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Circulating antibodies directed against conjugated fatty acids in sera of patients with multiple sclerosis

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Abstract

Using an adapted ELISA assay, we have tested sera from multiple sclerosis (MS) patients for antibodies directed against ten fatty acids conjugated to bovine serum albumin. In serum samples from 68 MS patients and 20 patients suffering from rheumatoid arthritis (RA), a significant antibody titer elevation to the ten tested fatty acids was found when compared to sera of 40 healthy subjects and 82 patients with other neurological and autoimmune diseases. G-200 purified IgM of MS patients reacted specifically with the aliphatic chains with an avidity of 3×10^{-7} M. These results suggest that in MS and RA, autoepitopes on cell membranes that are normally hidden from the immune system become immunogenic. This may arise because of previous membrane disruption by oxidative processes.

Keywords: Fatty acid hapten; Circulating IgM; Multiple sclerosis; Rheumatoid arthritis

1. Introduction

Multiple sclerosis (MS) is an autoimmune disease of unknown etiology, involving environmental factors, genetic susceptibility and immune dysfunctions (Poser, 1992). MS is characterized at the early stage by a perivascular infiltration of the central nervous system by T and B lymphocytes and macrophages leading to a patchy demyelination (Adams, 1977; Raine, 1991). Oligodendroglia is the cellular target and macrophages are the most probable effector cells (Prineas, 1985). However, the detailed mechanism of the demyelination process remains unknown (McFarland, 1995).

B and T cell responses directed against the myelin components have been identified in the spinal fluid and serum of MS patients. Peripheral T lymphocytes respond to myelin proteins, such as myelin oligodendrocyte glycoprotein, myelin basic protein, proteolipid and myelin associated glycoprotein (Kerlero de Rosbo et al., 1993) and to a small heat shock protein expressed only in MS lesion (van Noort et al., 1995). Circulating antibodies are directed against myelin proteins, i.e. myelin basic protein (Bernard et al., 1983), and myelin oligodendrocyte glycoprotein (Sun et al., 1991). Circulating antibodies directed against gangliosides (Stevens et al., 1992) recognized the carbohydrate determinant. Since 75% of the dry weight of myelin is constituted by lipids (Stoffel, 1990), we hypothesized that B cell immune responses might also target the fatty acid moiety.

In the present study, we have screened sera from MS patients against a panel of ten fatty acids conjugated to a carrier protein. We compared the sera of 68 MS patients with those of 40 healthy subjects and 82 patients suffering from other inflammatory autoimmune and neurological disorders.

2. Materials and methods

2.1. Patients

Informed consent was obtained from patients to be tested according to current French law and medical guidelines. Very few of the MS patients were receiving steroid treatment; most were being treated with azathioprine.

All MS patients were classified as definite, according to established criteria (Poser et al., 1983) on the basis of clinical and laboratory data. Patients were assigned to one

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of four groups according to the evolutional course of their disease:

- *relapsing-remitting form in acute relapse* (RRMS-AR, $n = 9$), with a neurological dysfunction lasting for more than 24 h and less than 6 months;
- *relapsing-remitting form in remission* (RRMS, $n = 17$), i.e. between two relapses;
- *primary chronic progressive form* (CPMS, $n = 18$), with neurological deterioration without a previous relapsing-remitting pattern over a period of more than 6 months since the onset of the disease; and
- *secondary slowly progressive form* (SPMS, $n = 24$), after several years of the relapsing-remitting pattern.

The control sera were from healthy subjects (HS, $n = 40$). The other neurological diseases tested were amyotrophic lateral sclerosis (ALS, $n = 20$), Parkinson's disease (PK, $n = 13$). Other autoimmune diseases tested were rheumatoid arthritis (RA, $n = 20$), insulin-dependent diabetes mellitus (IDDM, $n = 17$) and Chagas' disease in the chronic stage (Chagas, $n = 12$).

Ages of patients and controls were as follows (mean ± S.D.): RRMS-AR: 34.1 ± 8.7 years; RRMS: 40.9 ± 11.5 years; CPMS: 49.4 ± 9.0 years; SPMS: 52.1 ± 10.1 years; HS: 42.9 ± 9.5 years; other autoimmune diseases (RA, IDDM and Chagas): 46.8 ± 15.6 years; and other neurological diseases (ALS and PK): 63.7 ± 11.3 years. The autoimmune disease groups contained more women than men: RRMS-AR: 5 women, 4 men; RRMS: 11 women, 6 men; CPMS: 9 women, 9 men; SPMS: 13 women, 11 men; other autoimmune diseases (RA, IDDM and Chagas): 30 women, 19 men. The control groups had a majority of men: HS: 23 men, 17 women; and other neurological diseases (ALS and PK): 21 men, 12 women.

