Coadministration of Plasmid DNA Constructs Encoding an Encephalitogenic Determinant and IL-10 Elicits Regulatory T Cell-Mediated Protective Immunity in the Central Nervous System

Sagie Schif-Zuck, Gizi Wildbaum and Nathan Karin

_J Immunol_ 2006; 177:8241-8247; doi: 10.4049/jimmunol.177.11.8241
http://www.jimmunol.org/content/177/11/8241

References
This article cites 51 articles, 20 of which you can access for free at:
http://www.jimmunol.org/content/177/11/8241.full#ref-list-1

Subscription
Information about subscribing to _The Journal of Immunology_ is online at:
http://jimmunol.org/subscription

Permissions
Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts
Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts

The Journal of Immunology is published twice each month by
The American Association of Immunologists, Inc.,
1451 Rockville Pike, Suite 650, Rockville, MD 20852
Copyright © 2006 by The American Association of Immunologists All rights reserved.
Print ISSN: 0022-1767 Online ISSN: 1550-6606.
Coadministration of Plasmid DNA Constructs Encoding an Encephalitogenic Determinant and IL-10 Elicits Regulatory T Cell-Mediated Protective Immunity in the Central Nervous System

Sagie Schif-Zuck, Gizi Wildbaum, and Nathan Karin

We have previously shown that Ag-specific IL-10-producing regulatory T cells (Tr1) participate in the regulation of experimental autoimmune encephalomyelitis and that their specificity undergoes marked spread in a reciprocal manner to effector T cell specificity. The current study shows that coadministration of plasmid DNA vaccines encoding IL-10 together with a plasmid encoding a myelin basic protein (MBP) encephalitogenic determinant during an ongoing disease rapidly amplifies this Tr1-mediated response, in a disease-specific manner. Thus, coadministration of both plasmids, but not the plasmid DNA encoding MBP alone, rapidly suppresses an ongoing disease. Tolerance included elevation in Ag-specific T cells producing IL-10 and an increase in apoptosis of cells around high endothelial venules in the CNS after successful therapy. Tolerance could be transferred by MBP-specific primary T cells isolated from protected donors and reversed by neutralizing Abs to IL-10 but not to IL-4. Due to the nature of determinant spread in this model, we could bring about evidence implying that rapid and effective induction of Tr1-induced active tolerance is dependent on redirecting the Tr1 response to the epitope to which the effector function dominates the response at a given time. The consequences of these findings to multiple sclerosis, and possibly other inflammatory autoimmune diseases are discussed. The Journal of Immunology, 2006, 177: 8241–8247.

Experimental autoimmune encephalomyelitis (EAE) is a T cell-mediated autoimmune disease of the CNS that serves as an animal model for human multiple sclerosis (MS), given that in both diseases autoimmune cells home to the CNS to attack myelin components (1, 2). EAE is transferable by CD4+ T cells selected in response to CNS Ags (1). Depending on their cytokine profile, the CD4+ T cells fall into different subsets including: Th1 cells that produce large amounts of IFN-γ and TNF-α and low levels of IL-4; Th2 cells that mostly produce IL-4, IL-5, and IL-13 and, to a much lesser extent, IFN-γ and TNF-α (3–6); Th3 cells that produce high levels of TGF-β and to a much lesser extent other cytokines (7, 8); regulatory T cells (Tr1) that produce high levels of IL-10 (9); CD4+CD25+Tr1 (10–13); and the very newly defined Th17 cells, selected in response to IL-23 and producing high levels of the inflammatory cytokine IL-17 (14–20) and are likely to contribute to the pathogenic manifestation of inflammatory autoimmune diseases, including EAE (14, 17). The pivotal role of Th1 cells in the initiation and progression of the inflammatory process in EAE and other T cell-mediated autoimmune diseases has been well documented. Not only can these cells adoptively transfer EAE, but also blockade of their migratory properties rapidly suppresses an established disease (21, 22). Th17 cells are likely to be highly potent Ag-specific inflammatory cells as well (15–20). Th3 cells and Tr1 cells produce suppressor/regulatory cytokines (TGF-β, IL-10) in response to their target Ag (7, 9). Their ability to suppress different autoimmune diseases has been well documented in adoptive transfer experiments (7, 9, 23). CD4+CD25+ are natural suppressor T cells (12, 13). Their absence enhances the development of T cell-mediated autoimmunity, whereas their adoptive transfer suppresses these diseases (24, 25).

