to 4.4% of the intrathecally synthesized IgG are measles antibodies. The variation of F_S among the eight patients (0.14% to 4.42%) is several fold larger then the variation between lowest and highest AI value (1.7 to 10.5) (Table 3).

A statistical evaluation of median intensities of intrathecal synthesis in the whole groups is shown for MS-, SSPE- and HSV-E-related antibodies in Table 5. The intrathecally synthesized amount of IgG_{Loc} in CSF is highest for SSPE and HSV-E compared to the values in MS. The HSV-AI values with a median of HSV-AI=40.5 are the highest in the acute herpes simplex-virus encephalitis at a time between 10 and 18 days after start of the disease, followed by the SSPE with a smaller median measles-AI=21. The median values of about AI=3 for measles-, rubella-, varicella zoster and herpes simplex-antibodies in MS are consistent with the earlier reports (Reiber et al., 1998). The mean measles-AI of SSPE is relatively low with an only seven-fold larger value then for measles-AI in MS, in spite of the largest intrathecally synthesized amount, AB_{Loc}=27.2 mg/L 300-fold higher compared to 0.09 mg/L for measles in CSF of MS patients (Table 5). The corresponding measles-F_S is about 40-fold larger for SSPE then for MS. So, the intrathecally synthesized specific fraction in CSF, F_S, gives a more representative picture of the quantity of antibody synthesis in brain.

Regarding measle-AI versus measles-F_S (Table 5):

- The F_S represents best the amount of intathecal AB synthesis.
- No overlap for the F_S-values of both groups (MS and SSPE), different from the strong overlap of AI-values.
- The ranking of the intrathecal synthesis for different antibodies is correct, for F_S with 18.8 to 8.85 instead of inverted 21 to 40.5 for the AI.

As already reported (Reiber et al., 1998), the most relevant result of the comparison in Table 5 between MS, SSPE and HSV-E is the different intensity of intrathecal polyspecific antibody response in MS compared to the virus-driven antibody response against the causative antigen in SSPE or HSV-E: The median measles antibody synthesis is about 40-fold stronger in SSPE than the mean measles antibody response in MS, and the median herpes simplex antibody synthesis in HSV-E (H-F_S=8.85%) is about 60-fold stronger than the mean HSV antibody synthesis in MS (H-F_S=0.14%). Our measles data confirm the earlier report of Conrad et al. (1994) for the SSPE, that only the smaller part (18.8%) of the intrathecal IgG-class synthesis is a measles-specific response, leaving about 80% of the IgG antibodies to be detected with other specificities.

3.2. Relation between antibody concentrations in blood and intrathecally synthesized fractions in CSF of MS patients

Table 6 shows the fraction of specific antibodies of the total IgG in blood (R_S) as well as the corresponding intrathecal fractions of antibodies in CSF (F_S) of MS patients. The percentage of the blood antibodies for e.g. measles with a median of 0.15% (Table 6) is lower than the corresponding intrathecal percentage with 0.52%. In MS the mean relation between the F_S and R_S is similar for measles-, rubella- and varicella zoster-antibodies, i.e., mean MRZ-antibodies represent a 3-fold higher fraction in the intrathecal synthesis compared to blood.

Table 5
Median intensities of intrathecal synthesis of total IgG (IgG_{Loc} (mean)) and specific antibodies (AB_{Loc} (mean)) in patients with multiple sclerosis (MS, n=71), subacute sclerosing panencephalitis (SSPE, n=5) and Herpes simplex-encephalitis (HSV-E, n=4)

			IgG_{Loc} (mean)	AB_{Loc} (mean)	AB_S [a]	AI [b]	F_S
			[mg/L]	[mg/L]	[mg/L]		[%]
MS [c]	Measles-AB	Median	25.7	0.09	14.7	3.2	0.52
		Range	(0.9–181.7)	(0.002–2.06)	(0.7–89.6)	(1.5–16.9)	(0.04–4.42)
MS [c]	Rubella-AB	Median	27.3	0.13	17.0	2.6	0.53
		Range	(1.9–181.7)	(0.005–3.87)	(1.9–152.0)	(1.5–14.4)	(0.03–4.8)
MS [c]	VZV-AB	Median	21.4	0.05	6.7	3.4	0.23
		Range	(0.9–182)	(0.001–0.47)	(0.9–51.1)	(1.5–15.2)	(0.03–4.9)
MS [c]	HSV-AB	Median	35.6	0.07	23.1	2.3	0.14
		Range	(24.3–182)	(0.03–0.13)	(2.6–50.8)	(1.6–3.3)	(0.055–0.23)
SSPE [d]	Measles-AB	Median	154	27.2	665	21.0	18.8
		Range	(88–285)	(19.5–42.4)	(353–937)	(12.3–31.4)	(11.8–27.5)
HSV-E [e]	VZV-AB	Median	186.4	1.05	5.3	19.2	0.6
		Range	(92–302)	(0.675–1.42)	(3.9–5.4)	(12.0–29.4)	(0.5–0.7)
HSV-E [e]	HSV-AB	Median	186.4	16.5	48.1	40.5	8.85
		Range	(92–302)	(3.26–37.7)	(8.9–70)	(31.6–50.7)	(3.5–12.5)

[a] AB_S=serum antibody concentration. The corresponding median of n=28 controls was: 8.4 mg/L (measles-AB); 11.1 mg/L (rubella-AB); 5.2 mg/L (VZV-AB); 25.2 mg/L (HSV-AB).
[b] Mean of controls AI=0.9 (range 0.6–1.3).
[c] Subgroups of patients with AI≥1.5: n=60 measles-AB; n=62 rubella-AB; n=52 VZV-AB; n=3 HSV-AB.
[d] n=5.
[e] n=4.

