Suppression of Ongoing Disease in a Nonhuman Primate Model of Multiple Sclerosis by a Human-Anti-Human IL-12p40 Antibody

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Suppression of Ongoing Disease in a Nonhuman Primate Model of Multiple Sclerosis by a Human-Anti-Human IL-12p40 Antibody

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IL-12p40 is a shared subunit of two cytokines with overlapping activities in the induction of autoreactive Th1 cells and therefore a potential target of therapy in Th1-mediated diseases. We have examined whether ongoing disease in a nonhuman primate model of multiple sclerosis (MS) can be suppressed with a new human IgG1 Ab against human IL-12p40. Lesions developing in the brain white matter were visualized and characterized with standard magnetic resonance imaging techniques. To reflect the treatment of MS patients, treatment with the Ab was initiated after active brain white matter lesions were detected in T2-weighted images. In placebo-treated control monkeys we observed the expected progressive increase in the total T2 lesion volume and markedly increased T2 relaxation times, a magnetic resonance imaging marker of inflammation. In contrast, in monkeys treated with anti-IL-12p40 Ab, changes in the total T2 lesion volume and T2 relaxation times were significantly suppressed. Moreover, the time interval to serious neurological deficit was delayed from 31 ± 10 to 64 ± 20 days (odds ratio, 0.312). These results, in a disease model with high similarity to MS, are important for ongoing and planned trials of therapies that target IL-12 and/or IL-23.

a prolonged time (15, 17, 21). Similar to the situation in MS, clinical signs in the marmoset EAE model does not always reflect the pathological changes within the CNS, the so-called clinico-pathological paradox (21, 22). Hence, as in MS trials, we have used MRI as an outcome measure for the current study (23, 24).

The protocol of the current study comprised daily monitoring of clinical signs as well as in vivo brain imaging at ~2-wk intervals using clinically relevant MRI techniques to detect lesions (17), namely, 1) T2−weighted (T2W) MRI to assess the spatial distribution and size of lesions (25), 2) assessment of Ab leakage by contrast enhanced (with gadopentetate dimeglumine (Gd-DTPA)) T1W images (26), and 3) quantitative T2 relaxation time imaging to determine the extent of CNS inflammation (27). As soon as brain lesions were detected on T2W images, treatment with the Ab or the equivalent volume of solvent as a control was initiated. Brain MRI was continued until the end of the observation period to detect the effect of the Ab on existing lesions as well as new formation of lesions.

For ethical reasons, the number of monkeys in a preclinical trial of a new therapy is obviously much more limited than that in studies using mice or rats. Nevertheless, the results of the current study are clear, demonstrating that treatment with an Ab directed to the IL-12/23p40 subunit during ongoing disease within the CNS delays disease progression and/or reduces lesion activity.

Materials and Methods

Animals

Ten unrelated naive common marmosets (C. jaccus) were used for this study (see Table I). The monkeys were randomly selected from the purpose-bred colony that is kept at the Biomedical Primate Research Centre in Rijswijk, The Netherlands. During the studies the monkeys were individually housed within the Biomedical Primate Research Centre facilities in spacious cages with padded shelter provided on the bottom. The daily diet consisted of commercial food pellets for nonhuman primates (Special Diet Services) supplemented with rice and fresh fruit. Drinking water was provided ad libitum.

Before disease induction the 10 monkeys were randomly assigned to the two experimental groups: five monkeys to be injected with saline as sham treatment, and five to be injected with the anti-IL-12p40 Ab. As previously described, the disease incidence in this model is 100%, due to the presence of the MHC class II allele Caja-DRB*W1201 in each monkey from this species (28, 29).

Monkeys were excluded from the study when they showed one or more of the following criteria: 1) absence of MRI-detectable brain lesions; all monkeys in this study developed such lesions; 2) first appearance of brain lesions (tEAE = 0) outside the normal interval for this model, which is 140 days maximally; or 3) absence of detectable progression of clinical signs or lesion pathology within the observation period.

Monkey Mi089, which was originally assigned to the control group, developed a first lesion (tEAE = 0) only after 254 days. The maximum EAE score recorded in this monkey was 0.5 at 312 days after EAE induction, and no worsening of the lesion pathology could be detected with MRI. Hence, monkey Mi089 was excluded from the study as being an outlier.

