Página principal

Presentación

Equipo directivo y comité científico

Información para los autores

INDICE

Revistas Anteriores

Envio de artículos

Enlaces a revistas médicas

Congreso virtual de neurología

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Anti-Human Herpes Virus 6 Type B Antibodies Make Up the Oligoclonal Bands in Multiple Sclerosis.

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RESUMEN: Antecedentes: Existe evidencia epidemiológica considerable de que una infección viral latente con virus herpes simple tipo 6 esté asociada con el desarrollo de esclerosis múltiple. Sin embargo, las técnicas para determinar anticuerpos contra dicho virus en LCR han sido elusivas. Resultados preliminares de la elaboración de dichas técnicas fueron presentadas en la segunda conferencia internacional de herpesvirus humanos 6, 7 y 8 (Italia, Pisa, Mayo 8-11, 1997). Métodos: Adaptamos la prueba de ELISA para determinar anticuerpos contra virus herpes simple tipo 6 en LC.R. Estudiamos dichos anticuerpos en el LCR de dos poblaciones de enfermos: pacientes con esclerosis múltiples y un grupo control que incluyó pacientes con enfermedades neurológicas que no sean esclerosis múltiple. Se planteó la hipótesis que dichos anticuerpos se encontraban en el LCR de los pacientes con esclerosis múltiple pero no en los del grupo control. Resultados: Demostramos que anticuerpos del tipo IgG contra herpes simple tipo 6 se encontraban en las bandas oligoclonales de los pacientes con esclerosis múltiples pero no en los controles. Conclusiones: la significancia de nuestro hallazgo nos permite concluir que: i) es posible establecer con mayor certeza el diagnóstico de esclerosis múltiple en estos casos, ii) se fortalece la hipótesis de un rol causal del herpes virus tipo 6 en la esclerosis múltiple y iii) se vuelve plausible la idea de combatir la esclerosis múltiple con drogas que destruyan el herpes virus tipo 6.

ABSTRACT: Background: There is considerable Oepidemiological evidence that a latent or slow infection with human herpes virus 6 is associated with the etiology of multiple sclerosis. However, techniques for detecting anti-human herpes virus 6 antibodies in the cerebrospinal fluid had been lacking up to the time of this study. Preliminary results were presented at the Second International Conference on Human Herpesviruses 6, 7 and 8 (Italy, Pisa, May 8-11, 1997). Methods We therefore adapted the enzyme-linked immunosorbent assay in order to make this determination.

Cerebrospinal fluid was examined in two population samples: multiple sclerosis patients and a control group who had a disease other than multiple sclerosis. It was established that IgG antibodies to human herpes virus 6 type b were present in the cerebrospinal fluid of all of the multiple sclerosis patients but in none of the controls. Results with the adapted assay, we were also able to show that in the multiple sclerosis patients the nidus of the anti-human herpes virus 6 type b antibodies is the oligoclonal bands in the cerebrospinal fluid and that these bands contain no other antibodies. Conclusions The significance of this discovery is at least three-fold: i) it may now be possible to make firmer diagnoses of multiple sclerosis; ii) the hypothesis of a causal role of human herpes virus 6 type b in multiple sclerosis is strengthened, so that; iii) the idea of combating multiple sclerosis by developing drugs that target this virus becomes more plausible.

In Flanders (Belgium), multiple sclerosis (MS) is widespread. In fact, its prevalence is about 87.9 per per 100,000 inhabitants. The possibility that viral infection plays a role in MS is supported by data suggesting that an as yet undetermined childhood viral exposure somehow induces a susceptibility to the disease. These findings emerged from various migration and epidemiological studies. Viral infections may induce an exacerbation of MS and some MS patients have inappropiate immune responses to certain viruses, presenting higher antibody titers than controls against measles, herpes simplex, varicella, rubella and Epstein-Barr virus, as determined in the serum and CSF.

Human herpes virus 6 (HHV-6) causes exanthem sabitum (or roseola infantum), a benign disease and small children. Epidemiological data confirm that a substantial percentage of childrem become infected with HHV-6 by the age of 1-2 years. The infection is most probably transmitted via saliva the agent is readily detected in this fluid and the salivary glands. Recently, the possible role of HHV-6 type b (HHV-6b) specifically in MS has been investigated. In particular, Challoner et al established that HHV-6 was present in the oligodendrocyte nuclei of MS patients but not of controls.

