Autoantigen-Specific IL-10-Transduced T Cells Suppress Chronic Arthritis by Promoting the Endogenous Regulatory IL-10 Response[1]

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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 autoantigen-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. *The Journal of Immunology,* **2008, 180: 1373–1381.**

Rheumatoid arthritis (RA)[3] 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

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[3] Abbreviations used in this paper: RA, rheumatoid arthritis; Tg, transgenic; Treg, regulatory T cell; Tr1, T regulatory 1; PGIA, proteoglycan-induced arthritis; MSCV, murine stem cell virus; DC, dendritic cell; LN, lymph node; HPRT, hypoxanthine phosphoribosyltransferase; T_{IL-10}, IL-10/GFP-transduced T cell; T_{GFP}, GFP-transduced T cell; T_{resp}, responder T; T_{eff}, effector T; IRES, internal ribosomal entry site.

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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 $IL\text{-}10^+$ 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 be studied in this arthritis model (10, 11). We questioned if proteoglycan-specific T_{IL-10} cells would suppress a chronic arthritis and whether these cells would modulate by propagating a protective immune response in vivo.

The results indicated that proteoglycan-specific T_{IL-10} 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 T_{IL-10} cells depended on the presence of their proteoglycan-specific TCR. Interestingly, proteoglycan-specific T_{IL-10} cells could not reduce the inflammatory response in IL-10-deficient recipients, indicating that autoantigen-specific T_{IL-10} 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 Oosterhout, 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. DO11.10 (OVA-TCR Tg) and TCR-5/4E8-Tg (proteoglycan-TCR Tg) (10, 11) were bred and kept at the GDL under specific pathogen-free conditions. Human and murine proteoglycan were prepared as described elsewhere (11). All animal experiments were approved by the Animal Experimental Committee of the Veterinary Faculty of the University of Utrecht.

Constructs 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 3×10^6 cells in 10 ml of DMEM (Invitrogen Life Technologies) (+4500 mg/L glucose, +GlutaMAX I, -pyruvate) supplemented with 10% heat-inactivated FCS (Bodinco), 100 U/ml penicillin, 100 μg/ml streptomycin (Invitrogen Life Technologies) in 100-mm tissue -culture dishes (Nalge Nunc International) and cultured at 37°C, 6% CO_2. The next day, the culture medium was refreshed and 1–3 h thereafter, 500 μl of 0.25 M $CaCl_2$ 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 Na_2HPO_4, 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 thereafter, 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 microbeads (MACS; Miltenyi Biotec) and stimulated with magnetic M-450 tosyl-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 1×10^6 $CD4^+$ 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% CO_2. After 48 h, 750 μl 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 $930 \times g$ at 20°C for 2 h. Subsequently, 1 ml of supernatant was replaced with fresh medium and cells were cultured for another 48 h. 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). Acceptor mice received 1×10^6 sorted IL-10/GFP-transduced or GFP-transduced $CD4^+$ 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, decalcified 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 (M1/70), anti-MHC class-II (M5/114), and anti-CD8 (YTS169) mAbs and were subsequently labeled with CFSE (Molecular Probes as described elsewhere (38). At day 0, bone marrow cells from tibia and femurs were seeded at 2×10^6/100-mm suspension dish (Corning) in 10 ml of supplemented IMDM (Invitrogen Life Technologies) with 20 ng/ml rGM-CSF (Cytocen) 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 2×10^5 CFSE-labeled DO11.10 $CD4^+$ cells and 2.5×10^4 bone marrow-derived BALB/c DCs cultured in the presence of 0.32 μg/ml $OVA_{323-339}$ peptide in 200 μl during 4 days. To analyze the suppressive activity of culture supernatant, 50 μl of the final culture medium consisted of this conditioned supernatant. To test 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) at 2×10^5 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 $1\text{–}2 \times 10^6$ 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. After 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

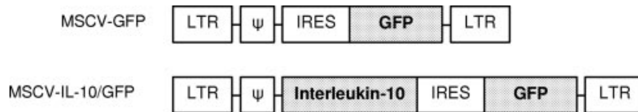

FIGURE 1. Murine IL-10 cDNA was inserted into the MSCV-GFP construct in front of the IRES sequence, enabling bicistronic translation of *IL-10* and *GFP* genes.

