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Experimental allergic encephalomyelitis in rhesus monkeys: II. Treatment of EAE with anti-T lymphocyte subset monoclonal antibodies

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SUMMARY

Experimental allergic encephalomyelitis was induced in rhesus monkeys by immunization with bovine brain homogenate emulsified in complete Freund's adjuvant. Four monkeys were treated with anti-CD4 (OKT4+4A) monoclonal antibodies after the onset of clinical signs. One monkey developed a chronic-progressive course of EAE and was killed after a significantly prolonged disease of 19 days. One monkey had a relapse and survived with stable neurological signs. Two monkeys fully recovered. OKT4+4A treatment resulted in a short-term clearance of $CD4^+$ lymphocytes and a reduction in granulocytes. Granulocytes may be attracted to the brain by chemotactic factors produced by $CD4^+$ lymphocytes and are responsible for the development of the lethal granulocytic lesions. Clearance of $CD4^+$ lymphocytes successfully prevented granulocytes from migrating to the brain. Nuclear magnetic resonance imaging showed extensive lesions during an acute attack, but these lesions became undetectable when the monkeys recovered. These results indicate that treatment with OKT4+4A can successfully reverse clinical signs of EAE. Four untreated monkeys and one monkey treated with OKT8F died of acute EAE within 3 days of the onset of clinical signs.

Keywords experimental allergic encephalomyelitis rhesus monkey monoclonal antibodies treatment.

INTRODUCTION

Experimental allergic encephalomyelitis (EAE) is an autoimmune disease of the central nervous system. It is induced by immunization with the autoantigen myelin basic protein or brain homogenate emulsified in complete Freund's adjuvant. There is ample evidence that EAE is mediated by T lymphocytes and in particular by the $CD4^+$ helper/inducer T lymphocyte subset (Swanborg, 1983; Hauser *et al.*, 1984a, b; Lando & Ben-Nun, 1984; Mokhtarian, McFarlin & Raine, 1984). Since EAE has been advocated as a preclinical model for studying the pathogenesis and treatment of human autoimmune diseases in which lymphocyte subset imbalances are involved (e.g. multiple sclerosis), it is of interest to investigate whether EAE can be modulated with monoclonal antibodies (MoAb) directed against these subsets. In a previous study it was shown that clinical signs of EAE in the mouse can be reversed with anti-CD4 MoAb (Waldor *et al.*, 1985). In the study presented here, rhesus monkeys were treated with anti-CD4 (OKT4+4A) and anti-CD8 (OKT8F) MoAb after the onset of clinical signs of EAE. The advantage of the rhesus monkey model is that MoAb can be used that crossreact with human lymphocytes, thus allowing a more direct extrapolation to the human situation. In addition, rhesus monkeys can be longitudinally

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monitored for immunological parameters, such as lymphocyte subsets and antibodies, in the peripheral blood and the cerebrospinal fluid (CSF). The results show that anti-CD4, but not anti-CD8, MoAb (only one monkey tested) can modulate the clincal course of EAE in the rhesus monkey.

MATERIALS AND METHODS

Induction of EAE. Fifteen young adult rhesus monkeys were immunized with 0·5 mg of protein from bovine brain white matter homogenate emulsified in complete Freund's adjuvant as previously described (Van Lambalgen & Jonker, 1987).

Treatment with monoclonal antibodies. Monkeys were treated with MoAb at the onset of clinical signs. One monkey received OKT8F (1 mg/kg i.v.; daily) and four monkeys received a mixture of OKT4 and OKT4A (1 mg/kg i.v. for 10 days). The OKT antibodies were kindly donated by Dr Goldstein, Ortho Pharmaceuticals, Raritan, NJ.

Blood and CSF sampling. Blood samples for haematological and serological studies and lymphocyte subset analysis were collected weekly and at the onset of clinical signs, and 10 min and 24 h after the first MoAb injection. Cerebrospinal fluid (CSF) was collected by lumbar puncture at weekly intervals and at the onset of clinical signs.

