Therapeutic potential of IFN-γ-modified dendritic cells in acute and chronic experimental allergic encephalomyelitis

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Keywords: dendritic cell, experimental allergic encephalomyelitis, IFN-γ, magnetic resonance imaging, tolerance

Abstract

Dendritic cells (DC) are antigen-presenting cells specialized to regulate immune responses. DC not only control immunity, but also maintain tolerance to self-antigens—two complementary functions that would ensure the integrity of the organism in an environment full of pathogens. Here we report that splenic DC that had been exposed in vitro to IFN-γ (IFN-γ-DC) exhibit therapeutic potential on acute experimental allergic encephalomyelitis (EAE) in Lewis rats, and on chronic-relapsing EAE in B6 and SJL/J mice. During incipient EAE [day 5 post-immunization (p.i.) in rats, day 7 p.i. in mice], IFN-γ-DC were injected s.c. Severity of clinical signs of EAE was dramatically inhibited in animals injected with IFN-γ-DC, showing normal magnetic resonance imaging (MRI) of the spinal cord and brain. In contrast, the EAE rats receiving PBS or naive DC had severe clinical signs with multiple and extensive MRI lesions in the spinal cord and brain. IFN-γ-DC triggered an antigen-specific IFN-γ production, and induced apoptosis of CD4+ T cells possibly through DC expressing indoleamine 2,3-dioxygenase and/or an IFN-γ-dependent pathway. As a result, infiltration of macrophages and CD4+ T cells within the spinal cords was dramatically reduced in animals injected with IFN-γ-DC as compared to animals injected with PBS or naive DC. This approach may represent a novel possibility of individualized immunotherapy using autologous, in vitro modified DC as a complement to conventional therapy in multiple sclerosis and other diseases with an autoimmune background.

Introduction

Multiple sclerosis (MS) is an inflammatory demyelinating disease of the central nervous system (CNS). Pathologically, MS is characterized by multiple, sharply demarcated foci of demyelination and mononuclear leukocyte infiltration. The latter indicates the immune-mediated character of demyelination. Traditional therapy of MS with immunosuppressive drugs and even with newer disease-modulating compounds (IFN-β, glatiramer acetate) is unsatisfactory. Based on advances in immunology and biotechnology, we have evaluated a variety of therapeutic regimes in experimental allergic encephalomyelitis (EAE), a model of human MS, including administration of IL-10, transforming growth factor-β1 and IL-4 as well as mucosal tolerance induced by autoantigens (1,2). However, these strategies only prevented the development of EAE when given before immunization, but did not treat ongoing EAE when given after immunization.

Dendritic cells (DC) are specialized antigen-presenting cells (APC) that capture antigen, migrate from the periphery to lymphoid organs and present antigens to naive T cells. They not only activate lymphocytes, but also tolerize T cells to antigens, thereby minimizing autoaggressive immune responses (3). In mice, in vivo treatment with Flt3L, a growth factor that expands DC, enhanced the induction of oral tolerance (4). Transfer of pancreas lymph node DC modulated autoaggressive immunity and limited the severity of diabetes in non-obese diabetic (NOD) mice by the induction of regulatory cells (5). Spontaneous autoimmune diabetes in NOD mice was also prevented by transferring human γ-globulin-pulsed DC (6).

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Transmitting editor: E. Simpson

Received 28 March 2003, accepted 22 September 2003
Recent reports have shown the suppression of experimental autoimmune uveoretinitis and experimental autoimmune thyroiditis by immature DC (7) or selective induction of DC (8). In humans, DC pre-cultured with IL-10 induced alloantigen-specific anergy in CD4+ T cells (9) and CD8+ T cells (10) by converting immunogenic DC into tolerogenic DC. The tolerogenic properties of DC are linked to their maturation state. Subcutaneous injection of immature DC can lead to peripheral tolerance by differentiation of regulatory T cells (11,12). Thus, the concept of ‘tolerogenic’ DC reflects an additional property of these important APC, which might be useful in transplantation and autoimmune diseases (13).

