Autoantigen-Specific IL-10-Transduced T Cells Suppress Chronic Arthritis by Promoting the Endogenous Regulatory IL-10 Response

Teun Guichelaar, Corlinda B. ten Brink, Peter J. van Kooten, Suzanne E. Berlo, Chris P. Broeren, Willem van Eden and Femke Broere

*J Immunol* 2008; 180:1373-1381; doi: 10.4049/jimmunol.180.3.1373

http://www.jimmunol.org/content/180/3/1373

**References**

This article cites 57 articles, 20 of which you can access for free at:
http://www.jimmunol.org/content/180/3/1373.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
Autoantigen-Specific IL-10-Transduced T Cells Suppress Chronic Arthritis by Promoting the Endogenous Regulatory IL-10 Response

Teun Guichelaar, Corlinda B. ten Brink, Peter J. van Kooten, Suzanne E. Berlo, Chris P. Broeren, Willem van Eden, and Femke Broere

Deficient T cell regulation can be mechanistically associated with development of chronic autoimmune diseases. Therefore, combining the regulatory properties of IL-10 and the specificity of autoreactive CD4⁺ T cells through adoptive cellular gene transfer of IL-10 via autologous-specific CD4⁺ T cells seems an attractive approach to correct such deficient T cell regulation that avoids the risks of nonspecific immunosuppressive drugs. In this study, we studied how cartilage proteoglycan-specific CD4⁺ T cells transduced with an active IL-10 gene (T_{IL-10}) may contribute to the amelioration of chronic and progressive proteoglycan-induced arthritis in BALB/c mice. TCR-transgenic proteoglycan-specific T_{IL-10} cells ameliorated arthritis, whereas T_{IL-10} cells with specificity for OVA had no effect, showing the impact of Ag-specific targeting of inflammation. Furthermore, proteoglycan-specific T_{IL-10} cells suppressed autoreactive proinflammatory T and B cells, as T_{IL-10} cells caused a reduced expression of IL-2, TNF-α, and IL-17 and a diminished proteoglycan-specific IgG2a Ab response. Moreover, proteoglycan-specific T_{IL-10} cells promoted IL-10 expression in recipients but did not ameliorate arthritis in IL-10-deficient mice, indicating that T_{IL-10} cells suppress inflammation by propagating the endogenous regulatory IL-10 response in treated recipients. This is the first demonstration that such targeted suppression of proinflammatory lymphocyte responses in chronic autoimmunity by IL-10-transduced T cells specific for a natural Ag can occur via the endogenous regulatory IL-10 response.

Rheumatoid arthritis (RA) is a progressive autoimmune disease, characterized by chronic inflammation of the articular joints. The inflammation results in irreversible destruction of cartilage and bone by enzymes produced by macrophages and fibroblast-like cells that have invaded the inflamed synovium. Different types of cells that are involved in arthritis and in other autoimmune disorders have been exploited as a tool to study or treat disease (1–4). T cells are promising candidates for immunological interventions in autoimmunity because of their Ag specificity and their ability to modulate other cells that are involved in autoimmune disease.

Although the actual triggers leading to a disease like RA are unknown, in vitro proliferation of T cells from RA patients in response to several autoantigens (5, 6) and restriction of the synovial T cell repertoire to common clonotypes (7, 8) are indicative of an autoantigen-driven T cell expansion in RA. Transfer and depletion studies using CD4⁺ T cells and the use of TCR-transgenic (TCR-Tg) animals in arthritis models have underscored that joint Ag-specific CD4⁺ T cells with a Th1-like phenotype mediate the induction and/or aggravation of arthritis (9–13).

In addition, B cells invade the inflamed synovium and are implicated in the pathogenesis of RA as producers of autoantibodies (14, 15) and as efficient APCs (14, 16, 17). Both autoreactive T and B cell responses are required to induce severe arthritis, suggesting that both lymphocyte subsets contribute to development of arthritis (9, 14).

A current hypothesis states that excessive development and/or function of (auto)aggressive CD4⁺ T cells is controlled by regulatory T cells (T_{reg} cells) (18–22). For example, a recent study by Lohr et al. (23) has shown that T_{reg} cells can control development of the IL-17-producing Th17 cells which are crucial for development of inflammation in several autoimmunity models (24). Several subsets of T_{reg} cells have been described to suppress inflammation. Of these subsets, naturally occurring CD4⁺CD25⁺ T cells, CD4⁺ T regulatory 1 (Tr1) cells, and CD4⁺ Th3 cells are the best studied T_{reg} cells. Although their exact phenotypes and mechanisms of suppression are still not fully understood and may vary between different subsets, most T_{reg} cells have been described to require IL-10 for successful suppression as summarized by Bluestone et al. (1).

IL-10 plays an important role in the homeostatic regulation of the autoreactive T cell repertoire (25). In addition, IL-10 depletion and IL-10 treatment in murine arthritis models (26–29) have demonstrated the anti-inflammatory properties of IL-10 in arthritis. Moreover, reduced IL-10 expression by CD4⁺ T cells is related to a higher frequency of Th1 cells and more severe disease in RA (30). Extensive studies (reviewed by Moore et al. (31)) have demonstrated that IL-10 inhibits the production of proinflammatory...
cytokines and chemokines in activated monocytes/macrophages and inhibits proliferation of CD4+ T cells by down-regulation of APC function. Moreover, IL-10 drives the generation of a population of IL-10-producing Tr1 cells that suppress Ag-specific T cell responses and prevent colitis (32, 33). Thus, exploiting Ag-specific IL-10+ T cells to propagate anti-inflammatory responses can be a promising method for therapy of autoimmune diseases.

Because peripheral blood of RA patients contains CD4+ T cell populations that are cartilage Ag-specific (5, 34, 35), ex vivo induction of a regulatory phenotype in such Ag-specific T cells may provide a tool for Ag-specific interventions in the chronic inflammation of RA. Therefore, we explored the potential mechanisms of targeting the inflammatory autoimmune response in cartilage proteoglycan-induced arthritis (PGIA) with proteoglycan-specific CD4+ T cells expressing IL-10 through retroviral transduction with an active IL-10 gene. PGIA is a chronic and progressive arthritis model induced by immunization with proteoglycan, representing many features of RA (36). A recently generated proteoglycan-specific TCR (proteoglycan-TCR)-Tg mouse served as donor of proteoglycan-specific CD4+ T cells to propagate anti-inflammatory responses can be a promising method for therapy of autoimmune diseases.