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 fluoresceinated 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 TTG A-3′), hypoxanthine phosphoribosyltransferase (HPRT; 5′-CTG GTG AAA AGG ACC TCT CG-3′ and 5′-TGA AGT ACT CAT TAT AGT CAA GGG CA-3′) and GFP (5′-GCA GTG CTT CAG CCG CTA-3′ and 5′-AAG AAG ATG GTG CGC TCC TG-3′). PCR (3 min at 95°C and 40 cycles of 10 s at 95°C and 45 s at 59,5°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 MyiQ iCycler. Per sample threshold values measured for the different genes were normalized to the threshold value for HPRT in that sample to determine relative gene expression.

Quantification of Ag-specific serum Abs

Serum murine and human proteoglycan-specific Abs were measured by ELISA as described elsewhere (37). In brief, 96-well ELISA plates (Corning) were coated overnight with human or murine proteoglycan in PBS. Free binding sites were blocked with 1% fat-free milk (Bio-Rad) in PBS. Sera were added at increasing dilutions in 1% fat-free milk in PBS. Proteoglycan-specific IgG1 and IgG2a were determined using peroxidase-conjugated rat mAb to IgG1 or IgG2a (BD Pharmingen). Serum Ab levels were calculated as OD relative to the OD measured for the corresponding isotypes of a standard of pooled sera from arthritic mice.

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 transduction rate, proteoglycan-specific CD4$^+$ T cells isolated from naive 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 (T_{GFP}) 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-IL-10/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 concentration between culture supernatants of T_{IL-10} cells and T_{GFP} cells (data not shown). When T_{IL-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 T_{GFP} cells.

Transfer of proteoglycan-specific T_{IL-10} cells ameliorates arthritis

To examine whether proteoglycan-specific T_{IL-10} cells could suppress chronic arthritis, proteoglycan-specific T_{IL-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 T_{IL-10} cells were transferred to acceptor mice. As a control, 1×10^6 proteoglycan-specific T_{GFP} cells were transferred. Another control group received PBS instead of transduced T cells.

Mice that received proteoglycan-specific T_{GFP} cells or PBS developed a chronic arthritis while recipients of proteoglycan-specific T_{IL-10} cells developed a significantly reduced form of arthritis (Fig. 2*A*). In addition, maximum arthritis severity and cumulative incidence were dampened by the proteoglycan-specific T_{IL-10} cells

Table I. *Phenotypes of TCR-Tg CD4$^+$ T cells transduced with IL-10/GFP or GFP*

TCR	Construct	MFI GFP[a]	IL-10[b]	IL-10[c]	Proliferation
PG-TCR	IL-10/GFP	644	62.0	31,999	14,896
PG-TCR	GFP	1,817	1.3	193	11,463
OVA-TCR	IL-10/GFP	630	50.0	38,651	ND
OVA-TCR	GFP	1,382	1.6	783	ND

[a] Mean fluorescence intensity (MFI) was determined for the GFP$^+$ population by flow cytometry.
[b] mRNA expression relative to HPRT expression in GFP$^+$ (sorted) cells as analyzed by quantitative real-time PCR 2 days after retroviral transduction.
[c] Secretion of IL-10 (picograms per milliliter) as measured in culture supernatant 2 days after transduction (60–70% of cells were GFP$^+$). Proliferation of GFP-sorted transduced T cells, shown as Δcpm, was measured by [^{3}H]thymidine incorporation in response to anti-CD3/-CD28-stimulation. ND, Not analyzed.

