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Prevention of Experimental Autoimmune Encephalomyelitis in the Common Marmoset (Callithrix jacchus) Using a Chimeric Antagonist Monoclonal Antibody Against Human CD40 Is Associated with Altered B Cell Responses

Louis Boon,†* Herbert P. M. Brok,‡† Jan Bauer,§ Antonio Ortiz-Buijssse,* Marc M. Schellekens,* Seema Ramdien-Murli,* Erwin Blezer,* Marjan van Meurs,** Jan Ceuppens,¶ Mark de Boer,* Bert A. ’t Hart,† and Jon D. Laman‡#**

Inhibition of CD40-CD40 ligand interaction is a potentially effective approach for treatment of autoimmune diseases, such as multiple sclerosis. We have investigated this concept with a chimeric antagonist anti-human CD40 mAb (ch5D12) in the marmoset monkey experimental autoimmune encephalomyelitis (EAE) model. Marmosets were immunized with recombinant human myelin oligodendrocyte glycoprotein (rMOG) and treated from the day before immunization (day −1) until day 50 with either ch5D12 (5 mg/kg every 2–4 days) or placebo. On day 41 after the induction of EAE, four of four placebo-treated monkeys had developed severe clinical EAE, whereas all animals from the ch5D12-treated group were completely free of disease symptoms. High serum levels of ch5D12 associated with complete coating of CD40 on circulating B cells were found. At necropsy placebo- and ch5D12-treated animals showed similar MOG-specific lymphoproliferative responses in vitro, but ch5D12 treatment resulted in strongly reduced anti-MOG IgM Ab responses and delayed anti-MOG IgG responses. Most importantly, treatment with ch5D12 prevented intramolecular spreading of epitope recognition. Postmortem magnetic resonance imaging and immunohistologic analysis of the CNS showed a markedly reduced lesion load after ch5D12 treatment. In conclusion, the strong reduction of clinical, pathological, and radiological aspects of EAE by ch5D12 treatment in this preclinical model points to a therapeutic potential of this engineered antagonist anti-CD40 mAb for multiple sclerosis.

C D40-CD40 ligand (CD40L) (CD154) interactions play an important role in B cell activation, APC activation, initiation of Ag-specific T cell responses, and induction of macrophage effector functions (1, 2). The central role of CD40-CD40L interaction in the initiation, amplification, and prolongation of immune responses justifies the choice of this cellular interaction as a target for immunotherapy in disorders based on unwanted immune responses, such as autoimmune diseases and transplant rejection. This has been illustrated in a large number of murine models of autoimmune diseases and transplantation (3). In addition, humanized anti-human CD40L mAbs targeting activated CD4+ T cells prevent kidney rejection in rhesus monkeys (4, 5) and rejection of pancreatic islets in rhesus monkeys and baboons (6, 7). To inhibit the CD40-CD40L interaction we have chosen the CD40 side of this interaction, targeting the APC, B cell, and macrophage activities. Therefore, we previously selected a mouse mAb (mu5D12) that, unlike most other anti-CD40 mAbs, potently inhibits CD40-CD40L-mediated activation in several cell types and is devoid of any CD40 stimulatory activity (8). μ5D12 strongly inhibits Ab production (both IgM and IgG) by human B cells cocultured with activated CD40L+ human T cells (9) as well as production of IL-12 and TNF-α by human monocytes induced by CD40L-CD40 interaction and IFN-γ (10). To reduce the potential immunogenicity and enhance the in vivo half-life of the parent 5D12 mAb for administration in humans, the murine parent Ab was re-engineered as a chimeric mouse-human mAb with a human IgG4 constant region (11).

Multiple sclerosis (MS) is an MHC-associated and T cell-dependent chronic inflammatory disorder of the CNS leading to white matter demyelination. The target of the cellular and humoral autoimmune reactivities in MS is the myelin sheath around axons, which is essential for pulse conduction. A number of myelin proteins, such as myelin basic protein (MBP), myelin oligodendrocyte glycoprotein (MOG), and proteolipid protein (PLP) have been described as possible target autoantigens (12). Active MS lesions in humans feature T cells and monocyte/macrophage accumulations around venules and venules and at plaque margins (13).
presence of activated T cells expressing CD40L in close contact with CD40-expressing macrophages in MS lesions, but not in brain sections, of patients with a non-demyelinating neurological disease (Alzheimer’s disease) suggests that lesion formation involves APC/T cell interactions (14). Functional evidence for the involvement of CD40-CD40L was provided in the SJL/J mouse model of PLP-induced experimental autoimmune encephalomyelitis (EAE), where injection with an mAb-blocking CD40L at the time of disease induction completely prevented disease. More importantly, treatment initiated well after disease induction strongly suppressed disease severity (14).