The results indicated that proteoglycan-specific Tn10 cells are suppressive in vitro, reduced the proteoglycan-specific inflammatory immune response in vivo, and promoted the endogenous IL-10 response. The observed arthritis-suppressive effect of Tn10 cells depended on the presence of their proteoglycan-specific TCR. Interestingly, proteoglycan-specific Tn10 cells could not reduce the inflammatory response in IL-10-deficient recipients, indicating that autoantigen-specific Tn10 cells regulated chronic arthritis via induction of the endogenous IL-10 response.

Materials and Methods

Mice and Ags

BALB/c mice (obtained from Charles River Laboratories) and IL-10 knockout BALB/c mice (a gift from Dr. A. van Oosterhoudt, University Medical Center Utrecht, Utrecht University, Utrecht, the Netherlands) were kept at the animal facility of the University of Utrecht (Gemeenschappelijk Dierenlaboratorium (GDL)) under standard conditions. Mice and Ags were kept at the animal facility of the University of Utrecht (Gemeenschappelijk Dierenlaboratorium (GDL)) under standard conditions. Human and murine proteoglycan were prepared as described elsewhere (11, 37). Limbs were dissected, fixed in 10% buffered formalin, and embedded in paraffin. Paraffin sections were stained with H&E and examined for histopathology of ankle joints.

Constructions and production of retrovirus

Murine IL-10 cDNA was obtained using specific primers (5′-AGA TCT TTG CAG AAA AGA GAG CTC CA-3′ and 5′-GTC GAC TGG AGT CCA GCA GAC TCA AT-3′) and cloned into the murine stem cell virus (MSCV) 2.2 plasmid (see Fig. 1). Ecotropic replication-deficient retrovirus was produced with a Phoenix Eco packager cell line. Packager cells were cultured per 3×105 cells in 10 ml of DMEM (Invitrogen Life Technologies) (+4500 mg/L glucose, +GlutaMAX I, -pyruvate) supplemented with 10% heat-inactivated FCS (Bodino), 100 U/ml penicillin, 100 μg/ml streptomycin (Invitrogen Life Technologies) in 100-mm tissue culture dishes (Nalgene Nunc International) and cultured at 37°C, 6% CO2. The next day, the culture medium was refreshed and 1–3 h thereafter, 500 μl of 0.25 M CaCl2, containing 20 μg of MSCV-plasmid and 5 μg of PCL-Eco plasmid was mixed with an equal volume HBS buffer (pH 7.02) (280 mM NaCl, 1.5 mM Na2HPO4, 12 mM glucose, 10 mM KCL, and 50 mM HEPES) by bubbling and added to the cells. At 20 h after transfection, the supernatant was replaced with fresh medium. Within 24 h, supernatant containing the retrovirus was harvested, filtered with a 0.45-μm filter, snap-frozen, and stored frozen until use. Again, fresh medium was added and virus was harvested the next day and pooled with the previous supernatant for infection.

Stimulation, retroviral transduction, and transfer of CD4+ T cells

CD4+ T cells of pooled spleens and lymph nodes of DO11.10 or TCR-5/4E8-Tg were isolated with anti-CD4 (L3T4) magnetic beads (Miltenyi Biotec) and stimulated with magnetic M-450 asylos-activated Dynabeads (Dynal Biotech) coated with anti-CD3 (145-2C11) and anti-CD28 (PV-1) mAbs (own production) in a 1:10 ratio. Anti-CD3/CD28-coated beads were added to 1×106 CD4+ T cells (in a 2:1 ratio) in 1 ml of supplemented DMEM in 24-well flat-bottom plates (Corning) and cultured at 37°C, 6% CO2. After 48 h, 750 μg of the culture supernatant was replaced with 1 ml of retroviral supernatant supplemented with 8 μg/ml hexadimethrine bromide (Sigma-Aldrich). Plates were then centrifuged at 930 × g at 20°C for 2 h. Subsequently, 1 ml of supernatant was replaced with fresh medium and cells were cultured for 4 days. Cells were removed from the stimulating beads and transduced cells (normally 60–80% before sorting) were sorted by GFP expression with a FACSVantage SE (BD Biosciences). Accepter mice received 1×106 sorted IL-10/GFP transduced or GFP-transduced CD4+ T cells, injected i.v. or i.p. in PBS.

Induction and assessment of arthritis

Arthritis was induced by i.p. injections of 2 mg of human proteoglycan emulsified in 2 mg of the synthetic adjuvant dimethyl-dioctadecyl-ammonium bromide (Sigma-Aldrich) in PBS (total volume of 200 μl) on days 0 and 21 as described elsewhere (11, 37). Paws were examined three times per week in a blinded set-up to determine onset and severity of arthritis using a standard visual scoring system based on swelling and redness of the paws (11, 37). Limbs were dissected, fixed in 10% buffered formalin, de-calced in 0.5 M EDTA, and embedded in paraffin. Paraffin sections were stained with H&E and examined for histopathology of ankle joints.

In vitro suppression assay

CD4+ responder cells were isolated from pooled spleen and lymph node (LN) cells of DO11.10 mice by negative selection with Dynabeads (Dynal Biotech) using an excess of anti-B220 (RA3-6B2), anti-CD11b (MI/70), anti-MHC class-II (M5/114), and anti-CD8 (YT8169) mAbs and were subsequently labeled with CFSE (Molecular Probes as described elsewhere (38). At day 0, bone marrow cells fromibia and femurs were seeded at 2×106/100-mm suspension dish (Corning) in 10 ml of supplemented IMDM (Invitrogen Life Technologies) with 20 ng/ml GM-CSF (Cyscogen) to generate dendritic cells (DCs). At day 3, 10 ml of supplemented IMDM with 20 ng/ml rGM-CSF was added. At day 6, 10 ng/ml rGM-CSF was added. At day 8, the nonadherent cells were harvested, washed, and used. The suppression assays were done in 96-well flat-bottom plates (Corning) with 2×104 CFSE-labeled DO11.10 CD4+ cells and 2.5×104 bone marrow-derived BALB/c DCs cultured in the presence of 0.32 μg/ml OVA323–339 peptide in 200 μl during 4 days. To analyze the suppressive activity of culture supernatant, 50 μl of the final culture supernatant of conditioned supernatant was tested. To the suppressive activity of transduced proteoglycan-specific T cells, indicated numbers of transduced cells and 0.30 μg/ml human proteoglycan 70–84 peptide were added to the culture.