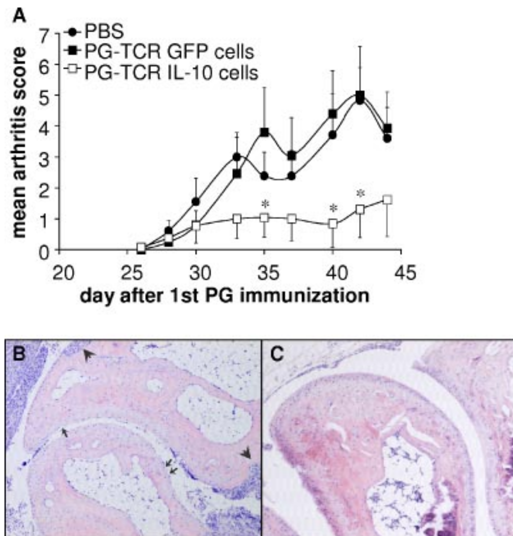

FIGURE 2. Proteoglycan-specific $CD4^+$ T cells retrovirally transduced to produce IL-10 ameliorate arthritis. *A*, Mice were immunized with proteoglycan on days 0 and 21 and received 1×10^6 proteoglycan-specific $T_{IL\text{-}10}$ cells (□, $n = 12$), proteoglycan-specific T_{GFP} cells (■, $n = 10$) or PBS (●, $n = 9$) on the day before the second proteoglycan immunization. Data represent pooled data of two experiments. *B* and *C*, Five weeks after transfer of (*B*) proteoglycan-specific T_{GFP} and (*C*) proteoglycan-specific $T_{IL\text{-}10}$ in PGIA histological examination of H&E-stained sections of ankle joints was performed. Large arrowheads indicate infiltrating cells, small arrowheads indicate cartilage damage. *, $p < 0.05$ for difference between recipients of proteoglycan-specific T_{GFP} cells and proteoglycan-specific $T_{IL\text{-}10}$ cells.

(Table II). Amelioration of arthritis in proteoglycan-specific $T_{IL\text{-}10}$ cell recipients was confirmed by histological examination of ankle joint sections showing less cellular infiltration in the ankle joints of the proteoglycan-specific $T_{IL\text{-}10}$ cell recipients (Fig. 2*C*), compared with proteoglycan-specific T_{GFP} cell recipients (Fig. 2*B*).

$T_{IL\text{-}10}$ 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 $T_{IL\text{-}10}$ 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 (T_{resp}) cells and stimulated in the presence of proteoglycan-specific $T_{IL\text{-}10}$ cells or proteoglycan-specific T_{GFP} control cells. DCs loaded simultaneously with $OVA_{323–339}$ peptide and human $proteoglycan_{70-84}$ peptide (the arthritogenic epitope that is recognized by proteoglycan-specific cells) were used to stimulate both the OVA-specific T_{resp} population and the transduced proteoglycan-specific ($T_{IL\text{-}10}$ or T_{GFP}) cells simultaneously. Varying numbers of proteoglycan-specific $T_{IL\text{-}10}$ cells or proteoglycan-specific T_{GFP} cells were added to this culture, with a T_{GFP}:T_{resp} or $T_{IL\text{-}10}$:T_{resp} ratio ranging from 2:1, 0.5:1 to 0.2:1. Within the population of T_{resp} cells, the percentage of cells that went into division was determined from their CFSE profiles and plotted in Fig. 3*A* as percentage suppression relative to the proliferation in the absence of $T_{IL\text{-}10}$ or T_{GFP} cells. Proliferation of T_{resp} cells was clearly suppressed in the presence of $T_{IL\text{-}10}$ cells as compared with proliferation in the presence of T_{GFP} (control) cells. This suppression was more pronounced when higher numbers of $T_{IL\text{-}10}$ cells were added to the T_{resp} cells.

To check whether suppression by $T_{IL\text{-}10}$ cells was mediated by secreted factors, a culture system was set up with $OVA_{323–339}$-loaded DC to stimulate CFSE-labeled OVA-specific T_{resp} cells in the presence of supernatant from cultured T_{GFP} or $T_{IL\text{-}10}$ cells. Division of these $CFSE^+$ T cells was used as a read out for the presence of such secreted factors. Thus, conditioned medium of cultured $T_{IL\text{-}10}$ or T_{GFP} cells taken 48 h after transduction was added to the DC-T_{resp} culture to check for the presence of secreted suppressive factors (Fig. 3*B*). Fresh culture medium was used as control. Conditioned medium of $T_{IL\text{-}10}$ cells suppressed proliferation of $CD4^+$ T_{resp} cells as shown by reduced CFSE dilution (Fig. 3*B*) compared with conditioned medium of T_{GFP} cells or fresh culture medium. This is shown for supernatant taken from both proteoglycan-specific (*upper panel*) and OVA-specific (*lower panel*) $T_{IL\text{-}10}$ cells, showing that the OVA-specific $T_{IL\text{-}10}$ cells that are used as a control for Ag-specificity for suppression in vivo (see next paragraph) secrete functional IL-10 to the same extend as proteoglycan-specific $T_{IL\text{-}10}$ cells. When IL-10 in supernatant taken from cultured $T_{IL\text{-}10}$ cells was neutralized with anti-IL-10 mAb (JES-2A5), the rate of proliferation of CFSE-labeled T_{resp} cells increased with 86% (data not shown). Blocking IL-10 function in conditioned medium taken from cultured T_{GFP} cells yielded an increase of proliferation of only 2%. This indicates that IL-10 secreted by $T_{IL\text{-}10}$ cells is the main secreted factor responsible for suppression.