Immunization of common marmosets (Callithrix jacchus), a neotropical primate species, with human myelin or recombinant human MOG (rhMOG) in CFA induces a demyelinating form of EAE that strongly resembles human MS in its clinical and pathological presentation (15–17). Anti-MOG Abs play an important role in the facilitation of demyelination in marmoset EAE, because adoptive transfer of MBP-specific T cells induced clinical signs of EAE, but no demyelination was observed (18). Interestingly, when T cells were co-transferred with anti-MOG Abs fully demyelinated lesions were formed (19). The accessible location of MOG at the outer surface of the myelin sheet may be an important contribution to this observation. The expression patterns of CD40 and CD40L are highly comparable between MS patients (14) and marmoset monkeys with active disease (20). CD40 is abundantly expressed by macrophages and microglia, and CD40L. T cells are present at lower frequencies in inflammatory CNS lesions in both MS and marmoset EAE. The high similarity of marmoset EAE and human MS and their close phylogenetic relationship justify the use of EAE in marmoset monkeys as a preclinical model for testing of the engineered antagonist anti-human CD40 mAb.

The major aims of the present study were to prevent clinical signs and symptoms of EAE in outbred marmoset monkeys using mAb ch5D12, and furthermore, we aimed to present functional biological data to confirm the antagonistic characteristics of mAb ch5D12 in vivo.

Materials and Methods

**Animals and EAE induction**

Eight marmoset monkeys (C. jacchus) were randomly selected from the purpose-bred colony at the Biomedical Primate Research Center (Rijswijk, The Netherlands). The body weight of the animals at the start of the study ranged between 213 and 381 g. During the experiments, the monkeys were individually housed in spacious cages with padded shelters provided at the bottom of the cage. The daily diet consisted of food pellets for New World monkeys (Special Diet Services, Witham, U.K.) supplemented with rice, peanuts, marshmallows, biscuits, fresh fruit, and vegetables. Drinking water was provided ad libitum. EAE was induced using Escherichia coli-derived rhMOG, representing the extracellular domain of human MOG (21). All animals were immunized with 100 µg rhMOG emulsified in CFA. Under ketamine anesthesia (15 mg/kg; AST Farma, Oudewater, The Netherlands), 1 ml of the mixture was injected with 400 µl emulsion into the dorsal skin divided over four locations: two in the inguinal and two in the axillary region. Bordetella pertussis was not included in the immunization protocol. The clinical course of EAE was recorded daily by a trained observer using a semiquantitative scoring system: 0 = no clinical signs; 0.5 = apathy, loss of appetite, altered walking pattern without ataxia; 1 = lethargy and/or anorexia; 2 = ataxia; 2.5 = mono- or paraparesis and/or sensory loss and/or brain stem syndrome; 3 = hemi- or paraplegia; 4 = quadriplegia; 5 = spontaneous death attributable to EAE. The highest per day scores in a week were averaged. For ethical reasons monkeys were sacrificed when the clinical EAE score of 3 was reached. Each monkey was weighed three times a week, and the body weight was used as a surrogate marker of clinical well-being. Animals were treated with 5 mg/kg ch5D12 or placebo on days −1 (before immunization), 0, 2, 4, 6, 8, and 10 and thereafter twice weekly. In the period after day 27 until day 50 the dose frequency was increased from twice weekly to three times per week. PBS was used as placebo. According to the Dutch law on animal experimentation, the experimental procedures of this study were reviewed and approved by the institute’s animal care and use committee.