Ex vivo antigenic stimulation of splenocytes

Single-cell suspensions of spleens of human proteoglycan-immunized mice that had received IL-10/GFP-transduced or GFP-transduced TCR-5/4E8-Tg CD4+ cells were cultured in 96-well flat-bottom plates (Corning) in 2×104 cells/well, in the presence or absence of human proteoglycan (10 μg protein/ml) in supplemented IMDM (Invitrogen Life Technologies) for 72 h.

Flow cytometry

Single-cell suspensions of spleen cells were cultured at 1–2×106 cells/ml supplemented IMDM (Life Technologies) with 50 ng/ml PMA (Sigma-Aldrich) plus 500 ng/ml ionomycin (Sigma-Aldrich) during 4–5 h for intracellular cytokine staining. Brefeldin A (Sigma-Aldrich) was added at 10 μg/ml after the first 2 h. Cells were washed, fixed in 4% PFA/PBS for 10 min, and washed again. For permeabilization in PBS/2% FCS/0.5% saponin (Sigma-Aldrich), cells were washed and stained with anti-IL-10-PE (BD Biosciences/BD Pharmingen) mAb and washed twice, all in the presence of 0.5% saponin. Cells were then washed and stained with anti-CD4-allophycocyanin or anti-CD4-PerCP mAbs (BD Pharmingen). After extensive washing, cells were analyzed on a FACSCalibur (BD Biosciences).

Analysis of migration of GFP+CD4+ cells with flow cytometry was done with unstimulated single-cell suspensions, stained with anti-CD4-allophycocyanin without permeabilization of the cell membrane. For analysis of CFSE-suppression assays cells were stained with anti-CD4-PerCP.
mAb in combination with biotinylated KJ1.26 mAb (Caltag Laboratories) and streptavidin-allophycocyanin (BD Pharmingen). Results were analyzed with CellQuest software (BD Biosciences).

**Cytokine (protein) quantification**

IL-2 was measured with fluorescein-conjugated microspheres coated with ELISA capture Abs (BD Pharmingen) as described elsewhere (11). In brief, coated beads were added to 50 μl of culture supernatant. After overnight incubation at 4°C, microspheres were washed and incubated with biotinylated detection Abs (BD Pharmingen) for 15 min. Subsequently, streptavidin-PE was added to the microspheres, which were incubated for another 15 min and then measured with a Luminex model 100. IL-10 and TNF-α in culture supernatant were measured with a comparable detection system using Lincoplex beads (LINCO Research) according to the manufacturer’s protocol.

**cDNA synthesis and quantitative real-time PCR for cytokine and GFP expression**

Total mRNA was extracted with the RNeasy kit (Qiagen Benelux) and treated with DNase (Qiagen) using the manufacturer’s protocol. Subsequently, RNA was reversely (RT) transcribed into cDNA using the iScript cDNA Synthesis kit (Bio-Rad). Quantitative real-time PCR was performed in a total volume of 25 μl using IQ SYBR Green Supermix (Bio-Rad). A total of 0.25 μM of primers specific for IL-10 (5'-GGT TGC CAA GCC TTA TCG GA-3' and 5'-ACC TGC TCC ACT GCC TTG CT-3'), IL-17a (5'-GCT CCA GAA GCC CCT CAG A-3' and 5'-AGC TTT CCC TCC GCA TGG A-3'), hypoxanthine phosphoribosyltransferase (HPRT; 5'-AGC TTT CCC TCC GCA TGG A-3' and 5'-AAG AAG ATG GTC CGC TGG T-3'), and GFP (5'-GCA GTG CTT CAG CCG CTA-3' and 5'-AAG AAG ATG GTC CGC TGG T-3'). PCR was performed at 95°C and 40 cycles of 10 s at 95°C and 45 s at 60.2°C or, for GFP, 50 cycles of 10 s at 95°C and 45 s at 60.2°C) and real-time detection was done with a Bio-Rad iCycler. Mean fluorescence intensity (MFI) was determined for the GFP population by flow cytometry.

**Statistics**

Data are expressed as mean ± SEMs unless stated otherwise and statistical evaluation was done with nonparametric Mann-Whitney’s U test (two-tailed) or with one-way ANOVA when more than two test groups were compared. A value of p < 0.05 was considered significant.

**Results**

**Retroviral transduction with the IL-10 gene is efficient and results in nonanergic CD4+ T cells producing a high amount of IL-10**

To generate functionally modulated cartilage Ag-specific CD4+ T cells ex vivo, proteoglycan-specific CD4+ T cells were stably transduced with the murine IL-10 gene using MSCV. GFP was used as a marker to select transduced cells. To obtain a high-dose transduction, proteoglycan-specific CD4+ T cells isolated from naïve proteoglycan-TCR Tg mice were stimulated with anti-CD3/CD28 coated beads before infection with MSCV-IL10/GFP (Fig. 1). As a control CD4+ T cells were transduced with the GFP gene alone (TGFP) by MSCV-GFP. Due to an internal ribosomal entry site (IRES), the IL-10 gene and the GFP gene are translated into separate proteins.

CD3/CD28 stimulation of proteoglycan-specific CD4+ T cells typically resulted in an increased number (1.5 – 2 times increase of number of starting population at 2 days posttransfection) of CD4+ cells of which 60 – 80% were transduced as analyzed for GFP expression by flow cytometry. After transduction with MSCV-IL10/GFP, CD4+ T cells showed a substantial increase in IL-10 expression compared with MSCV-GFP-transduced CD4+ T cells (Table I). This increase was observed at both mRNA level by quantitative real-time PCR and protein level measured in culture supernatants of transduced cells 2 days after transduction. At this moment, no difference was found in IFN-γ, IL-2, or IL-4 secretion between culture supernatants of TIL-10 cells and TGFP cells (data not shown). When TIL-10 cells were restimulated with anti-CD3 and irradiated APCs (data not shown) or with anti-CD3/CD28 coated beads (Table I), these cells were fully able to proliferate as compared with TGFP cells.

**Transfer of proteoglycan-specific TIL-10 cells ameliorates arthritis**

To examine whether proteoglycan-specific TIL-10 cells could suppress chronic arthritis, proteoglycan-specific TIL-10 cells were transferred in the PGIA model. Arthritis was induced by two immunizations with proteoglycan in the synthetic adjuvant dimethyldioctadecyl-ammonium bromide with an interval of 3 wk. One day before the second proteoglycan immunization, 1 × 10^6 proteoglycan-specific TIL-10 cells were transferred to acceptor mice. As a control, 1 × 10^6 proteoglycan-specific TGFP cells were transferred. Another control group received PBS instead of transduced T cells. Mice that received proteoglycan-specific TGFP cells or PBS developed a chronic arthritis while recipients of proteoglycan-specific TIL-10 cells developed a significantly reduced form of arthritis (Fig. 2A). In addition, maximum arthritis severity and cumulative incidence were dampened by the proteoglycan-specific TIL-10 cells.
PBS, press proliferation of CD4+ bone marrow-derived DCs in vitro. For this purpose, CFSE-labeled TIL-10 cells.