$T_{IL\text{-}10}$ cells require recognition of the cartilage Ag proteoglycan in vivo to ameliorate arthritis

Subsequently, we asked whether $T_{IL\text{-}10}$ cells require recognition of proteoglycan to exert their anti-inflammatory properties. To this end, $T_{IL\text{-}10}$ cells expressing a Tg TCR specific for OVA (OVA-TCR) were transferred in PGIA as described in the previous section. Unlike proteoglycan-specific $T_{IL\text{-}10}$ cells, OVA-specific $T_{IL\text{-}10}$ cells could not reduce severity of arthritis (Table II). In addition, OVA-specific $T_{IL\text{-}10}$ cells did not reduce the incidence of arthritis whereas proteoglycan-specific $T_{IL\text{-}10}$ cells did. Although a

Table II. *Arthritis onset, arthritis incidence, and maximum arthritis severity in mice receiving PG-TCR T_{GFP} cells, PG-TCR $T_{IL\text{-}10}$ cells, OVA-TCR $T_{IL\text{-}10}$ cells or PBS*[a]

	PBS	PG-TCR GFP	PG-TCR IL-10	OVA-TCR IL-10
Day of onset	31.3 (±1.5)	33.0 (±1.1)	31.1 (±0.9)	33.9 (±1.9)
Incidence	9/9 (100%)	24/26 (92%)	18/30 (60%)	11/12 (92%)
Maximum severity	5.3 (±1.2)	6.3 (±1.0)	3.2 (±0.8)[b]	5.8 (±1.6)

[a] 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.

[b] Value of $p < 0.05$ for difference with PG-TCR T_{GFP} cell-recipient group and with OVA-TCR $T_{IL\text{-}10}$ cell-recipient group.

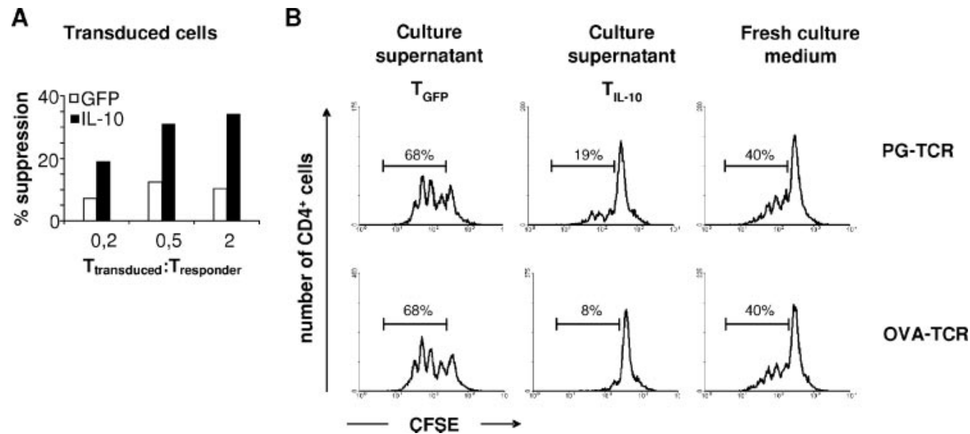

FIGURE 3. $CD4^+$ T cells retrovirally transduced with the murine *IL-10* gene suppress proliferation of freshly isolated $CD4^+$ T cells in vitro. *A*, CFSE-labeled $CD4^+$ T_{resp} cells were stimulated by peptide-loaded DC in the presence of different numbers of $T_{IL\text{-}10}$ cells (■) or T_{GFP} cells (□) as indicated. Suppression is plotted as the percent of suppression compared with proliferation in the absence of transduced T cells. *B*, CFSE-labeled OVA-specific $CD4^+$ T_{resp} cells were stimulated by $OVA_{323–339}$-loaded DC in the presence of supernatants taken from cultured IL-10-transduced or GFP-transduced (~70% of population transduced) proteoglycan-specific $CD4^+$ T cells (*upper panel*) or OVA-specific $CD4^+$ T cells (*lower panel*). Numbers indicate the percent of cells in dividing population.