We have previously shown that during inflammatory autoimmune diseases the immune system mounts a beneficial autoimmune response against selected number of inflammatory mediators that direct the pathogenesis of each disease and that these responses could be rapidly amplified by targeted DNA plasmids encoding each relevant mediator (26–34). One of these studies has also been extended to humans (28). In this particular study, we have also shown that this response is directed exclusively to inflammatory, but not regulatory mediators such as IL-4, IL-10, or TGF-β and that under these conditions administration of plasmid DNA encoding a regulatory mediator would not lead to production of autoantibodies to this mediator (28). Tentatively, under these conditions, the function of this mediator encoded by plasmid DNA would not be neutralized and could even be functional. Independently, the group of Lawrence Steinman, at Stanford University (Stanford, CA), developed a novel therapeutic strategy in which the DNA plasmid encoding the self-autoimmune determinant is coadministered with another plasmid encoding IL-4 (35). Under these conditions, the IL-4 produced at the site of immunization is not neutralized and therefore could effectively shift the polarization of Ag T cells activated in response to the target autoimmune Ag into IL-4-producing T cells to suppress the disease (35). In clinical trials, Steinman, Garren and colleagues have accomplished...
a similar feat in early phase 1/2 studies in patients with relapsing remitting MS (36).

We have recently shown that shifting the Ag-specific Th1/Th2 balance into Th2 suppresses EAE by an indirect mechanism because Ag-specific Th2 cells are incapable of transferring the beneficial effect resulting from driving the T cell balance into Th2 (37). In another study, we have shown that Ag-specific IL-10-producing T cells participate in the regulation of EAE and that their specificity undergoes determinant spread in a reciprocal manner to effector T cell specificity (23). The current study explores for the first time the possibility of using combined DNA vaccine strategy to selectively amplify the regulatory function of these Ag-specific T11 cells during ongoing EAE.

Materials and Methods

Animals

Female Lewis rats ~6 wk old were purchased from Harlan Biotech Israel and maintained under special pathogen-free conditions in our animal facility.

Peptide Ags

Myelin basic protein (MBP) peptide 68–86 (YGSLPQKSSQDSQDENPV) and P257–81 (FKNTENIKLQGEEFEETTADNKRKT) were synthesized on a MilliGen 9050 peptide synthesizer by standard 9-fluorenylmethoxycarbonyl chemistry. Peptides were purified by HPLC. Sequence was confirmed by amino acid analysis and mass spectroscopy. Only peptides that were >95% pure were used in our study.

Active induction of EAE or experimental autoimmune neuritis (EAN)

MBP<sub>68–86</sub> or P257–81 at a concentration of 1 mg/ml were dissolved in PBS and emulsified with an equal volume of IFA supplemented with 4 mg/ml heat-killed Mycobacterium tuberculosis H37Ra in oil (Difco Laboratories). Rats were immunized s.c. in the hind footpads with 0.1 ml of the emulsion and maintained under special pathogen-free conditions in our animal facility.

Immunohistochemistry

Immunohistochemical detection of active caspase 3 was conducted using a commercially available kit (APO active 3TM; Cell Technology) according to the manufacturer’s protocol.

Statistical analysis

Significance of differences was examined using Student’s t test. A value of p < 0.05 was considered significant. The Mann-Whitney sum of ranks test was used to evaluate significance of differences in mean of maximal clinical score. A value of p < 0.05 was considered significant.