Table 6
Correlations between relative antibody fractions of total IgG in blood (R_S) and in the CSF (F_S) of MS patients ($n=71$)

	R_S [%]		F_S [%]		F_S:R_S	
	Median	Range	Median	Range	Median	Range
$n=71$						
Measles	0.15	0.01–1.0	0.52	>0.0–4.4	3.5	0.5–27
Rubella	0.19	0.02–1.4	0.53	>0.0–4.8	3.0	>0.0–47.4
VZV	0.06	0.01–0.3	0.23	>0.0–4.9	3.5	>0.0–27.2

Due to the large variability in the wide range of this relation in the different patients (Table 6) it makes sense to look more detailed on the individual variation of the intrathecal antibody synthesis. In a subgroup of MS patients, in which all three antibodies (MRZ) are synthesized intrathecally (32 cases, Table 1) we made a ranking for the antibody concentration in blood (AB_S) and the ratio AB_{Loc}/AB_S (Table 3). We counted the number of cases with the same ranking and found that two third of the patients (67%) had a different ranking, indicating, that there is no strong coupling between relative amount in blood and the intrathecally synthesized fraction in CSF.

4. Discussion

4.1. Methods

We introduced the specific fraction, F_S, in CSF as a quantitative measure for the mean intrathecal antibody synthesis. F_S corresponds to a previously reported value (Conrad et al., 1994), called percent measles intrathecal synthesis rate of total IgG synthesis rate. The main difference, beside a minor modification of the ELISA method (methods) is our reference to a non-linear reference range of blood derived antibodies in CSF, established as Reibergrams in routine CSF analysis (Reiber and Peter, 2001). The approach of Conrad et al. with reference to the linear Tourtellotte formula would lead to wrong interpretations in cases of a barrier dysfunction as shown in Reiber and Peter (2001).

The calculation of F_S refers to Q_{mean}, the mean of the hyperbolic reference range for the single patients albumin quotient Q_{Alb}, (Reiber, 1994). This reference is necessary to calculate individual intrathecal fractions for the specific antibody and the total IgG and is more suitable as a reference for the normal value the patient would have had in the healthy condition than Q_{Lim}, the upper discrimination line of the reference range, suitable for the discrimination of an intrathecal synthesis from a purely blood derived IgG fraction in CSF. This is the reason why F_S is not suitable to detect correctly an intrathecal synthesis for cases with small CSF/serum quotients for the antibodies. The Antibody-Index, AI, is still the best, clinically evaluated method (Reiber and Lange, 1991; Felgenhauer and Reiber, 1992) to detect an intrathecal antibody synthesis and was therefore used as a precondition to calculate F_S in the patients involved in this study. A few patients with $Q_{IgG}<Q_{mean}$ are lost in this evaluation as the calculated value for their intrathecal IgG fraction would be negative. Nevertheless F_S represents the more reliable evaluation than AI where absolute quantities of intrathecal synthesis needs to be compared e.g. between different species or different groups of diseases (Quentin and Reiber, 2004). There is some discussion about the earliest and most reliable method to detect an intrathecal antibody synthesis which can not be ignored. There are still proposals to compare optical densities (OD) measured in CSF and serum. This earlier proposal (Felgenhauer et al., 1982; Johnson et al., 1984) to dilute CSF and serum sample to the same IgG concentration does not help to match the concentrations of the antibodies in the samples in case of any pathological intrathecal synthesis (Table 4). This is the reason why the correction of the AI for $Q_{IgG}>Q_{Lim}$ was invented on the base of arbitrary concentration units (Reiber and Lange, 1991). In general it is not reasonable for routine analysis to compare OD instead of (arbitrary) concentration units, as the ratio between concentration and OD is not linear, so that if CSF and serum samples are exactly in the same range of the standard curve they can not be compared correctly, leading to a wrong quotient. The example of a standard curve in Table 7 shows this change in ratios of almost a factor of ten between the low and the high concentration range. So the proposed reference to a standard curve needs also the control that a serial dilution of a sample gives the same recovery in all ranges of the standard curve regarded as reliable (0.1 OD to 2.0 OD, in particular the range 0.2 to 1.0 OD). In case of double estimations at two different concentrations in CSF and serum, for calculation those values should be paired which are next to each other in their OD.

