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# 11. NEUROIMMUNOLOGY: IMMUNOGLOBULINSANDTHE INTRATHECAL POLYSPECIFIC IMMUNE RESPONSE INACUTE,SUBACUTEAND CHRONIC NEUROLOGICAL DISEASES

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Acute, subacute and chronic inflammatory diseases of the central nervous system (CNS) are often accompanied by humoral immune response characterized by the following methods:

· The disease-related immunoglobulin class pattern (1)

· The specific antibodies against the causative antigen (IgG-, IgA-, IgM-class) (2)

· Oligocloncal IgG (IgG-class) (3)

· Polyspecific immune response against a large variety of non-causative antigens (4)

Intrathecally synthesized antibodies, detected in cerebrospinal fluid (CSF), can have two different sources: a causative persisting antigen or a polyspecific concomitant immune response. The classical view of the immune response is the clonal selection of a B lymphocyte clone producing specific antibodies against the invading microorganism. As always several clones are found to fit the specific antigen, we speak about an "oligoclonal" immune reaction. In addition to the oligoclonal antibodies against the specific causative antigen each immune reaction produces a wide spectrum of different antibody-species not connected with the causative antigen. This "polyspecific immune response" does not depend on the presence of a corresponding persisting antigen (13) and is of lower intensities than for the causative antigen (4, 5).

The detection of oligoclonal immunoglobulin G (IgG) in CSF is a basic part of a laboratory supported diagnosis of MS (3). An unexplained high frequency of combined intrathecal measles, rubella and varicella zoster virus (VZV) antibodies in CSF of patients with (MS) (4) or autoimmune diseases with involvement of the CNS (6) allows the diagnosis of a chronic inflammatory process (autoimmune type) at the time of the first clinical manifestation (1, 4). This is also important for diagnosis of cases with a monosymptomatic start of the disease like an optical neuritis or an uveitis intermedia and periphlebitis retinae (7).

It was a long way to understand that antibodies, which are synthesized in brain and in blood, are not only directed against a causative antigen (clonal selection) but also against other antigens, which are not involved in the cause of the disease. The first detection of intrathecal measles antibody synthesis (8) in multiple sclerosis (MS) led to the hypothesis of a virus aetiology of MS. Later observations showed a polyspecific antibody synthesis against rubella, varicella zoster, herpes simplex, mumps viruses (9) in the single MS-patient. Meanwhile there are also reports about the intrathecal synthesis of autoantibodies against ds-DNA, observed in a fraction of MS patients (4, 6).

It is the recognition of an immunological network (10), which gives the clue to understand the polyspecific, oligoclonal immune response.

The detection of intrathecal antibody synthesis in CSF has a long tradition. The linear Goldmann-Witmer-Index (11) frequently used in ophthalmology (GW-I= Qspec / QIgG) is improved by the corrected "Antibody-Index", AI (2), established in CSF analysis to avoid a false negative interpretation (7) in cases with a strong intrathecal IgG synthesis.

The AI presents a relative value for the quantity of intrathecally synthesized specific antibodies. With the invention of the measurement of absolute antibody concentrations (5) the evaluation of quantitative intrathecal antibody synthesis became possible. With an improved calculation of the specific antibody fraction in CSF, Fs, 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 QIgG, QIgA, QIgM with a nonlinear, hyperbolic discrimination function, QLim (2), 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 (Ref. in (1)), which lead to false interpretations in cases of a blood/CSF barrier dysfunction as demonstrated in detail (1).

The polyspecific, oligoclonal IgG response is seen in many chronic, acute and subacute inflammatory diseases of the CNS, but there are basic differences in quantity (Tab. 1) and the frequency of an intrathecally synthesized antibody species (4, 7).

In MS (4) as well as in autoimmune diseases with involvement of the CNS (6), i.e., in chronic inflammatory diseases, we observe a high frequency of measles-, rubella- and VZV intrathecal antibody synthesis among antibodies against other virotropic viruses or other microorganisms, like toxoplasma gondii (4), or chlamydia pneumonia (14). This frequency is not seen in any other acute or subacute chronic disease.