The point of departure of our work was the circumstance that although epidemiological evidence pointed clearly to the implication of HHV-6 in MS, the technical limitations of current detection methods posed a severe obstacle to investigation of the CSF. Our answer to this problem was to adapt the enzyme-linked immunosorbent assay (ELISA) (Biotrin, Ireland), thereby opening up to research the role of HHV-6 in MS. In the light of the above-noted findings on the association of HHV-6 with the etiology of MS, we reasoned that the corresponding antibodies should be present in the CSF of persons with the disease and possibly these persons only. We therefore set out to examine, in a prospective study, the CSF of MS patients and of (non-MS) controls, which was now possible owing to the adapted ELISA. In addition, in a later phase of our investigation we decided it would be useful to determine whether the nidus of these antibodies, and these antibodies alone, was the oligoclonal bands; such a finding could increase current diagnostic power in MS. Finally, given the above-noted exacerbating effect of viruses other than HHV-6 in MS, we wanted to examine the CSF of MS patients and controls for antibodies to these pathogens. We reasoned that since the blood-brain barrier is defective in MS, the titers of these antibodies might be higher in this population.

METHODS

This study was carried out on two population samples: MS patients, and patients with a disease other than MS, who served as controls. There were out-patients and inpatients in both groups. Out-patients were approached to participate in the study in the order in which they presented, and in-patients in the order in which we happened on them in the hospital. The MS profiles fell

into three categories: chronic progressive, relapsing-remitting and first flare-up. At least one lumbar punction (LP) had been performed in each patients entering in the first two categories, whereas no LP had been carried out in the third category of MS patients. The investigator who perform the LP was different from the investigator who perform the CSF analyses, and in all but two of the 78 patients under study the former did not communicate any medical information whatsoever to the latter. The controls were randomly selected among patients for whom an LP was required for their condition. A local ethics committee approved the investigation and ruled that to be admitted, a patient had to fully understand its nature and purpose.

To be entered to the MS group patients had to meet the following diagnostic criteria: history of clinical symptoms; a Kurtzke expanded disability status scale score of at least 1.0; presence of one or more oligoclonal band in the CSF on IEF. These patients were recruited from the Multiple Sclerosis and Rehabilitation Center in Overpelt (Belgium). The control group were recruited from various Belgian hospitals from among patients who had a disease other than MS. To be entered, these patients had to be negative for MS, although they were allowed to present one or more oligoclonal bands, and in addition, either MS clinical symptoms or a Kurtzke expanded disability status scale score of at least 1.0.

Two assays were carried out on each CSF sample. First, an ELISA was done to identify CSF antibodies and to determine their concentration. For this technique, the CSF antibody concentration was considered adequate. The optical density (OD) was automatically calculated from the microplate results. ODs below 0.200 were considered as indicating that no antibodies were present (diagnostic cut-off). This criterion is also applied for the results of dosing techniques used in serology and endocrinology. In the ELISA, antibodies of the IgG class, when present, combine with HHV-6b antigen attached to the polystyrene surface of the microwell test strips. Residual CSF is removed by washing and peroxidase-conjugated anti-human IgG was added. The microwells were washed and a colorless substrate system (tetramethylbenzidine/hydrogen peroxide) was added. The substrate was hydrolyzed by the enzyme and the chromogen turns blue. After the reaction was stopped with acid, the tetramethyl-benzidine turned yellow. The concentration of anti-HHV-6b antibodies in the test sample was proportional to the intensity of the color and this intensity was, in turn, proportional to the OD of the antibodies. As controls, we used serum with 36 IU antibodies/ml diluted 100-fold and also a CSF with an OD > 1.000.

The second test performed on each CSF sample was an IFA, which was carried out after a five-fold dilution. In this system the indirect immunofluorescent method of antibody detection and titer determination was used. Patient CSF sample were incubated with immobilized HHV-6b antigen and stabilized on a glass slide. If anti HHV-6b IgG antibodies were present in the sample, a stable complex formed with the antigen. Bound antibody was then reacted with a fluorescein-conjugated goat anti-human IgG and this complex was visualized with the aid of a fluorescence microscope. A positive antibody reaction was indicated by bright green fluorescence at the antigen sites. As controls, we used the same samples as in the ELISA.