difference was found for arthritis suppressive capacity in vivo, OVA-specific $T_{IL\text{-}10}$ cells showed a similar phenotype as proteoglycan-specific $T_{IL\text{-}10}$ cells when analyzed for IL-10 expression (Table I) and suppressive activity in vitro (Fig. 3B) 2 days post-transduction. This indicates that differences found for regulation in vivo may be ascribed to the different specificities of TCRs.

The need of their specificity for cartilage Ags to regulate arthritis suggests that $T_{IL\text{-}10}$ cells may act locally and thus migrate to where the Ag is processed and presented. To address whether transferred proteoglycan-specific $T_{IL\text{-}10}$ cells and OVA-specific $T_{IL\text{-}10}$ cells migrate differentially, GFP expression was analyzed in spleen, joint-draining LN, joints and in cervical LN and nonlymphoid tissue. 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^+ $T_{IL\text{-}10}$ cells. Up to 2 wk after transfer, both OVA-specific and proteoglycan-specific $T_{IL\text{-}10}$ cells were found in all lymphoid organs analyzed (up to 0.66% of the total $CD4^+$ population), indicating that, irrespective of the specificity of the TCR, the $T_{IL\text{-}10}$ cells migrate throughout the whole lymphoid compartment. However, at 2 wk after transfer the number of proteoglycan-specific $T_{IL\text{-}10}$ cells tended, compared with OVA-specific $T_{IL\text{-}10}$ cells, to preferentially be sustained in joint-draining LN and spleen as shown by a higher ratio of GFP^+ 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 (=0.002% in arthritic 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 $T_{IL\text{-}10}$ cells (50% of all animals analyzed), in most animals that received proteoglycan-specific $T_{IL\text{-}10}$ 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 $T_{IL\text{-}10}$ cells preferentially migrate to the inflamed tissue (joints).

Proteoglycan-specific $T_{IL\text{-}10}$ 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 $T_{IL\text{-}10}$ cells suppress proliferation of $CD4^+$ T cells. This suggests that $T_{IL\text{-}10}$ 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 $T_{IL\text{-}10}$ cells on proteoglycan-specific T_{eff} activation. Therefore, spleen cells were taken from animals 2 wk after transfer of proteoglycan-specific $T_{IL\text{-}10}$ cells or proteoglycan-specific T_{GFP} control cells in PGIA, stimulated with or without human proteoglycan ex vivo and secreted IL-2 was measured subsequently. Fig. 4*A* shows that the human proteoglycan specific IL-2 response was

Table III. *Migration of PG-TCR $T_{IL\text{-}10}$ cells and OVA-TCR $T_{IL\text{-}10}$ cells at different time points after transfer in PGIA*[a]

	1 wk		2 wk			4 wk		
	Draining LN/spleen[b]	Draining LN/cervical LN[b]	Draining LN/spleen[b]	Draining LN/cervical LN[b]	Joints[c]	Draining LN/spleen[b]	Draining LN/cervical LN[b]	Joints[c]
PG-TCR $T_{IL\text{-}10}$	0.24 (±0.02)	0.63 (±0.26)	0.21 (±0.04)	1.11 (±0.52)	2/3 (66%)	None detected	None detected	3/3 (100%)
OVA-TCR $T_{IL\text{-}10}$	0.22 (±0.03)	0.88 (±0.06)	0.61 (±0.18)	0.76 (±0.24)	2/4 (50%)	None detected	None detected	2/4 (50%)

[a] The presence of transferred PG-TCR $T_{IL\text{-}10}$ and OVA-TCR $T_{IL\text{-}10}$ 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.

