INTRATHECAL HUMORAL IMMUNE RESPONSE IN CORONA VIRUS INDUCED ENCEPHALOMYELITIS OF LEWIS AND BN RATS

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INTRODUCTION

Dependent on the variant of virus, the age and the strain of animals intracerebral infection of rats with the murine Corona virus JHM results in different diseases, ranging from acute lethal encephalitis (AE) to subacute demyelinating encephalomyelitis (SDE) (Wege et al., 1982). The delayed type of disease, SDE, can be observed most frequently after infection of weanling Lewis rats with JHM wild type virus (JHM-WT) or suckling rats with TS 43, a temperature sensitive mutant of JHM (JHM-TS43) (Wege et al., 1983). Clinically, SDE is characterized by hindleg paralysis, ataxic gait and severe impairment of growth. Histopathological changes include plaques of primary demyelination in the brain and spinal cord and perivascular cuffs of mononuclear cells. Viral antigens are demonstrable in phases of clinically apparent disease (Nagashima et al., 1978, 1979; Sorensen et al., 1980; Koga et al., 1984). Most likeley T lymphocytes as effector cells contribute to the pathogenesis of SDE, since Watanabe et al. (1983) demonstrated that lymphocytes from SDE animals, stimulated in vitro with basic myelin protein and transfered to syngeneic animals cause perivascular cuffing in the brain of the recipients comparable to the typical histopathological changes in animals suffering from experimental allergic encephalitis (EAE).

In contrast to the Lewis rat, the Brown Norway (BN) rat, which is known not to be susceptible to EAE, almost never reveals clinical signs of an acute or delayed type of disease although marked demyelination in periventricular areas of the brain can be observed frequently. Viral antigen is seen up to several weeks past infection (p.i.) and plasma cell infiltrates dominate in demyelinated areas (Watanabe et al., in prep.).

The significant plasma cell infiltration in the context of a clinically inapparent infection in BN rats and the lack of these cells in demyelinated plaques of SDE diseased Lewis rats made it very likely that the analysis of the humoral immune response might give some clues on the role of antibodies in SDE. Therefore, serum and cerebrospinal fluid (CSF) specimens were taken from Lewis and BN rats 4 to 8 weeks p.i in order to examine the state of the blood brain barrier (BBB) and the JHM specific antibody response with respect to titers, site of synthesis and clonal distribution. The selected Lewis rats suffered from severe SDE after intracerebral infection with JHM-TS43, whereas the BN rats, although clinically healthy after infection with

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JHM-WT, exhibited small foci of demyelination in the central nervous system (CNS) at the time being sacrificed.

RESULTS

JHM-virus specific antibodies in serum and CSF

In a first approach virus-specific antibody titers were determined in serum and CSF from both rat populations by an enzyme immuno assay (EIA). The results are summarized in figures 1 and 2. Remarkable differences were detectable between Lewis and BN rats. In serum, BN rats revealed more frequently virus-specific antibodies (10/10) than Lewis rats (7/10) (fig.1) and in CSF, 8 out of 10 BN rats were detected with JHM specific antibody titers compared to only 4 animals out of 7 in the Lewis group (fig.2).