EAE induction

EAE was induced by immunization with rhMOG as an emulsion with CFA (29, 30), because this model develops the large and relatively homogeneous lesions needed for MRI analysis (31). Notably, the rhMOG-induced EAE model reproduces the essential clinical and pathological signs of myelin-induced EAE (17, 21).

Clinical signs of EAE were recorded twice daily, using a previously described scoring system (32). The substantial variation in the time of onset (5–20 wk) and the severity of clinical signs is inherent to the outbred nature of this model (15) (P. Smith, manuscript in preparation). For ethical reasons, the monkeys were killed once they had reached clinical score 3.0 (hemiparesis or paraplegia). For autopsy, monkeys were deeply sedated with ketamine (1 mg in 100 µl/kg as i.m. injection; ASP Pharma), after which 400 mg of sodium-pentobarbital (Euthesate; APharma) was infused. At necropsy, the brain and spinal cord were removed and processed for histological examination.

Anti-IL-12p40 Ab

The tested Ab is a human IgG1 anti-IL-12p40 monoclonal antibody (Ab) (clone 1D11, kindly provided by Dr. S. Ichii, National Cancer Center Research Institute, Tokyo, Japan). The Ab is specific for the human IL-12p40 subunit but does not bind to mouse IL-12p40. The Ab is purified from culture supernatants by protein G affinity chromatography; its purity and concentration are confirmed by SDS-PAGE and absorbance at 280 nm. The Ab is a human IgG1κ Ab, which binds to human IL-12p40 and prevents its interaction with IL-12Rβ1. The neutralization of marmoset IL-12p40 by the anti-human IL-12p40 mAb (IgG1κ) has previously been shown to block LPS-stimulated mononuclear cells, using measurement of IFN-γ production with ELISA as a readout. In this way it was shown that human and marmoset IL-12p40 produced by LPS-stimulated plastic adherent cells are neutralized with similar efficacy (20).

The objective of the current set of experiments was to investigate the effect of the Ab on existing lesions in the brain. We have focused on lesions within the cerebral white matter, because these can be well visualized and characterized with a set of newly implemented MRI techniques (17). Administration of the Ab was started once T2 lesions of sufficient size for

<table>
<thead>
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<th>Table I. Treatment with anti-IL-12p40 Ab delays disease progressiona</th>
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<tr>
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a The table summarizes relevant individual data of each monkey, namely the identity code, gender, and birth date (day/month/year). Moreover, the time points (days after immunization) are given when the first lesion was detected with MRI, when serious coordination defects were detected (score 2.0), and when an animal showed serious impairment of motor functions (score 3.0) indicating the sacrifice of that monkey for ethical reasons.

b Monkey Mi092 had disease score 2.0 already at the time the first lesion was detected. Hence, this monkey was excluded from the means calculation of the time interval between first lesion detection and disease score 2.0.

c Monkey Mi089 developed only very mild EAE within the observation period. While the experimental end-point of the other monkeys was disease score 3.0, it was only 0.5 in monkey Mi089 (reached at 312 days after EAE induction). The monkey was therefore excluded from the calculation of the mean clinical scores of the sham control group.
quantitative evaluation were detected. The time points in individual ani-
mals when treatment was started (defined as \( t_{\text{EAE}} \)) are given in Table
I. Five animals were injected once weekly into the vena saphena with 10
mg/ml/kg anti-IL-12p40 Ab. The administered dose resulted in a mean
trough level of the IL-12p40 Ab measured 3 days after Ab injection of
23.4 ± 5.9 μg/ml. Five control animals received once weekly i.v. injec-
tions with the solvent only (sterile PBS; 1 ml/kg) as a sham treatment.
In a previous study the administered dose of anti-IL-12p40 Ab was found to
protect marmosets against the clinical and neuropathological consequences
of EAE (20).

MRI
High resolution MR images were recorded in a 4.7 T horizontal bore nu-
clear magnetic resonance (NMR) spectrometer (Varian Instruments),
equipped with a high performance gradient insert (12-cm inner diameter;
maximum gradient strength, 220 mT/m). A Helmholtz volume coil (Ø, 85
mm) and an inductively coupled surface coil (Ø, 35 mm) were used for
radiofrequency transmission and signal reception, respectively. Baseline
measurements of each animal were collected before EAE induction. After
EAE induction, the animals were scanned every 2 wk until they reached a
disability score 3.0 (hemiparaplegia), at which stage they were killed.