Paired sera and spinal fluid from 6 MS and 6 ALS patients were kindly provided by Prof. D. Bernard (hôpital de la Timone, Marseille, France). The group of MS patients (39.6 ± 9.7 years) consisted of 5 women and 1 man, and the ALS group (65 ± 9.9 years) of 4 women and 2 men.

2.2. Purification of blood immunoglobulins (Ig)

The crude Ig fraction of four MS patients sera was obtained by precipitation in a solution of 50% ammonium sulfate (Merck). The IgM fraction was purified by chromatography on a Sephadex G-200 gel filtration column. Purified IgMs (30 μg per well) were separated by SDS-PAGE electrophoresis (Laemmli, 1970) on 5% polyacrylamide gel and stained by Coomassie blue. No band at the level of human serum albumin (10 μg per well) was seen. The purified IgMs were aliquoted and stored at −80°C.

2.3. Delipidated bovine serum albumin

A solution of 1% bovine serum albumin (BSA, Sigma) in distilled water was added vol/vol to the polar solvent 1,1,2-trichlorotrifluoroethane (Merck). The mixture was vigorously stirred for 3 min and centrifuged for 10 min at 2000 × *g*. The aqueous supernatant containing the delipidated BSA (dBSA) was then lyophilized. The delipidation efficiency was evaluated by addition of [^{14}C]palmitic acid (NEN Research Products) before 1,1,2-trichlorotrifluoroethane treatment and found to be over 90%.

Table 1
Ten fatty acids conjugated to dBSA (FA-dBSA)

Fatty acid		FA-dBSA
lauric acid	12:0	Laur-dBSA
palmitic acid	16:0	Pal-dBSA
palmitoleic acid	16:1	Po-dBSA
stearic acid	18:0	Ste-dBSA
oleic acid	18:1	Ole-dBSA
linoleic acid	18:2, $n-6$	Lin2-dBSA
linolenic acid	18:3, $n-3$	Lin3-dBSA
arachidonic acid	20:4, $n-6$	AA-dBSA
eicosapentaenoic acid	22:5, $n-3$	EPA-dBSA
docosahexaenoic acid	22:6, $n-6$	DHA-dBSA

2.4. Synthesis of conjugated fatty acid

Acylation of the carrier dBSA was performed with the ten different fatty acids (Sigma) presented in Table 1. For this reaction, 1 mg of each fatty acid was dissolved in 1 ml of anhydrous methanol (Merck) containing 10 μl of triethylamine (Merck) and 1 μl of 74–370 MBq/mmol [^{14}C]palmitic acid. Activation of the carboxylic group was initiated by addition of 200 μl of anhydrous dimethylformamide (Merck) solution containing ethylchloroformate (Fluka) diluted 1/16 and incubated for 3 min at 4°C. Then, a solution of 20 mg dBSA dissolved in 1 ml of 1 mM $CaCl_2$ phosphate buffer (pH 6.8) containing 10 μl of triethylamine was added. The conjugates were purified by dialysis, first against a mixture of dimethylformamide, methanol and 1 mM $CaCl_2$ phosphate buffer (pH 6.8) vol/vol/vol, then against 1 mM $CaCl_2$ phosphate buffer (pH 6.8) for 24 h at 4°C. Beta-counting was performed on 100 μl aliquots, both before and after dialysis in order to calculate the concentration of conjugated fatty acid in each conjugate. The concentration of the dBSA carrier was evaluated by measuring the optical density (OD) at 280 nm. Molar coupling ratios were calculated and found to be between 4 and 7. To prevent oxidation, the polyunsaturated fatty acids were kept under a N_2 enriched atmosphere. *C*9-dicarboxylic azelaic acid (Aze, Aldrich) used in competition studies was conjugated to dBSA (Aze-dBSA) according to the FA-dBSA procedure, except that the dialysis was against distilled water (Daverat et al., 1989). The determined molar coupling ratio was 9.