**Flow cytometry**

Whole blood samples were obtained preimmunization and on days 0, 2, 6, 10, 16, 25, 37, and 44 after immunization. For this purpose, 100 µl blood was drawn via a needle prick into the vena saphena and collected in 3 ml EDTA. Cells were collected by centrifugation and re-suspended, and aliquots were incubated with mAbs for 30 min at 4°C. Subsequently, RBC were removed with lysis buffer (Beckman Coulter, San Jose, CA) for 10 min at room temperature, and the remaining leukocytes were washed twice with PBS. Abs used for staining were anti-CD20, clone B1, directly labeled with R-6G (Beckman Coulter); anti-human IgM (to detect ch5D12 coated cells), directly labeled with FITC (Jackson ImmunoResearch Laboratories, West Grove, PA); anti-CD4, clone MT310 directly labeled with PE (Dako, Glostrup, Denmark); and anti-CD8 clone MT1004 (Connex, Munich, Germany), indirectly labeled with anti-mouse IgM-PE (Southern Biotechnology Associates, Birmingham, AL). To determine the ch5D12 coating of CD40 expressed by circulating B cells in vivo, incubations were performed with R1D-labeled anti-CD20 mAb in combination with FITC-labeled anti-human IgG mAb. The absence of CD20+/B cell staining with the anti-human IgG mAb might be due to either low ch5D12 serum levels or low CD40 expression. To discriminate between these options extra ch5D12 was added to a parallel FACs incubation.

**MOG-specific cellular response at necropsy**

Lymph node cell suspensions were prepared from experimentally induced inguinal and axillary lymph nodes, and lymphocytes were isolated using Lymphocyte Separation Medium (ICN Biomedical, Aurora, OH). Cultures were set up in medium (HEPES-buffered RPMI 1640; Life Technologies, Glasgow, U.K.) supplemented with 10% FCS (Flow Laboratories, McLean, VA), 10 mM MEM-nonessential amino acids, 2 mM l-glutamine, 100 U/ml penicillin G, 100 µg/ml streptomycin, and 2 × 10−4 M 2-ME (all obtained from Life Technologies). PBMC or lymph node cells (2 × 106/well) were seeded into 96-well flat-bottom plates (catalog no. 665180; Greiner, Solingen, Germany) and cultured with rhMOG (10 µg/ml) or lMBP (25 µg/ml). After 48 h, 0.5 µCi/well 3Hthymidine was added, and incorporation of radioactivity was determined 18 h later using a Matrix 9600 beta-counter (Packard, Meriden, CT).

**ELISA for ch5D12, anti-ch5D12, and anti-MOG**

Before each administration of ch5D12 or placebo, serum samples were prepared from venous blood obtained via a needle prick into the vena saphena to determine ch5D12 trough levels, anti-ch5D12 responses, and anti-MOG responses. The ch5D12 concentrations were determined in a competition ELISA. Briefly, plates were coated with Ag (10 ng/ml CD40-Fc) in PBS. For detection a mu5D12-HRP conjugate was used to compete with free ch5D12 for CD40-Fc. Simultaneous addition of ch5D12 and the mu5D12-HRP conjugate decreased absorbance compared with that observed with mu5D12-HRP alone. To circumvent the influence of serum in the ELISA, the standard matrix contained 1% human serum. For quantification, triplicate standard serial dilutions of mAb ch5D12 (4000–40 ng/ml) were added to each plate.

For the detection of Abs against the variable regions of ch5D12, plates were coated with mu5D12 at a concentration of 2 µg/ml, and for detection an alkaline-phosphatase labeled rabbit anti-mouse IgG Ab was used (Sigma, St. Louis, MO). Marmoset serum samples were serially diluted (1/1) from a starting dilution of 1/100 to a final dilution of 1/12,800. Responses were scored positive for the anti-ch5D12 variable region when the signal exceeded the background signal by a factor of 2. Background was determined in prestudy serum samples of the animal at the same dilution on the same plate.

Anti-MOG IgM and IgG responses were detected with ELISA on plates coated with mROG. Sera were tested at a 1/50 dilution. Bound Ab was detected using a polyclonal alkaline phosphatase-conjugated goat anti-mouse IgM µ-chain (Rockland, Gilbertsville, PA) or an alkaline phosphatase-labeled swine anti-human IgG y-chain (BioSource International, Camarillo, CA).