Recipient of proteoglycan-specific TGFP cells and proteoglycan-specific TIL-10 cells or proteoglycan-affected ankles was performed. Large arrowheads indicate infiltrating cells, small arrowheads indicate cartilage damage. *p < 0.05 for difference between recipients of proteoglycan-specific TGFP cells and proteoglycan-specific TIL-10 cells.

One of the regulatory functions of IL-10 is suppression of proliferation of effector CD4+ T cells in their response to antigenic stimulation. Therefore, we tested whether TIL-10 cells could suppress proliferation of CD4+ T cells responding to Ag presented by bone marrow-derived DCs in vitro. For this purpose, CFSE-labeled OVA-specific CD4+ T cells from OVA-TCR-Tg mice were used as a model for responder CD4+ T (TResp) cells and stimulated in the presence of proteoglycan-specific TIL-10 cells or proteoglycan-specific TGFP control cells. DCs loaded simultaneously with OVA323–339 peptide and human proteoglycan70–84 peptide (the arthritogenic epitope that is recognized by proteoglycan-specific TIL-10 in PGIA) were used to stimulate both the OVA-specific TResp population and the transduced proteoglycan-specific (TIL-10 or TGFP) cells simultaneously. Varying numbers of proteoglycan-specific TIL-10 cells or proteoglycan-specific TGFP cells were added to this culture, with a TIL-10:TResp or TGFP:TResp ratio ranging from 2:1, 0.5:1 to 0.2:1. Within the population of TResp cells, the percentage of cells that went into division was determined from their CFSE profiles and plotted in Fig. 3A as percentage suppression relative to the proliferation in the absence of TIL-10 or TGFP cells. Proliferation of TResp cells was clearly suppressed in the presence of TIL-10 cells as compared with proliferation in the presence of TGFP (control) cells. This suppression was more pronounced when higher numbers of TIL-10 cells were added to the TResp cells.

To check whether suppression by TIL-10 cells was mediated by secreted factors, a culture system was set up with OVA323–339-loaded DC to stimulate CFSE-labeled OVA-specific TResp cells in the presence of supernatant from cultured TGFP or TIL-10 cells. Division of these CFSE-labeled T cells was used as a readout for the presence of such secreted factors. Thus, conditioned medium of cultured TIL-10 or TGFP cells taken 48 h after transduction was added to the DC-TResp culture to check for the presence of secreted suppressive factors (Fig. 3B). Fresh culture medium was used as a control. Conditioned medium of TIL-10 cells suppressed proliferation of CD4+ T Resp cells as shown by reduced CFSE dilution (Fig. 3B) compared with conditioned medium of TGFP cells or fresh culture medium. This is shown for supernatant taken from both proteoglycan-specific (upper panel) and OVA-specific (lower panel) TIL-10 cells, showing that the OVA-specific TIL-10 cells that are used as a control for Ag-specificity for suppression in vivo (see next paragraph) secrete functional IL-10 to the same extent as proteoglycan-specific TIL-10 cells. When IL-10 in supernatant taken from cultured TIL-10 cells was neutralized with anti-IL-10 mAb (JES-2A5), the rate of proliferation of CFSE-labeled TResp cells increased by 86% (data not shown). Blocking IL-10 function in conditioned medium taken from cultured TGFP cells yielded an increase of proliferation of only 2%. This indicates that IL-10 secreted by TIL-10 cells is the main secreted factor responsible for suppression.

TIL-10 cells suppress proliferation of CD4+ T cells

One of the regulatory functions of IL-10 is suppression of proliferation of effector CD4+ T cells in their response to antigenic stimulation. Therefore, we tested whether TIL-10 cells could suppress proliferation of CD4+ T cells responding to Ag presented by bone marrow-derived DCs in vitro. For this purpose, CFSE-labeled OVA-specific CD4+ T cells from OVA-TCR-Tg mice were used as a model for responder CD4+ T cells (TResp) cells and stimulated in the presence of proteoglycan-specific TIL-10 cells or proteoglycan-specific TGFP control cells. DCs loaded simultaneously with OVA323–339 peptide and human proteoglycan70–84 peptide (the arthritogenic epitope that is recognized by proteoglycan-specific TIL-10 cells) were used to stimulate both the OVA-specific TResp population and the transduced proteoglycan-specific (TIL-10 or TGFP) cells simultaneously. Varying numbers of proteoglycan-specific TIL-10 cells or proteoglycan-specific TGFP cells were added to this culture, with a TIL-10:TResp or TGFP:TResp ratio ranging from 2:1, 0.5:1 to 0.2:1. Within the population of TResp cells, the percentage of cells that went into division was determined from their CFSE profiles and plotted in Fig. 3A as percentage suppression relative to the proliferation in the absence of TIL-10 or TGFP cells. Proliferation of TResp cells was clearly suppressed in the presence of TIL-10 cells as compared with proliferation in the presence of TGFP (control) cells. This suppression was more pronounced when higher numbers of TIL-10 cells were added to the TResp cells.

To check whether suppression by TIL-10 cells was mediated by secreted factors, a culture system was set up with OVA323–339-loaded DC to stimulate CFSE-labeled OVA-specific TResp cells in the presence of supernatant from cultured TIL-10 or TGFP cells. Division of these CFSE-labeled T cells was used as a readout for the presence of such secreted factors. Thus, conditioned medium of cultured TIL-10 or TGFP cells taken 48 h after transduction was added to the DC-TResp culture to check for the presence of secreted suppressive factors (Fig. 3B). Fresh culture medium was used as a control. Conditioned medium of TIL-10 cells suppressed proliferation of CD4+ T Resp cells as shown by reduced CFSE dilution (Fig. 3B) compared with conditioned medium of TGFP cells or fresh culture medium. This is shown for supernatant taken from both proteoglycan-specific (upper panel) and OVA-specific (lower panel) TIL-10 cells, showing that the OVA-specific TIL-10 cells that are used as a control for Ag-specificity for suppression in vivo (see next paragraph) secrete functional IL-10 to the same extent as proteoglycan-specific TIL-10 cells. When IL-10 in supernatant taken from cultured TIL-10 cells was neutralized with anti-IL-10 mAb (JES-2A5), the rate of proliferation of CFSE-labeled TResp cells increased by 86% (data not shown). Blocking IL-10 function in conditioned medium taken from cultured TGFP cells yielded an increase of proliferation of only 2%. This indicates that IL-10 secreted by TIL-10 cells is the main secreted factor responsible for suppression.