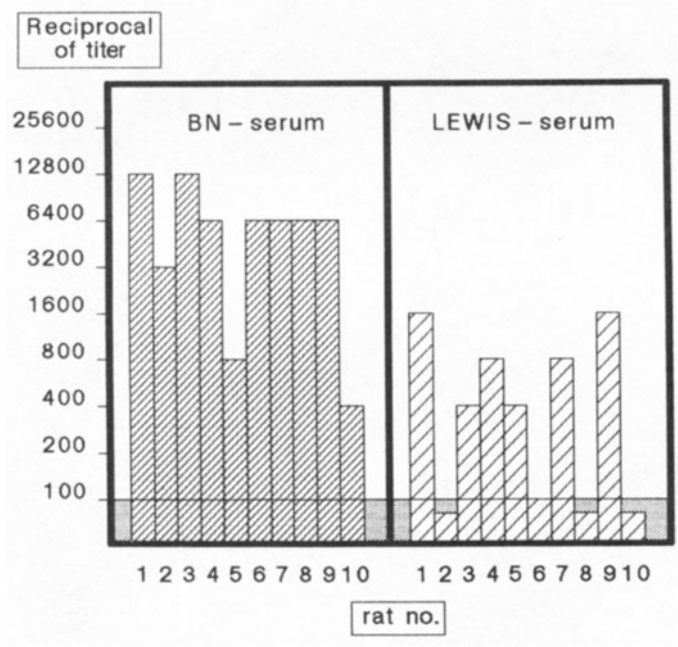

Fig. 1. JHM specific antibody titers in serum specimens from clinically inapparent BN and SDE diseased Lewis rats. An EIA as described by Wege et. al (1984) was used to titrate antibodies specific for JHM virus in serum specimens. The titer of a sample was determined graphically by extrapolation of the linear part of the titration curve to a cut-off line indicating a control antigen corrected absorbance at 496 nm of 0.2. The last dilution of the specimen closest to this intersection was defined as the titer. Titers lower than 1:100 were scored as negative (shaded box).

Additionally, JHM specific antibody titers generated by BN rats were clearly higher than titers found in Lewis rats (approx. 5 fold in serum and 4 fold in CSF). These findings strongly suggest that the virus specific antibody response is most likely of protective nature and not an immunopathological effector mechanism in JHM virus induced primary demyelination of the CNS.

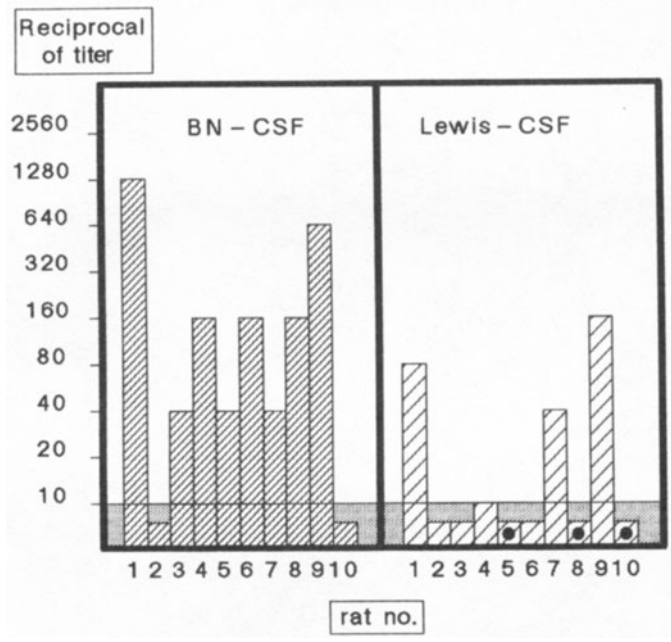

Fig. 2. JHM specific antibody titers in CSF specimens from clinically inapparent BN and SDE diseased Lewis rats. Methods see fig. 1. Titers lower than 1:10 were scored as negative (shaded box). Due to a shortage of CSF, titers could not be determined in rats 5, 8, and 10 (black dots). These CSF specimens turned out to be negative in affinity mediated immunoblot, a finding that usually corresponds to a titer below 1:10.

The appearance of JHM specific antibodies in the CSF of both rat populations led to the question for the site of synthesis of these antibodies. To decide wether JHM specific antibodies entered the CNS by diffusion or have been synthesized by CSF resident B Lymphocytes, specific antibody indices (SAB-indices) were calculated from the CSF/serum ratios of JHM specific antibodies and albumin (Arnadottir et al., 1982). The CSF/serum ratio of albumin is introduced into this calculation in order to control the permeability of the blood brain barrier (BBB). As a reference for normal diffusion of antigen specific antibodies from blood to CSF these indices were calculated in specimens sampled from rats which have been hyperimmunized intraperitoneally with JHM virus and unrelated antigens as Measles

virus and keyhole limpet hemocyanine(KLH). The results shown in figure 3 can be summarized as follows: JHM specific antibodies present in the CSF of SDE diseased Lewis rats as well as of clinically inapparent BN rats have been synthesized to a significant extent in the CSF. Especially BN rats revealed strongly enhanced SAB-indices up to 170 fold over the reference value.