Results

Coadministration of plasmid DNA vaccines encoding MBP<sub>68–86</sub> and IL-10 suppresses EAE in a disease-specific manner

Groups of six Lewis rats were subjected to active induction of EAE induced by MBP<sub>68–86</sub>/CFA. At the onset of disease, these rats were treated with a plasmid DNA encoding MBP<sub>68–86</sub> or plasmid DNA encoding IL-10, or both plasmids coadministered. Control rats were treated with PBS or with an empty vector. Of these groups only rats given coadministration of the IL-10-encoding DNA plasmid together with the MBP-encoding plasmids went into fast remission (Fig. 1A; mean maximal score of 0.3 ± 0.166 compared with 4 ± 0.66 in control group; p < 0.001). Within the other groups, only those treated with plasmid DNA encoding IL-10 displayed a lower mean maximal score (2.6 ± 0.5, compared with 4 ± 0.66 in the control group; p < 0.05), which was significantly higher than the one recorded in the group subjected to coadministration of plasmids (0.3 ± 0.166 compared with 2.6 ± 0.5; p < 0.001). On day 14 (peak of disease in the control group; Fig. 1A), representative rats were sacrificed (three per group). Lumbar spinal cord samples were subjected to histological evaluation (Fig. 1B). The mean histological score of control EAE rats (Fig. 1Bc) was 2.1 ± 0.66 and did not differ from that of rats injected with an empty vector (2 ± 0.5, Fig. 1Bb). Both were significantly higher (p < 0.001) than the histological score observed in rats treated with MBP and IL-10-encoding plasmids (Fig. 1Bd; 0.66 ± 0.166; p < 0.001), which closely resembled sections from naïve healthy rats (Fig. 1Ba). Thus, histological evaluation could verify the beneficial effect that was clinically observed in the group subjected to
FIGURE 1. Lewis rats were subjected to active induction of EAE. On day 10, these rats were separated into five groups of equally sick animals (six rats per group) and repeatedly administered (two times, days 10 and 12; 300 μg per rat per injection) with either plasmid DNA (pcDNA) encoding MBP<sub>68–86</sub> (C), plasmid DNA encoding IL-10 (D), or coadministered with both plasmids (A). Control rats were treated with PBS (□) or with an empty vector (●). Rats were monitored daily for clinical signs by an observer blind to the experimental protocol. A, Results of one of three independent experiments with similar data are shown as mean maximal score ± SE. B, Histological analysis of lumbar spinal cord section of naive healthy rats (a), rats treated with an empty vector (b), PBS (c), or the combined vaccine comprised of MBP- and IL-10-encoding plasmids (d). C, Immunohistochemical staining of active caspase 3 of naive healthy rats (a), rats treated with an empty vector (b), rats treated with plasmid DNA encoding IL-10 alone (c), rats treated with plasmid DNA encoding MBP alone (d), or the combined vaccine comprised of MBP and IL-10 encoding plasmids (e). D, Primary cultures of spleen cells, isolated at this time, were subjected to in vitro stimulation with the MBP<sub>68–86</sub> target Ag, and levels of IL-4 and IL-10 were determined by ELISA. E, Additional groups (six rats each) were subjected to the induction of active EAE with MBP (MBP<sub>87–99</sub>, a) or to EAN (b). On days 10 and 12, both groups were subjected to the codelivery of plasmid DNA vaccines encoding MBP<sub>68–86</sub> + IL-10 (C). Control rats were treated with PBS (□) or empty vector (●). In a reciprocal experiment (c), rats (six per group) were subjected to EAE induced by MBP<sub>87–99</sub> and at the onset of disease to combined DNA vaccines including: plasmid DNA encoding MBP<sub>68–86</sub> + plasmid DNA encoding IL-10 (●), plasmid DNA encoding MBP<sub>87–99</sub> + plasmid DNA encoding IL-10 (■), plasmid DNA encoding whole MBP + plasmid DNA encoding IL-10 (●), or PBS (□). Results of one of three independent experiments with similar data are shown as mean maximal score ± SE.