Recent studies demonstrate that IFN-γ plays a critical role in EAE by suppressing the expansion of activated CD4+ T cells (14-17). DC exposed in vitro to IFN-γ resulted in T cell tolerance in vitro to self-antigen by initiating T cell apoptosis (18). DC treated with IFN-γ also down-modulated autoimmune diabetes in NOD mice (19). Here, we reported that splenic DC that had been exposed in vitro to IFN-γ (IFN-γ-DC) have therapeutic potential on acute EAE in Lewis rats and on chronic-relapsing EAE in B6 and SJL/J mice.

**Methods**

**Animals and reagents**

Female Lewis rats and SJL/J mice were purchased from Zentralinstitut fur Versuchstierzucht (Hannover, Germany). Female B6 mice were obtained from the Jackson Laboratory (Bar Harbor, ME). Rats were used at 6–8 weeks of age and mice were used at 8–12 weeks of age. All animals were housed under pathogen-free conditions.

Guinea pig myelin basic protein (MBP) peptide 68–86, mouse proteolipid peptide 139–151 and mouse myelin oligodendrocyte glycoprotein (MOG) peptide 35–55 were synthesized in an automatic Tecan/Syro Synthesizer (Multisyntech, Bochum, Germany). Recombinant rat and human myelin basic protein (MBP) peptide 68–86, mouse proteolipid peptide 139–151 and mouse myelin oligodendrocyte glycoprotein (MOG) peptide 35–55 were synthesized in an automatic Tecan/Syro Synthesizer (Multisyntech, Bochum, Germany). Recombinant rat and mouse IFN-γ was from Innogenetics (Ghent, Belgium). mAb rat CD4, ED1, OX-62 and phycoerythrin (PE)-conjugated anti-rat CD3 were from Serotec (Oxford, UK). PE-conjugated anti-rat CD80 and CD86 were from PharMingen, (PharMingen, San Diego, CA). Modified Griess reagent was from Sigma (St Louis, MO). Annexin-V-FLUOS and propidium iodide were from Boehringer (Mannheim, Germany).

**Induction of EAE**

Lewis rats were immunized in footpads with 200 µl inoculum containing 25 µg MBP68–86 and 2 mg Mycobacterium tuberculosis (strain H37RA; Difco, Detroit, MI). To induce chronic EAE, SJL/J and B6 mice were inoculated along their back with 100 µl inoculum containing 150 µg PLP139–151 or 50 µg MOG35–55, and 0.4 mg M. tuberculosis. Immediately after immunization and 2 days after immunization the mice received an i.v. injection of 200 ng of pertussis toxin (List Biological, Campbell, CA) in 200 µl PBS. Animals were weighed and evaluated in a blind fashion by at least two investigators for clinical signs. Clinical scores of EAE were graded according to the following criteria: 0, asymptomatic; 1, complete loss of tail tone; 2, hind limb paraparesis; 3, complete hind limb paralysis; 4, hind limb paralysis with forelimb involvement; and 5, moribund/dead.

**Magnetic resonance imaging (MRI)**

MRI recordings were performed on a 4.7-T magnet with a bore diameter of 40 cm (Biospec Advance 47/40 spectrometer; Bruker, Karlsruhe, Germany). The system was equipped with a 12-cm self-shielded gradient coil capable of switching 200 mT/m in 250 µs. For the MRI experiments, rats were anesthetized with isoflurane, induction 0.3% in air, maintenance 0.5%. A temperature-regulated warm air stream maintained the rat body temperature at 37.5°C. A RF coil 35-mm (inner diameter) birdcage resonator was used for the brain. A rapid acquisition with relaxation enhancement (RARE) protocol was used with the following parameters: TR = 3000 ms, TE = 21 ms, RARE factor 32, matrix dimensions = 256 × 256, FOV = 4 cm, 8 averages, 1.0 mm slice thickness. For spinal cord, a homemade surface coil with a length of 12 cm was used. The parameters were: TR = 3358.12 ms, TE 63.5 ms, RARE factor 32, matrix dimensions = 256 × 256, FOV = 3 cm, 32 averages, 1.0 mm slice thickness.