**Intramolecular spreading of MOG Ab responses**

The.Ab responses of individual monkeys to intact MOG and linear MOG were determined by biotin assay. Biotinylated hMOG and synthetic 23-mer peptides overlapping by 10 aa spanning the extracellular domain of MOG were spotted onto a polyvinyl difluoride membrane (Hybond; Amersham, Little Chalfont, U.K.) using a Bio-Dot SF
blotting apparatus (catalog no. 170-6542; Bio-Rad, Hercules, CA). To ensure that all peptides remained bound, the blots were immersed in 2.5% glutaraldehyde in PBS for 15 min and washed with PBS for 15 min and the remaining binding sites were blocked by incubation for at least 2 h in PBS containing 3% BSA (PBS/BSA). The blots were then incubated for 1 h with the relevant serum at a 1/1000 dilution in PBS/BSA (1%) and washed four times for 10 min each time with PBS containing 0.05% Tween 20. Blots were developed by incubation for 1 h with rabbit anti-human IgA, IgG, and IgM (DAKO); diluted 1/1/4,000 in PBS/BSA (1%); washed as described above, and processed for ECL detection according to the manufacturer’s instructions (Amersham).

Magnetic resonance imaging (MRI) of brain lesions
MRI was performed at the Image Sciences Institute of Utrecht University (Utrecht, The Netherlands). High resolution postmortem T2-weighted MR images were recorded from one formalin-fixed hemisphere of each animal using a 11-cm inner diameter gradient insert (22 gauge/cm). First, a sagittal scout scan was made, and the posterior and anterior positions of the corpus callosum were chosen as orientation markers for precise localization of the axial plane. Slices in the coronal direction were set perpendicular to the axial plane. A T2-weighted (TE/TR 25/7000 ms) multislice scan (50–70 slices of 0.5 mm thickness) was obtained. Each slide was recorded with a matrix of 512 × 256 data points and a field of view of 4 × 4 cm. The datasets were analyzed on an I-MAC G3 using the public domain National Institutes of Health program.

Neuropathology
Ketamine-anesthetized monkeys were euthanized by an i.v. injection of 400 mg sodium-pentobarbital (Euthesate; Aparhmo, Duiven, The Netherlands). The brain and spinal cord were excised in toto, fixed in 4% buffered formalin, and processed for neuropathologic examination. Fixed tissues were rinsed with PBS containing 0.05% sodium azide and embedded in paraffin. The extent of inflammation, demyelination, and axonal pathology was evaluated on tissue sections stained for H&E to visualize infiltrated cells with Klüver Barrera (Luxol fast blue combined with periodic acid Schiff) staining myelin and myelin degradation products and with Bielschowsky silver impregnation staining axons. The degree of demyelination (percent loss of total white matter) was quantified on Klüver Barrera-stained spinal cord cross sections (16–21) using an ocular morphometric grid. The degree of inflammation was expressed as an inflammatory index, i.e., the number of inflamed blood vessels per spinal cord cross section (10–15 sections). Immunocytochemistry was performed with a biotin-avidin system. T cells were stained with anti-human CD3 Abs (Dako); diluted 1/14,000 in PBS/BSA; washed as described above, and processed for ECL detection according to the manufacturer’s instructions (Amersham).

Results

Clinical course
The eight monkeys were randomly distributed over two groups of four monkeys and received injections of ch5D12 at 5 mg/kg body weight or the same volume of PBS two or three times per week for a period of 50 days. Immunization with rhMOG/CFA was performed 1 day after the first dose administration. Fig. 1 shows that all animals in the placebo group had developed clinical signs of EAE by day 41 (disease onset on days 30, 34, 38, and 41). In contrast, all ch5D12-treated animals remained free of clinical signs for the complete 50-day treatment period. In the placebo-treated group, two animals were sacrificed before day 50 according to Institutional Animal Care and Use Committee regulations, because of the severity of their disease. At the end of the 50-day treatment period, the remaining two animals of the control group and two blindly chosen ch5D12-treated animals were sacrificed for neuropathologic examination and postmortem MRI analysis. The two other ch5D12-treated animals (QN and QO) were further followed for possible occurrence of rebound disease. Animal QO developed EAE 9 days after cessation of treatment and was terminated with stage 3 EAE on day 65 after immunization (15 days after cessation of treatment with ch5D12). Animal QN remained asymptomatic for 100 days after cessation of treatment. Then a short episode of visual problems occurred (maximum score, 2.0), which fully remitted. Only after 200 days did this monkey finally develop signs of incomplete paralysis (score of 2.5), at which stage the monkey was sacrificed for neuropathologic examination.