coadministration of both plasmids (Fig. 1A). We have subjected the lumbar spinal cord sections of each group to active caspase 3, a whole mark of apoptosis, immunohistochemistry. Our results (Fig. 1C) clearly show that: 1) during EAE apoptosis of cells around high endothelial venules (HEV) is apparent; 2) the combined therapy with plasmids DNA encoding IL-10 and MBP significantly increases the number of caspase 3<sup>+</sup> cells around HEV as compared with control EAE, EAE rats treated with IL-10-encoding plasmid alone, or MBP-encoding plasmid alone. Fig. 1C shows a representative section from each group. Statistical analysis of 18 sections per group, 3 fields per section, showed significant differences between mice subjected to combined therapy with DNA plasmids encoding IL-10 and MBP (Fig. 1C), as compared with control EAE, EAE rats treated with IL-10-encoding plasmid alone (Fig. 1Ce) or MBP-encoding plasmid alone (Fig. 1Cd; 20.6 ± 2.4 compared with 5.2 ± 0.6 and 7.4 ± 0.8 and 5.66, respectively; p < 0.01). These data suggest that rapid apoptosis of invading leukocytes within the CNS contributes to the rapid recovery from the disease. Spleen cells from each group were subjected to in vitro stimulation with the MBP<sub>68–86</sub> target Ag. Levels of IL-4 and IL-10 were then recorded (Fig. 1D). We show here that primary cultures from EAE rats treated with the combined constructs (encoding MBP and IL-10) displayed an elevated production of IL-10 in response in comparison with each of the other groups (Fig. 1D; 2.33 ± 0.5 μg/ml compared with 1.2 ± 0.2 μg/ml in the control group; p < 0.001). No significant difference could be observed in IL-4 production between these groups.

In a complementary experiment (Fig. 1E), rats were subjected to either active EAE induced by the secondary determinant of MBP (peptide 87–99) or to EAN, a paralytic disease of the peripheral nervous system (41). Both groups were subjected to the codelivery of plasmid DNA vaccines encoding MBP<sub>68–86</sub> together with a plasmid DNA encoding IL-10. Fig. 1E clearly shows that this targeted DNA therapy provides protective immunity in a disease-specific manner.
That is, rats subjected to disease induction with one determinant of MBP acquire resistance to tolerance induced by a plasmid DNA encoded by the other determinant (Fig. 1Ea; mean maximal score of 0.66 ± 0.166 compared with 2.5 ± 0.5 in the control group; p < 0.01), but not to a disease resulting from an autoimmune response against an irrelevant target Ag (i.e., P2 in Fig. 1Eb). Very similar results were obtained using plasmid DNA encoding the whole MBP together with plasmid DNA encoding IL-10 (data not shown). In a reciprocal experiment (Fig. 1Ec) rats were subjected to EAE induced by MBP$_{68–86}$ and at the onset of disease to combined DNA vaccines including: plasmid DNA encoding MBP$_{68–86}$ + plasmid DNA encoding IL-10; plasmid DNA encoding MBP$_{68–86}$ + plasmid DNA encoding IL-10; plasmid DNA encoding whole MBP + plasmid DNA encoding IL-10; or PBS. We have previously shown that in this model of disease at the onset of disease the immune response spreads from the secondary epitope (MBP$_{47–99}$) to the major one (MBP$_{68–86}$), but not vice versa (23, 42, 43). Fig. 1Ec shows that at this time combined DNA plasmids encoding the major encephalitogenic determinant and IL-10, but not the secondary determinant, and IL-10 rapidly suppress the disease. This strongly suggests that rapid and effective induction of Tr1-induced active tolerance is dependent on redirecting the Tr1 response to the epitope to which the effecter function dominates the response.

The therapeutic effect acquired by coadministration of plasmid DNA vaccines encoding MBP$_{68–86}$ and IL-10 is IL-10 dependent