The frequency of measles antibodies in MS is 78%, for rubella 60%, for VZV 55% and for HSV 28%. In other acute or subacute inflammatory diseases (HSV-E, SSPE, neurosyphilis,

neurotuberculosis) we find the antibody response for these single species with the frequency below 5% and for a combination of two antibody species (e.g., measles and rubella) below 0.1%.

In MS patients we observed with an increasing intrathecal IgG fraction an increasing frequency of the combination of all three antibody species (measles, rubella, zoster = M, R, Z) compared with those patients who had a low intrathecal IgG response with only one or a combination of two of these three antibodies (4). With increasing intrathecal IgG synthesis there is also an increasing amount of antibodies produced for these MRZ species, indicated by an increasing AI (4).

With the invention of the quantitation, i.e., characterization of the intensity of the intrathecal antibody response (5) and the improvement of the calculation (7) for FS (Methods), we get new perspectives for detection of intrathecally synthesized antibodies, regarding its diagnostic as well as its pathophysiological relevance.

**Table 1. Mean intensity of the intrathecal virus-specific antibody synthesis in acute, subacute and chronic inflammatory diseases nof the CNS. Comparison of the Antibody-index (AI) and the specific intrathecal antibody fraction (FS) against the causative antigen in subacute sclerosing panencephalitis (SSPE), herpes simplex encepahalitis (HSV-E) and the Fuchs heterochromic cyclitis of the eye (FHC) besides multiple sclerosis (MS) with a polyspecific immune reaction against non-causal antigens.**

| | AB-Species | Fs(%) | | AI | |
|---|---|---|---|---|---|
| | | MV | (Range) | MV | (Range) |
| SSPE | Measles | 20 | 12-28 | 21 | 12-31 |
| HSV-E | HSV | 9 | 4-13 | 41 | 32-51 |
| FHC[1] | Rubella | 2.6 | 0.14-46 | 21 | 1.5-309 |
| MS | Measles | 0.5 | 0.04-4.4 | 3.3 | 1.5-46 |
| MS[2] | Rubella | 0.5 | 0.03-4.8 | 2.6 | 1.5-14 |
| MS | VZV | 0.23 | 0.06-4.9 | 3.4 | 1.5-15 |
| MS | HSV | 0.14 | 0.06-2.3 | 2.3 | 1.6-9.3 |

1 Data in the aqueous humor of the eye in the Fuchs heterochromic cyclitis (7)
2 Correspondingly, in MS patients with uveitis or periphlebitis the following data are found in aqueous humor: Rubella-AI = 3.0 (0.7-35); Rubella-FS = 0.06 (0.01-0.25)%

In Table 1 we compare directly the Antibody-Index (AI) with the specific antibody fraction (FS) for chronic, acute and subacute diseases. From these data we learn that also in acute or subacute diseases with a persistent causative antigen, only less than 30% of the intrathecally synthesized total IgG represents the specific antibodies (20% measles antibodies in SSPE, 9% herpes simplex antibodies in HSV-E, or 2.6% rubella antibodies in aqueous humor of the Fuchs heterochromic cyclitis (FHC) of the eye). But it is clear by these data that the intensity of antibody synthesis against the causative antigen is up to 60-fold higher than the intensity of the polyspecific antibody synthesis in a chronic inflammatory process, like MS (FS < 0.5%). The specific fraction, FS, allows a better discrimination between these both causes of the antibody response than the AI. AI is a relative value depending on the ratio of the amounts of the antibodies in CSF, which derive from blood and brain FS contributes an important diagnostic aspect to discriminate the polyspecific antibody synthesis from the antibody synthesis against a causative antigen. Nevertheless, for the general diagnostic approach, AI remains the most sensitive parameter to detect an intrathecal antibody synthesis.

As a second important information from these data in Tab. 1, we learn that also in case of a specific antibody response against the causative antigen the larger fraction of the intrathecally synthesized IgG represents a polyspecific antibody response against the non-causative antigens, which do not need the persistence of an antigen in the immune system (13).