Assays. Histological, haematological and serological studies and lymphocyte subset analysis (CD4 with OKT4+4A MoAb; CD8 with GM9 MoAb which is equivalent to OKT8) were carried out as previously described (Van Lambalgen & Jonker, 1987). Antibody levels of OKT4+4A, anti-OKT4+4A and anti-brain homogenate in the serum and the CSF were determined by ELISA. For detecting OKT4+4A, plates were coated with goat-anti-mouse IgG (H+L), incubated with serial dilutions of serum or CSF and developed with peroxidase conjugated goat-anti-mouse IgG (H+L) and the substrate orthophenyldiamide (OPD). For the detection of anti-OKT4+4A, produced in response to the injected OKT4+4A, plates were coated with OKT4+4A, incubated with serum or CSF and developed with peroxidase conjugated rabbit-anti-monkey IgG and OPD. The ELISA for anti-brain homogenate antibodies was carried out as previously described (Van Lambalgen & Jonker, 1987).

Nuclear magnetic resonance (NMR) imaging was carried out using a Technicare NMR system with an operating field strength of 0·6 T. Images were made with TR = 1500 msec, TE = 38 msec and EN = 2/2.

RESULTS

Fifteen rhesus monkeys were immunized with bovine brain homogenate. Nine of these developed EAE. Four untreated monkeys and one monkey treated with OKT8F died of acute EAE within 3 days of the onset of clinical signs (Fig. 1). Four monkeys were treated with OKT4+4A. One of these (monkey CP) initially showed some improvement but then developed a chronic-progressive course of the disease. This monkey was killed when moribund, after a significantly prolonged disease of 19 days. Two monkeys (M1 and M2) completely recovered from the acute attack. One monkey (RR) suffered a relapse after an initial improvement. This monkey recovered from the relapse without further treatment and survived with stable neurological signs. Three monkeys (CP, M1 and RR) became blind in both eyes at 3–5 days of the start of OKT4+4A treatment. This blindness was reversible in one monkey, M1.

Effect of OKT8F treatment

The single monkey treated with OKT8F showed a permanent depletion of the $OKT8F^+$ subset. The clinical course of EAE further developed as in the untreated animals. When this animal was moribund a significant granulocytosis in the peripheral blood and high levels of antibodies specific for the brain homogenate in the CSF were observed.

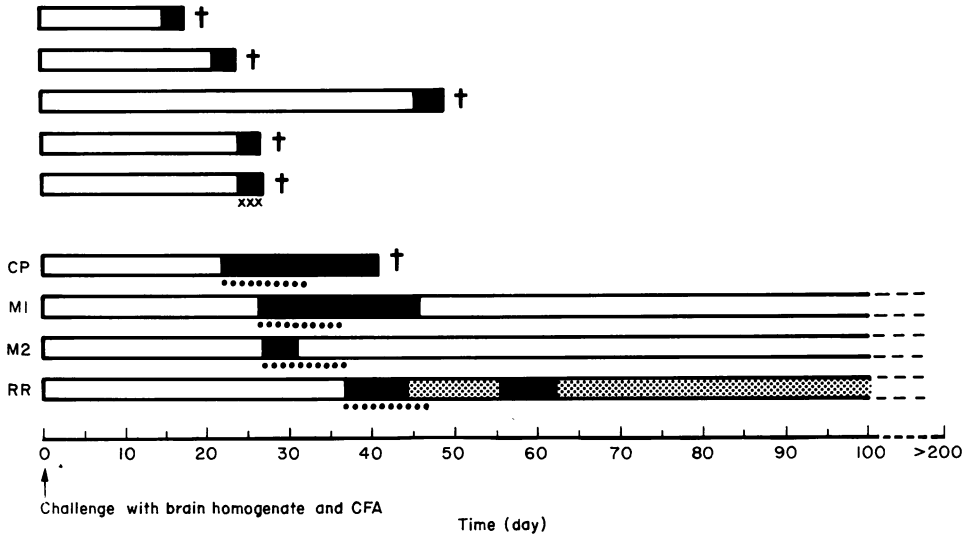

Fig. 1. The effect of OKT4+4A and OKT8F treatment on EAE in rhesus monkeys. Monkeys treated with OKT4+4A developed a chronic-progressive (CP), a monophasic (M1 and M2) or a relapsing-remitting (RR) course of the disease. (□) no symptoms; (■) EAE symptoms, deteriorating; (□) EAE symptoms, stable; (...) OKT4+4A treatment; (× × ×) OKT8F treatment.

Effect of OKT4+4A treatment

The four monkeys treated with OKT4+4A were longitudinally followed for lymphocyte subsets, granulocytes, and for antibodies in the serum and the CSF.

