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The experimental autoimmune encephalomyelitis (EAE) model in the common marmoset approximates recognized features of the human disease multiple sclerosis (MS) with regard to its clinical presentation as well as neuropathological and radiological aspects of the lesions in brain and spinal cord. IL-12 is a proinflammatory cytokine that is produced by APC and promotes differentiation of Th1 effector cells. IL-12 is produced in the developing lesions of patients with MS as well as in EAE-affected animals. Previously it was shown that interference in IL-12 pathways effectively prevents EAE in rodents. In this study we report that in vivo neutralization of IL-12p40 using a novel Ab has beneficial effects in the myelin-induced EAE model in common marmosets. The Ab was injected i.v. at 7-day intervals starting well after immunization (day 14) and was continued until the end of the study (day 86). Stable levels of the Ab were measured 3 days after each injection throughout the study period. During this period anti-Ab responses could not be detected. We demonstrate that anti-IL-12p40 treatment has a protective effect on the neurological dysfunction as well as on neuropathological changes normally observed in the brain and spinal cord of EAE-affected individuals. The Journal of Immunology, 2002, 169: 6554–6563.

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Multiple sclerosis (MS) is a chronic inflammatory demyelinating disease of the CNS. The pathological hallmark of MS is the CNS white matter lesion, a focal area of infiltrated mononuclear cells with a variable degree of demyelination, axonal loss, and gliosis. Although susceptibility to MS is thought to be a multifactorial trait, it is generally accepted that disease progression is driven by autoimmune reactions directed against Ags of the CNS white matter (1, 2). A broad pathological analysis revealed that in MS at least four fundamentally different neuropathological patterns can be discerned (3). Patterns I and II lesion pathologies are modeled in the current virus- and autoimmune-based animal models of encephalomyelitis that have been established in susceptible rodent strains (4–6) or non-human primates (7).

IL-12 is the predominant cytokine for triggering Th1-mediated (autoimmune) responses; it is induced when CD4+ T cells and APC interact (8–10) and abrogated when CD40-CD154 ligation is disturbed (11–13). Evidence is accumulating that IL-12 plays a pivotal role in the induction of the critical autoimmune responses involved in the initiation of experimental autoimmune encephalomyelitis (EAE), lesion formation, and progression of the disease (11–22). Therapies directed at the neutralization of IL-12 or prevention of production by abrogation of CD40-CD154 interaction have proven effective in rodent (20, 21, 23–27) as well as marmoset (28) models of EAE, while excess IL-12 reverses these effects and enhances EAE severity (15–17, 22, 29). Furthermore, local expression of IL-12 within the CNS of rodents (14, 30, 31) and common marmosets (32) during active EAE has been demonstrated.

Although the situation is less clear in MS, IL-12 is locally expressed within the CNS of affected individuals (33–35), and levels of IL-12 in cerebrospinal fluid and plasma are increased during active disease (36–38). Moreover, it has been argued that the beneficial effect of IFN-β on MS is exerted via suppression of IL-12 production (39–41). Finally, lower baseline levels of IL-12p35 and p40 mRNA seem to predict clinical responsiveness to IFN-β treatment (42, 43).

In its radiological and neuropathological presentation, the chronic progressive EAE model in the common marmoset approximates the most prevalent lesion type, pattern II, in MS patients (7, 44, 45). These aspects added to the MS-like clinical expression of the disease (7, 46) and the close immunological similarity with humans (47–49) make this model an excellent test system for preclinical evaluation of new therapies for chronic MS. The model is particularly important for the safety and effectivity testing of biotechnology engineered reagents, which by their species specificity cannot be evaluated in rodent EAE models. The present study demonstrates that i.v. injected mAb directed against human IL-12p40 significantly reduces the lesion load, as detected by magnetic resonance imaging (MRI) and neuropathological examination.
Materials and Methods

Animals

Ten nonrelated healthy common marmosets (Callithrix jacchus) were selected from the experimental stock of the Biomedical Primate Research Center (Rijswijk, The Netherlands). Before the monkeys entered the experiment, a full physical, hemoculture, and biochemical check-up was performed. Individual data of the monkeys, which were identified with an implanted transponder, are summarized in Table I.