Coating of CD40 on circulating B cells and serum levels of ch5D12 and anti-ch5D12 Abs
Throughout the study, the saturation of CD40 on PBMC by ch5D12 was monitored by flow cytometric analysis. Before day 27 all CD40 on CD21+ B cells was completely saturated. B cell

FIGURE 1. ch5D12 prevents EAE in marmoset monkeys. EAE score (ranging from 0 to 5) is shown for placebo-treated (left) and ch5D12-treated (right) marmoset monkeys. Treatment of ch5D12 was performed every 2–4 days for a period of 50 days. Individual animals are identified by the code in the upper right corner. Asterisks indicate the time of sacrifice due to the severity of EAE.
CD40 molecules in animal QN remained fully coated for the duration of treatment. However, it was observed that the ch5D12 coating in vivo decreased in the three other animals on day 27 (data not shown). To determine whether this was due to low serum levels of ch5D12 or to a decrease in B cell CD40 expression, a parallel incubation of PBMC pulsed with extra ch5D12 was included. All animals showed positive staining for CD40 on CD20⁺ B cells (data not shown). Thus, on day 27 ch5D12 serum levels in animals QQ, QP, and QT were insufficient to saturate all CD40 molecules. Therefore, the dosing frequency was increased to three times per week for all animals from day 28 onward. The increased dosing frequency did not completely restore the CD40 coating of B cells. The serum levels of free ch5D12 paralleled the incomplete saturation of CD40 (Fig. 2). Serum concentrations of ch5D12 ranged from 10 to 40 μg/ml during the first 2 wk and subsequently dropped below 10 μg/ml in QO, QP, and QT. Sustained circulating ch5D12 concentrations >10 μg/ml could be measured only in QN, probably explaining the continuous CD40 saturation on CD20⁺ B cells with ch5D12 in this animal. Apparently, a serum concentration of 10 μg/ml is a threshold for CD40 saturation. Increasing the dosing frequency resulted in only a slight restoration of circulating ch5D12 concentrations in QO, QP, and QT, probably because at that time neutralizing Abs were already being formed (see below). Treatment with ch5D12 had no effect on the percentage of either CD4⁺ or CD8⁺ T cells (data not shown). A transient decrease in the percentage of CD20⁺ B cells from peripheral blood was observed. In three of four animals anti-ch5D12 Abs were not detectable throughout the study. In monkeys QO, QP, and QT anti-ch5D12 IgG Abs were first detectable between days 17 and 27. A correlation was found between the circulating ch5D12 concentration, saturation of CD40 with ch5D12, and the development of an anti-ch5D12 IgG response (Fig. 2). The correlation between the magnitude of the anti-ch5D12 response and the area under the curve during the first 3 wk of the study suggests a dose-response relationship. Whereas monkeys QO, QP, and QT developed anti-ch5D12 IgG Abs in the same order at the time when their ch5D12 levels dropped below the 10 μg/ml threshold, monkey QN failed to develop an anti-ch5D12 response after cessation of ch5D12 treatment on day 50 until day 200.