Lymphocyte subsets. The first injection of OKT4+4A resulted in a complete clearance of the $CD4^+$ subset from the peripheral blood within 10 min. All T lymphocytes had the CD8 phenotype. After 24 h normal percentages of $CD4^+$ cells were found. These cells were coated *in vivo* by the injected OKT4+4A as was shown by the fact that they could be directly labelled with a FITC conjugated goat-anti-mouse antiserum. Seven days after the onset of treatment the $CD4^+$ subset was not coated *in vivo* although OKT4+4A antibodies were detected in the serum using an ELISA. It is likely that at this time the serum concentration of OKT4+4A was so low due to anti-OKT4 and 4A antibodies (see below) that the coating *in vivo* was below the detection threshold of the FACS. After the termination of the treatment the CD4/CD8 ratios levelled off at $\pm$ 50% of the ratios at the start of the experiment (Fig. 2). This coincided with an increase in the absolute numbers of lymphocytes, indicating that there was both a relative and an absolute increase in the numbers of $CD8^+$ cells.

Granulocytes. Three of the four monkeys (CP, RR and M1) had a granulocytosis at the onset of clinical signs. The number of granulocytes were strongly reduced within 10 min after the first injection of OKT4+4A. Thereafter it gradually returned to higher values. The rapid decrease and subsequent increase ran parallel to the numbers of $CD4^+$ lymphocytes. OKT4 and 4A antibodies do not crossreact with granulocytes, so the changes in granulocyte numbers must be secondary to those in the $CD4^+$ lymphocyte numbers. Monkey M2 had no granulocytosis and the OKT4+4A treatment did not affect the number of granulocytes.

Antibodies in the serum. Antibodies specific for the injected OKT4+4A were detected 8 days after the first injection and maximum levels were found after 14 days. All monkeys had high titres of anti-brain homogenate antibodies at the onset of clinical signs. OKT4+4A treatment had no significant effect on these antibody titres (Fig. 3).

Antibodies in the CSF. The first CSF sample after the onset of treatment was taken after 7 days. Neither at this time nor at any time later, could OKT4+4A be detected in the CSF. Low levels of anti-OKT4+4A antibodies were found 14 days after the onset of treatment in the CSF of the CP

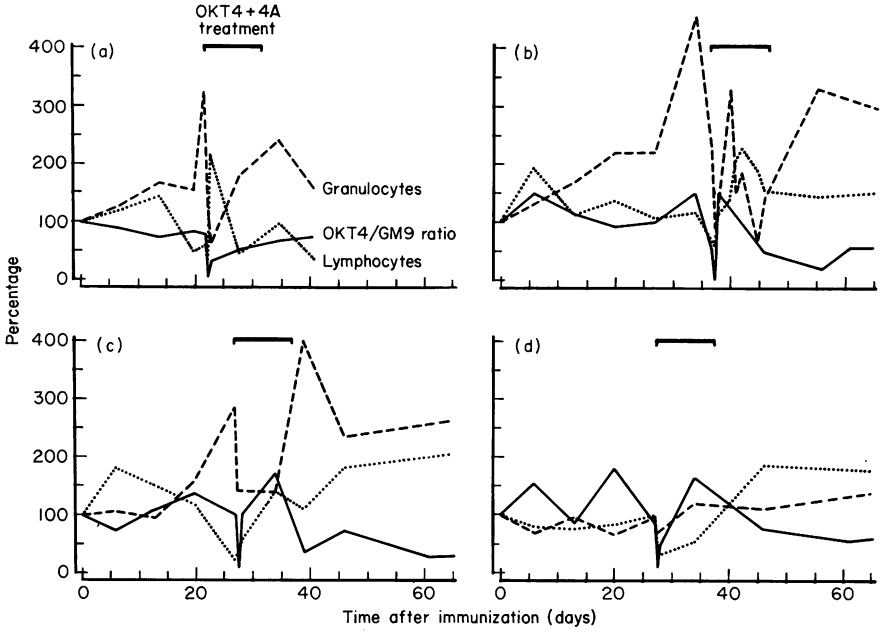

Fig. 2. Longitudinal study of granulocytes (broken lines), lymphocytes (dotted lines) and OKT4/GM9 ratios (solid lines) in monkeys treated with OKT4+4A. (a) CP EAE (b) RR EAE (c) monophasic EAE (M1) (d) monophasic EAE (M2). Values at day 0 were taken as 100%.