**Preparation, modification and injection of DC**

Splenic DC were prepared from healthy Lewis rats, and SJL/J and B6 mice for different EAE models. Spleens were removed under aseptic condition, and cell suspension was prepared by forcing through a 40-µm nylon mesh (Falcon, Franklin Lakes, NJ). After erythrocytes were osmotically lysed, the cell suspension was filtered to remove debris. DC were obtained as the non-adherent component after overnight culture of plastic adherent cells as previously described with minor modifications (20), while macrophages represent the persistently adherent to flask (21,22). Briefly, DC were enriched by differential adherence by incubating cells in 75-mm² culture flasks (Falcon, Becton Dickinson, Mountain View, CA) in serum-free DMEM (Gibco, Paisley, UK) containing 50 IU penicillin, 50 µg/ml streptomycin, 1% minimum essential medium and 10 mM HEPES at 37°C in 5% CO₂. After 2 h, non-adherent cells were gently removed by swirling the flasks and aspirating the medium. Flasks were washed 5 times with serum-free medium to remove remaining non-adherent cells. New medium containing 10% FCS (Gibco) was added to the flasks. After 18 h, re-floating cells were collected as a DC-enriched fraction, while adherent cells mostly consisted of macrophages. The DC-enriched population contained ~85% DC by staining with OX-62 mAb, which recognizes the αE2 subunit of an integrin specifically expressed on rat DC (22). Anti-rat CD3 (T cells), CD45RA (B cells) and CD161 (NK cells) were used to detect contamination of other cells (<10%).

DC were exposed to recombinant rat or mouse IFN-γ (100 U/ml) (IFN-γ-DC) or to medium (naive DC) for 48 h at 37°C. DC were then washed with serum-free medium and injected s.c. into the back of animals that had been immunized 5 days earlier with MBP68–86 (in Lewis rats; 2 × 10⁶ DC/rat) or 7 days earlier with PLP139–151 or MOG35–55 (in both SJL/J and B6 mice; 0.5 × 10⁶ DC/mouse).

**Separation of T cells**

Rats were immunized with MBP68–86 as described in 'Induction of EAE' and sacrificed on day 9 post-immunization (p.i.)
Mononuclear cells (MNC) from draining inguinal and mesenteric lymph nodes were prepared by forcing through a 40-µm nylon mesh and T cells (MBP<sub>68-86</sub>-reactive T cells) were isolated by using a rat T cell-enrichment column (R & D Systems, Minneapolis, MN). Purity was routinely >98% for CD3<sup>+</sup> T cells.

**Preparation of MNC**

Peripheral blood and spleens from rats were obtained on day 14 p.i. and spleens from SJL/J mice were obtained on day 70 p.i. Blood MNC were isolated by centrifugation over a Lymphoprep density gradient (Nycomed, Oslo, Norway). Splenic MNC were obtained by grinding the organs through a 40-µm nylon mesh. After erythrocytes were osmotically lysed, cells were then washed 3 times and re-suspended in medium.

**IFN-γ ELISA assay**

Splenic MNC from rats on day 14 p.i. or from SJL/J mice on day 70 p.i. were cultured in the absence and presence of either MBP<sub>68-86</sub> (10 µg/ml) or PLP<sub>139-151</sub> (10 µg/ml) for 48 h at 37°C. The supernatants were collected and the levels of IFN-γ were measured by a sandwich ELISA kit (PharMingen). Determinations were performed in duplicate and results expressed as pg/ml.

**Phenotyping**

Rat splenic DC from healthy rats were exposed in vitro to recombinant rat IFN-γ (100 U/ml) or lipopolysaccharide (1 µg/ml) for 48 h. After washing with PBS, DC were stained with PE–MHC class II, PE–CD80 and PE–CD86 for 30 min at 4°C, and analyzed with a FACScan. The expression of these molecules on DC was analyzed by mean fluorescence intensity (MFI).

**Immunohistochemistry**

Rats were sacrificed and spinal cords were dissected on day 14 p.i. Cryostat sections were cut at 10 µm and fixed in acetone for 10 min. Non-specific binding sites were further blocked with 2% horse serum (Serotec). The sections were incubated overnight with primary mouse anti-rat CD4 and ED1 antibodies. After washing with PBS, sections were incubated with biotinylated anti-mouse IgG and PE–streptavidin. Thereafter, slides were permeabilized with 0.1% Triton X-100 in 0.1% sodium citrate for 5 min on ice. TUNEL reaction mixture was added to samples for 60 min at 37°C in the dark. Then the sections were directly analyzed under a fluorescence microscope.