Ethical regulations limit the total blood volume that can be collected and the frequency of MR imaging. Hence, the monkeys were randomly paired and assigned to the groups receiving placebo or anti-IL-12p40 mAb treatment before the immunization (see Table I). Paired indiviuals were handled identically throughout the experiment. The time points for collection of larger blood volumes for immunological tests and MRI were determined on the basis of the clinical stage of EAE in one individual of each pair.

During the experiments the monkeys were individually housed in spacious cages with padded shelters provided at the bottom of the cage and were under constant veterinary care. The daily diet consisted of commerical food pellets for New World monkeys (Special Diet Services, Witham, U.K.), supplemented with rice, raisins, peanuts, marshmallows, biscuits, and fresh fruit. Drinking water was provided ad libitum. According to Dutch law on animal experimentation, the protocol of this study was reviewed and approved by the institute’s animal care and use committee.

Disease induction and clinical read-out

EAE was induced by a single immunization with 300 μl human myelin in water (10 mg/ml) emulsified with 300 μl CFA (Difco, Detroit, MI) under ketamine anesthesia (6 mg/kg; AST Farma, Oudewater, The Netherlands) as described previously (50). Bordetella pertussis was not used for reasons discussed previously (50).

Twice daily, clinical signs of EAE were scored blind by a trained observer using a previously described semiquantitative scale (50): 0, no clinical signs; 0.5, apathy, loss of appetite and altered walking pattern without ataxia; 1, lethargy and/or anorexia; 2, ataxia, sensory loss/blindness, 2.5, hemi- or paraparesis; 3, hemi- or paraplegia; 4, quadriplegia; and 5, spontaneous death attributable to EAE. Body weights were determined once weekly as a surrogate disease marker. Monkeys were sacrificed for ethical reasons once a monkey had reached an EAE score of 3.0 or on day 86 after immunization, which was the planned end point of the study.

Reactivity, dosing regimen, plasma levels, biodistribution, and immunogenicity of anti-IL-12p40 Ab

The neutralization of marmoset IL-12 by the anti-human IL-12p40 mAb (IgG1k) was tested using LPS-stimulated cells. Briefly, plastic-adherent cells from common marmosets and human PBMC were stimulated for 24 h with LPS (1 μg/ml). Cell-free supernatant was collected and titrated onto 4-day cultured cells, with plastic-adherent cells from RPrs (experiment with the yield of the cytokines. Furthermore, the surface area of all rodent cells sections stained with H&E to visualize inminated tissues. Cells were fixed in 4% paraformaldehyde for 1 h at room temperature. The tissue sections were rehydrated in PBS and counterstained with 0.1% crystal violet and 0.1% methylene blue.

Expression profiles of pathogenic effector molecules in the CNS

Immunohistochemistry was performed essentially as previously described (32, 52) with minor modifications. Frozen sections of 6 μm thickness were cut, thaw-mounted on glass slides, and kept overnight at room temperature in a humidified atmosphere. After air-drying for 1 h slides were fixed in fresh acetone containing 0.02% H2O2 (v/v), air-dried for 10 min, washed with PBS, and incubated overnight at 4°C with primary Ab. Incubations with secondary and tertiary reagents were performed for 1 h at room temperature. Between the incubation steps slides were washed twice with PBS. Detection of primary antibody was achieved with biotinylated anti-mouse Ig HRP (Dako, Glostrup, Denmark) or, in the case of three-step staining, with rabbit anti-mouse IgG (Dako) and HRP-labeled avidin–biotin complex (Dako). Rabbit polyclonal Ab was detected with biotin-labeled goat anti-rabbit Ig (Amersham, Little Chalfont, U.K.) as a second step. HRP activity was revealed by incubation for 10 min at room temperature with 3-amino-9-ethylcarbazole (Sigma, St. Louis, Missouri), resulting in a bright red translucent precipitate.

For detection of IFN-γ and TNF-α, mAb MD-2 and 61E71 were used, respectively (U-CYTECH, Utrecht, The Netherlands). mAb against IL-4 and IL-6 were obtained from Genzyme (Cambridge, MA). CR2, a mouse Ab used for detection of IL-12p40, was obtained from BD PharMingen (San Diego, CA). IL-10 was visualized using B-S10 mAb (Innogenics, Hilversum, The Netherlands). The Ab against IL-18, M318, was obtained from R&D Systems (Abingdon, U.K.). ZD9, a mouse mAb against matrix metalloproteinase 9 (MMP-9; gelatinase B), was a gift from Dr. G. Opdenakker (REGA Institute, Leuven, Belgium). Rabbit polyclonal Ab against CD3 and inducible NO synthase (iNOS) were obtained from Dako and Calbiochem (San Diego, CA), respectively. Finally, CD40 was detected using a mouse anti-human CD40 mAb (mAb SD12; Tanox, Houston, TX), which is known to be cross-reactive with marmoset CD40 (28, 32).