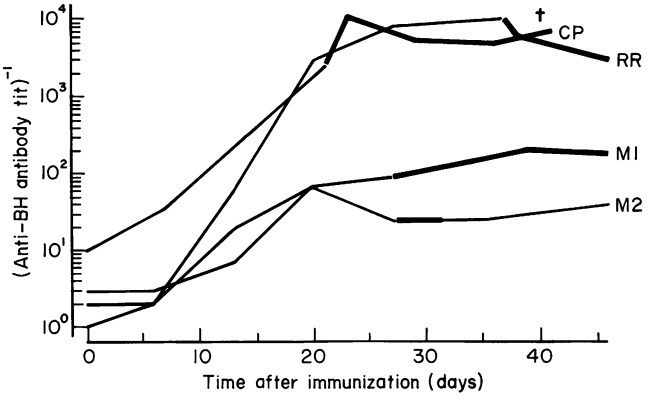

Fig. 3. Anti-brain homogenate antibodies in the serum of rhesus monkeys with EAE treated with OKT4+4. Thick lines show the presence of EAE symptoms.

and the RR monkeys but not in the CSF of the two monkeys which had a monophasic disease (M1 and M2). Anti-brain homogenate antibodies were detected in the CSF of three monkeys (CP, RR and M1) at the onset of clinical signs (Fig. 4). Subsequent changes in the levels of anti-brain homogenate in the CSF correlated with the clinical evolution of the disease. Anti-brain homogenate antibodies became undetectable in the CSF of one monkey (M1) that clinically recovered. High antibody levels were maintained in the RR and the CP monkeys, although the latter showed a temporary reduction after the onset of treatment.

Histology and NMR imaging

Untreated monkeys had a few large, predominantly granulocytic lesions in the white matter, which

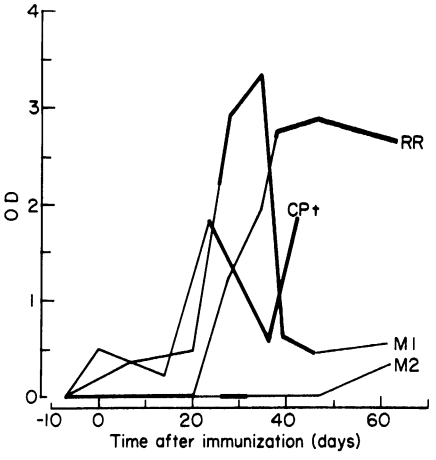

Fig. 4. Anti-brain homogenate antibodies in the CSF of rhesus monkeys with EAE treated with OKT4+4A. Thick lines show the presence of EAE symptoms. CSF was diluted 1 in 100.

were often haemorrhagic. In addition, very little demyelination was found. This was in contrast with the extensive pathology of the CP monkey which was killed after a disease of 3 weeks. Many lesions containing lymphocytes and relatively few granulocytes with little or no admixture of erythrocytes were found throughout the white matter of the cerebrum and cerebellum. Both fresh, predominantly lymphocytic lesions and older lesions with demyelination and necrosis were observed. The most pronounced pathology was found in the visual system with both lateral geniculate bodies, the optic chiasm and the optic nerves severely affected. The grey matter contained many diffuse lymphocytic infiltrates. The spinal cord was unaffected except for a few lymphocytic infiltrates in the white matter of the caudal roots.

NMR imaging revealed high intensity areas in the brains of two of the three surviving monkeys (monkeys RR and M1) treated with OKT4+4A. One monkey with a monophasic disease (M1) had a lesion in the right hemisphere 2 days after the onset of signs. Six weeks later this lesion could not be detected anymore. The monkey with a relapse had large symmetrical lesions in both hemispheres during the first attack. At the time of the relapse (RR) these lesions were undetectable, but instead a single lesion in the right hemisphere was found (Fig. 5). No lesions were found in monkey M2.

DISCUSSION

This study showed that EAE can effectively be treated with OKT4+4A but not OKT8F MoAb (although only one monkey was tested), indicating that $CD4^+$ lymphocytes are responsible for mediating EAE in the rhesus monkey. This is in line with previous studies in the mouse showing that EAE can be transferred by $CD4^+$ cell lines (Hauser *et al.*, 1984b; Lando & Ben-Nun, 1984; Mokhtarian *et al.*, 1984) and signs of EAE can be treated with anti-CD4 but not anti-CD8 MoAb (Waldor *et al.*, 1985). In contrast, anti-CD4 treatment in the rat was not successful (Brinkman, Ter Laak & Hommes, 1985), but it is likely that the intramuscular route of administration, as used in these experiments, was ineffective.