Anesthesia for MRI recording was induced by i.m. injection of ket-
amine, after which the animals were instrumented for mechanical ventila-
tion by endotracheal intubation. The tail vein was cannulated for injection
of the contrast agent gadopentetate dimeglumine (GdDTPA) to probe the
BBB permeability. The head was immobilized in a metal-free device
based on a stereotactic frame for rats, which was placed in an animal
cradle, after which the construction with the animal was inserted into the
NMR spectrometer (33). During the NMR recordings the animals were
ventilated with isoflurane (1.5–2%) in \( N_2O/O_2 \) (70/30). Expiratory \( CO_2 

was monitored, and the body temperature was maintained at 37°C with a
heated water pad. On a sagittal scout image, 35 contiguous coronal slices
of 1 mm were defined covering the complete brain. From these 35 slices,
MRI datasets were collected (field of view, 4 × 4 cm²; matrix, 128 × 128;
zero-filled to 256 × 256; in-plane resolution, 312.5 μm²; two transitions).

Quantitative T1WI maps. These maps were obtained by a monoeponential
fit of five multiecho images (repetition time = 5000 ms; echo time = 17.5,
35, 52.5, 70, and 87.5 ms). The T1WI images with a TE of 35 ms were used as
for region of interest determination (see below).

Quantitative GdDTPA-enhanced T1WI maps (GE-T1WI). These maps were
calculated from two T1WI images (repetition time = 650 ms; echo time = 11.5 ms) before and after a bolus of 0.3 mmol/kg GdDTPA i.v.,
12.5 min in circulation) with GE-T1WI = 100 × [\( T1_{\text{pre GDTPA}} - T1_{\text{pre GDTPA}} \)]/(\( T1_{\text{pre GDTPA}} \)). Pixel intensities thus display the percent
increase in \( T1 \) signal intensity due to GdDTPA leakage. Calculations of \( T1 \) and GE-T1WI maps were made with a homemade
software package developed in Interactive Data Language (IDL version
5.3; Research Systems).

MRI data evaluation. The first appearance of a lesion on the T1WI images
was defined as \( t_{\text{EAE}} = 0 \). The last recorded set of MR images before a
monkey had to be withdrawn from the experiment for reason of serious
neurological deficit (score of 3.0) was defined as \( t_{\text{EAE}} = \text{final} \). Both hemi-
spheres of the 35 slices were analyzed. Regions of interest were defined on
the individual slices of T1WI images with the help of the marmoset brain
atlas (34): cortex, white matter, lesions, normal-appearing white matter
(NAWM). The latter area was defined as the total white matter area without
the lesions and was calculated as white matter – lesions. The change in
MRI characteristics of individual lesions present at \( t_{\text{EAE}} = 0 \) was
determined.

Histology
After formalin fixation, parts of the brain and spinal cord were embedded
in paraffin and processed as described previously (32). In brief, the cere-
brum and cerebellum were divided into seven or eight coronal-cut parts,
and the spinal cord was dissected transversely. The extent of inflammation,
demyelination, and axonal pathology was evaluated on 3- to 5-μm tissue
sections stained with H&E to visualize infiltrated cells, with Klüver-Bar-
dera Luxol Fast Blue (LFB) combined with periodic acid-Schiff (PAS) for
myelin and myelin degradation products, and with Bielschowsky silver
staining for axons.

Histological quantification was performed as previously described (19,
20, 32). Inflammation in the spinal cord was quantified in H&E-stained
sections and expressed as an inflammatory index, calculated as the average
number of inflamed blood vessels per spinal cord section (n = 10–15
sections). Furthermore, the area of demyelination was quantified on 10–15
spinal cord fields of LFB-stained sections using a monomorphic grid. The
white matter pathology of the brain is more difficult to quantify, being
much more heterogeneous than that of the spinal cord with regard to lo-
cation and activity of the lesions, with many lesions located in both white
as well as gray matter (M. K. Storch et al., manuscript in preparation).
Hence, we normally use a semiquantitative measure for the brain. Inflam-
mation was scored as: −, absent; +, and +, present; and demyelination as: +,
zero, small to middle sized lesions; and ++, large lesions. The activity of the lesions was assessed as previously described (32).