In situ detection of i.v. administered anti-IL-12p40 Ab

To determine whether the Ab traverses the blood-brain barrier and gains access to lesions in the CNS, 1 mg of biotinylated anti-IL-12p40 Ab was i.v. injected into a PBS-treated monkey with an EAE score of 3.0 (Mi-031). One hour after injection of the Ab the monkey was sacrificed. Frozen tissue sections of brain and spleen were incubated with SA-HRP (Jackson ImmunoResearch) for 1 h at 0°C temperature, followed by tyramide signal

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amplification (NEN Life Science Products, Boston, MA). HRP activity was revealed as described above. To assess whether the biotinylated Ab bound to IL-12p40 produced by astrocytes, tissue sections were double stained with the C8.6 mAb against IL-12p40 and donkey anti-human polyclonal Ab (Sanbio) directed toward glial fibrillary acidic protein characteristic for astrocytes. A combination of HRP and alkaline phosphatase-labeled conjugates was used, giving a red precipitate for 3-amino-9-ethyl-carbazole and a bright blue precipitate using Fast Blue BB base and napthol AS-MX phosphate for alkaline phosphatase, as described in detail previously (32, 52).

T and B cell functions

Just before necropsy, heparinized venous blood was drawn via a needle puncture from the vena saphena, after which PBMC were isolated using lymphocyte separation medium (ICN Biomedical, Aurora, OH). Cell suspensions were prepared from aseptically removed lymph node (LNC) and spleen (SC) and cultured in the presence of recombinant human myelin oligodendrocyte glycoprotein (rMOG; 10 µg/ml) or human myelin basic protein (hMBP; 25 µg/ml) throughout the observation period of 86 days (Fig. 2). This is an ~50-fold excess of the IC50 determined in vitro. The assay used to measure anti-IL-12p40 Ab required the plasma Ab to bind to IL-12p40 to be detected. Therefore, the assay was designed to measure only active anti-IL-12p40, which may, in fact, be less than the total plasma concentration. In one animal (Mi-026) an unexplained disappearance and subsequent reappearance of anti-IL-12p40 Ab were observed. No anti-Ab responses were detectable, and no alterations in hematological and biochemical parameters were observed during the study period.

Statistics

The χ² test was used to determine statistically significant treatment-related effects on the progression to an EAE score of 3.0; the Mann-Whitney U test was used for anti-MOG and anti-MBP Ab levels and MRI scores. In all cases, p < 0.05 was considered statistically significant.

Results

Effect of in vivo neutralization of IL-12p40 on EAE

The EAE course in placebo- and Ab-treated monkeys is given as the day of disease onset, when the animal showed clear neurological signs (EAE score of 2.0), and when the disease score of 3.0 was reached (Table I). The percentages of maximal weight loss during the experiment are depicted in the same table. The results show a beneficial effect of Ab treatment on both aspects of EAE. Four PBS-treated monkeys developed severe progressive EAE, and one had a period of mild EAE during the observation period of 86 days. In the Ab-treated group only one monkey, Mi-019, developed clinical signs of EAE (p < 0.001 vs PBS treatment, by χ² test). It should be noted, however, that the time interval between disease onset and EAE score of 3.0 in this animal was considerably longer than that in the placebo-treated monkeys (see Table I).

Cross-reactivity, serum levels, and anti-Ab responses

As shown in Fig. 1, the IFN-γ-inducing properties of conditioned medium derived from LPS-stimulated marmoset adherent cells were neutralized by anti-IL-12p40 Ab. The IC50 was comparable with conditioned medium from human cells. The Ab levels measured at 3 days after each administration were generally maintained at a concentration of 50–75 µg/ml throughout the observation period of 86 days (Fig. 2). This is an ~50-fold excess of the IC50 determined in vitro. The assay used to measure anti-IL-12p40 Ab required the plasma Ab to bind to IL-12p40 to be detected. Therefore, the assay was designed to measure only active anti-IL-12p40, which may, in fact, be less than the total plasma concentration. In one animal (Mi-026) an unexplained disappearance and subsequent reappearance of anti-IL-12p40 Ab were observed. No anti-Ab responses were detectable, and no alterations in hematological and biochemical parameters were observed during the study period.