**Measurement of nitric oxide (NO) production**

Cells were washed with PBS and lysed in 1 ml of TRIzol reagent (Gibco/BRL). Total RNA was isolated and synthesis of single-strand cDNA was carried out using SuperScript II RNase H<sup>−</sup> reverse transcriptase (Invitrogen, Carlsbad, CA) according to the manufacturer’s instructions. Briefly, 20 µl of reaction mixture contains 4 µl 5 × Superscript first-strand buffer, 2 µl Superscript DTT, 2 µl pd(N)₆ random hexamer (50 µM; Amersham, Uppsala, Sweden), 1 µl dNTP (10 mM), 0.25 µl recombinant RNasin ribonuclease inhibitor (Promega, Madison, WI) and 0.5 µl Superscript enzyme. The reaction was incubated at 25°C for 10 min, at 43°C for 60 min and at 70°C for 15 min. Primers for rat IDO and β-actin were designed using the computer program Primer Express, a software program specially provided with the 7700 SDS (Applied Biosystems, Foster City, CA). The primers for IDO were
We evaluated the therapeutic potential of splenic IFN-γ-modified DC in acute and chronic EAE.

(i) forward primer: 5'-AGCTCCGAGAAAGTGCGAGA-3';
(ii) reverse primer: 5'-TGTAACCTGTGCCCCTGAATTC-3'.
The primers for β-actin were (i) forward primer: 5'-TGCTGACAGGAGAAAGAAGA-3'; (ii) reverse primer: 5'-CGCTAGGAGAAGCATGAT-3'. Real-time RT-PCR assay on the basis of SYBR Green I (24,25) was performed with the ABI Prism 7700 sequence detection system (Perkin-Elmer Applied Biosystems). For both IDO and β-actin, different concentrations of primers (100–200 nM) are tested to determine the optimal PCR condition. Briefly, Master Mix (90 µl) containing 200 nM primers, 45 µl 2 x qPCR Mastermix Plus for SYBR Green I (Eurogentec, Seraing, Belgium) and 2 µl cDNA was mixed before aliquoting into triplicate to a 96-well microtiter plate. The cDNA was amplified under the following universal conditions: one cycle at 50°C for 2 min and 95°C for 10 min, followed by 40 cycles at 95°C for 15 s and 60°C for 1 min. At the end of PCR, the dissociation curve analysis was conducted to make sure the specificity of the amplification. For each sample, the normalized Ct (cycle threshold) value of IDO was got by subtracting the Ct value of β-actin from the Ct value of IDO. The fold change versus one sample of the control group (taken as 1) was calculated.

Statistics
Statistical analysis was performed using ANOVA and Tukey-Kramer multiple comparisons when ANOVA showed significant differences (P < 0.05).

Results
General characteristics of IFN-γ-DC
Compared to lipopolysaccharide-DC, both naive DC and IFN-γ-DC exhibited rather low MHC class II, CD80 and CD86 expression (Fig. 1a), indicating that IFN-γ-DC are still in an immature state.

As expected, IFN-γ-DC produced high levels of nitrite, as compared to naive DC (P < 0.001; Fig. 1b). Because NO production may contribute to cell death, we examined the survival of DC. There were no differences in proliferation, viability and apoptosis of DC between naive DC and IFN-γ-DC (Fig. 1b), suggesting that NO production of DC induced by IFN-γ did not influence the proliferation and survival of DC.

IFN-γ-DC reduce MRI lesions in brains and spinal cords
In preliminary experiments, we observed that clinical score and inflammatory cell infiltration within spinal cords can reach a peak on day 14 p.i. in MBP68-86-induced Lewis rat EAE, while the damage of MRI became more severe on day 18 p.i. and can last 1 week, regardless of the recovery phase of EAE. Thus we chose MRI analysis of rats on day 18 p.i. Brain MRI performed in Lewis rat EAE on day 18 p.i., i.e. during the recovery phase of EAE, revealed enlargement of both lateral ventricles and of the third ventricle in both PBS- or naive DC-injected rats (Fig. 3A and B). In contrast, the ventricles in the IFN-γ-DC-injected rats were of normal size (Fig. 3C). MRI of the lumbar spinal cord revealed scattered T2 lesions in both PBS- or naive DC-injected rats (Fig. 3D and E), while IFN-γ-DC-injected rats showed normal T2 images of the lumbar spinal cord (Fig. 3F).