In a subsequent experiment, conducted under the same conditions as described in the legend to Fig. 1A, groups of six Lewis rats each were subjected to active induction of EAE induced by MBP$_{68–86}$/CFA. At the onset of disease, these rats were injected with plasmid DNA encoding MBP$_{68–86}$/plasmid DNA encoding IL-10, or both plasmids coadministered. On day 15 (peak of disease in control groups), the anterior muscle of the titib was subjected to RT-PCR of IL-10 transcription (Fig. 2A) and cervical lymph node cells for Western blot analysis for the expression of STAT-3 and pSTAT-3 (Fig. 2B), which has been well implicated with IL-10 production (44, 45). Our results clearly show a significant elevation in pSTAT-3 expression as well as IL-10 transcription in rats subjected to the administration of plasmid DNA encoding IL-10, either alone (Fig. 2B, lane b) or together with the MBP-encoding plasmid (Fig. 2B, lane d), but not in control (Fig. 2B, lane a), or following the administration of plasmid DNA encoding MBP alone (Fig. 2B, lane c). Primary cultures from the cervical lymph node cells of each group were cultured with the target MBP$_{68–86}$ epitope followed by intracellular flow cytometry analysis (gated on CD4$^+$ T cells) of intracellular cytokine production. Fig. 2C shows analysis of one of three independent experiments with similar results. It shows that: 1) during EAE, the number of IL-10$^+$ IL-4$^{low}$-producing cells in the control EAE group elevates (Fig. 2C; 1.2% compared with 0.7%); 2) administration of either plasmid DNA encoding IL-10 alone, or MBP$_{68–86}$ alone (but not an empty vector; data not shown) significantly increases the relative number of these cells (3.7 and 3.4% compared with 1.2%), albeit not enough to suppress an ongoing disease. One possibility is that the MBP-encoding plasmid further promotes the proliferation of MBP-specific Tr1 cells, among other MBP-specific T cells. As for the IL-10-encoding plasmid, IL-10 transcribed by this plasmid may provide an enrichment milieu for Tr1 cells (9), and therefore their relative number increases; 3) only within the group subjected to the coadministration of both plasmids does the number of CD4$^+$ IL-10$^+$ IL-4$^{low}$-producing cells substantially increase (Fig. 2C; 16.9% compared with 3.7%, 3.4% in groups treated with single plasmid DNA vaccines). We could not observe increased intracellular expression of TGF-β in either CD4$^+$ IL-10$^{high}$ or CD4$^+$ IL-10$^{low}$-producing T cells (Fig. 2D). A direct ELISA further substantiated this observation (data not shown). Cytokine levels in supernatants of cultured primary cervical lymph node T cells proliferating in response to their target Ag were 10.5 ± 0.5, 12.7 ± 0.7, 11.6 ± 0.4, and 11.9 ± 0.6 pg/ml in control EAE rats, rats

![FIGURE 2.](http://www.jimmunol.org/) Lewis rats were subjected to active induction of EAE. On day 10, these rats were separated into four groups of equally sick animals (six rats per group) and repeatedly treated with PBS (Aa and Ba), plasmid DNA encoding IL-10 (Ab and Bb), plasmid DNA encoding MBP$_{68–86}$ (Ac and Bc), or coadministration of plasmid DNA encoding MBP$_{68–86}$ and plasmid DNA encoding IL-10 (Ad and Bd). Fifteen days after disease induction, rats were sacrificed. The anterior muscle of the titib was subjected to PCR for IL-10 transcription (A). Cervical lymph nodes were harvested and either subjected to Western blot analysis for the expression of STAT-3 and pSTAT-3 (B) or cultured with MBP$_{68–86}$ epitope and then subjected to intracellular flow cytometry analysis (gated on CD4$^+$ T cells) for IL-4 and IL-10 production (C) and for TGF-β and IL-10 production (D) as follows: a, naive rats; b, EAE rats; c, EAE rats treated with plasmid DNA encoding MBP; d, EAE rats treated with plasmid DNA encoding IL-10; e, EAE rats treated with plasmid DNA encoding MBP + plasmid DNA encoding IL-10. These cells were then tested for their ability to transfer disease resistance (E). Three groups of EAE rats (six rats per group) were subjected to the administration of 3 × 10$^7$ MBP-activated spleen cells isolated from PBS (□), control EAE rats (○), or those coadministered with plasmid DNA encoding MBP$_{68–86}$ and plasmid DNA encoding IL-10 (■). An observer blind to the experimental protocol monitored clinical manifestation of disease. Results of one of three independent experiments with similar data are shown as mean maximal score ± SE. F, On day 15, cervical lymph node cells from representative rats from each group were tested for their ability to proliferate in response to the target Ag (MBP$_{68–86}$). Results are shown as mean stimulation index ± SE.
treated with plasmid DNA encoding MBP_{68-86} alone, plasmid DNA encoding IL-10 alone, or their combination, respectively.