In vivo and postmortem MRI

The a priori condition set before the experiment was to subject paired monkeys to MRI analysis once one of the animals had reached an EAE score of 2.0 (ataxia) regardless of the clinical condition of the second monkey. Because of the acute onset of the disease in Mi-032 and Mi-043, both animals were euthanized for ethical reasons before an in vivo MRI could be made. Consequently, the in vivo MRIs of Mi-026 and Mi-023 were recorded on day 55 a.i. ND, not done. The number of infiltrates in the brain was quantified using immunohistochemistry. The number of infiltrates per section was scored as: −, no infiltrates; +, infiltrates; +++, 4–10 infiltrates; and +++++, >10 infiltrates. Results represent the mean of two sections. The size of the largest infiltrates found in two sections was scored as: −, small (<30 cells); +, medium (>30 cells); and +++, large (>100 cells). The inflammatory index (Infl. index) in the spinal cord was quantified as being the average number of inflamed blood vessels per spinal cord cross-section (10–15 sections). Furthermore, the surface area of demyelination (Demyel (%)) was quantified for 10–15 spinal cord cross-sections using a monomorph grid. Inflammation and demyelination in the brain are expressed as present (+) or absent (−).

Table I. Individual data of the monkeys

<table>
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<tr>
<th>Name</th>
<th>Gender</th>
<th>Pair</th>
<th>Birth Date (month-year)</th>
<th>Max. Weight Loss (%)</th>
<th>Disease Onset (day a.i.)</th>
<th>EAE Score 2.0 (day a.i.)</th>
<th>Time of Sacrifice (day a.i.)</th>
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*Clinical signs of EAE were scored as 0, no clinical signs; 0.5, apathy, loss of appetite and altered walking pattern without ataxia; 1.0, lethargy and/or anorexia; 2.0, ataxia, sensory loss/blindness; 2.5, hemi- or paraparesis; 3.0, hemi- or paraplegia; 4.0, quadriplegia; and 5.0, spontaneous death attributable to EAE. Body weight was determined from day 14 after immunization (a.i.) onward and either sacrificed when an EAE score 3.0 was reached or at the end of the study period (day 86 a.i.). T1-w (pre- and postcontrast) and T2-w MRI datasets were acquired and scored as described in Materials and Methods. MRI were made once one of the animals had reached an EAE score of 2.0 (ataxia) regardless of the clinical condition of the second monkey. Because of the acute onset of the disease in Mi-032 and Mi-043, both animals were euthanized for ethical reasons before an in vivo MRI could be made. Consequently, the in vivo MRIs of Mi-026 and Mi-023 were recorded on day 55 a.i. ND, not done. The number of infiltrates in the brain was quantified using immunohistochemistry. The number of infiltrates per section was scored as: −, no infiltrates; +, infiltrates; +++, 4–10 infiltrates; and +++++, >10 infiltrates. Results represent the mean of two sections. The size of the largest infiltrates found in two sections was scored as: −, small (<30 cells); +, medium (>30 cells); and +++, large (>100 cells). The inflammatory index (Infl. index) in the spinal cord was quantified as being the average number of inflamed blood vessels per spinal cord cross-section (10–15 sections). Furthermore, the surface area of demyelination (Demyel (%)) was quantified for 10–15 spinal cord cross-sections using a monomorph grid. Inflammation and demyelination in the brain are expressed as present (+) or absent (−).
same day (Fig. 3B). Instead multiple, very small, hyperintense foci were found in the T2-w brain images of this monkey, yielding an in vivo MRI score of 4.0 as defined by Jordan et al. (51). In the postmortem scans, however, these foci were not visible, suggesting that they may represent patches of edema rather than demyelinated lesions. Scores of in vivo and postmortem MRI are given in tabular form. The table shows that postmortem MRI scores of Ab-treated animals tended to be lower compared with the scores of control animals (p = 0.08, by Mann-Whitney U test).