2.5. Enzyme-linked immunosorbent assay (ELISA)

Polystyrene well plates (Polysorp, Nunc) were coated with 200 μl of a solution containing either FA-dBSA or

dBSA at a concentration of 50 μg/ml in 1 mM $CaCl_2$, 50 mM carbonate buffer (pH 9.6) for 16 h at 4°C under agitation. Antibody binding was tested in duplicate. For this, plates were rinsed twice with 1 mM $CaCl_2$, 150 mM NaCl phosphate-buffered saline (pH 7.2) (Ca-PBS_1), the well plates were filled with 200 μl of human serum samples at a dilution of 1:250 in 1 mM $CaCl_2$, 450 mM NaCl phosphate-buffered saline (pH 7.2) (Ca-PBS_2) containing 0.1% dBSA, and left for 2 h at 37°C. Samples of spinal fluid were tested at a dilution of 1:2 or 1:4 and the sera were tested at a dilution 1:250, 1:500, 1:1000 to match their IgM and IgG levels. After two washes with Ca-PBS_1, the well plates were incubated for 1 h at 37°C with 200 μl of horseradish peroxidase-labeled goat anti-human IgM and anti-human IgG secondary antibodies (Institut Pasteur Diagnostics), diluted 1:5000 in Ca-PBS_1 containing 0.1% dBSA. The well plates were washed 3 times with PBS containing 0.05% Tween 20, before the immunobinding was revealed by peroxidase assay with 0.5 g/l of orthophenylenediamine (OPD, Sigma) in 0.2 M phosphate buffer (pH 5) containing 0.1 M citrate and 0.025% H_2O_2 (Merck). Well plates were incubated for 10 min in the dark and the reaction was stopped by adding 50 μl of 4N H_2SO_4. The OD at 492 nm was read in a multiscan spectrophotometer (MR 610, Dynatech). Experimental values were corrected by subtracting OD read on well plates coated with the carrier protein dBSA: these blank values were less than 0.05. OD binding obtained to FA-dBSA and dBSA without primary antibodies was 0.05. The OD variability between two wells is $\pm$10%. Sera and spinal fluid were collected over a period of months, aliquoted and stored at −80°C until tested in three independent assays.

2.6. Control of ELISA reproducibility

One serum sample was tested 10 times on Ole-dBSA. The coefficient of reproducibility was calculated as follows: mean/standard deviation was 11%. However, as the OD level depends on the temperature and the FA-dBSA, the control group of HS were tested with the pathological groups on each FA-dBSA. Thus the intertest variations were not taken into account by the statistical analysis.

2.7. Determination of antibody avidity

The avidity of antibody binding to FA-dBSA was determined by competition experiments. G-200 purified IgMs at 10 μg/ml in Ca-PBS_2 were preincubated for 16 h at 4°C with various competitors: Ole-dBSA, Laur-dBSA, EPA-dBSA and Aze-dBSA at concentrations varying between 10^{-5} and 10^{-11} M. After centrifugation at 10000 × g for 30 min the supernatants were applied on well plates according to the ELISA described above. The avidity was defined as the concentration of competitor required for 50% inhibition of competition (IC_{50}) to immobilized Ole-dBSA.

2.8. Statistical analysis

As the populations were not normally distributed, the non-parametric tests Mann Whitney and Kruskall-Wallis (InStat 2.01 software) were used.

3. Results

In a preliminary study, blood circulating antibodies to Ole-dBSA were tested for both IgG and IgM isotypes in 11 MS patients and 6 healthy subjects (HS). Antibody binding of the IgM isotype from MS patients but not the IgG isotype showed a significant OD elevation when compared to HS. A dilution study of 6 MS and 5 HS sera was performed on Ole-dBSA. The greatest relative difference in OD level between MS and HS sera was found at a dilution of 1:250.

In our present work, sera of 68 MS patients, 40 HS and 82 patients suffering from other diseases were tested at this dilution for the IgM isotype to ten different FA-dBSAs.