IFN-γ-DC reduce cell infiltration within the CNS and augment cell apoptosis in the periphery
Using immunohistochemistry staining, we examined infiltration of macrophages and CD4+ T cells in Lewis rats sacrificed on day 14 p.i., i.e. at the time point of the peak of clinical EAE. Infiltration of ED1+ macrophages and CD4+ T cells in spinal cords from IFN-γ-DC-injected rats (mean levels 0.4 ± 0.3 and 0.2 ± 0.2 respectively) was clearly lower than in control rats injected with naive DC or PBS (P < 0.001 for both comparisons; Fig. 4A). In healthy rats, there are no ED1+ cells in spinal cords, indicating that resting microglia cannot be stained by ED1 antibody. It is possible that activated microglia may be ED1+. Thus, it cannot be excluded that increased ED1+ cells may include infiltrating macrophages and activated microglia.

To define a possible mechanism behind the reduction of infiltrating cells within the CNS after injection of IFN-γ-DC, we examined apoptotic cells among blood MNC and lymph node MNC. Rats injected with IFN-γ-DC had higher numbers of CD4+ apoptotic cells among blood MNC (Annexin-V+CD4+ cells) and lymph node MNC (TUNEL+CD4+ cells) as compared to rats injected with PBS or naive DC (P < 0.001 and P < 0.05 respectively) (Fig. 4b and c).

IFN-γ-DC induce antigen-specific IFN-γ production
To assess whether IFN-γ-DC have an impact on their potential to induce an antigen-specific immune response under the inflammatory environment of immunization with MBP68-86, we examined the ability of IFN-γ-DC to elicit antigen-specific IFN-γ production. Rats injected with IFN-γ-DC showed 18.8- and 5.1-fold increase of MBP68-86-induced IFN-γ secretion in supernatants of splenic MNC on day 14 p.i., as compared to rats injected with PBS or naive DC respectively (Fig. 5a). In rats injected with IFN-γ-DC, antigen-induced IFN-γ production was higher than spontaneous IFN-γ production (3.6-fold, P < 0.01, Fig. 5a). In accordance with the rat EAE model, SJL/J mice injected with IFN-γ-DC exhibited 13- and 6-fold increase of PLP139-151-induced IFN-γ production in supernatants of splenic MNC on day 70 p.i., as compared to SJL/J mice injected with naive DC (mean clinical score = 1.1; P < 0.05) and mice receiving naive DC (mean clinical score = 2.7; P < 0.05), and did not develop the clinical relapse of EAE on day 59 p.i. (mean clinical score = 0.2) compared to mice receiving PBS (mean clinical score = 1.4; P < 0.01) and mice receiving naive DC (mean clinical score = 1.1; P < 0.05) (Fig. 2c).

IFN-γ-DC induce protection from EAE
We evaluated the therapeutic potential of splenic IFN-γ-modified DC during the incipient phase of EAE in the different EAE models. In acute Lewis rat EAE, injection of IFN-γ-DC reduced severity of clinical symptoms on day 14 p.i. (mean clinical score = 0.5) as compared to rats receiving PBS (mean clinical score = 4.5; P < 0.01) and rats receiving naive DC (mean clinical score = 4; P < 0.05) (Fig. 2a). In chronic relapsing EAE of SJL/J mice, injection of IFN-γ-DC prevented clinical relapse of EAE on day 14 p.i., i.e. at the time point of the peak of clinical EAE. Using immunohistochemistry staining, we examined infiltration of ED1+ macrophages and CD4+ T cells in spinal cords, indicating that resting microglia cannot be stained by ED1 antibody. It is possible that activated microglia may be ED1+. Thus, it cannot be excluded that increased ED1+ cells may include infiltrating macrophages and activated microglia.
injected with PBS and naive DC respectively (Fig. 5b). Similarly, antigen-induced IFN-\(\gamma\) production in SJL/J mice injected with IFN-\(\gamma\)-DC was higher than spontaneous IFN-\(\gamma\) production (3-fold, \(P < 0.05\), Fig. 5b).