**Neuropathology**

All PBS-treated control animals displayed cellular infiltrates in the brain and spinal cord. Four PBS-treated animals showed extensive demyelination, which was mainly localized in the spinal cord, while in the animal with the mild EAE (Mi-038) the CNS pathology was mainly localized in the brain (see Table I). Although inflammation and demyelination of the CNS were detected in two Ab-treated individuals, three animals (Mi-003, Mi-023, Mi-026) were completely devoid of CNS pathology in spinal cord and brain (p = 0.03 for inflammation and demyelination, by Mann-Whitney U test). The PBS-treated animal Mi-032 displayed large areas with infiltrated mononuclear cells and specific demyelination of the CNS in the spinal cord (Fig. 4, A and C), while no such pathological changes were observed in the Ab-treated animal of this pair, Mi-026 (Fig. 4, B and D). Macrophages actively involved in demyelination were present in the lesions as shown in Fig. 4, E and F, while B and T cells also could be detected (Fig. 4, G and H, respectively). The immunoreactivity for APP points at degeneration of the demyelinated axons (Fig. 4I).

**CNS lesion load and expression of pathogenic molecules**

Immunological aspects of the brain lesions were assessed in cryosections on the basis of the number and size of the infiltrates as defined by hematoxylin counterstaining, as well as the expression of the pan-T cell marker CD3 and of acid phosphatase as a marker of infiltrating macrophages. Both the number and size of infiltrates in the brains of Ab-treated animals were markedly reduced compared with those in control animals (Table I). While CD3+ T cells were present in the cellular infiltrates of all EAE-affected monkeys, these were undetectable in the brains of three of four Ab-treated animals that remained asymptomatic. Together these data indicate that neutralization of IL-12p40 affects recruitment of mononuclear cells from the circulation into the CNS.

Next we assessed whether IL-12p40 neutralization would interfere with CNS expression of Th1 or Th2 cytokines (IFN-γ, IL-4, IL-10, IL-12p40, IL-18, TNF-α) or the inflammatory mediators iNOS and MMP-9. IL-12p40 was expressed by some mononuclear cells within brain infiltrates and by many astrocytes (Fig. 5, A and B). The number of IL-12p40-expressing astrocytes decreased with distance from the infiltrates. Similar expression of IL-12p40 was found in both groups of animals (Fig. 5, A vs B). In control animals lacking CNS inflammation, naive animals, or monkeys immunized with OVA emulsified in CFA, no IL-12p40 was detectable within the CNS (Fig. 5C).

The staining is specific, as controls omitting the specific Ab step were shown to be negative (Fig. 5D). Mononuclear cell infiltrates in the brain of PBS-treated animals displayed clear expression of IL-4, TNF-α, and MMP-9 (Fig. 5, E, G, and I). In asymptomatic Ab-treated animals CNS expression of IL-4, TNF-α, and MMP-9 was substantially reduced (Fig. 5, F, H, and J). IFN-γ was also expressed at lower frequency in nonaffected mAb-treated animals (data not shown). The two Ab-treated animals that showed CNS inflammation with histology displayed staining patterns similar to those in the EAE-affected animals in the PBS-treated group. No marked effect of Ab treatment on the numbers of IL-6-, IL-10-, IL-18-, and iNOS-expressing cells was observed (results not shown).

**Access of anti-IL-12p40 Ab to lesions within the CNS**

We assessed whether i.v. administered Ab extravasates into CNS lesions, allowing the capture of locally produced IL-12p40. To this end, biotinylated anti-IL-12p40 Ab was injected i.v. into two PBS-treated animals with clinically manifest EAE (score of 3.0) at 1 h

---

**Table I.**

<table>
<thead>
<tr>
<th>In Vivo MRI Score</th>
<th>Postmortem MRI Score</th>
<th>Infiltrates in the Brain</th>
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<th>Brain</th>
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**FIGURE 1.** Anti-IL-12p40 Ab neutralizes marmoset IL-12. We tested the neutralization of IL-12 in culture supernatant obtained from LPS-stimulated marmoset or human PBMC. As shown, the IL-12 neutralization profiles in conditioned media of marmoset and human origin were comparable.
before sacrifice. The subsequent immunohistochemical analysis revealed granular staining adjacent to the ventricles as well as in the cellular infiltrates within the brain of animals injected with the Ab (Fig. 6, A and B). No such staining was detected in the brains of naive animals (Fig. 6C), or OVA/CFA-immunized marmosets (not shown). Biotinylated Ab was found attached to the astrocytes in the immediate environment of the inflammatory infiltrates. This is in conformity with the immunohistochemical demonstration of IL-12p40 by brain astrocytes in EAE-affected animals (Fig. 5A).