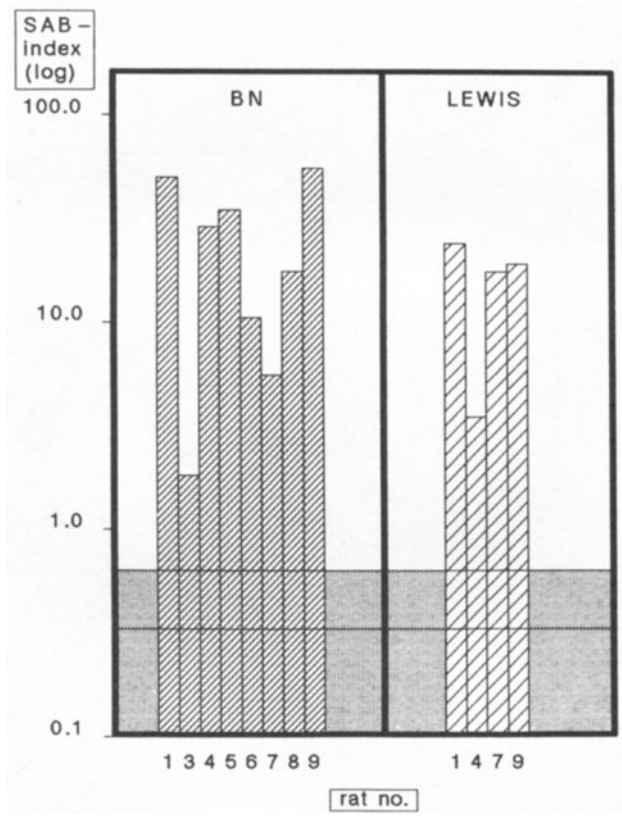

Fig. 3. JHM specific antibody indices (SAB) in clinically inapparent BN and SDE diseased Lewis rats. The shaded box represents the mean SAB-index (+ 2x the standard deviation) calculated from rats immunized intraperitoneally with measles virus, JHM virus and KLH.

State of the blood brain barrier and intrathecal Ig-synthesis

In order to evaluate the complex interactions between intrathecal synthesis of JHM specific antibodies and the CSF/serum ratio of total immunoglobulin (Ig) the CSF protein profile for albumin and immunoglobulin was analysed in a graph according to Reiber (1980) (fig. 4). Albumin concentrations in serum and CSF were determined by rocket immune electrophoresis and Ig concentrations by an EIA published previously (Dörries et al. 1986). In this two-dimensional graph each individual animal is represented by the intersection of the CSF/serum ratios of albumin (A) and immunoglobulin (I).

On the basis of 15 non-inoculated, healthy BN and Lewis rats a normal range forthese ratios was established by adding 2x the standard deviation to the mean value. Animals not included in this "normal box" can be grouped into 5 classes. Animals in area I are characterized by a disproportional increase of the BBB for small proteins like albumin. Area II contains animals revealing a proportional increase of the BBB for large and small proteins (Ig and

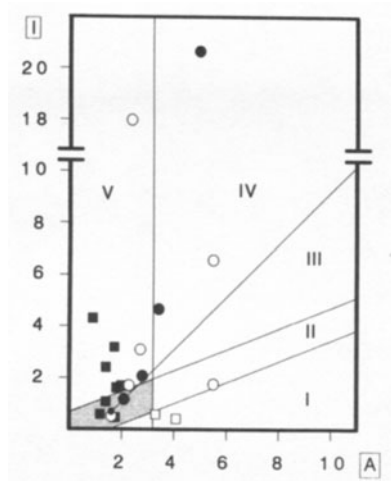

Fig. 4. CSF protein-profile of clinically inapparent BN and SDE diseased Lewis rats according to Reiber (1980). I = CSF/serum ratio x 1000 for immunoglobulin. A = CSF/serum ratio x 1000 for albumin. Black squares refer to BN rats revealing JHM virus-specific intrathecal antibody synthesis; open squares refer to BN rats lacking JHM specific antibody titers in the CSF. Black and open circles refer to the same properties in the Lewis rat group. For explanation of I-V see results.

albumin). Area III either indicates a disproportional increase of the BBB permeability for immunoglobulins or intrathecal Ig synthesis in the context of an increased leakiness of the BBB for albumin. The line separating area III and IV theoretically represents a BBB after total loss of its capability to control protein diffusion into the CNS indicated by identical CSF/serum ratios for Ig and albumin.