**IFN-\(\gamma\)-DC trigger antigen-dependent apoptosis of CD4 T cells in vitro**

In view of the fact that injection of IFN-\(\gamma\)-DC induced apoptosis of CD4 T cells in vivo, we further examined whether IFN-\(\gamma\)-DC-induced apoptosis of CD4 T cells is antigen dependent. In the absence of specific antigen, both naive DC and IFN-\(\gamma\)-DC only induced a low frequency of CD4 T cell apoptosis (Fig. 6). The number of apoptosis of CD4 T cells was increased in coculture of IFN-\(\gamma\)-DC with MBP-reactive T cells in the presence of MBP\(_{68-86}\) (12.7%), as compared to naive DC (3.9%) or coculture of IFN-\(\gamma\)-DC with MBP-reactive T cells in the absence of antigen (1.9%) (Fig. 6).

**IFN-\(\gamma\)-DC mediate IDO-dependent hypoproliferation and apoptosis of T cells in vitro**

The results obtained from in vitro studies using real-time PCR suggest that IFN-\(\gamma\) (100 U/ml) induced the up-regulation of IDO mRNA by DC (3.8 -fold, \(P < 0.01\)), while the competitive inhibitor of IDO, 1-methyl-D-tryptophan (1-MT), partly inhibited up-regulation of IDO mRNA expression (Fig. 7a). Addition of IFN-\(\gamma\)-DC to MBP-reactive T cells significantly inhibited
proliferation of T cells compared to naive DC ($P < 0.01$). The suppressive effect was abolished by the addition of the competitive inhibitor 1-MT to the culture of DC (Fig. 7b). In additional experiments, we observed that IFN-$\gamma$-DC may enhance apoptosis of T cells after co-culture with MBP-reactive T cells ($P = 0.058$). There were no differences between naive DC and 1-MT-treated DC (Fig. 7a±c).

Therefore, IFN-$\gamma$-DC appear to mediate proliferative suppression and apoptosis of T cells via IFN-$\gamma$-induced IDO activation under in vitro experimental condition.

**Discussion**

The main finding of this study is that injection of IFN-$\gamma$-DC demonstrates therapeutic potential on acute EAE in Lewis rats and chronic EAE in mice, accompanied by an increase of T cell apoptosis in the periphery, and reduction of macrophage and CD4$^+$ T cell infiltration within the CNS, possibly through an antigen-specific IFN-$\gamma$ pathway and/or IDO$^+$ DC. In view of the established $T_{H1}$-mediated autoimmune pathogenesis of EAE, the therapeutic effect of IFN-$\gamma$-DC is unexpected. However, the traditional view has been challenged recently by a number of studies describing unexpected disease-ameliorating effects by IFN-$\gamma$ in EAE (14–17,26), as well as in lethal autoimmune myocarditis (27) and collagen-induced arthritis (28). In autoaggressive immunity, it has been proposed that...
pro-inflammatory cytokines may be required at an early stage to induce self-responses by priming inflammatory T$_{h}$1 responses. The late expression of the same cytokines could drive the terminal differentiation and death of T cells (29). In IFN-$\gamma$-deficient mice, 10- to 16-fold more activated CD4$^+$ T cells were accumulated in the CNS during EAE than in wild-type mice (15), providing evidence that IFN-$\gamma$ may limit the extent of EAE by inducing apoptosis of activated CD4$^+$ T cells. Intrathecal delivery of IFN-$\gamma$ inhibited EAE by inducing apoptosis of infiltrating T cells (24). IFN-$\gamma$ also mediates apoptosis of activated CD4$^+$ T cells via NO induction (14,17,30). Recent studies demonstrate that adjuvant [complete Freund’s adjuvant (CFA)] immunotherapy against EAE inhibited EAE in wild-type mice, but failed in NOS2$^{-/-}$ mice (31). More interestingly, 100% of recovered Lewis rats developed a relapse of acute EAE if treated with $N$-methyl-$L$-arginine acetate, a NOS inhibitor (32). Thus, antigen-induced IFN-$\gamma$ production in rats injected with IFN-$\gamma$-DC may play an important role in inducing T cell apoptosis and preventing cell infiltration.