Anti-myelin T cell reactivity
To investigate the effect of anti-IL-12p40 Ab on anti-myelin T cell activity we determined the proliferative responses of PBMC, SC, and LNC to rhMOG and hMBP in Ab- and placebo-treated animals. The results in Fig. 7 show that in all cases the T cell response to MBP was low, while high responses were found to rhMOG (MOG vs MBP, \( p < 0.01 \), by Mann-Whitney U test). In the animals that developed EAE relatively early after disease induction, significant proliferative responses to rhMOG were detectable in SC as well as LNC (Fig. 7, A–C). In contrast, in all animals sacrificed at the end point of the study (both PBS- and Ab-treated animals), proliferative responses were mainly restricted to the spleen, while those in LNC were very low or undetectable (Fig. 7, E–J). As a similar profile was found in the placebo monkey of pair 5, we assume that the number of autoreactive T cells in the spleen is not affected by the Ab treatment.

Anti-myelin B cell reactivity
A critical role of anti-MOG Ab in the marmoset EAE model, mediating demyelination in particular, has been reported (45, 53–56). We therefore determined the serum levels of anti-MBP and anti-MOG Ab in PBS- and Ab-treated animals using ELISA. Anti-MOG and anti-MBP IgM Ab were hardly detectable in the sera of the animals from both groups at any of the evaluated time points. As shown in Fig. 8, increased anti-MOG (A) and anti-MBP (B) IgG serum levels were found in PBS-treated animals at an EAE score of 2.0 compared with IgG Ab levels in sera from the paired Ab-treated animals (\( p = 0.05 \), by Mann Whitney U test). At necropsy, only serum levels of MBP-specific IgG were significantly reduced in the asymptomatic Ab-treated animals (\( p = 0.03 \)). Taken together, neutralization of IL-12p40 seems to have a suppressive effect on the production of autoantibodies.

Discussion
In this article we report that Ab-mediated neutralization of IL-12p40 results in profound protection from EAE in the common marmoset model. While the role of IL-12p40 has been demonstrated in murine EAE, this is the first report that IL-12p40 plays a key role in this non-human primate model of chronic MS in the human population. Besides its beneficial effect on the EAE course the Ab has some other promising capacities for the application in MS. First, stable plasma levels of the active anti-IL-12 Ab were measured 3 days after each injection during the entire study period, indicating that the Ab is probably not very immunogenic. However, for technical reasons we have been unable to directly measure monkey Abs to the anti-IL-12 Ab. In the past years we have tested a variety of extensively engineered Abs in non-human primate models of autoimmune arthritis and encephalomyelitis.
Although by extensive engineering up to 99% of the original mouse IgG was replaced with human Ig sequences, the neutralizing immune response toward the remaining 1% mouse part of the molecule abolished a long term therapeutic effect. For example, treatment of EAE-affected marmosets with a chimeric anti-CD40 Ab resulted in substantial neutralizing Ab activity within 2 wk of the first administration (28). Similar findings have been published by other groups (56–58). A second important capacity is that the injected Ab can be detected inside lesions, indicating that it can also act locally (see below).

All PBS-treated monkeys in this study developed clinical EAE. In contrast, four of five Ab-treated animals remained asymptomatic. The disease progression in the one Ab-treated animal that developed clinical EAE was substantially delayed compared with that in the PBS-treated monkeys. Our results also show a diminution of MRI-detectable changes in CNS white matter in Ab-treated monkeys compared with those in paired placebo-treated monkeys. A clear effect of the treatment was that the periventricular inflammatory reactions, as observed in all PBS-treated monkeys, were absent in all anti-IL-12p40 Ab-treated monkeys. The beneficial effect of Ab treatment on in vivo MRI scores is less significant than that on the scores based on postmortem images. This is probably because T2-w MR images detect mainly altered water content of tissues, such as due to vasogenic edema, while with T2-w images of fixed tissue areas of demyelination can be visualized (50). Indeed, the postmortem MRI scans showed clearly reduced MRI scores in the Ab-treated monkeys (with the exception of Mi-019) compared with the placebo group (Table I). More advanced MRI parameters are needed to reveal the beneficial effects of IL-12p40 neutralization in vivo. Quantitative MRI parameters are now being developed and implemented for serial imaging, in particular T1, T2, and MTR maps (E. L. A. Blezer, H. P. M. Brok, K. Nicolay, and B. A. t’Hart, manuscript in preparation). The beneficial effect of anti-IL-12p40 Ab on CNS white matter pathology was confirmed with histology; three of five Ab-treated monkeys remained completely devoid of CNS infiltration by CD3⁺ T cells, while inflammation and demyelination were also prevented.
IL-12 is expressed at sites where T cells and APC interact (8–10). In the common marmoset EAE model, such sites are the secondary lymphoid organs and the developing lesions within the CNS white matter (32, 52). While microglia is an important source of IL-12p40 (red) within the CNS (10, 59), astrocytes have also been found to produce IL-12p40 (60). Our present finding that astrocytes in EAE-affected marmosets indeed express intracellular IL-12p40 confirm this observation.