Thus, animals falling in area IV show intrathecal synthesis of Ig accompanied by an increased BBB permeability for albumin, whereas animals in area V reveal intrathecal Ig synthesis and no disturbance in the BBB permeability for small proteins. The linkage of this type of analysis with the data concerning the intrathecal synthesis of JHM specific antibodies gave some interesting results. (1) SDE diseased Lewis rats suffered slightly more often from increased BBB permeability than BN rats, most likely due to their severe encephalomyelitis. (2) Although the frequency of intrathecal Ig synthesis was almost the same in both populations one should note that 8/10 BN rats have shown to synthesize JHM specific antibodies intrathecally but only 5 of them displayed an increased CSF/serum ratio for total Ig. It seems as if a clinically inapparent state and a normal CSF protein profile does not exclude a significant agent specific intrathecal antibody production. (3) Looking into the Lewis rat group 4 rats were detected with increased CSF/serum ratio for total Ig but no detectable virus-specific antibody production, a phenomenon usually not detected in BN rats. To date the antigenic specificity of these immunoglobulins is unknown but it cannot be ruled out that during the course of a clinically severe SDE antibody reactions of autoaggressive nature are induced.

Isoelectric patterns of CSF derived immunoglobulins and JHM-specific antibodies

Local synthesis of high titer antibodies in the CSF of our rats carrying virus as well as unknown antigen-specificity gave reason to ask for the electrophoretic distribution of CSF and serum derived immunoglobulins. On the basis of a technique published earlier (Dörries and ter Meulen, 1984) the isoelectric pattern of virus specific as well as of total Ig was developed in small aliquots of unconcentrated CSF and the corresponding serum specimen. Briefly, identical amounts of CSF and serum derived total Ig were focused isoelectrically in an agarose gel. Immediately after electrophoretic separation of the proteins, a print was taken from the gel by nitrocellulose filters, coated with either rabbit-anti-rat Ig (Rab-a-Rat-Ig) or with JHM virus (EIA grade). The pattern of total Ig on the Rab-a-Rat-Ig coated filter and of JHM-specific antibody clones bound to the viral antigen coated filter, was detected by incubation of both filters with rabbit-anti-Rat Ig labeled with horseraddish peroxidase (Rab-a-Rat-Ig-POD). Filters were developed in 4-chloro-naphthol, a colourless substrate which is converted by the secondary antibody bound peroxidase to a water-insoluble blue-violet precipitate, thereby indicating the isoelectric distribution of either total Ig (Rab-a-Rat Ig coated filter) or JHM-specific Ig (JHM antigen coated filter).

Examples of this technique displaying the typical picture of CSF specimens from SDE Lewis rats and clinically healthy BN rats are shown figure 5. In Lewis rats the intrathecal virus-specific antibody response was always of oligoclonal nature (fig. 5, track 1). Corresponding tracks of total Ig indicated that not all Ig bands visible were necessarily of JHM specificity (fig 5, track 2) and in some rare cases Lewis rats were detectable with restricted isoelectric distribution of Ig even in the total absence of JHM virus-specific clones (fig 5, tracks 3 and 4), supporting the idea that presumably B-lymphocytes with specificity for autoantigens are induced during severe SDE. In contrast, CSF specimens of BN rats revealed vigorous JHM specific antibody synthesis in form of a broad isoelectrical pattern containing clusters of oligoclonal bands (fig 5, track 5), a finding in good agreement with the fact that these animals did rise high titers of JHM specific antibodies. Although in these cases individual Ig bands were difficult to assign to distinct virus-specific clones (fig 5, track 6) it is obviously that most if not all immunoglobulin carried specificity for JHM antigens. The weak or mostly absent staining of JHM specific bands in the paired serum samples (data not shown) diluted to the same Ig concentration

as seen in CSF strengthened the observation that JHM specific antibodies have been synthesized locally.

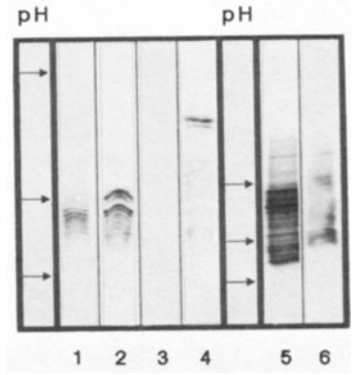

Fig. 5. Isoelectric distribution of total Ig and JHM specific antibodies in selected CSF specimens of clinically inapparent BN and SDE diseased Lewis rats. Track 1: JHM-specific antibodies rat no.7. Track 2: Total Ig rat no.7. Track 3: JHM-specific antibodies rat no.5. Track 4: Total Ig rat no.5. Track 5: JHM-specific antibodies rat no.1. Track 6: Total Ig rat no.1. Black arrows indicate the measured pH gradient from bottom to top: pH 5.2, 6.6 and 8.2.