Table 2
Antibody binding of MS sera to ten FA-dBSAs

FA-dBSA	HS 1 $n = 20$	MS $n = 68$	RRMS-AR $n = 9$	RRMS $n = 17$	CPMS $n = 18$	SPMS $n = 24$
Laur-dBSA	0.08 ± 0.08	0.12 ± 0.10 *	0.17 ± 0.12 *	0.14 ± 0.12	0.14 ± 0.08 **	0.08 ± 0.06
Pal-dBSA	0.19 ± 0.13	0.28 ± 0.18 **	0.33 ± 0.22 *	0.31 ± 0.22	0.31 ± 0.18 **	0.23 ± 0.10
Po-dBSA	0.19 ± 0.11	0.27 ± 0.16 **	0.30 ± 0.19	0.29 ± 0.20	0.29 ± 0.16 **	0.22 ± 0.10
Ste-dBSA	0.09 ± 0.09	0.16 ± 0.12 **	0.22 ± 0.16 *	0.17 ± 0.16	0.18 ± 0.10 ***	0.12 ± 0.07 *
Ole-dBSA	0.09 ± 0.10	0.15 ± 0.12 **	0.19 ± 0.13 *	0.17 ± 0.16 *	0.18 ± 0.12 ***	0.11 ± 0.06
Lin2-dBSA	0.05 ± 0.07	0.10 ± 0.08 ***	0.13 ± 0.10 *	0.11 ± 0.11 *	0.11 ± 0.07 ***	0.07 ± 0.04 **
Lin3-dBSA	0.08 ± 0.08	0.13 ± 0.10 **	0.18 ± 0.14 *	0.15 ± 0.14	0.15 ± 0.09 **	0.10 ± 0.06
AA-dBSA	0.33 ± 0.13	0.46 ± 0.18 **	0.50 ± 0.24	0.49 ± 0.23 *	0.47 ± 0.15 **	0.41 ± 0.12 *
EPA-dBSA	0.18 ± 0.11	0.29 ± 0.17 ***	0.33 ± 0.22 *	0.32 ± 0.22 *	0.31 ± 0.16 ***	0.25 ± 0.12 *
DHA-dBSA	0.20 ± 0.12	0.30 ± 0.17 **	0.33 ± 0.21	0.32 ± 0.22	0.32 ± 0.15 **	0.25 ± 0.12

Values are expressed as mean OD ± standard deviation. MS sera were compared to HS1 sera using Mann-Whitney test (* $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$).

OD of the total 68 MS sera showed a significant elevation ($P < 0.05$) to the ten FA-dBSA tested (Table 2). Analysis according to the clinical form of MS revealed that the CPMS group showed a significant binding to the ten FA-dBSA ($P < 0.01$ and $P < 0.001$). However, the variation among the four MS groups analysed by the Kruskal-Wallis test did not reveal any significant differences.

Sera of patients suffering from other neurological and autoimmune diseases were tested against the ten FA-dBSA (Table 3). Among the groups of other autoimmune diseases, sera of RA patients showed a significant elevation of antibody binding when compared to healthy controls ($P < 0.05$) and when compared to IDDM, Chagas and ALS sera ($P < 0.05$).

Sera and paired spinal fluid of 6 MS patients and 6 ALS patients were tested for both IgM and IgG to Ole-dBSA. IgM level of MS sera was increased (0.188 ± 0.106) when compared to that of ALS patients, which was under the detection threshold of OD 0.05, like the IgM level found in CSF of four MS patients and six ALS patients. Only two MS patients showed an elevated OD of IgM in their CSF (0.191 and 0.116), which did not exceed the OD of corresponding serum (respectively 0.199 and 0.274). This means a passive transfer of circulating IgM to CSF. No elevation of IgG binding was found either in sera, or in CSF of MS and ALS patients.

To assess the specificity of the antibody binding to FA-dBSA, G-200 purification of blood IgM was performed from sera of MS patients 54, 64, 72 and 82 which showed strong binding to FA-dBSA. Then binding inhibition of purified IgM from patient 82 was done in various conditions with the competitor Ole-dBSA. No binding inhibition occurred at the ionic strength of 150 mM NaCl PBS, whereas an avidity in the range of 10^{-7} M was revealed when an ionic strength of 450 mM NaCl PBS was used (Fig. 1). After SDS-PAGE electrophoresis, purified IgMs were assessed to be free of human serum albumin. In competition experiments we determined the influence of human serum albumin on the binding of purified IgM from

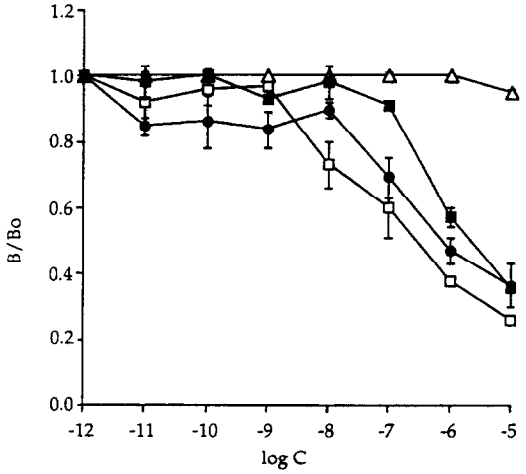

Fig. 1. Inhibition of binding of G-200 purified IgM of patient 82 to immobilized Ole-dBSA by soluble Ole-dBSA in $PBS_{450mM\ NaCl}$ (□), with 0.1mg/ml (●), or with 1.0 mg/ml (■) human serum albumin, and in $PBS_{150mM\ NaCl}$ (△). *Log C* is the $\log_{10}$ of competitor concentration in mole/l of conjugated haptens. The ratio between the absorbances with (*B*) and without (*Bo*) competition is plotted, and each point with standard deviation represents the mean value of 3 experiments.