Another view is that IDO$^+$ DC induced by IFN-$\gamma$ can inhibit T cell responses. Stimulation with exogenous or T cell-derived IFN-$\gamma$ promoted DC to generate functional IDO (33), which participates in the regulation of T cell responses and may control autoreactive T cells by the depletion of tryptophan (34). In vitro studies showed that T cells are specifically sensitive to tryptophan deprivation (35). IDO$^+$ DC enhanced their tolerogenic activity toward CD4 T cells (36). This action preferentially affects activated T cells and increased gradually with exposure time (37). Indirect proof for the role of IDO in suppression of alloreactive T cells was obtained by Munn et al. (38), who showed that inhibition of IDO resulted in rejection of the fetus in pregnant mice. IFN-$\gamma$ also enhanced the regulatory activity of CD8$^+$ DC through inducing IDO expression and tryptophan degradation, leading to apoptosis of T cells in vitro (39). Treatment of DC with IFN-$\gamma$ increased their IDO activity and conferred tolerogenic properties apparently by initiating apoptosis of antigen-specific CD4 T cells (36,40,41). It is of interest that IFN-$\gamma$ induces IDO expression of DC and that IFN-$\gamma$-induced IDO$^+$ DC cause peripheral tolerance by inhibiting T cell expansion or proliferation. Subsequent experiments demonstrate the requirement of IFN-$\gamma$ treatment of the
In vitro experiments, we observed that IFN-γ-induced IDO mRNA expression of DC, and that IDO+ DC exhibited an IDO-dependent proliferative suppression and apoptosis of T cells. These data represent a possible mechanism that IDO+ DC may trigger apoptosis of CD4 T cells in vivo by tryptophan metabolites.

Is the immunotherapeutic strategy mediated by IFN-γ-DC antigen specific? In principle, IFN-γ-DC from healthy rats, upon injection to rats with incipient EAE, can capture, process and present antigen to T cells when specific antigen stimulation is delivered. Our data support that injection of IFN-γ-DC induced an antigen-specific immune response in vivo. In animals injected with IFN-γ-DC, antigen-induced IFN-γ production was significantly increased, as compared to spontaneous IFN-γ production. As discussed above, IFN-γ can result in apoptosis in multiple cells by different pathways, including promoting caspase-8-dependent apoptosis (43), up-regulating Fas (44), down-regulating Bcl-2 and Bcl-xL (45), and inducing NO production (46). It is thus proposed that injection of IFN-γ-DC induce antigen specific IFN-γ production, which influences expansion and infiltration of activated T cells by initiating apoptosis of T cells. Our further experiments indicate that IFN-γ-DC can cause apoptosis of MBP-reactive T cells in the presence of antigen. In addition to an apoptotic mechanism mediated by IFN-γ, it may be consistent with the idea that treatment of DC with IFN-γ increases IDO expression and confers tolerogenic properties of DC, which may induce antigen-specific T cell tolerance by blocking T cell-cycle progression and by rapid induction of T cell activation-induced cell death (37).

In conclusion, IFN-γ-DC, when given s.c., effectively suppressed clinical signs of acute and chronic EAE in rats and mice, showing normal MRI of the spinal cord and brain, enhanced apoptosis of CD4+ T cells in the periphery, and reduced infiltration of inflammatory cells within the CNS. Apoptosis of CD4+ T cells may be mediated by IDO+ DC and/or an IFN-γ pathway in an antigen-dependent manner. Our observations provide the possibility that autologous DC from the individual MS patient might be modified with IFN-γ in vitro and then re-infused to the patient for therapeutic purposes.

Acknowledgements
This work was supported by grants from the Swedish Medical Research Council, Karolinska Institute Research Funds and an unrestricted research grant from Biogen (Cambridge, MA).
Abbreviations

1-MT  1-methyl-D-tryptophan
APC  antigen-presenting cell
CFA  complete Freund's adjuvant
CNS  central nervous system
DC  dendritic cell
EAE  experimental autoimmune encephalomyelitis
IDO  indoleamine 2,3-dioxygenase
MBP  myelin basic protein
MFI  mean fluorescence intensity
MNC  mononuclear cell
MOG  myelin oligodendrocyte glycoprotein
MRI  magnetic resonance imaging
MRS  multiple sclerosis
NOD  non-obese diabetic
PE  phycoerythrin
PLP  proteolipid peptide
RARE  rapid acquisition with relaxation enhancement

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