We have previously shown that Tr1 cells selectively accumulate in the cervical lymph nodes that drain the CNS and to a much lesser extent in spleen and other lymph nodes (23). Nevertheless, the total number of these cells in cervical lymph nodes is not sufficient for adoptive transfer experiments. Before extending our study to adoptive transfer experiments, we have recorded the relative number of these cells following combined DNA plasmid therapy in cervical lymph nodes, in comparison with spleen and inguinal lymph nodes. These organs were removed 2 days after the second administration of the combined DNA plasmid therapy. The relative number of IL-10^highCD4^+ T cells in cervical lymph nodes was \(\sim16\%\), and that in spleen and inguinal lymph nodes was \(\sim6\%\). The relative number of these cells in control EAE rats was \(\sim1\%\)–1.5\% in each organ (data not shown). Thus, accumulation of regulatory cells is selective. However, due to their limited total number in cervical lymph nodes, continuing adoptive transfer experiments were conducted using primary spleen cells (see below).

We have then tested the ability of these primary T cells, activated in vitro with MBP_{68-86} to affect the kinetics of ongoing EAE, in an adoptive transfer experiment (Fig. 2E). Just after the onset of disease, three groups of equally sick EAE rats (six rats per group) were subjected to the administration of \(3 \times 10^7\) MBP-activated spleen cells isolated from either control EAE rats or those treated by coadministration of plasmid DNA encoding MBP_{68-86} and plasmid DNA encoding IL-10. Only the adoptive transfer of MBP-specific primary T cells from protected donors, subjected to coadministration of plasmid DNA encoding MBP_{68-86} and plasmid DNA encoding IL-10 rapidly suppressed ongoing EAE (Fig. 2E; mean maximal score of 0.66 ± 0.16 compared with 2.33 ± 0.3; \(p < 0.01\)). On day 15, cervical lymph node cells from representative rats from each group were tested for their ability to proliferate in response to the target Ag (MBP_{68-86}). Fig. 2F shows a significant decrease in the proliferative response of T cells from protected donors, as compared with both control groups (mean stimulation index ± SE, 2.5 ± 0.4 compared with 5.8 ± 0.8 and 7.2 ± 1.2, respectively; \(p < 0.01\)).

Finally, to learn whether therapy is indeed IL-10 dependent, five groups of Lewis rats (six rats each) were subjected to the induction of active EAE and at the onset of disease were, or were not, treated by coadministration of plasmid DNA encoding MBP_{68-86} and plasmid DNA encoding IL-10. These rats were then subjected to repeated administration of 100 \(\mu\)g/ml commercially available PeproTech rabbit anti-rat IL-10, rabbit anti-rat IL-4 polyclonal Abs, or polyclonal Abs from preimmunized rabbit (PeproTech). Our results (Fig. 3) show that only the administration of neutralizing Abs to IL-10 could reverse the tolerance induced by the above combined plasmid DNA therapy.

**Discussion**

The cascade of events leading to the development of MS is not fully understood. Yet, it is believed that the disease involves an autoimmune destructive process (46), and as such nonspecific and specific approaches for interfering in the multistep events initiating and promoting the inflammatory process of the disease may lead to disease regression. Fourteen years ago, we have demonstrated that the \(\alpha_4\beta_2\) integrin VLA-4 is a key adhesion molecule that directs inflammatory leukocyte trafficking to the CNS and that Ab blockade of this molecule may reverse EAE (21). This particular study has been successfully extended in clinical trials (47) and became a Food and Drug Administration-approved drug for the disease (47). Nevertheless, it has recently been found that such a successful nonselective inhibition of T cell and monocyte migration to the CNS may apparently lead to fatal infections (48). This experience further demonstrates the importance of developing novel Ag-specific means of therapy. The need to develop Ag-specific therapy is compelling and motivated us to explore DNA vaccination-based therapy that would select highly specific Tr1 capable of suppressing the disease. Ag-specific Tr1 cells were discovered by Groux et al. (9) 9 years ago. We have later shown that these IL-10-producing Tr1 cells participate in the natural regulation of EAE and that their function could be amplified in a beneficial manner by soluble peptide therapy (23). The promising use of DNA vaccines as future means of intervention in various diseases motivated us to investigate whether codelivery of plasmid DNA encoding IL-10 together with a plasmid encoding the self-autoimmune Ag would effectively select IL-10 producing Tr1 capable of suppressing the disease. The data presented in the current manuscript show that indeed these combined vaccines select Ag-specific Tr1 cells capable of suppressing EAE, and their beneficial function is reversible by anti IL-10 Abs. Three questions may arise from the data presented in Fig. 1. Why does the combined vaccine affect the manifestation of disease so rapidly? Why is it selective against disease induced by the other encephalitogenic determinant, but not against an irrelevant autoimmune disease (EAN; Fig. 1E)? And why do the combined vaccines encoding one target epitope and IL-10 suppress disease induced by the other epitope and not vice versa? We have previously shown that during EAE the immune system mounts a Tr1-mediated response, not only against the determinant with which disease was induced, but also against the determinants to which the pathogenic response spreads (23), and that this regulatory response restrains the severity, albeit not totally suppress its manifestation (23). The data presented here clearly show, once again, an apparent increase in IL10^high^IL-4^low^-producing T cells in cervical lymph nodes during EAE (Fig. 2C) that is profoundly increased following coadministration of plasmid DNA encoding MBP + plasmid DNA encoding IL-10. Tolerance was associated with marked cell apoptosis around HEV (Fig. 1C). These data suggest that rapid apoptosis of invading leukocytes within the CNS either contributes, or is the outcome, of the rapid recovery from the disease. A positive association between successful therapy of EAE, including Tr1-based therapy, had previously been observed by others (49, 50). We have previously shown that leukocytes apoptosis at the autoimmune site is accelerated before and during spontaneous remission from the disease, and that neutralization of Fas ligand inhibits this process and delays recovery (33). It has
also been suggested that during recovery from EAE CD4+ T cells do not leave the CNS but rather undergo apoptosis there (51, 52). Following tolerance induction of apoptosis is likely to be amplified. This may explain, in part, the mechanistic basis of increased apoptosis in treated mice (Fig. 1C).