MS sera (Fig. 1). Human serum albumin added in buffer at the concentration equivalent to a serum dilution 1:250, i.e. 0.1 mg/ml, inhibited the binding of purified IgM from patient 82 ($IC_{50} = 7 \times 10^{-7}$ M) less than the competitor Ole-dBSA alone ($IC_{50} = 3 \times 10^{-7}$ M). At 1 mg/ml, human serum albumin had more effect than at 0.1 mg/ml in increasing the IC_{50} to 1.8×10^{-6} M.

Discrimination of antibody binding was evaluated by three different FA-dBSA: Laur-dBSA, Ole-dBSA and EPA-dBSA, varying in their carbon chain length and in the number of their unsaturation. A control specificity was done by a short *C*9 dicarboxylic acid Aze-dBSA. Few differences in avidity were found between the three FA-dBSA competitors, whereas Aze-dBSA was not recognized at all by purified IgMs of the four MS patients. The purified IgM of patient 82 recognized Ole-dBSA ($IC_{50} = 3 \times 10^{-7}$ M) best, followed by EPA-dBSA ($IC_{50} = 1 \times$

Table 3
Antibody binding of sera from other autoimmune and neurological diseases to ten FA-dBSAs

FA-dBSA	HS 2 $n = 20$	RA $n = 20$	IDDM $n = 17$	Chagas $n = 12$	PK $n = 13$	ALS $n = 20$
Laur-dBSA	0.04 ± 0.03	0.13 ± 0.12 ***	0.06 ± 0.05	0.04 ± 0.03	0.11 ± 0.15	0.07 ± 0.05
Pal-dBSA	0.05 ± 0.04	0.13 ± 0.11 **	0.05 ± 0.04	0.04 ± 0.03	0.11 ± 0.15	0.06 ± 0.05
Po-dBSA	0.03 ± 0.03	0.10 ± 0.09 ***	0.03 ± 0.02	0.02 ± 0.02	0.07 ± 0.10	0.04 ± 0.04
Ste-dBSA	0.02 ± 0.02	0.08 ± 0.07 **	0.03 ± 0.03	0.01 ± 0.01	0.05 ± 0.08	0.03 ± 0.03
Ole-dBSA	0.06 ± 0.04	0.14 ± 0.12 *	0.05 ± 0.03	0.03 ± 0.03	0.11 ± 0.14	0.07 ± 0.06
Lin2-dBSA	0.10 ± 0.06	0.27 ± 0.18 ***	0.14 ± 0.06	0.09 ± 0.07	0.22 ± 0.20	0.13 ± 0.09
Lin3-dBSA	0.07 ± 0.05	0.19 ± 0.13 ***	0.08 ± 0.04	0.05 ± 0.04	0.14 ± 0.15	0.08 ± 0.07
AA-dBSA	0.10 ± 0.05	0.21 ± 0.14 *	0.10 ± 0.04	0.06 ± 0.05	0.17 ± 0.16	0.10 ± 0.08
EPA-dBSA	0.06 ± 0.04	0.15 ± 0.11 ***	0.06 ± 0.03	0.03 ± 0.03	0.10 ± 0.11	0.06 ± 0.06
DHA-dBSA	0.13 ± 0.06	0.25 ± 0.16 **	0.11 ± 0.05	0.08 ± 0.06	0.20 ± 0.19	0.12 ± 0.08

Values are expressed as mean OD ± standard deviation. Sera of other autoimmune and neurological diseases were compared to HS2 sera using Mann-Whitney test (* $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$).