TIL-10 cells suppress proliferation of CD4+ T cells

One of the regulatory functions of IL-10 is suppression of proliferation of effector CD4+ T cells in their response to antigenic stimulation. Therefore, we tested whether TIL-10 cells could suppress proliferation of CD4+ T cells responding to Ag presented by bone marrow-derived DCs in vitro. For this purpose, CFSE-labeled OVA-specific CD4+ T cells from OVA-TCR-Tg mice were used as a model for responder CD4+ T cells (TResp) cells and stimulated in the presence of proteoglycan-specific TIL-10 cells or proteoglycan-specific TGFP control cells. DCs loaded simultaneously with OVA323–339 peptide and human proteoglycan70–84 peptide (the arthritogenic epitope that is recognized by proteoglycan-specific TIL-10 cells) were used to stimulate both the OVA-specific TResp population and the transduced proteoglycan-specific (TIL-10 or TGFP) cells simultaneously. Varying numbers of proteoglycan-specific TIL-10 cells or proteoglycan-specific TGFP cells were added to this culture, with a TIL-10:TResp or TGFP:TResp ratio ranging from 2:1, 0.5:1 to 0.2:1. Within the population of TResp cells, the percentage of cells that went into division was determined from their CFSE profiles and plotted in Fig. 3A as percentage suppression relative to the proliferation in the absence of TIL-10 or TGFP cells. Proliferation of TResp cells was clearly suppressed in the presence of TIL-10 cells as compared with proliferation in the presence of TGFP (control) cells. This suppression was more pronounced when higher numbers of TIL-10 cells were added to the TResp cells.

To check whether suppression by TIL-10 cells was mediated by secreted factors, a culture system was set up with OVA323–339-loaded DC to stimulate CFSE-labeled OVA-specific TResp cells in the presence of supernatant from cultured TIL-10 or TGFP cells. Division of these CFSE-labeled T cells was used as a readout for the presence of such secreted factors. Thus, conditioned medium of cultured TIL-10 or TGFP cells taken 48 h after transduction was added to the DC-TResp culture to check for the presence of secreted suppressive factors (Fig. 3B). Fresh culture medium was used as a control. Conditioned medium of TIL-10 cells suppressed proliferation of CD4+ T Resp cells as shown by reduced CFSE dilution (Fig. 3B) compared with conditioned medium of TGFP cells or fresh culture medium. This is shown for supernatant taken from both proteoglycan-specific (upper panel) and OVA-specific (lower panel) TIL-10 cells, showing that the OVA-specific TIL-10 cells that are used as a control for Ag-specificity for suppression in vivo (see next paragraph) secrete functional IL-10 to the same extent as proteoglycan-specific TIL-10 cells. When IL-10 in supernatant taken from cultured TIL-10 cells was neutralized with anti-IL-10 mAb (JES-2A5), the rate of proliferation of CFSE-labeled TResp cells increased by 86% (data not shown). Blocking IL-10 function in conditioned medium taken from cultured TGFP cells yielded an increase of proliferation of only 2%. This indicates that IL-10 secreted by TIL-10 cells is the main secreted factor responsible for suppression.

**Table II. Arthritis onset, arthritis incidence, and maximum arthritis severity in mice receiving PG-TCR TGFP cells, PG-TCR TIL-10 cells, OVA-TCR TIL-10 cells or PBS**

<table>
<thead>
<tr>
<th></th>
<th>PG-TCR</th>
<th>PG-TCR</th>
<th>OVA-TCR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GFP</td>
<td>IL-10</td>
<td>IL-10</td>
</tr>
<tr>
<td>Day of onset</td>
<td>31.3 ± 1.5</td>
<td>33.0 ± 1.1</td>
<td>31.1 ± 0.9</td>
</tr>
<tr>
<td>Incidence</td>
<td>9/9 (100%)</td>
<td>24/26 (92%)</td>
<td>18/30 (60%)</td>
</tr>
<tr>
<td>Maximum severity</td>
<td>5.3 ± 1.2</td>
<td>6.3 ± 1.0</td>
<td>3.2 ± 0.8</td>
</tr>
</tbody>
</table>

*Day of arthritis onset (mean ± SEM), arthritis incidence (cumulative until day 44), and maximum severity (mean of maximum scores ± SEM) are shown for pooled data of four experiments analyzed for 44 days upon the primary PG immunization. Transduced T cells or PBS as a control were transferred on day 20. The second PG immunization was given on day 21 to induce arthritis.

Value of p < 0.05 for difference with PG-TCR TGFP cell-recipient group and with OVA-TCR TIL-10 cell-recipient group.
A specific and proteoglycan-specific TIL-10 cells were found in all lymphoid organs. Detection of GFP + T cells in the lymphoid organs was done by flow cytometry for GFP + cells within the CD4 + population. In joints and pancreas, this was done by RT-PCR for mRNA expression of GFP. In Table III, the ratio of CD4 + GFP + cells within the joint-draining LN over the CD4 + GFP + cells in spleen or cervical LN is shown to depict preferential migration of the GFP + TIL-10 cells. Up to 2 wk after transfer, both OVA-specific and proteoglycan-specific TIL-10 cells were found in lymphoid organs analyzed (up to 0.66% of the total CD4 + population), indicating that, irrespective of the specificity of the TCR, the TIL-10 cells migrate throughout the whole lymphoid compartment. However, at 2 wk after transfer the number of proteoglycan-specific TIL-10 cells tended, compared with OVA-specific TIL-10 cells, to preferentially be sustained in joint-draining LN and spleen as shown by a higher ratio of GFP +7 cells in joint-draining LNs over cervical LNs and a lower ratio of these cells in draining LNs over spleen. However, at 4 wk after transfer no GFP + CD4 + cells could be distinguished from background (3.002% in arthritis mice receiving no GFP + cells) in these lymphoid organs anymore. In addition, Table III shows that although GFP expression was found in joints of some animals that received OVA-specific TIL-10 cells (50% of all animals analyzed), in most animals that received proteoglycan-specific TIL-10 cells (83% of all animals analyzed) GFP expression was found in the joints. Moreover, GFP expression was not found in pancreatic tissue (data not shown), indicating that TIL-10 cells preferentially migrate to the inflamed tissue (joints).