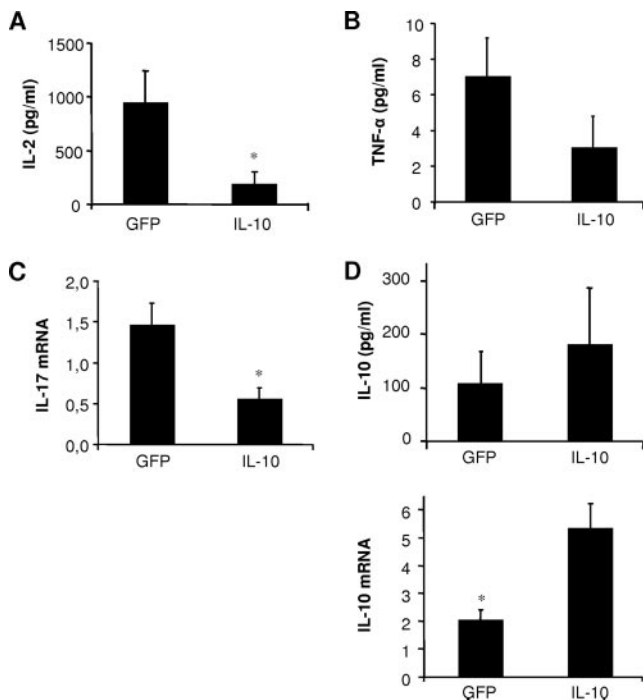

FIGURE 4. Proteoglycan-specific T_{IL-10} $CD4^+$ T cells propagate an anti-inflammatory cytokine response in vivo. Spleen cells were isolated after transfer of proteoglycan-specific T_{IL-10} or proteoglycan-specific T_{GFP} cells in PGIA as described for Fig. 2 and were either cultured in vitro (*A*, *B*, and *D*, *upper graph*) or used for RNA isolation directly (*C* and *D*, *lower graph*). Protein concentrations were measured in supernatants after 72 h, cultured in the presence or absence of human proteoglycan, 2 wk after transfer. IL-2 (*A*), TNF-α (*B*), and IL-10 (*D*) is plotted as the amount (picograms per milliliter) of protein human proteoglycan specifically produced and is representative for two experiments. IL-17 (*C*) and IL-10 (*D*) mRNA expression was quantified by quantitative PCR on reversely transcribed mRNA, 4 or 2 wk after transfer, respectively. Expression of mRNA is normalized to HPRT-mRNA expression ($\times 10^3$). All data are expressed as means $\pm$ SEM (n = 5/group); $*$, $p < 0.05$.

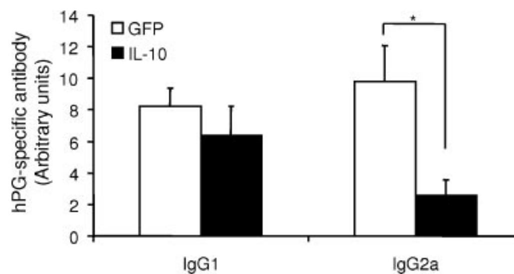

FIGURE 5. Proteoglycan-specific $CD4^+$ T_{IL-10} cells induce a shift in proteoglycan-specific B cell immunity toward a reduced IgG2a response in vivo. Human proteoglycan-specific Abs of the IgG1 and IgG2a isotype were measured in sera by ELISA at 4 wk after transfer of proteoglycan-specific $CD4^+$ T_{IL-10} cells (■) or T_{GFP} cells (□) to proteoglycan-immunized mice as described for Fig. 2. Concentrations are shown as units relative to a standard of pooled sera of arthritic mice $\pm$ SEM (n = 5 mice/group). The results are representative for two experiments. $*$, $p <$ 0.05 for the human proteoglycan-specific IgG2a response in proteoglycan-specific T_{IL-10} cell recipients compared with proteoglycan-specific T_{GFP} cell recipients.

significantly lower in the group that had received proteoglycan-specific T_{IL-10} cells compared with the proteoglycan-specific T_{GFP} cell recipients. In addition, reduced Ag-specific expression of the proinflammatory cytokine TNF-α in splenocytes from T_{IL-10} cell recipients paralleled the observed protection by proteoglycan-specific T_{IL-10} cells (Fig. 4*B*).

Recently, the proinflammatory cytokine IL-17 has been described as a pathogenic T cell-derived cytokine in autoimmune inflammatory disorders. Therefore, we wondered whether T_{IL-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 T_{IL-10} cells or proteoglycan-specific T_{GFP} control cells in PGIA (Fig. 4*C*). After transfer of proteoglycan-specific T_{IL-10} cells, IL-17 mRNA expression was significantly reduced compared with the proteoglycan-specific T_{GFP} 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 T_{IL-10} cells in PGIA.