Statistics
The risk ratio for the development of serious neurological deficit (EAE
score, ≥3.0) was estimated by the Cox proportional hazards model, with
time to EAE as the dependent variable and treatment as the independent
variable. MRI data were evaluated by two-way ANOVA for repeated measure-
ments, followed by a two-tailed multiple comparison procedure (Student-
Newman-Keuls method) for only those time points with at least three con-
trary animals surviving (i.e., \( t_{\text{EAE}} = 42 \)). Data are presented as the mean ±
SEM. A value of \( p < 0.05 \) was considered statistically significant.

Ethics
According to The Netherlands’s law on animal experimentation, the pro-
cedures of this study were reviewed and approved by the institute’s animal
care and use committee. The housing, care, and all biotechnical and ex-
perimental handleings were performed in conformity with guidelines set by
the committee.

Results
Brain lesion characteristics on MRI and histology
Abnormalities developing in the brain white matter of rhMOG-
immunized monkeys were assessed with T1WI images. On the basis of the
histological aspect and the presentation on T2 relaxation time and GE-T1WI images, two types of brain lesions were distin-
guished in this model (Fig. 1). The vast majority of the lesions (type A) display a high T2 relaxation time as well as GdDTPA
enhancement of the T1WI images. Histology showed that this lesion type contains many MRP-14-positive macrophages filled
with early myelin degeneration products. In these typically early active lesions (32), we found only a few macrophages with late myelin
degradation product and well-conserved axonal structures. A much smaller group of lesions (type B) displayed only a slight or no
increase in the T2 relaxation time and contained a low number of macrophages filled with late (PAS-positive) myelin degeneration
products and only few macrophages with early (LFB-positive) my-
elin degradation products. In these typically late inactive lesions, the vast majority of the present macrophages were MRP-14 neg-
sitive, and significant axonal damage was present.

MRI characteristics of the lesions present at the start of
treatment
The first columns of each panel in Fig. 2 show the slice in T1WI brain images from each monkey that contained the first T2 lesions
(\( t_{\text{EAE}} = 0 \)). The average number of brain T2 lesions per monkey

were detected at \( t_{\text{EAE}} = 0 \) was 2.6 ± 1.2, with a mean volume of 4.6 ±
2.1 mm³. The mean total lesion load (the sum of all individual
lesion volumes) in the monkeys at \( t_{\text{EAE}} = 0 \) was 11.6 ± 8.7 mm³.

The mean T2 relaxation times of all brain lesions present at \( t_{\text{EAE}} = 0 \)
was 57 ± 1.3 ms, and the mean GE-T1WI value was 4.8 ±
0.4/μm².

Effect of anti-IL-12p40 Ab treatment on clinical signs of EAE
The time interval between the rhMOG/CFA inoculation and the
appearance of the first MRI-detectable brain white matter lesion
(\( t_{\text{EAE}} = 0 \)) ranged from post sensitization days 35 to 136, with
a mean duration of 57 ± 32 days (Table I). This variation between
individual animals is within the normal range of this model (15).
As explained in Materials and Methods, monkey Mi089 was excluded as being an outlier. In monkeys treated with anti-IL-12p40 Ab, the progression of EAE from t_{EAE} = 0 (initiation of treatment) to t_{EAE} = final (EAE score, 3.0; i.e., the ethical end point) was clearly delayed (Table I and Fig. 3). This time interval was 31 ± 10 days in saline-treated monkeys and 64 ± 20 days in Ab-treated monkeys. The incidence ratio to EAE score 3.0 in the nine monkeys that qualified for the study (four controls and five Ab treated) was reduced 3-fold for the monkeys treated with anti-IL-12p40 Ab (odds ratio, 0.312; 95% confidence interval, 0.056–1.750).

The beneficial effect of the Ab was mainly exerted during the time interval from t_{EAE} = 0 to score 2.0 (ataxia), which was 12.8 ± 5 days in the sham group and 46 ± 23 days in the anti-IL-12p40 Ab-treated group (p = 0.21). The subsequent progression from a score of 2.0 (ataxia) to 3.0 (complete paralysis below the waist) occurred within the same time interval in both groups, namely, 18 ± 7 days in the control group and 18 ± 4 days in the anti-IL-12p40-treated group. This suggests that disease progression from a score of 2.0 to 3.0 is less dependent on IL-12p40 activity.