As a particular clue of these investigations, e.g., about the rubella antibody synthesis in the eye of the FHC patients, we get a biological example for theoretical approaches (15), which try to explain the dynamics, which in the immune response can lead to a chronic inflammatory process.

With these approaches we are on the way to a new understanding of an immunological network-based immune response in chronic inflammatory diseases.

## 11.2 Methods

### 11.2.1 Oligoclonal IgG

According to the international consensus, oligoclonal IgG is detected with isoelectric focussing and immune detection (Andersson et al. 1994). As interpretation criteria the 5 types shown in figure 1 are accepted as state of the art.

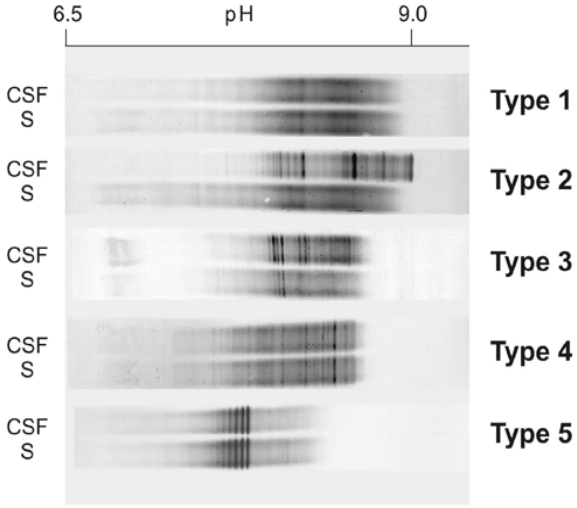


**Figure 1.** *Isoelectric focussing on agarose gels with immunoblot: The figure represents the classical types 1 - 5 (Andersson et al. 1994):*

· Type 1: No bands in CSF and serum.

· Type 2: Oligoclonal IgG-bands in CSF, not in serum.
Interpretation: Intrathecal IgG-synthesis.

· Type 3: Oligoclonal bands in CSF (like type 2) and additional identical oligoclonal bands in CSF and serum (like type 4).
Interpretation: Intrathecal IgG-synthesis

· Type 4: Identical oligoclonal bands in CSF and serum. Interpretation: No intrathecal IgG-synthesis but systemic immune reaction.

· Type 5: Monoclonal bands in CSF and serum. Interpretation: Systemic paraproteinaemia.

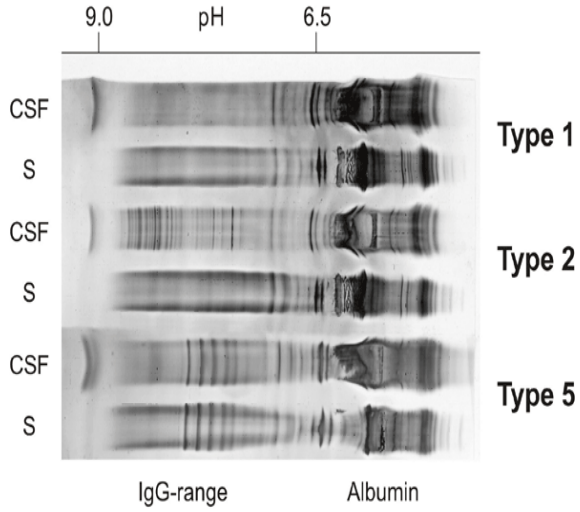


**Figure 2.** *Isoelectric focussing on polyacrylamidgel with silverstain: In contrast to the immune detection (Fig. 1) we find in the protein stain the albumin range (also place for application of the samples) at about pH = 5.0. In the alcaline range (at pH 9.3) we find cystatin C (gamma-trace-protein) as a single band in CSF (CSF marker).*

*The pH-range of the gradient starts on the right side with pH 3.5 (anode (+)) and reaches pH 10.5 (catode (-)).*
*In case of immunoblot (Fig 1) the pattern is inversed. The nitro-cellulose-acetate foil is attached beginning at pH 6.5.*

Figure 2 shows an isoelectric focussing with subsequent protein detection by silver stain. Both methods have the same sensitivity, but the silver stain is more capricious as an immune detection. Other electrophoretic techniques are not sufficient to identify olicoclonal IgG. It depends on the method how many bands (at least 2 bands) are necessary to detect reliably oligoclonal IgG. A single isolated band in CSF is per definition not oligoclonal IgG.