Proteoglycan-specific T_{IL-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 T_{IL-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 T_{IL-10} cells or proteoglycan-specific T_{GFP} (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. 4*D*). Culture supernatants of cultured spleen cells of proteoglycan-specific T_{IL-10} cell recipients contained higher concentrations of proteoglycan-specific IL-10 than that of proteoglycan-specific T_{GFP} cell recipients. Furthermore, spontaneous IL-10 production by unstimulated spleen cells (medium control) was also slightly enhanced in the proteoglycan-specific T_{IL-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 T_{IL-10} cell recipients (Fig. 4*D*). Flow cytometry to determine numbers of IL-10-producing splenocytes of mice that received proteoglycan-specific T_{IL-10} cells showed an increase in the number of IL-10^+ cells in the $CD4^-$ population (1.3 $\pm$ 0.31% in T_{IL-10} recipients and 0.9 $\pm$ 0.09% in T_{GFP} recipients). An increase was also found in the $CD4^+$ population (2.2 $\pm$ 0.30% in T_{IL-10} recipients and 1.9 $\pm$ 0.43% in T_{GFP} recipients) compared with proteoglycan-specific T_{GFP} recipients. As described previously, no GFP^+ (from transferred T_{IL-10}) cells could be detected by flow cytometry at this time point anymore, indicating that the increased IL-10^+ population consisted of endogenous cells. Together, these data indicate that the proteoglycan-specific T_{IL-10} cells enhance the IL-10 response in vivo.

Proteoglycan-specific T_{IL-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 T_{IL-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 T_{IL-10} cells or proteoglycan-specific T_{GFP} 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 was significantly

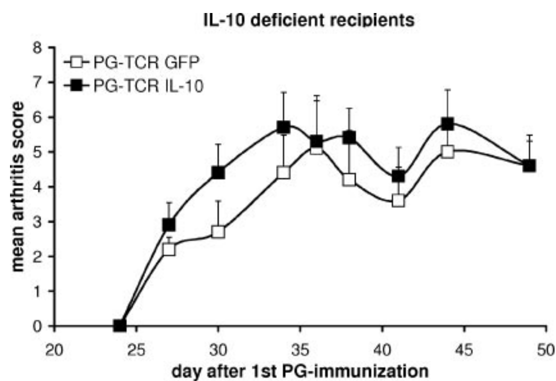

FIGURE 6. Proteoglycan-specific CD4$^+$ T_{IL-10} cells do not suppress arthritis in IL-10-deficient recipients. Mice were immunized with proteoglycan and received 1×10^6 proteoglycan-specific T_{IL-10} cells (■) or proteoglycan-specific T_{GFP} (□) cells on the day before the boosting proteoglycan immunization as described for Fig. 2. The graph shows the mean arthritis severity (score) per group (n = 5/group) per day ± SEM and is representative for two separate experiments. No significant differences were observed at any time point.

suppressed by the proteoglycan-specific T_{IL-10} cells compared with T_{GFP} cells. A similar trend was found for the anti-murine proteoglycan-specific IgG2a response; 364.1 (±171.5, n = 6) in T_{GFP} cell recipients and 130.1 (±40.3, n = 7) in T_{IL-10} cell recipients. Collectively, these data demonstrate that proteoglycan-specific T_{IL-10} cells act on the proteoglycan-specific B cell response by inhibiting proteoglycan-specific IgG2a Ab production.