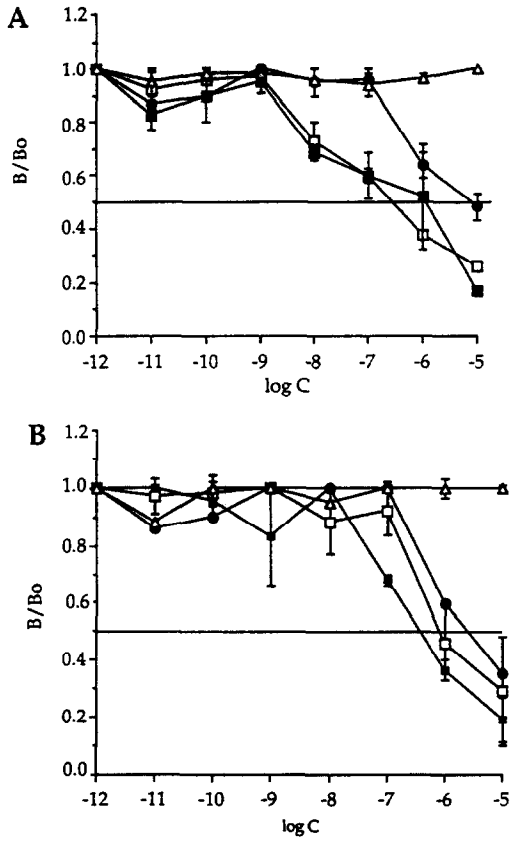

Fig. 2. Inhibition of binding of G-200 purified IgM of patient 82 (**A**) and patient 64 (**B**) to immobilized Ole-dBSA by soluble Ole-dBSA (□), Laur-dBSA (●), EPA-dBSA (■) and Aze-dBSA (△). *Log C* is the $\log_{10}$ of competitor concentration in mole/l of conjugated haptens. The ratio between the absorbances with (*B*) and without (*Bo*) competition is plotted, and each point with standard deviation represents the mean value of 3 experiments.

10^{-6} M), and Laur-dBSA ($IC_{50} = 8 \times 10^{-6}$ M) (Fig. 2). Purified IgM of patient 64 showed a slightly different pattern of discrimination because EPA-dBSA was the best recognized ($IC_{50} = 3 \times 10^{-7}$ M), followed by Ole-dBSA ($IC_{50} = 8 \times 10^{-7}$ M) and Laur-dBSA ($IC_{50} = 2.5 \times 10^{-6}$ M).

4. Discussion

The immunogenicity of fatty acids is weak. They have not, therefore, been considered as important antigens. The FA-dBSA used in our study for screening the IgM binding represent major fatty acid compounds of the cell membrane. The lack of knowledge about the manipulation of fatty acids, especially with respect to immunochemistry, was a major obstacle in studying their immunogenicity. In our experiments particular attention has been paid to adapt an ELISA test, using dBSA and elevated ionic strength. Serum albumin can absorb significant amounts of lipids (Glatz and Veerkamp, 1983; Mailman and Rose, 1990). We showed that non-delipidated human serum albumin, added in buffer competition experiments, caused a weaker binding inhibition of the competitor Ole-dBSA (Fig. 1), and so we used only delipidated serum albumin as saturating agent in the tests.

The ionic strength played a role in the binding of antibodies to FA-dBSA, as shown by our inhibition experiments. IgG directed against FA-dBSA have been detected in sera of HIV patients in an ELISA assay using PBS with the same ionic strength as that used in our experiments (Amara et al., 1994). The three-fold increase of ionic strength may reinforce hydrophobic interactions arising between antigen and antibody, probably by elimination of non-specific binding. Thus, the choice of an appropriate ionic strength appears to be important in the detection of anti-FA-dBSA antibody to minimize background labeling.

Purified human IgM showed a weak discrimination between the different FA-dBSAs, but reacted specifically with the long aliphatic chains, since no inhibition was observed by Aze-dBSA, a short *C*9 dicarboxylic acid. The same absence of discrimination was reported for a rabbit antiserum raised against oleic acid linked to BSA which recognized similarly oleic acid and linoleic acid (Maneta-Peyret et al., 1992). Given the interaction of free fatty acid with other proteins such as purified enzymes and ionic channels, it is noteworthy that these proteins did not discriminate between the chain length and the degree of unsaturation (Ordway et al., 1991).

Apparently no relation was found between the binding of anti-fatty acid antibodies and the level of their respective fatty acid antigens, because no significative difference in binding was found between oleic acid (24.6%), stearic acid (14.9%) and palmitic acid (9.8%) which constitute nearly half of the total fatty acids in the white matter (Wilson and Tocher, 1991).

The main myelin protein proteolipid is acylated with palmitic, stearic and oleic acids (Bizzozero et al., 1986; Bizzozero and Good, 1990). It is possible that the presence of anti-FA-dBSA antibodies in MS sera may be an autoimmune response to these three fatty acids, whereas anti-proteolipid antibodies were rarely found in spinal fluid of MS patients in acute relapse (Warren et al., 1994). Paired serum and spinal fluid of six MS patients tested against Ole-dBSA did not show evidence of intrathecal synthesis of IgM or IgG. However the small number of samples does not allow one to conclude that no intrathecal synthesis of anti-fatty acid antibodies occurred.