4.2. AI versus F_S

The size of an AI-value does not correlate well with the amount of intrathecally synthesized antibodies, as the AI depends also from the individual antibody concentration in blood (AI=AB(CSF)/AB(ser):(IgG(CSF)/IgG(ser)). With a large value of AB (ser) the AB(CSF) will also become larger (increased blood derived fraction) and the brain-derived fraction represents a relatively smaller part of total AB(CSF). As an example: the large mean measles serum concentration (median 665 mg/L) is the reason why in SSPE (Table 5) in spite a larger amount of intrathecal antibody synthesis then in HSV Encephalits (mean $F_S=18.8$ versus 8.85%) the mean AI values (21.0) are smaller than those for HSV encephalitis (AI=40.5) with a median serum value of 48.1 mg/L Still more striking is the fact that the measles-AI in MS with a very small intrathecal antibody synthesis (mean $F_S=0.52\%$) can reach AI-values (up to 16.9 in Table 5 or still larger in another group of MS patients (>40) reported in Reiber et al., 1998) which overlap strongly with the measles-AI in SSPE (12.3–31.4).

This effect does not influence the sensitivity of detection of an intrathecal AB-synthesis (AI=1.5), but leads to a weaker

Table 7
Changing ratio between concentration (AU, arbitrary units) and optical density (OD) in a standard curve in the measles antibody assay (ELISA)

OD	1.71	1.33	0.67	0.34	0.13	0.06
AU measles	92.8	36.9	10.2	4.1	1.2	0.3
OD/AU measles	0.02	0.04	0.07	0.08	0.10	0.17

discrimination between cases with antibody synthesis against the causative antigen (SSPE) and the polyspecific co-synthesis (MS). This in fact is different for measles-F_S with no overlap at all between the groups for MS (0.04–4.42%) and SSPE (11.8–27.5%).

There is a further aspect where F_S can improve the information got from AI calculation. In a follow up, e.g. to be observed in a therapy control study, the faster reduction of the antibody concentration in blood compared to the brain can lead to an increase of AI (like the change from line 3 to line 4 in Table 4 with AB(ser) = 13.7 to 5.4 mg/L). Regarding the AI (2.2 to 3.1) this would give the wrong impression of an increasing intrathecal synthesis in contrast to the true condition shown in the decreasing values of F_S (0.39 to 0.19 % of the intrathecal total IgG synthesis).

The data in Table 4, assorted for increasing intrathecal IgG synthesis also show clearly that there is no correlation of total IgG synthesis with the amount of the intrathecal measles antibody synthesis. This documents one of the reasons why the CSF and serum samples, matched for the same IgG concentration for analysis, do not match correspondingly for the concentrations of the specific antibody.

4.3. Causative antigen and/or polyspecific immune response

With our improvement and extension of the quantitation concept of Conrad et al. (1994) to other viruses, like HSV or rubella, we established the concept that the immune response against the causative antigen is 40- to 60-fold higher than in the corresponding polyspecific immune response, observed in MS. As a particular result of this approach we provided plausibility that the rubella virus, persisting in the eye of patients with a Fuchs heterochromic cyclitis, which is causative for the disease (Quentin and Reiber, 2004).

For these examples of acute or subacute diseases with 10–20% intrathecal antibodies against the causative microorganism we also demonstrate a particular property of all immune reactions, which are often ignored or source of wrong interpretations: in acute or subacute inflammatory diseases a high amount of the antibodies synthesized are directed to non-causative antigens.

4.4. Multiple sclerosis

As a contribution to neuroimmunology we gained with this concept of quantitation also a particular tool to understand connections between the systemic and the intrathecal antibody response in MS. The data in Table 6 show a similar mean ratio between measles-, rubella- and VZV-antibody fractions in % of total IgG in serum (R_S) and CSF (F_S). The mean ratio, F_S:R_S, was about 3.5 (Table 6). This indicates a mean 3-fold higher preference of the B-cell clones, producing antibodies against the neurotropic viruses, compared to the total number of other B-cell clones invading the CNS in case of multiple sclerosis. The large variability of this ratio F_S:R_S indicate that there is no strong correlation between blood concentration and intrathecal synthesis. This can be directly shown by a ranking of the MRZ antibodies in blood and in CSF, if ranked according to the size of the AB concentrations in serum (AB_S) and the ratio AB_{Loc}/AB_S. The comparison shows that two third (67 %) of the MS patients with a combined M–R–Z response have a sequence of antibody concentrations in blood (AB_S) different from the ratio AB_{Loc}/AB_S.

The rational behind this ratio is the idea that the AB_S related B-cells in the systemic immune system corresponds to the number of intrathecal (perivascular) B-cells producing AB_{Loc} and that their ratio is constant for all AB species if the passage refer to an arbitrary barrier transfer/diffusion process. This idea is proposed by the similar mean ratio of about 3 for all three AB species (Table 6). But the ranking shows that the large variation (Table 6) is due to a more complex condition than just a simple correlation with the actual blood ratio of the different AB species and their B-cells. This clearly points out that the intrathecal antibody synthesis against the neurotropic viruses in CNS of MS patients does not simply depend on their arbitrary blood concentrations.

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