Proteoglycan-specific T_{IL-10} 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 regulatory capacities in APCs (39) and in CD4$^+$ 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_{IL-10} cells suggests that T_{IL-10} cells stimulate a protective endogenous IL-10 response. To test whether the proteoglycan-specific T_{IL-10} cells indeed need to boost the endogenous IL-10 response of the recipient mice to accomplish suppression of the arthritic immune response we transferred proteoglycan-specific T_{IL-10} cells in PGIA in IL-10 deficient BALB/c mice. Proteoglycan-specific T_{IL-10} cells or proteoglycan-specific T_{GFP} 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. Although proteoglycan-specific T_{IL-10} 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_{GFP} cell recipient control group at any time point in IL-10-deficient mice (Fig. 6), showing that proteoglycan-specific T_{IL-10} cells could not suppress arthritis in IL-10-deficient mice. Taken together, these data indicate that proteoglycan-specific T_{IL-10} 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 homeostatic balance between autoaggressive T_{eff} cells and autoreactive T_{reg} cells. Although several immunoregulatory mechanisms have been described, numerous studies indicate IL-10 being crucial for several populations of T_{reg} cells to maintain this balance (31). The requirement for an IL-10 response to control excessive autoreactive immune responses in arthritis is underscored by studies showing that reduced numbers of CD4$^+$ 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 systemic IL-10 administration has not been particularly successful so far (44). Most likely, for IL-10 to have a regulatory effect, targeting of this cytokine to sites of relevant cell-cell interaction is essential. In addition, the in vivo administration of ex vivo expanded non-Ag-specific T_{reg} cells, although seemingly attractive, is probably 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 cells. In the present study, we show that administration of IL-10-transduced cartilage proteoglycan-specific CD4$^+$ T cells can ameliorate chronic PGIA by amplifying a regulatory endogenous IL-10 response.

The CD4$^+$ T cells used in this study are specific for the arthritogenic immunodominant T cell epitope of cartilage proteoglycan and have been shown previously to induce the Th1-dominated proteoglycan-induced arthritis when activated in vivo (10). Although stimulation of CD4$^+$ T cells via CD3 plus CD28 that was used for efficient retroviral transduction has been shown to induce a Th1-like phenotype (45), 1×10^6 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 transduced T cells are effective in suppressing disease. This disease-suppressive effect was paralleled by a reduced proteoglycan-specific production of both IL-2 and TNF-α, indicating that proteoglycan-specific T_{IL-10} cells specifically control the inflammatory autoimmune response. In addition, the finding that OVA-specific T_{IL-10} cells did not suppress arthritis indicated that IL-10 produced by T_{IL-10} cells was targeted to the proteoglycan-specific inflammatory response.

Adoptive transfer studies with transduced Ag-specific T cells in arthritis (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_{IL-10} cells migrated 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_{IL-10} cells could be found in the inflamed joints and joint-draining LNs, presumable as part of a steady-state influx of cells into the site of inflammation, migration of proteoglycan-specific T_{IL-10} cells was found in these organs at an increased rate. Moreover, proteoglycan-specific T_{IL-10} cells could not be found in irrelevant nonlymphoid tissue (pancreas) indicating that proteoglycan-specific T_{IL-10} cells preferentially migrated to the inflamed joint tissue. Furthermore, 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 findings in this study indicate that proteoglycan-specific T_{IL-10} 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. Altogether, these results suggest that autoantigen-specific CD4$^+$ T_{IL-10}

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 $CD4^+$ T_{IL-10} cells. In our study, in situ IL-17 mRNA expression in a mature stage of disease was reduced by T_{IL-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 T_{IL-10} or T_{GFP} 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 T_{IL-10} cells would influence autoantibody production. T_{IL-10} cells reduced the human and mouse proteoglycan-specific IgG2a response. This is in line with the effect of T_{IL-10} cells in reducing Th1 responses, as IgG2a is considered 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 T_{IL-10} recipients may indicate that T_{IL-10} cells control the proinflammatory B cell response by preventing interaction of harmful autoreactive 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 concurrent immunosuppressive features in $CD4^+$ T cells (32) and DCs (39). Therefore, the immune modulatory potency of IL-10 produced by T_{IL-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 T_{IL-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 T_{IL-10} cell recipients was not solely produced by the transferred T_{IL-10} cells, but, at least in part, by endogenous cells of the recipient. Furthermore, the finding that proteoglycan-specific T_{IL-10} cells did not protect IL-10-deficient recipients from arthritis indicated that propagation of the endogenous regulatory IL-10 response by these T_{IL-10} cells was indeed required to generate regulation of arthritis. Considering their Ag-specific interaction with T_{IL-10} cells, APCs such as DCs or Ag-specific B cells are good candidates in which T_{IL-10} cells might Ag specifically propagate IL-10 expression. These cells have, in turn, shown to propagate the IL-10-producing regulatory Tr1 population (39) and to suppress the generation of a pathogenic T_{eff} 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 T_{IL-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 T_{IL-10} cells may restore immunological homeostasis by suppressing the proinflammatory response and promoting the regulatory endogenous IL-10 response.

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Disclosures

The authors have no financial conflict of interest.

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