Proteoglycan-specific TIL-10 cells suppress the proinflammatory cytokine response in vivo

CD4 + effector T cells (T eff ) contribute to the pathogenesis of arthritis. In vitro data show that TIL-10 cells suppress proliferation of CD4 + T cells. This suggests that TIL-10 cells might reduce the activation of the proteoglycan-specific T eff cells in arthritis. Because activated T cells produce IL-2, we measured the proteoglycan-specific IL-2 response to determine the effect of proteoglycan-specific TIL-10 cells on proteoglycan-specific T eff activation. Therefore, spleen cells were taken from animals 2 wk after transfer of proteoglycan-specific TIL-10 cells or proteoglycan-specific TGFP control cells in PGIA, stimulated with or without human proteoglycan ex vivo and secreted IL-2 was measured subsequently. Fig. 4A shows that the human proteoglycan specific IL-2 response was

| Table III. Migration of PG-TCR TIL-10 cells and OVA-TCR TIL-10 cells at different time points after transfer in PGIA |
|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|
| 1 wk                                         | 2 wk                                         | 4 wk                                         |
|                                              | Draining LN/spleen                          | Draining LN/cervical LN                        | Draining LN/spleen                          | Draining LN/cervical LN                        | Joints                                      |
| PG-TCR TIL-10                                | 0.24 (±0.02)                                | 0.63 (±0.26)                                 | None detected                              | None detected                              |
| OVA-TCR TIL-10                               | 0.22 (±0.03)                                | 0.88 (±0.06)                                 | None detected                              | None detected                              |
|                                              | OVA/PG-TCR TIL-10                          |                                              |                                              | None detected                              | 3/3 (100%)                                 |

a The presence of transferred PG-TCR TIL-10 and OVA-TCR TIL-10 cells in different organs was determined by their expression of their transduced GFP gene at 1, 2, and 4 wk after transfer in PGIA.

b Ratio of transferred T cells within the CD4 + population of joint draining LNs to spleens or cervical lymph nodes as analyzed by flow cytometry for GFP expression (mean of three to five animals per group ± SEM).

c Presence of transferred T cells in joints was determined by RT-PCR for GFP mRNA expression. Number of positive animals out of the number of animals tested is shown.
significantly lower in the group that had received proteoglycan-specific TIL-10 cells compared with the proteoglycan-specific TGFp control recipients. In addition, reduced Ag-specific expression of the proinflammatory cytokine TNF-α in splenocytes from TIL-10 cell recipients paralleled the observed protection by proteoglycan-specific TIL-10 cells (Fig. 4B).

Recently, the proinflammatory cytokine IL-17 has been described as a pathogenic T cell-derived cytokine in autoimmune inflammatory disorders. Therefore, we wondered whether TIL-10 cells could suppress the IL-17 response in vivo. To this end, we quantified IL-17 (IL-17a) expression by real-time PCR in spleen cells taken at 4 wk after transfer of proteoglycan-specific TIL-10 cells or proteoglycan-specific TGFp control cells in PGIA (Fig. 4C). After transfer of proteoglycan-specific TIL-10 cells, IL-17 mRNA expression was significantly reduced compared with the proteoglycan-specific TGFp recipient group.

Together with the antiproliferative effects, this cytokine profile suggests that regulation of growth and/or activation of proinflammatory effector CD4+ T cells may be part of the protective effect by proteoglycan-specific TIL-10 cells in PGIA.

**Proteoglycan-specific TIL-10 cells propagate the IL-10 response in vivo**

Because IL-10 has been shown to propagate the expression of IL-10 in vitro (32, 39), we wondered whether the transferred proteoglycan-specific TIL-10 cells would cause a substantial increase in the IL-10 response in vivo during PGIA. To this end, IL-10 expression in spleen cells of recipient mice was analyzed 2 wk after transfer of proteoglycan-specific TIL-10 cells or proteoglycan-specific TGFp control cells in PGIA. Spleen cells were cultured ex vivo with human proteoglycan or medium as a control and IL-10 in culture supernatants was quantified (Fig. 4D). Culture supernatants of cultured spleen cells of proteoglycan-specific TIL-10 cell recipients contained higher concentrations of proteoglycan-specific IL-10 than that of proteoglycan-specific TGFp control cell recipients. Furthermore, spontaneous IL-10 production by unstimulated spleen cells (medium control) was also slightly enhanced in the proteoglycan-specific TIL-10 cell recipients (data not shown). In addition, quantitative real-time PCR with unstimulated spleen cells taken directly after isolation showed a significant increase of IL-10 expression in the proteoglycan-specific TIL-10 cell recipients (Fig. 4D). Flow cytometry to determine numbers of IL-10-producing splenocytes of mice that received proteoglycan-specific TIL-10 cells showed an increase in the number of IL-10+ cells in the CD4+ population (1.3 ± 0.31% in TIL-10 recipients and 0.9 ± 0.09% in TGFp recipients). An increase was also found in the CD4+ population (2.2 ± 0.30% in TIL-10 recipients and 1.9 ± 0.43% in TGFp recipients) compared with proteoglycan-specific TGFp recipients. As described previously, no GFP+ (from transferred TGFp cells) could be detected by flow cytometry at this point anymore, indicating that the increased IL-10+ population consisted of endogenous cells. Together, these data indicate that the proteoglycan-specific TIL-10 cells enhance the IL-10 response in vivo.

**Proteoglycan-specific TIL-10 cells suppress the Ag-specific IgG2a Ab response**

A proteoglycan-specific B cell response is required to cause severe PGIA and depends on the interaction between B and T cells (14, 40). To examine whether proteoglycan-specific TIL-10 cells would suppress the proteoglycan-specific B cell response, proteoglycan-specific IgG1 and IgG2a Ab responses were analyzed. Therefore, sera were taken from mice 4 wk after they had received proteoglycan-specific TIL-10 cells or proteoglycan-specific TGFp control cells in PGIA and human proteoglycan-specific Abs of the IgG1 and IgG2a isotypes were analyzed by ELISA. Fig. 5 shows that the human proteoglycan-specific IgG2a response in proteoglycan-specific TIL-10 cell recipients compared with proteoglycan-specific TGFp cell recipients.
suppression of the arthritic immune response we transferred pro-
tive Treg cells. Although several immunoregulatory mechanisms
were done in parallel with transfers of the same T cell populations
mice one day before the second proteoglycan immunization of the
were found for the anti-murine proteoglycan-specific IgG2a response; 364.1 (±171.5, n = 6) in T<sub>GFP</sub> cell recipients and 130.1 (±40.3, n = 7) in T<sub>IL-10</sub> cell re-
cipients. Collectively, these data demonstrate that proteoglycan-
specific T<sub>IL-10</sub> cells act on the proteoglycan-specific B cell re-
sponse by inhibiting proteoglycan-specific IgG2a Ab production.