These two test techniques were applied in triplicate in the same laboratory on the same day. In addition, for each CSF sample the protein and IgG concentrations were determined and the intrathecal production of HHV-6b IgG antibodies was confirmed.

The literature states that in the normal population the ratio of a mean concentration of IgG in the CSF to a mean concentration of IgG in the serum is 1/289. Such a low concentration in both MS patients and persons with a disease other than MS exclude false positive results.

Up to the time of the study, the Biotrin technique was used only for serum analyses, for which Biotrin recommends a 100-fold dilution of the serum. If we had used the Biotrin technique on CSF, we would have had to concentrate the CSF by a factor of 3 in order to bring it to the level recommended by Biotrin for serum, but that is technically impossible with the ELISA. We therefore used unconcentrated CSF for the ELISA, and despite Biotrin´s recommendation, managed in this way to determine the OD of the anti-HHV-6b antibodies in the CSF, as validated by the diagnostic index.

With the IFA, on the other hand, concentrating is possible; with the AMICON filter, this can be done 5-fold or a multiple thereof. Accordingly, we concentrated the CSF 5-fold and obtained a concentration of 1/60, which approximates the 100-fold dilution recommended by Biotrin. We were thus able to detect whether there were anti HHV-6b antibodies in the CSF.

In a second phase of the investigation, after the oligoclonal bands had been isolated by IEF, we used the ELISA to calculate the OD of the anti-HHV-6b antibodies making up these bandsl. This determination was carried out only on patients for whom the OD of the CSF was $\geq$ 0.800. The reason for the cut-off was that diluting CSF of a lower OD would have resulted in an inadequate volume of supernatant. In this application of the ELISA, after focusing and before fixation, oligoclonal bands on the agarose gel were removed with a scalpel, subsequently submerged in 1.5 ml physiological saline (9.0 g NaCl/l, pH 5.9) and incubated for 2 hours at 37°C with regular stirring at 5-minute intervals. After centrifugation of the CSF, the ELISA was carried out on the supernatant.

The eluate of the agarose gel (pH 7) was used as a control. Each agarose gel was filled with 8 CSF samples from each patient, so that a total of 120 **ul** of CSF was required (8 times 15 **ul**).

RESULTS

A total of 46 MS patients were recruited (mean age 38.0 yrs, range 25 – 64). There were 31 women and 15 men. The mean Kurtzke expanded disability status scale score for the group was 2.7 (range 1.0-3.5) and isoelectric focusing (IEF) disclosed a mean of 4.7 oligoclonal bands per patient (range: 1-15). The MRI yielded a mean of 7.1 lesions per patient (range 5-11). In all, 28 controls were entered to the study (mean age 43.9 yrs, range 20-67). There were 18 women and 10 men. In this group, the diseases broke down as follow: 20 patients-radiculopathy, for which a LP was performed for myelography; 3 patients-cerebellar degeneration; 2 patients-Friedreich´s ataxia; 1 patient-amyotrophic lateral sclerosis; 2 patients-rheumatoid arthritis. No control patient presented oligoclonal bands, 6 patients had two or more anomalies on clinical neurological examination, and for these patients the mean Kurtzke expanded disability status scale sore was 4.8 (range: 3-6). In both study groups each patient presented an IgG formation in the CSF. In the MS group the mean IgG concentration was 8.6 mg/dl (range 1.4-19.7) (Table 1) vs. 2.0mg/dl in the controls (range 0.5-5.6) (Table 2).

There was a higher production of IgG antibodies to HHV-6b in the serum of MS patients (mean 25.2 IU/l) than in that of the controls (mean 18 IU/l).

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Tabla 2. Analysis of cerebrospinal fluid by IEF, ELISA, IFA in a control group of patients who had various neurological diseases other than MS (n=28).