In RA, an autoimmune disease which is not characterized by targeting the PLP, we observed anti-FA-dBSA antibodies. This may be due to the fact that MS and RA share a common autoimmune inflammatory feature with possible involvement of the free radical, nitric oxide (NO). Enhanced expression of inducible NO synthase has been shown in MS brain (Bö et al., 1994). Sera of MS patients have been shown to contain IgM directed against the *S*-nitrosocysteine epitope indicating an indirect involve-

ment of NO in the pathology (Boullerne et al., 1995). In RA, massive NO production has been evidenced by the increased level of nitrite in the synovial fluid of patients (Farrell et al., 1992).

Furthermore, lipid peroxidation in rat brain has been shown to enhance the autoimmune response by increasing the sera level of antibodies directed against brain antigens (Prilipko et al., 1983). A possible mechanism may be the oxidation of membrane lipids caused by free radicals (Radi et al., 1991) which lead to membrane disorganization (Freeman and Crapo, 1982; Lamba et al., 1991). This kind of mechanism might be implicated in MS and RA by exposing immunogenic autoepitopes normally hidden from humoral and cellular immune interactions.

Several reports have revealed lipid peroxidation products in MS. A raised level of malondialdehyde has been demonstrated in spinal fluid (Hunter et al., 1985) and serum (Korpela et al., 1989) of MS patients. Pentane, a specific marker of linoleic acid peroxidation, dosed in breath of MS patients correlated with acute relapse (Toshniwal and Zarling, 1992). The marker of peroxidation uric acid was found to be elevated in lesions of MS patients (Langemann et al., 1992).

Our data provide evidence of a specific circulating antibody response to fatty acid haptens found in the diseases MS and RA with inflammatory and autoimmune features. This particular response may be due to oxidative events occurring at membrane level and may act synergistically with other components of the immune system (i.e. T cell and macrophages). However, we do not know if this circulating antibody response to fatty acid haptens plays a causal or secondary role in the autoimmune pathogenesis.

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References

Adams, C.W.M. (1977) Pathology of multiple sclerosis: progression of the lesion. Br. Med. Bull. 33, 15–19.

Amara, A., Chaugier, C., Ragnaud, J.M. and Geffard, M. (1994) Circulating autoantibodies directed against conjugated fatty acids in sera of HIV-1-infected patients. Clin. Exp. Immunol. 96, 379–383.

Bernard, C.C.A., Townsend, E., Randell, V.B. and Williamson H.G. (1983) Do antibodies to myelin basic protein isolated from multiple sclerosis cross-react with measles and other common virus antigens? Clin. Exp. Immunol. 52, 98–106.

Bö, L., Dawson, T.M., Wesselingh, S., Mörk, S., Choi, S., Kong, P.A., Hanley, D., Trapp, B.D. (1994) Induction of nitric oxide synthase in demyelinating regions of multiple sclerosis brains. Ann. Neurol. 36, 778–786.

Bizzozero, O.A., McGarry, J.F. and Lees, M.B. (1986) Acylation of rat brain myelin proteolipid protein with different fatty acids. J. Neurochem. 47, 772–778.

Bizzozero, O.A. and Good, L.K. (1990) Myelin proteolipid protein contains thioester-linked fatty acids. J. Neurochem. 55, 1986–1992.

Boullerne, A.I., Petry, K.G., Meynard, M. and Geffard, M. (1995) Indirect evidence for nitric oxide involvement in multiple sclerosis by characterization of circulating antibodies directed against conjugated S-nitrosocysteine. J. Neuroimmunol. 60, 117–124.

Daverat, P., Geffard, M. and Orgogozo, J.M. (1989) Identification and characterization of anti-conjugated azelaic acid antibodies in multiple sclerosis. J. Neuroimmunol. 22, 129–134.

Farrell, A.J., Blake, D.R., Palmer, R.M.J., Moncada, S. (1992) Increased concentrations of nitrite in synovial fluid and serum samples suggest increased nitric oxide synthesis in rheumatic diseases. Ann. Rheu. Dis. 51, 1219–1222.

Freeman, B.A. and Crapo, J.D. (1982) Biology and free radicals and tissue injury. Lab. Invest. 47, 412–426.

Glatz, J.F.C. and Veerkamp, J.H. (1983) A radiochemical procedure for the assay of fatty acid binding by proteins. Anal. Biochem. 132, 89–95.

Hunter, M.I.S., Nlemadim, B.C. and Davidson, D.L.W. (1985) Lipid peroxidation products and antioxidant proteins in plasma and cerebrospinal fluid from multiple sclerosis patients. Neurochem. Research 10, 1645–1652.

Kerlero de Rosbo, N., Milo, R., Lees, M.B., Burger, D., Bernard, C.C.A. and Ben-Nun, A. (1993) Reactivity to myelin antigens in multiple sclerosis. J. Clin. Invest. 92, 2602–2608.