Effect of anti-IL-12p40 Ab treatment on lesion load and lesion activity

Lesion load. Fig. 2 shows the same slices at the start of treatment and in the last recorded T2W images before that monkey was killed (t_{EAE} = final) in monkeys treated with saline (A) or anti-IL-12p40 Ab (B). The calculated volumes of all individual lesions at the consecutive time points are depicted in graphic form. Volume measurements show that the individual lesion volumes varied considerably between monkeys, but that lesions developing in the Ab-treated monkeys (Fig. 2B) enlarged less rapidly than those developing in the monkeys that received sham treatment (Fig. 2A). At t_{EAE} = final, the average number of lesions in sham-treated monkeys was 8.3 ± 1.4, with a mean volume of 19.9 ± 4.0 cm³ and
an average brain lesion load sum of 160.4 ± 38.8 cm³. The average lesion number in anti-IL-12p40 Ab-treated monkeys was 17.0 ± 5.1, but despite the much longer disease duration, the mean volume remained substantially lower, namely, 9.1 ± 4.0 cm³. The average total lesion load at tEAE final in Ab-treated monkeys was slightly lower than that in the placebo-treated animals (151.6 ± 82.3 cm³), but this is mainly caused by monkey Mi093, which already contained a very high lesion load at the initiation of treatment.

Lesion enlargement and activity. To assess the effect of anti-IL-12p40 treatment on lesion enlargement, we analyzed only lesions present at tEAE = 0 (controls, seven lesions; anti-IL-12p40 Ab, 16 lesions; Fig. 3). Notably, the bell shape of the curves in Fig. 3 does not reflect normal lesion development, but is determined by the sequential withdrawal of monkeys with severe EAE and serious CNS pathology from the experimental groups.

Between tEAE = 0 and tEAE = final, the mean volume of the selected lesions increased 14-fold in the group of sham-treated monkeys (from 2.2 ± 0.9 to 30.7 ± 5.4 mm³) and only 4.1-fold in the Ab-treated animals (from 5.9 ± 1.7 to 19.3 ± 5.7 mm³). Fig. 3b shows the markedly reduced increase of the lesion volume between tEAE = 0 and tEAE = 28 in Ab-treated compared with placebo-treated monkeys.

**T2.** We have determined the relative change in T2 relaxation times as a measure of the treatment effect of anti-IL-12p40 Ab on lesion inflammation (Fig. 3c). Predictably, the T2 relaxation times of the lesions in sham-treated monkeys increased significantly with disease progression. This increase in T2 relaxation times was virtually absent in the anti-IL-12p40 Ab-treated animals, indicating almost complete suppression of inflammatory activity in the lesions. At no time point did we detect significant differences in T2 relaxation times of the perilesional NAWM between anti-IL-12p40- or sham-treated monkeys (data not shown).

**GE-T1W.** The relative change in GE-T1W values was calculated as a measure of BBB leakage. The data in Fig. 3d show that the peak of BBB permeability occurred at tEAE = 14 days in the control group and at tEAE = 28 days in the Ab-treated group.

**Histopathological characterization of end-stage lesions**

Because all monkeys were killed with severe clinical EAE, we expected to find many lesions with active inflammation and demyelination in the brains of both controls and Ab-treated animals. The degrees of inflammation and demyelination in the spinal cord were quantified as described previously (32). As in the brain, we did not find a markedly different degree of demyelination between...
A prophylactic treatment effect of this Ab has previously been shown in a myelin-induced EAE model in marmosets (20), but the effect of treatment during established disease is unknown. In the current study we have treated animals with established disease, as assessed by the presence of active brain white matter lesions. The critical clinical parameters in the study were 1) the time interval between the detection of the first lesion and score 2.0, and 2) the time interval between score 2.0 and 3.0. An EAE score of 2.0 (ataxia; i.e., difficulty with keeping balance indicating cerebellar disturbance) can be compared with a score of 4.0–5.0 on the Expanded Disability Status Scale that rates functional impairment in MS on a scale of 1–10 (36). A score of 3.0, which is the clinical end point of the study, is given to monkeys with one- or two-sided paralysis below the waist, which is to some extent comparable with an Expanded Disability Status Scale score of 8.0. The critical MRI parameters were those detecting BBB leakage, lesion inflammation, and lesion enlargement.