Untreated monkeys died within 3 days of the onset of clinical signs and had only a few, but large, haemorrhagic lesions which consisted mainly of granulocytes. The formation of these lesions may proceed in two steps (Van Lambalgen & Jonker, 1987). They are initiated by infiltrating $CD4^+$ lymphocytes and an influx of granulocytes follows. The rapid expansion of small lymphocytic infiltrates into the large granulocytic infiltrates, with still very little demyelination, may be the lethal

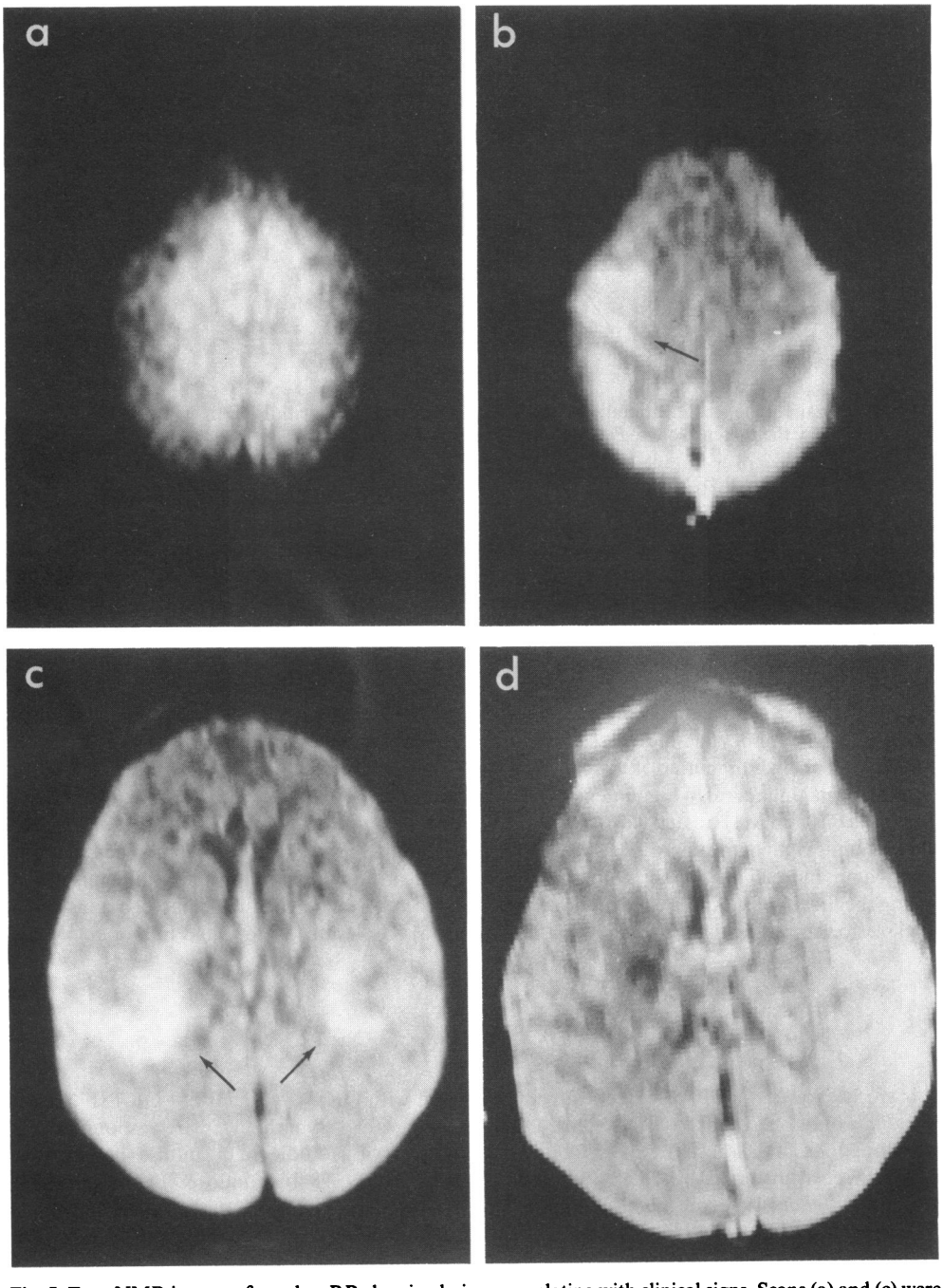

Fig. 5. Four NMR images of monkey RR showing lesions correlating with clinical signs. Scans (a) and (c) were made two days after the onset of clinical signs. Two lesions (arrows) can be seen in scan (c). Scans (b) and (d) (corresponding with (a) and (c) respectively) were made 6 weeks later after a relapse. Scan (b) shows a lesion in the right hemisphere (arrow), whereas the lesions of the first relapse (scan (c)) were undectable 6 weeks later (scan (d)).

event in acute EAE in the rhesus monkey. Thus, in case of successful treatment, the migration of granulocytes to the brain lesion must also be prevented. OKT4+4A treatment initially resulted in a depletion and later in coating of $CD4^+$ lymphocytes. Simultaneously, a depletion of granulocytes was observed, although granulocytes do not crossreact with these antibodies. It may well be that $CD4^+$ lymphocytes, apart from initiating lesions, also produce chemotactic factors which trigger the granulocytosis and attract the granulocytes to the lesion. This suggests that both steps of lesion formation could be inhibited by the inactivation of $CD4^+$ lymphocytes with OKT4+4A. In contrast, the granulocytosis in the monkey treated with OKT8F was not affected and this monkey died with large granulocytic lesions similar to those of untreated monkeys. It remains speculative as to why the OKT4+4A treatment was not equally successful in the four treated monkeys, but it is likely that the extent of brain damage at the onset of treatment may influence the outcome of the treatment.

The successful treatment of EAE with OKT4+4A was not parallelled by a decrease of anti-brain homogenate antibodies in the serum, thus emphasizing the T cell mediated nature of EAE. The reduction in antibody titre in the CSF of one monkey with monophasic EAE may signify a healing of the blood–brain barrier.