Korpela, H., Kinnunen, E., Juntunen, J., Kumpulainen, J. and Koskenvuo, M. (1989) Serum selenium concentration, glutathione peroxidase activity and lipid peroxides in a co-twin control study on multiple sclerosis. J. Neurol. Sci. 91, 79–84.

Laemmli, V.K. (1970) Cleavage of structural proteins during the assembly of head bacteriophage T4. Nature 227, 680–685.

Lamba, O.P., Lal, S., Yappert, M.C., Lou, M.F. and Borchman, D. (1991) Spectroscopic detection of lipid peroxidation products and structural changes in a sphingomyelin model system. Biophysica Acta 1081, 181–187.

Langemann, H., Kabiersch, A., Newcombe, J. (1992) Measurement of low-molecular weight antioxidants, uric acid, tyrosine and tryptophan in plaques and white matter from patients with multiple sclerosis. Eur. Neurol. 32, 248–252.

Mailman, D. and Rose, C. (1990) Binding and solubility of oleic acid to laboratory materials: a possible artifact. Life Sci. 47, 1737–1744.

Maneta-Peyret, L., Picard, J.P., Bezian, J.-H. and Cassagne, C. (1992) Fatty acids rendered immunogenic. Immunol. Lett. 31, 227–232.

McFarland, H.F. (1995) The multiple sclerosis lesion. Ann. Neurol. 37, 419–421.

Ordway, R.W., Singer, J.J. and Walsh, J.V. (1991) Direct regulation of ion channels by fatty acids. Trends In Neurosc. 14 (3), 96–100.

Poser, C.M., Patty, D.W., Scheinberg, G L., MacDonald, I., Davis, F., Ebergs, G., Johnson, K., Sibley, W.A., Silbergerg, D.H. and Tourtelotte, W. (1983) New diagnostic criteria for multiple slcerosis. Ann. Neurol. 13, 227.

Poser, C.M. (1992) Multiple Sclerosis: observations and reflections — a personal memoir. J. Neurol. Sci. 107, 127–140.

Prilipko, L.L., Savov, V.M. and Kagan, V.E. (1983) Activation of lipid peroxidation: a mechanism triggering the autoimmune response. Acta Physiologica et Pharmacologica Bulgaria 9, 14–20.

Prineas, J.W. (1985) The neuropathology of multiple sclerosis. In: J.C. Koetsier (Eds.), Handbook of Clinical Neurology, Vol. 3:47, Demyelinating Disease, Elsevier Science Publishers B.V., Amsterdam, pp. 213–257.

Radi, R., Beckman, J.S., Bush, K.M. and Freeman B.A. (1991) Peroxynitrite-induced membrane lipid peroxidation: the cytotoxic potential of superoxide and nitric oxide. Arch. Biochem. Biophys. 288 (2), 481–487.

Raine, C.S. (1991) Demyelinating diseases. In: R.L. Davis, D.M. Robertson, (Eds.), Textbook of Neuropathology, Williams and Wilkins, Baltimore, pp. 535–620.

Stevens, A., Weller, M., Wiethölter, H. (1992) CSF and serum ganglioside antibody patterns in MS. Acta Neurol. Scand. 86, 485–489.

Stoffel, W. (1990) The myelin membrane of the central nervous system — essential macromolecular structure and function. Angew. Chem. Int. Engl. 29, 958–976.

Sun, J., Link, H., Olsson, T., Xiao, B.-G., Andersson, G., Ekre, H.-P., Linington, C. and Diener, P. (1991) T and B cell responses to myelin-oligodendrocyte glycoprotein in multiple sclerosis. J. Immunol. 146, 1490–1495.

Toshniwal, P.K. and Zarling, E.J. (1992) Evidence for increased lipid peroxidation in multiple sclerosis. Neurochem. Res. 17, 205–207.

van Noort, J.M., van Sechel, A.C., Bajramovic, J.J., Ouagmiri, M.E., Polman, C.H., Lassmann, H. and Ravid, R. (1995) The small heat-shock protein aB-crystallin as candidate autoantigen in multiple sclerosis. Nature 375, 798–801.

Warren, K.G., Catz, I., Johnson, E., Mielke, B. (1994) Anti-myelin basic protein and anti-proteolipoid specific forms of multiple sclerosis. Ann. Neurol. 35, 280–289.

Wilson, R. and Tocher, D.R. (1991) Lipid and fatty acid composition is altered in plaque tissue from multiple sclerosis brain compared with normal brain white matter. Lipids 26, 9–15.