SUMMARY AND DISCUSSION

The intrathecal humoral immune response and the state of the blood-brain-barrier were analysed in Lewis and BN rats after intracerebral infection with the murine Corona virus JHM. The selected animals were of particular interest with respect to their immunological make up, since the outcome of an intracerebral JHM infection differs significantly between the two rat strains. Lewis rats often suffer from a severe subacute demyelinating encephalomyelitis accompanied by relevant clinical symptoms as paralysis of the extremeties (Wege et al., 1982), whereas BN rats in general remain clinically healthy, although significant periventricular demyelination is seen in the brain (Watanabe et al. in prep.). Like in EAE involvement of an autoagressive cellular immune response with specificity for MBP in the pathogenesis of SDE seems very likely in Lewis rats (Watanabe et al., 1983), a phenomenon which is never seen in the EAE resistent BN rat strain. Since much less is known about the humoral immune response in these two rat strains after intracerbral infection with JHM virus a series of experiments was carried out to gain more information about the importance of antibodies in JHM virus induced demyelination.

Titration of JHM specific antibodies in paired serum and CSF samples by EIA and calculation of specific antibody indices revealed that Lewis and BN rats respond with the intrathecal synthesis of virus-specific antibodies upon intracerebral infection. Comparable findings have been made by Sorensen et al (1984), who noticed intrathecal synthesis of virus-specific antibodies in Lewis-Wistar rats after intracerebral infection with Corona virus JHM.

However, marked differences between SDE diseased Lewis rats und clinically inapparent BN rats were noticable: Frequency as well as titers of JHM-specific antibodies were clearly higher in the BN rat group. This finding makes it very unlikely that virus-specific antibodies contribute to the pathogenesis of SDE. On the contrary, the presence of high titer antibodies in the clinically healthy BN group argues strongly for a protective function of the antibody response. An intermediate agent specific antibody response in diseased animals as described here, has also been noticed in another animal model of primary virus-induced demyelination (Krakowa et al., 1975), supporting the idea of the non-pathological nature of the virus-specific response in this type of disease.

The analysis of the blood brain barrier with respect to CSF/serum ratios of total immunoglobulin and linkage of these data to presence or absence of a virus-sepecific, intrathecal antibody response resulted in two interesting observations: (1) Intrathecal synthesis of JHM specific antibodies is not correlated to an overall increase of the CSF/serum ratio of total immunoglobulins. High titer antibodies with specificity for JHM virus could be noticed in BN rats with a perfectly controled BBB and a clinically healthy state of the animal. Obviously, the amount of virus-specific antibodies synthesized in these animals is not always high enough to be reflected in an increase of the total immunoglobulins, an observation well known from virus-induced complications in the CNS of men (Ukkonen et al., 1981). (2) Absence of JHM-specific antibodies but increased CSF/serum ratios for total Ig is usually not seen in the BN rat population but happens in SDE diseased Lewis rats. Although the antigenic specificity of these intrathecally synthesized immunoglobulins is not identified one could assume that, additionally to MBP-specific T cell clones, B-lymphocyte clones with specificity for autoantigens might be induced in the course of a severe disease like SDE. This is not unlikeley, since in canine distemper virus induced demyelination of dogs, myelin specific autoantibodies have been identified (Koestner and Krakowa, 1977; Vandevelde et al., 1986).

Examination of the isoelectric distribution of virus-specific antibodies and total immunoglobulin in CSF and serum specimens revealed that Lewis and BN rats respond with JHM-specific antibody clones of restricted heterogeneity, a phenomenon detected very frequently in virus-induced disorders of men. The distinct differences between BN and Lewis rats with respect to frequency and intensity of JHM specific antibody responses as measured by EIA, were reflected perfectly in the electrophoretic antibody patterns. Lewis rats usually were characterized by a low number of JHM-specific bands with faint staining intensity. BN rats most often were detected with a broad pattern of virus-specific antibodies including 2 or 3 clusters of strong staining oligoclonal bands again supporting the idea that the intense production of agent-specific antibodies will protect the animal from getting diseased clinically. A possible mechanism of protection by JHM-specific antibodies has to be discussed in view of recent findings of Massa et al (1986) who demonstrated in vitro induction of class II antigens on rat astrocytes by UV-inactivated JHM virus, an event which was prevented by neutralizing monoclonal antibodies directed to the E2 peplomerprotein of JHM. Since expression of Ia on epithelial cells seems to play an important role in the induction of autoimmunity (Bottazzo et al., 1984; Todd et al., 1986), JHM specific antibodies may prevent to a certain extent the induction of autoaggressive immune reactions in the CNS by neutralization of virus particles.

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