Patient no.	IEF (no. Of) bands	IgG (mg/dl)	IFA	ELISA (OD)	Age (yrs)	Disease
1	0	1.4	negative	0.035	30	N
2	0	0.5	negative	0.135	20	N
3	0	2.8	negative	0.142	48	N
4	0	0.7	negative	0.072	43	CD
5	0	0.5	negative	0.117	66	N
6	0	2.5	negative	0.080	58	RA
7	0	1.0	negative	0.000	36	N
8	0	2.2	negative	0.128	62	N
9	0	1.9	negative	0.078	47	F
10	0	2.5	negative	0.041	70	N
11	0	2.7	negative	0.170	45	F
12	0	2.6	negative	0.080	38	N
13	0	2.8	negative	0.113	48	ALS
14	0	2.5	negative	0.096	60	N
15	0	2.7	negative	0.128	67	N
16	0	1.1	negative	0.090	40	RA
17	0	0.7	negative	0.050	48	CD
18	0	1.6	negative	0.184	45	N
19	0	1.4	negative	0.096	32	N
20	0	2.2	negative	0.025	50	N
21	0	5.6	negative	0.124	49	N
22	0	4.0	negative	0.097	32	N
23	0	1.0	negative	0.048	28	N
24	0	1.9	negative	0.194	36	CD
25	0	1.9	negative	0.167	32	N
26	0	2.0	negative	0.135	43	N
27	0	1.7	negative	0.125	21	N
28	0	1.6	negative	0.075	35	N

N: no central neurological disease. **CD:** cerebellar degeneration; **RA:** rheumatoid arthritis; **F:** Friedreich's ataxia; **ALS:** amyotrophic lateral sclerosis; **IEF:** inmunofluorescent assay; **ELISA** enzymelinked inmunosorbent assay.

The immunofluorescent assay (IFA) was positive for all MS patients and negative for all controls. In the MS group the mean OD of the antibodies to HHV-6b in the CSF as determined by the ELISA was 0.475 (range: 0.228-2.206) (Table I) vs. 0.101 (range: 0.000-0.194) in the controls (Table 2). The repetability of this measurement was high (SD: 0.039)

A diagnostic index was calculated so as to validate the ELISA and IFA results. This index is defined by the following expression: S (Sensitivy) + SP (Specificity), where S=TP/(TP+FN) and SP=TN/(TN+FP), where in turn TP=true positives, FN=false negatives, TN=true negatives and FP=false positives. For a resultto be valid the value of the diagnostic index has to lie within a range of 1 to 2. For both the ELISA and the IFA, the sensitivy was 1.0, the specificity 1.0 and the diagnostic index 2.0, so that the results for these parameters were valid.

In the MS patients antibodies to viruses other than HHV-6b were present in the CSF as follows: against measles 41.3%, against mumps 47.8%, against rubella 34.8% and against varicella 39.1% (Table 3). In 15 of these patients antibodies were present against two viruses, in 6 patients against three viruses and in 4 patients against four viruses. In the controls, no antibodies were detected against measles, mumps, rubella or varicella.

In the second phase of the study, the oligoclonal bands in the alkaline phase of the CSF of the MS patients were found to consist entirely of antibodies against HHV-6b. Antibodies against other viruses, like measles, mumps, rubella and varicella, were found in the acidic phase of the CSF. A total of 6 patients met the criterion of a CSF OD$\geq$0.800 for the determination of the OD of the isolated oligoclonal band with the ELISA, and the mean OD obtained was 0.360.

Tabla 3. Presence of antibodies in the cerebrospinal fluid of multiple sclerosis patients (n = 46)

Patients no.	Measles	Mumps	Rubella	Varicella
1	+	+	-	+
2	-	-	+	-
3	-	-	-	-
4	+	+	-	-
5	+	-	+	-
6	-	-	-	-
7	-	-	-	-
8	+	+	+	+
9	-	-	-	-
10	+	+	+	-
11	+	-	+	-
12	-	-	-	-
13	+	-	+	-
14	-	-	-	-

15	-	+	-	+
16	+	+	-	-
17	+	+	-	-
18	-	+	-	-
19	-	-	+	+
20	-	-	-	-
21	-	+	+	+
22	-	-	-	-
23	+	-	-	+
24	-	+	-	-
25	-	+	+	-
26	+	+	+	+
27	-	+	+	-
28	+	-	-	-
29	-	-	-	-
30	+	-	+	+
31	-	-	+	+
32	-	+	-	+
33	+	+	+	+
34	-	+	-	-
35	+	-	-	+
36	-	-	-	+
37	-	+	-	-
38	+	+	+	-
39	-	+	-	-
40	+	-	-	+
41	-	-	-	-
42	-	-	-	+
43	+	+	-	+
44	-	-	-	+
45	+	+	+	+
46	-	+	-	-