Intravenously injected biotinylated Ab was retrieved at peripheral (spleen/lymph nodes) as well as central (brain) locations, confirming our previous finding that astrocytes in EAE-affected marmosets indeed express intracellular IL-12p40 confirm this observation.

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In view of the 100% susceptibility of outbred common marmosets to myelin-induced EAE (7, 44), it is highly unlikely that the observed beneficial effects of anti-IL-12p40 Ab can be explained by the possibility that the Ab-treated monkeys that remained asymptomatic were all nonresponders to the disease induction. Hence, the conclusion is warranted that anti-IL-12p40 Ab treatment protects marmoset monkeys immunized with human myelin in CFA against the clinical and neuropathological expression of EAE. Our present results suggest that anti-IL-12p40 Ab may modulate autoimmune responses.
Anti-myelin Ab are considered a critical factor in the marmoset EAE model as mediators of CNS demyelination (44, 53–55, 62). It is therefore of great interest that during the development of EAE, circulating autoantibody levels were consistently lower in anti-IL-12p40 Ab-treated animals than in the paired PBS-treated animals. This suggests that besides preventing T cell recruitment into the CNS, autoantibody responses are also mediated by IL-12p40. Similar observations have been made in marmosets that were protected from EAE by anti-CD40 mAb treatment. In that study the protective effect of the mAb to clinical EAE was associated with abrogation of broadening of the epitope response against MOG (28).

The different reactivity patterns of T cells present in PBMC, LNC, and SC to MBP and MOG between PBS- and Ab-treated monkeys can be explained by the different disease durations. Our data are in line with observations by Targoni and coworkers (63) that T cell autoreactivity in mice wanes in time from draining lymph nodes and the circulation during EAE development, but persists in the spleen. All Ab-treated monkeys displayed a similar reaction pattern as the one PBS-treated monkey sacrificed at the same time point (day 86). We assume, therefore, that IL-12p40 modulates the development of autoreactive T cells, yet does not interfere with cell circulation kinetics.

A likely result of IL-12p40 neutralization is deviation of the encephalitogenic myelin-reactive T cells into the Th2 direction, as was observed in mice (21). However, we are presently unable to prove this, since reagents for specific detection of Th2 cytokines in common marmosets with ELISA are lacking. Instead, using immunostaining we determined cytokine expression patterns within the CNS of both groups of monkeys.

In three of five Ab-treated animals neither T cell infiltrates nor IFN-γ- or IL-4-producing cells could be detected. Moreover, inflammatory mediators such as TNF-α and MMP-9 were not expressed in the brains of these monkeys. In contrast, in all PBS-treated animals we found brain infiltrates expressing each of the tested inflammatory mediators. The patterns of intracellular IL-12p40 expression within the CNS white matter did not differ between PBS- and Ab-treated animals. A possible explanation for this lack of difference is that the anti-IL-12p40 mAb acts on the effector mechanisms induced by released IL-12, but not on the actual production of IL-12p40.

In conclusion, anti-IL-12p40 Ab has direct access to secondary lymphoid organs and the CNS. Since Ab treatment was initiated at a late stage (day 14 after immunization), it is less likely that the Ab interferes with initial activation of autoreactive T and B cells. However, by neutralization of APC-derived IL-12, it affects the influx of autoreactive T cells into the CNS, inhibits the inflammatory response, and suppresses the autoantibody response against myelin proteins, resulting in prevention of EAE development.
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