Proteoglycan-specific T<sub>IL-10</sub> cells suppress inflammation via the
endogenous IL-10 response
IL-10 has been shown to promote expression of IL-10 not only in several cell types, but IL-10 also has been shown to induce regu-
atory capacities in APCs (39) and in CD4<sup>+</sup> cells (32, 41) during
their activation, as a mechanism for infectious tolerance (42). The
boosted IL-10 response that was observed in animals which had received proteoglycan-specific T<sub>IL-10</sub> cells suggests that T<sub>IL-10</sub>
cells stimulate a protective endogenous IL-10 response. To test
whether the proteoglycan-specific T<sub>IL-10</sub> cells indeed need to boost the
endogenous IL-10 response of the recipient mice to accomplish suppression of the arthritic immune response we transferred proteo-
glycan-specific T<sub>IL-10</sub> cells in PGIA in IL-10 deficient BALB/c mice. Proteoglycan-specific T<sub>IL-10</sub> cells or proteoglycan-specific T<sub>GFP</sub>
control cells were transferred to IL-10-deficient BALB/c mice one day before the second proteoglycan immunization of the
PGIA induction protocol. Transfers to IL-10-deficient animals were
done in parallel with transfers of the same T cell populations to
wild-type recipients that were used as a positive control. Al-
though proteoglycan-specific T<sub>IL-10</sub> cells ameliorated arthritis in these wild-type recipients, as shown by data integrated in Table II, no difference in the arthritis score could be observed compared with the proteoglycan-specific T<sub>GFP</sub> cell recipient control group at any time point in IL-10-deficient mice (Fig. 6), showing that proteo-
glycan-specific T<sub>IL-10</sub> cells could not suppress arthritis in IL-
10-deficient mice. Taken together, these data indicate that proteo-
glycan-specific T<sub>IL-10</sub> cells regulate the arthritic immune response via propagation of the endogenous IL-10 response in vivo.

Discussion
Inflammatory autoimmune disease may result from a disturbed ho-
meostatic balance between autoaggressive T<sub>T</sub> eff cells and autoreac-
tive T<sub>reg</sub> cells. Although several immunoregulatory mechanisms
have been described, numerous studies indicate IL-10 being cru-
cial for several populations of T<sub>reg</sub> cells to maintain this balance (31). The requirement for an IL-10 response to control excessive autoaggressive immune responses in arthritis is underscored by stud-
ies showing that reduced numbers of CD4<sup>+</sup> T cells producing IL-
10 in RA patients (30) and a genetic predisposition to low IL-10
production in juvenile idiopathic arthritis seemed to correlate with
enhanced disease severity (43). Despite these facts, treatment of
existing autoimmune inflammatory diseases like arthritis with sys-
temic IL-10 administration has not been particularly successful so
far (44). Most likely, for IL-10 to have a regulatory effect, target-
ing of this cytokine to sites of relevant cell-cell interaction is es-
ential. In addition, the in vivo administration of ex vivo expanded
non-Ag-specific T<sub>reg</sub> cells, although seemingly attractive, is prob-
ably risky as it may lead to uncontrolled immune suppression. For
these reasons, we endeavored to combine Ag-directed targeting and
delivery of IL-10 by the use of Ag-specific IL-10-transduced T

The CD4<sup>+</sup> T cells used in this study are specific for the arthri-
togenic immunodominant T cell epitope of cartilage proteoglycan
and have been shown previously to induce the Th1-dominated pro-
goxygen-induced arthritis when activated in vivo (10). Although
stimulation of CD4<sup>+</sup> T cells via CD3 plus CD28 that was used for
efficient retroviral transduction has been shown to induce a Th1-
like phenotype (45), 1 × 10<sup>6</sup> CD3/CD28-stimulated proteoglycan-
specific T cells significantly suppressed clinical arthritis when transduced with an active IL-10 gene. In addition, analysis for the
presence of transduced T cells by flow cytometry and RT-PCR
after transfer in vivo indicated that rather low numbers of trans-
duced T cells are effective in suppressing disease. This disease-
suppressive effect was paralleled by a reduced proteoglycan-spe-
cific production of both IL-2 and TNF-α, indicating that proteoglycan-specific T<sub>IL-10</sub> cells specifically control the inflam-
matory autoimmune response. In addition, the finding that OVA-
specific T<sub>IL-10</sub> cells did not suppress arthritis indicated that IL-10
produced by T<sub>IL-10</sub> cells was targeted to the proteoglycan-specific inflammatory response.

Adoptive transfer studies with transduced Ag-specific T cells in
arthritics (46, 47) indicated requirement for autoantigen specificity of transduced T cells to exert their suppressive functions locally at the
site of inflammation. Proteoglycan-specific T<sub>IL-10</sub> cells mi-
gated to joints and draining lymph nodes. This suggests that these
cells may interact with proinflammatory cells at locations where
cartilage Ags are presented. Although OVA-specific T<sub>IL-10</sub> cells
could be found in the inflamed joints and joint-draining LNs, presu-
malble as part of a steady-state influx of cells into the site of inflamma-
tion, migration of proteoglycan-specific T<sub>IL-10</sub> cells was found
in these organs at an increased rate. Moreover, proteogly-
can-specific T<sub>IL-10</sub> cells could not be found in irrelevant nonlym-
phoid tissue (pancreas) indicating that proteoglycan-specific T<sub>IL-10</sub>
cells preferentially migrated to the inflamed joint tissue. Further-
more, the IL-10 concentration in blood was below detection level in
protected animals (data not shown), suggesting that the systemic
level of IL-10 was not substantially increased. Though systemic
administration of IL-10 is known to suppress PGIA (27), the find-
ings in this study indicate that proteoglycan-specific T<sub>IL-10</sub> cells
target IL-10 to the actual site of the autoimmune response rather
than through a systemic IL-10 response. Recognition of the Ag is
required for adequate regulation and only the ability to home to the
inflamed organ is not sufficient to regulate inflammation. Alto-
gether, these results suggest that autoantigen-specific CD4<sup>+</sup> T<sub>IL-10</sub>

FIGURE 6. Proteoglycan-specific CD4<sup>+</sup> T<sub>IL-10</sub> cells do not suppress ar-
thritis in IL-10-deficient recipients. Mice were immunized with proteogly-
can and received 1 × 10<sup>6</sup> proteoglycan-specific T<sub>IL-10</sub> cells (■) or proteo-
glycan-specific T<sub>GFP</sub> (□) cells on the day before the boosting proteoglycan
immunization as described for Fig. 2. The graph shows the mean arthritis
severity (score) per group (n = 5/group) per day ± SEM and is represen-
tative for two separate experiments. No significant differences were ob-
served at any time point.
cells depend on recognition of their cognate autoantigen to sustain interactions of these T cells at locations where they counteract inflammatory cells.