DISCUSSION

Using various techniques, a number of researchers have detected human herpes virus-6 in MS patients. The percentages of patients in question were considerably iower than 100%. In our study, however, antibodies against HHV-6b were found in 100% of the MS patients. A possible explanation of the presence of antibodies against HHV-6b in the totaly of the MS patients is that our adaptation of the ELISA made it possible to detect antibodies against the total HHV-6b rather than an epitope or a particle of the virus. The strength of our techniques is that it can be carried out in living persons and that is more sensitive to viral activity than, for example, the polymerase chain reaction. With the latter and other direct techniques, many viruses go undetected since one does not know if they are to be found in the nerves, between the nerves, in the cerebrum, etc. On the other hand, antibodies always circulate freely in the CSF, so that they are much more accessible than viruses. Furthermore, antibodies are a firm index of viral activity, whereas a virus by itself is not, witness Challoner's post mortem finding of HHV-6 in more than 70% of the brain specimens from non-MS controls. In the MS group the virus was found in the oligodendrocytes, which produce myelin, whereas in the controls it nestled in other parts of nervous system. It is quite likely, then, that in persons without MS, HHV-6b is a commensal organism, whereas in MS patients it is activated and therefore aggressive. This difference in functioning remains a mystery.

Although other viruses may be involved in the pathogenesis of MS our finding that the oligoclonal bands in multiple sclerotic CSF is made up exclusively of HHV-6b antibodies, together with the findings of Challoner et al and those reported in numerous publications on the associations between MS and HHV-6, strongly suggests that HHV-6b is a privotal aggressor of the central nervous system. There is now sufficient evidence to justify the design of drugs that protect the CNS against viral aggression and to kill HHV-6b.

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REFERENCES

1. van Ooteghem P, D'Hooghe MB, et al. Prevalence of Multiple Sclerosis in Flanders, Belgium. Neuroepidemiology 1994; 13:220-225.
2. Alter M, Leibowitz U, et al. Risk of múltiple sclerosis related to age at immigration to Israel. Archives of Neurology 1966; 15:234-237.
3. Levy JA, Ferro F. Frequent isolation of HHV-6 from saliva and high seroprevalence of the virus in the population. Lancet 1990; 335:1047-1050.
4. Oksenberg JR, Hauser SL. Phatogenesis of Multiple Sclerosis: Relationship to Therapeutic Strategies in Multiple

Sclerosis. Advances in Clinical Trial Design, Treatment and Future Perspectives. Goodkin DE and Rudick RA (eds). Springer Verlag London 1996; 17-46.
5. Perron H, Lalande B. Isolation of retrovirus from patients with MS. Lancet 1991; 1:862-863.
6. Kurtzke JF. Epidemiological evidence for multiple sclerosis as an infection. Clinical Microbiological Reviw 1993; 6:382-427.
7. Okuno T, Tatahashi K. Seroepidemiology of human herpes virus 6 in normal children and adults. Journal of Clinical Microbiology 1989; 27:651-653.
8. Sada E, Yasukawa M. Detection of HHV-6 and HHV-7 in the submandibular gland, parotid gland and lip salivary gland byPCR. Journal of Clinical Microbiology 1996; 34:2320-2321.
9. Declercq P. Medische Biochemie 1993; Catholic University of Leuven, Belgium.
10. Challoner PB, Smith KT, et al. Plaque-associated expression of human herpes virus 6 multiple sclerosis. Proceeds of the Natural Academy of Sciences 1995; 92:7440-7444.
11. Haahr S, Sommerlund M. Is MS caused by dual infection with a dual infection with retrovirus and Epstein-Barr virus? Neuroepidemiology 1992; 11:299-303.
12. Jacobson S, Flerlage ML, et al. Impaired measles virus specific cytotoxic T-cell responses in multiple sclerosis. Journal of Experimental Medicine 1985; 162:839-850.
13. Sumaya CV, Myers LW. Increased prevalence and titer of EBV antibodies in patients with MS. Annals of Neurology 1985; 35:371-377.

Wilborn F, Schmidt CA. A potential role for human herpes virus 6 in nervous system disease. Journal of Neuroimmunology 1994; 49:213-214.