**Treatment effect on clinical scores**

In accordance with our historical data (15), 100% of the monkeys developed EAE, although the disease course varied considerably between individual animals. It is inherent to the outbreed character of this model that lesions develop after a variable period of time, in the current study after 57 ± 32 days (t_EAE = 0), which is also in the normal range for this model. The random assignment of monkeys to either of the treatment groups resulted in an asymmetrical distribution of EAE cases; the most severely affected monkeys were in the Ab-treated group. One monkey in the placebo group had to be withdrawn from the experiment because it did not comply with the inclusion criteria (see Materials and Methods). In contrast, the Ab group contained one monkey (Mi092) that at the start of treatment already had clinical score of 2.0 and a second monkey (Mi093) that at t_EAE = 0 lacked clinical signs, but displayed a dramatic brain lesion load. Nevertheless, we observed a clear delay in the disease progression to an EAE score of 2.0. The fact that the disease progression from an EAE score of 2.0 to 3.0 is less influenced by the Ab was not entirely unexpected, because pathologicial processes causing the progressive neurological deficit late in the disease, such as axonal degeneration, are probably less sensitive to the inhibitory activity of the Ab. The clinical effects were compatible with the histological findings, because we observed suppression of inflammation in the lesions, but no effect on demyelination and axonal pathology. It is therefore highly remarkable that monkey Mi092 survived >100 days, although it already had a clinical score of 2.0 at the start of the treatment. Monkey Mi093 survived 24 days despite the dramatic brain lesion load present at the start of the treatment.

**Treatment effect on MRI**

Predictably, the T2 lesions in the sham-treated monkeys showed strong enlargement associated with a significant increase in T2 relaxation times due to inflammatory edema. Both parameters were substantially reduced in the anti-IL-12p40 Ab-treated monkeys. From treatment day 28 onward, we observed increased leakage of the BBB in Ab-treated monkeys. Moreover, after treatment day 14, new lesions became detectable. However, our calculations show that newly formed lesions remained substantially smaller than those formed in sham-treated monkeys, although the total lesion volume was comparable in the two groups. These data indicate that the anti-IL-12p40 Ab has a strong inhibitory effect on the model, but does not completely block disease progression.

In summary, the treatment of rhMOG-immunized marmosets with anti-IL-12p40 Ab delays the clinical expression of existing lesions. However, once neurological deficit has become manifest...

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**FIGURE 3.** Effect of Ab treatment MRI parameters during the course of rhMOG-induced EAE in marmosets (C). From 10 rhMOG-immunized monkeys, qualitative and quantitative MR images were recorded at 14-day intervals (see Materials and Methods). When a brain white matter lesion was detectable on T2W MRI (t_EAE = 0; see Fig. 2), weekly i.v. injections with saline () or IL-12p40 Ab (○) were started. Note that the time interval (t_EAE in days) between the immunization and t_EAE = 0 differs between individual monkeys. The graphs depicted show: a, time to reach serious impairment of motor functions (neurological score, ≥3); b, the mean total lesion load per animal; c, the mean T2 relaxation times of all individual lesions; and d, the mean increment in T1W values by i.v. injected Gd-DTPA. All results are expressed relative to t_EAE = 0. The decline in the sham graphs beyond t_EAE = 28 is due to the withdrawal from the experiment of monkeys with strong lesion enlargement and early onset of EAE, but does not reflect the natural course of the lesions. The outlier monkey Mi089 has been excluded from the graphs. *p < 0.05 vs control. Only lesions present at t_EAE = 0 were included, and all values are expressed relative to t_EAE = 0.

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**Discussion**

We have examined the efficacy of a fully human IgG1κ mAb directed against the IL-12/23p40 subunit during ongoing disease in a nonhuman primate model of MS. Because the Ab is ineffective in rodents, it can only be preclinically tested in nonhuman primates. A phase I study in psoriatic patients has shown a very promising safety, pharmacokinetic, and clinical profile of this Ab (35).
References


Table II. Extent of spinal cord and brain inflammation and demyelination

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*With the exception of monkeys Mi089, marmosets were sacrificed with severe clinical EAE (see Table I). Routinely, the extent of inflammation, demyelination, and axonal loss was expressed semiquantitatively in the brain and quantitatively in the spinal cord (see Materials and Methods). The lesions were classified on basis of their overall activity as: early active (EA), late active (LA), inactive (IA).*