**Postmortem MRI and immunohistologic analysis, and neuropathology of marmoset brain**

To assess whether ch5D12 treatment affects CNS lesion load, animals were analyzed by postmortem MRI and immunohistochemistry (Table I). The two animals in the placebo group that were sacrificed during the 50-day period due to the severity of their disease, had a very high lesion load in the CNS and overt demyelination in the spinal cord. Also, the two animals from the placebo group that were sacrificed at the end of treatment period day 50 had a very high lesion load in both immunohistochemical and MRI analysis, indicating high inflammatory activity in the CNS. Demyelination was present mainly in the spinal cord, but it was less severe than in the two placebo-treated animals that were sacrificed before day 50 (Table I). These observations were in sharp contrast to those in the two animals from the ch5D12 group (QT and QP) that were sacrificed at the end of the treatment period according to protocol. These two animals that had the lowest ch5D12 serum levels had only minor inflammatory lesions in MRI analysis. In the immunohistochemical analysis no infiltrates were observed in animal QT; besides the inflammation, some limited demyelination in spinal cord and brain was found (Table I). Because ch5D12 levels were higher in the other two animals of the ch5D12 group that were kept alive (QO and QN), this may suggest that the lesion load in these two animals on day 50 was comparable to or less than that in animals QT and QP. Animal QO that developed EAE shortly after cessation of treatment and was sacrificed due to severity of disease had a lesion load comparable to that of the placebo-treated animals. Immunopathology revealed that in this animal large demyelinating infiltrates were present. These infiltrates consisted of T cells and actively demyelinating macrophages and were identical with the early infiltrates in placebo animals QQ and QR, indicating that a rebound EAE developed after cessation of ch5D12 treatment on day 50. There was a complete absence of lesions and demyelination in animal QN.

**MOG-specific cellular response, anti-MOG Ab response, isotype distribution, and peptide specificity**

To control for the presence of a cellular autoimmune reaction in both groups, MOG- and MBP-specific cellular responses were measured in cultures isolated from peripheral lymph nodes collected at necropsy. Although no response was detectable against MBP, clear T cell proliferative responses against MOG were detected in all placebo- and ch5D12-treated animals (Fig. 3).

Fig. 4 shows that with the exception of monkey QP, treatment with ch5D12 resulted in lower levels of anti-MOG IgM Abs. In the placebo-treated animals anti-MOG IgM Abs first became detectable on day 16 after immunization and remained above background level throughout the treatment period in all animals. Although in the ch5D12-treated animals a small increase in anti-MOG IgM Abs was observed on day 16, the magnitude...
of this response overall remained lower than that in the placebo group. Treatment with ch5D12 delayed the appearance of the anti-MOG IgG response in all animals (Fig. 5). However, from day 38 onward, most treated animals developed MOG-specific IgG levels comparable to placebo-treated animals. The reduction of anti-MOG IgM and IgG responses demonstrated in vivo functional biological activity of ch5D12 on humoral immunity as predicted by the crucial role of CD40-CD40L interaction in thymus-dependent B cell responses.

To assess the effect of ch5D12 treatment on intramolecular epitope spreading, the reactivity of anti-MOG Abs with MOG protein and MOG peptides was determined in a dot-blot assay. Fig. 6 shows the reactivity of sera with intact rhMOG and a set of 23-mer peptides overlapping by 10, spanning residues 1–116 of the N-terminal extracellular part of human MOG. All monkeys from both groups showed reactivity against rhMOG, confirming the ELISA data. In necropsy sera of three of four monkeys from the placebo group, broad reactivity with the peptide panel was found (Fig. 6) as described previously (22). Only in monkey QQ, which was the first to develop severe EAE and was sacrificed on day 35, was no reactivity with the overlapping peptides found, suggesting that epitope spreading takes place thereafter. In three of four ch5D12-treated animals no reactivity with the peptide panel was found (Fig. 6). Interestingly, the only ch5D12-treated animal (QP) that showed reactivity in its necropsy serum against some of the peptides was also the only ch5D12-treated animal that developed high anti-MOG IgM responses during treatment. Possibly this monkey has escaped from the immunosuppressive effects of ch5D12, which is confirmed by the early development of an anti-ch5D12 response. These results demonstrate that ch5D12 treatment not only suppresses anti-MOG Ab production, but also prevents intramolecular spreading of the peptide reactivity of anti-MOG Abs.

**Discussion**

We have previously demonstrated that interruption of CD40-CD40L interaction by antagonist mAbs is an effective target of therapy in mice (13). This study was undertaken to test the efficacy of a clinically relevant mAb in an animal model closely reflecting MS. The chosen animal model was rhMOG-induced EAE in the common marmoset, a well-established nonhuman primate model in terms of clinical, neuropathologic, and immunologic features (22). This model is a valid preclinical model of inflammatory demyelination of the CNS; therefore, it enabled us not only to obtain proof of our concept that interruption of CD40-CD40L interaction with ch5D12 effectively prevents disease symptoms of EAE in marmoset monkeys. The postmortem MR images and neuropathologic examination confirm the beneficial effect of the Ab with suppression of inflammation and demyelination.