Combined plasmid DNA vaccines encoding MBP{from 68 to 86} + IL-10 could rapidly suppress disease induced by the major epitope (MBP{from 68 to 86}) and the other, noncross-reactive epitope (MBP{from 87 to 99}), but not EAN (Fig. 1E). One possible explanation is that both the MBP{from 87 to 99}-specific effector T cells, initially activated after active induction of disease, and the MBP{from 68 to 86}-specific Treg, induced after protective therapy, home to the CNS and cointact, in the microenvironment there, in part via IL-10 functioning as a suppressor factor (bystander suppression). This may well explain why the combined DNA vaccine protects from EAE but not EAN. The other option is that the combined vaccine acts, during determinant spread, on nonpolared MBP{from 68 to 86}-specific T cells, undergoing Ag-specific T cell activation, and redirects their polarization into Ag-specific regulatory, rather than effector T cells. On this subject, we have previously shown in numerous studies that the MBP{from 68 to 86}, as a major epitope, is extremely dominant and that the MBP-specific response spreads very fast from the secondary to the major epitope, but not the opposite, and that the onset of disease is dependent on the response to the primary, but not necessarily the secondary epitope (23, 42, 43). In two consecutive experiments, we tried to subject rats with MBP{from 68 to 86}-induced disease to MBP{from 87 to 99} and IL-10-encoding DNA plasmids and failed to induce tolerance (data not shown). Nevertheless, in these rats we could not record any significant response in cervical lymph nodes to MBP{from 87 to 99} (data not shown). Therefore, this model is probably not the best model for distinguishing direct from bystander effects of active tolerance. A possible implication for human MS could be that a tolerance induction during determinant spread should focus on the determinant to which the immune response spreads, at a given time point.

STAT3 was elevated in rats subjected to plasmid DNA encoding IL-10 alone as well as to those subjected to the combined plasmids. Yet only the administration of the combined plasmid was highly protective. It is likely that only the combination would provide the immune system to favor the polarization of Ag-specific T cells into regulatory cells, which may also explain the specificity of therapy (Fig. 1E).

Finally, we do not exclude the possibility that other types of Tr, particularly (CD25+ T cells, are also being activated and contribute to the resistant state achieved by plasmid DNA therapy. Nevertheless, we think that the Lewis rats system is well set up for studying the biological functions of Tr1 cells (23), and so much as extend the biological functions of CD25+ cells. For this purpose, we intend to extend our studies, in the near future, to myelin oligodendrocyte glycoprotein-induced disease in C57BL/6 mice.

Disclosures
The authors have no financial conflict of interest.

References