The CP monkey progressively deteriorated and was killed. Of interest was the difference in brain pathology between this treated monkey and the untreated monkeys. Most of the white matter of the CP monkey contained small perivascular demyelinating lesions containing predominantly lymphocytes. This was in sharp contrast with the few but large haemorrhagic and granulocytic lesions of the untreated monkeys. Thus, the disease duration of the CP monkey may have been significantly prolonged because the development of the lethal granulocytic lesions (step 2) was prevented. However, the development of fresh, lymphocytic lesions (step 1) was not successfully inhibited in this monkey.

OKT4+4A treatment did not directly abrogate a further progression of the disease. Up to several days after the onset of treatment the monkeys still presented with new neurological signs of which the most prominent was the blindness as observed in three of the four treated monkeys. This blindness is not likely to be a side effect of the OKT4+4A treatment since on the one hand damage to the visual system in EAE in the guinea pig (Raine *et al.*, 1980) and the rhesus monkey (Van Lambalgen *et al.*, 1984) has also been observed in untreated animals and on the other hand, anti-rejection therapy with OKT4+4A in organ transplantation studies has never resulted in any side effects (Jonker *et al.*, 1985). Furthermore, the blindness in one animal was reversed during the treatment.

One of the monkeys with monophasic EAE (monkey M2) displayed the general malaise which always precedes clinical EAE and treatment was initiated. However, in retrospect, this monkey did not have the granulocytosis nor anti-brain homogenate antibodies in the CSF, which are characteristics of EAE at the onset of disease. Furthermore, NMR imaging did not reveal any brain lesions. Possibly this monkey was treated for the wrong reason.

NMR imaging showed extensive lesions in the brains of monkeys RR and M1 during the acute attack. The lesions of the acute attack in the relapsing-remitting monkey were undetectable at the time of the relapse when another lesion was observed. This indicates that the relapse was induced by a fresh lesion rather than by the reactivation of old lesions.

The reversal of EAE symptoms in mice treated with anti-CD4 MoAb has led to speculations about the possible treatment of multiple sclerosis (Waldor *et al.*, 1985), for which EAE is regarded as a preclinical model (Alvord, Kies & Suckling, 1984). The results presented here further substantiate this idea, in particular since the MoAb used in this study (OKT4 and OKT4A) are specific for human lymphocytes and crossreact with rhesus monkey $CD4^+$ cells.

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