The results of this study clearly show the therapeutic potential of ch5D12 in EAE. Importantly, although treatment with ch5D12 was started before the immunization, the similar MOG-specific cellular responses at necropsy in both groups of animals clearly demonstrate that the therapeutic effect is not due a general immune suppression. The absence of clinical symptoms of EAE appears to be associated with reduced anti-MOG IgM and IgG responses. In an
animal that had sustained elevated anti-MOG IgM Ab levels, intramolecular epitope spreading in Ab responses against MOG and lesions in the CNS were observed. In the ch5D12-treated animals in which anti-MOG IgM Abs were reduced, clinical signs, CNS white matter lesions, and epitope spreading were lacking. It is tempting to hypothesize a causal relation between these phenomena.

The altered profile of anti-MOG Ab reactivity in ch5D12-treated monkeys is of particular interest in view of the important role of this Ab specificity in the facilitation of demyelination (18, 19). The pathogenic mechanism by which anti-MOG Abs mediate demyelination is thought to be mediated by complement activation and subsequent immune activation (23, 24). Anti-MOG mAbs can mediate opsonization of myelin dependent on the isotype, the recognized epitope, and the ability to fix complement (25). Notably, IgM Abs are particularly capable of classical route complement fixation. Therefore, the abolishment of anti-MOG IgM Abs associated with the absence of clinical signs in ch5D12-treated monkeys may explain the absence of clinical signs. We have found a similar mechanism in another primate autoimmune disease model, namely the rhesus monkey model of collagen-induced arthritis. We have demonstrated that disease susceptibility is directly correlated to the capacity to produce anti-type II collagen IgM autoantibodies (26, 27). In addition to the altered isotype distribution of anti-MOG Abs, we found a different reactivity of the necropsy sera with the peptide panel between placebo- and ch5D12-treated monkeys.

What do the modulatory effects of ch5D12 on anti-MOG autoantibodies and the suppression of clinical and pathological aspects in the marmoset model of EAE imply for the possible future treatment of MS patients? The higher incidence of MOG-specific T cell and Ab reactivity in MS compared with non-MS patients or healthy individuals (21, 28, 29) and the localization of anti-MOG Abs in MS brain areas with myelin disintegration underline the relevance of anti-MOG autoimmunity for the MS pathogenesis (29, 30). An issue that is receiving increasing interest in MS is the progressive broadening of the anti-myelin T and B cell reactivity, a phenomenon called epitope spreading. A causal relation between the chronicity of MS and epitope spreading has been suggested (31). Our present results show that the broad serum reactivity with the panel of MOG peptides found in placebo-treated monkeys is absent in the majority of ch5D12-treated animals. Although this assay does not provide formal proof of epitope spreading, the results at least suggest that induction of B cell neoreactivity is abolished in ch5D12-treated monkeys.

The new therapeutic ch5D12 was generated to increase the serum half-life of the mAb and to reduce its potential immunogenicity in humans (11). However, it was observed in this study that the serum half-life of ch5D12 was unexpectedly low and was only
sacrificed after immunization with MOG on day

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In conclusion, in the presence of adequate serum levels of administered ch5D12 (10 μg/ml), the expression of clinical and pathological signs of EAE in outbred marmoset monkeys is prevented. The protective effect of the Ab is probably not due to a generalized immune suppression, as similar MOG-specific cellular responses could be detected at necropsy in placebo- and Ab-treated monkeys. After the arrest of treatment, induction of disease may occur, but only after a variable period of time. CD40-expressing macrophages are abundant in lesions (17, 20), an observation that is indicative for the involvement of macrophages as effector cells in lesions and the role of ch5D12 for treatment of established lesions. In addition, it was observed that the mAb gains access not only to secondary lymphoid organs, but also to the CNS. These observations clearly demonstrate the therapeutic value of ch5D12 in this nonhuman primate model. Extrapolation of these results to MS, indicates that ch5D12 may be effective to reduce the duration and severity of exacerbations and, furthermore, may inhibit progression of the disease by preventing the induction of neuauto-reactivities. Thus, disease exacerbations and the concomitant progressive decline of neurological functions may be arrested.

### References