Moreover, it is now becoming clear that a distinct T cell population, Th17 cells (24, 48), contributes to (auto)inflammatory responses. This population is characterized as a source of the proinflammatory IL-17, which has been described to be a crucial cytokine for development of autoimmunity and destruction of cartilage in arthritis (49). The reduced IL-17a response we observed in the protected animals during the chronic phase of arthritis would therefore be in line with the idea of IL-17 as a proinflammatory cytokine in arthritis and suggests that CD4+ T cells that produce IL-10 dampen the proinflammatory IL-17 response.

Early generation of Th17 cells, by stimulation in the presence of TGF-β and IL-6, has been shown to be restricted by IL-2 (50). However, other recent studies have demonstrated that IL-2 will ultimately lead to expansion of the Th17 cell population (51, 52). Therefore, the reduction of IL-17 in our study has to be noted in the context of suppression of the proteoglycan-specific IL-2 response by CD4+ TIL-10 cells. In our study, in situ IL-17 mRNA expression in a mature stage of disease was reduced by TIL-10 cells, indicating that the reduced proteoglycan-specific IL-2 response does not sustain development of Th17 cells. These data would rather indicate the opposite; reduction of human proteoglycan-specific IL-2 in the arthritic immune response may help to reduce the expansion of Th17 cells, which would be in line with the recent studies mentioned above (51, 52) showing final expansion of Th17 cells by IL-2. The suppression of IL-2 and IL-17 we found may therefore be placed in the context of IL-10 in the cytokine milieu and further study is needed to help elucidating differentiation and growth of Th17 cells. In addition to IL-17 mRNA expression in situ, proteoglycan-specific secretion of IL-17 by splenocytes was analyzed at 4 wk after transfer of proteoglycan-specific TIL-10 or Tgpp cells in PGIA. However, splenocytes stimulated with proteoglycan did not produce IL-17 amounts that were significantly different from the unstimulated (medium) controls (data not shown), which is indicative of a rather small population of proteoglycan-specific Th17 cells.

Because B cells and Abs are essential for the pathogenesis of (proteoglycan-induced) arthritis and CD4+ T cells are determinants of Ag-specific Ab responses, we studied how TIL-10 cells would influence autoantibody production. TIL-10 cells reduced the human and mouse proteoglycan-specific IgG2a response. This is in line with the effect of TIL-10 cells in reducing Th1 responses, as IgG2a is considered as a Th1-induced isotype (53) and proteoglycan-specific IgG2a autoantibodies correlate with severity of Th1-mediated PGIA (27, 40, 54).

It has been speculated that IgG2a autoantibodies may elicit a pathogenic effect through FcγRIII-mediated mechanisms (40, 55) indispensable for development of PGIA (56, 57). During the effector phase of inflammation, FcγR-immune complex interaction is supposedly required for the expression of proinflammatory cytokines and β-chemokines in ankle joints to stimulate the influx of lymphocytes, macrophages, and neutrophils into the joint (57). Therefore, the reduced proteoglycan-specific IgG2a response in proteoglycan-specific TIL-10 recipients may indicate that TIL-10 cells control the proinflammatory B cell response by preventing interaction of harmful autoantecive Th1 cells with B cells.

Besides suppressing the inflammatory response, another notable feature of IL-10 is its property to promote IL-10 expression and concomitant immunosuppressive features in CD4+ T cells (32) and DCS (39). Therefore, the immune modulatory potency of IL-10 produced by TIL-10 cells may not just be the inhibition of proinflammatory mediators, such as TNF-α and IL-17, but may be found even more in the amplification of IL-10 expression and concomitant transfer of regulatory qualities. Indeed, expression of IL-10 was elevated in TIL-10 cell recipients not only at the mRNA level and cytokine level, but also relative numbers of cells producing IL-10 in situ were elevated as analyzed by flow cytometry, which was most pronounced within the CD4+ population. Moreover, because within the IL-10+ cell population no transduced cells were detected, these data indicate that the elevated IL-10 level measured in TIL-10 cell recipients was not solely produced by the transferred TIL-10 cells, but, at least in part, by endogenous cells of the recipient. Furthermore, the finding that proteoglycan-specific TIL-10 cells did not protect IL-10-deficient recipients from arthritis indicated that propagation of the endogenous regulatory IL-10 response by these TIL-10 cells was indeed required to generate regulation of arthritis. Considering their Ag-specific interaction with TIL-10 cells, APCs such as DCS or Ag-specific B cells are good candidates in which TIL-10 cells might Ag specifically propagate IL-10 expression. These cells have, in turn, shown to propagate the IL-10-producing regulatory Th1 population (39) and to suppress the generation of a pathogenic Teff cell response (3, 39).

In summary, this study shows that IL-10-transduced CD4+ T cells may control the chronic autoimmune response in arthritis, and that their specificity for a cartilage Ag is essential. Besides controlling the autoantigen-specific proinflammatory cytokine response, a suppressive effect was found at the level of B cell immunity. Moreover, it was shown for the first time that one of the crucial mechanisms by which such TIL-10 cells control inflammation in arthritis is the spreading of expression of IL-10 and concomitant regulatory properties to the endogenous immune response. Therefore, autoantigen-specific TIL-10 cells may restore immunological homeostasis by suppressing the proinflammatory response and promoting the regulatory endogenous IL-10 response.

Acknowledgments
We thank Dr. G. Arkesteijn for assistance with flow cytometric cell sorting, Dr. A. van Oosterhout for providing the IL-10 knockout mice, and Drs. B. Prakken, M. Oosterwegel, A. Sijts, and R. van der Zee for helpful suggestions and comments. We thank Dr. A. Marsman of the Hospital Hilversum (Hilversum, The Netherlands) for providing essential materials. Dr. Chris P. Broeren is deceased.

Disclosures
The authors have no financial conflict of interest.

References