**Clinical sensitivity**

Oligoclonal IgG is more sensitive than the Antibody-Index (AI) against the specific antigen in case of chronic diseases (4), but in cases of acute inflammatory processes the Antibody-Index of the antibody species against the causative antigen is more sensitive than the oligoclonal IgG (16) e.g., in varicella zoster caused facial nerve palsy, with 100% increased Antibody-Index, only 50% of the patients had oligoclonal IgG detectable.

### 11.2.2 Antibody Index (AI)

**Definition of the (corrected) Antibody-Index (AI)**

AI = Qspec /QIgG (QIgG < QLim)

AI = Qspec /QLim (QIgG > QLim)

| | |
|---|---|
| Qspec = | AB(CSF) / AB(ser), specific antibody-CSF/serum quotient |
| QIg = | IgG(CSF) / IgG(ser), empirical immunoglobulin CSF/serum quotient for IgG, IgA or IgM |
| QLim = | upper hyperbolic discrimination line of the reference range for blood-derived immunoglobulins (IgG, IgA or IgM) |

**Reference range and interpretation**

| | |
|---|---|
| Method-related range of precision( x± 2s): *Clinically defined reference range* | AI = 1.0 ± 0,3 |
| Normal | AI = 0.7 - 1.3 |
| Intrathecal synthesis | AI = 1.5 |

Values of AI < 0.5 are an indication of non-matched CSF/serum samples or of analytical faults. Values reach a higher sensitivity by combined evaluation of several Antibody-Index values as shown in the three cases in the table, where in case I a rubella-AI = 1.4 is the clear indication of an intrathecal antibody synthesis with reference to the other normal Antibody-Index values. Less reliability is found in the case II with a rubella-AI = 1.5 compared to the three other high Antibody-Index values. Case III represents a typical combination of non-matched CSF/serum samples (in spite of repetition the measles-AI remains < 0.5).

**Table 2 Evaluation examples of the Antibody-Index (AI)**

| | Case I | Case II | Case III |
|---|---|---|---|
| Measles-AI | 0.8 | 1.2 | 0.2 |
| Rubella-AI | 1.4 | 1.5 | 1.1 |
| VZV-AI | 0.8 | 1.2 | 2.1 |
| HSV-AI | 0.7 | 1.1 | 0.7 |

### 11.2.3 Fraction of specific intrathecal antibodies in CSF.

The specific fraction, F, in % is the ratio of the intrathecally synthesized concentration of specific antibodies (ABLoc), and the intrathecally synthesized concentration of total IgG (IgGLoc). This calculation of F, for comparison of means in different groups, refers to Qmean, the mean function (12) of the reference range instead of the upper limit QLim used for AI: F= ABLoc(mean) / IgGLoc(mean) · 100 in %. With Qmean (IgG) = (0.65 (QAlb2 +8)0.5 - 1.4) · 10-3, we calculate IgGLoc (mean) = (QIgG - Qmean ) · IgG(ser) in mg/L or ABLoc (mean) = (Qspec - Qmean ) · AB(ser) in mg/L.
The antibody concentrations are determined as quantitative values in mg/l.

**Quantitation of IgG class antibodies in CSF and serum.** The absolute amount of measles-, rubella-, VZV- and HSV antibodies was measured with a modified ELISA,(5): The microtiter plate was divided into five stripes: coated with anti-Human IgG, measles antigen, rubella antigen, varicella zoster antigen and herpes simplex antigen, respectively.

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