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Antigen-Specific T Cells Transduced with IL-10 Ameliorate Experimentally Induced Arthritis Without Impairing the Systemic Immune Response to the Antigen

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For the treatment of rheumatoid arthritis, efficient drug delivery methods to the inflamed joints need to be developed. Because T cells expressing an appropriate autoantigen-specific receptor can migrate to inflamed lesions, it has been reasoned that they can be employed to deliver therapeutic agents. To examine the ability and efficiency of such T cells as a vehicle, we employed an experimentally induced model of arthritis. Splenic T cells from DO11.10 TCR transgenic mice specific for OVA were transduced with murine IL-10. Adoptive transfer of the IL-10-transduced DO11.10 splenocytes ameliorated OVA-induced arthritis despite the presence of around 95% nontransduced cells. Using green fluorescent protein as a marker for selection, the number of transferred cells needed to ameliorate the disease was able to be reduced to $10^6$. Preferential accumulation of the transferred T cells was observed in the inflamed joint, and the improvement in the disease was not accompanied by impairment of the systemic immune response to the Ag, suggesting that the transferred T cells exert their anti-inflammatory task locally, mainly in the joints where the Ag exists. In addition, IL-10-transduced DO11.10 T cells ameliorated methylated BSA-induced arthritis when the arthritic joint was coinjected with OVA in addition to methylated BSA. These results suggest that T cells specific for a joint-specific Ag could be useful as a therapeutic vehicle in rheumatoid arthritis for which the arthritic autoantigen is still unknown. The Journal of Immunology, 2000, 165: 5980–5986.

Rheumatoid arthritis (RA) is a systemic chronic inflammatory disease characterized by persistent synovial cell proliferation with inflammatory cell infiltration and destruction of joints (1). Although the pathogenesis of RA remains to be elucidated, it has been proposed that blocking of proinflammatory cytokines is a promising strategy for controlling RA (2, 3). Various anti-inflammatory biological agents, including anti-IL-6 Ab, anti-TNF Ab, soluble TNF receptors, and IL-1R antagonist, have been demonstrated to exert considerable beneficial effects in patients as well as in experimental animal models (4–11). However, unexpected adverse reactions have also been reported, and one of the critical problems is serious infection due to immunosuppression (6, 7, 12, 13). To avoid these undesirable events without losing the therapeutic effects, there is a need to develop more efficient systems for delivery of effective biological agents into inflammatory arthritic lesions instead of systemic delivery.

It has been suggested that T cells recognizing organ-specific autoantigens are involved in the pathogenesis of autoimmune diseases (2). These autoreactive T cells migrate to and accumulate in the target organs, inducing inflammation and leading to the destruction of tissues. Because it is the Ag-specific TCR that endows autoreactive T cells with the ability to recognize the autoantigen and to migrate into the target organs, circulating T cells with specificity for the autoantigen would be one of the most effective ways for transporting therapeutic agents.

The concept of employing T cells recognizing an organ-specific autoantigen as vehicles for delivering therapeutically useful agents was first demonstrated in experimental autoimmune encephalomyelitis (14). A T cell line specific for P2 protein, which is a peripheral myelin protein, is neuritogenic when transferred adoptively. A P2 protein-specific T cell line transduced with nerve growth factor was no longer neuritogenic, because locally released nerve growth factor exhibited an anti-inflammatory effect. This concept was confirmed by a more refined study in which an anti-myelin basic protein T cell clone transfected with TGF-β1 protected against experimental autoimmune encephalomyelitis induced by immunization with either myelin basic protein or proteolipid protein (15). These successes prompted us to test the concept that T cells specific for a joint Ag might be an efficient therapeutic vehicle in experimental arthritis.

IL-10 is known to mediate immunosuppressive effects predominantly through down-regulation of macrophage functions and inhibition of proinflammatory cytokines produced by Th1 cells, such as IL-1β, IL-6, IL-8, TNF-α, IFN-γ, and GM-CSF (16). Earlier studies demonstrated that IL-10 was able to prevent disease expression and development in collagen-induced arthritis by i.p. injection of mouse IL-10 (mIL-10) or an adenovirus vector encoding...
murine IL-10 (17–20). Viral IL-10, which is a biological homologue of mIL-10, was demonstrated to exert similar effects (18). It has also been demonstrated that anti-collagen type II (anti-CII) T cells transfected with viral IL-10 can reduce disease severity (19). These abilities of IL-10 in ameliorating disease seem to depend on systemic suppression of the immune response to the induced Ag, because, for example, anti-CII Ab was suppressed in these manipulated mice. Systemic immunosuppression could present the risk of severe infection. Therefore, it is desirable that the immunosuppressive effect of IL-10 is exerted only at the inflammatory lesion as a result of an efficient local delivery system.

In the present study to evaluate the effect of Ag-specific T cells transduced with IL-10 on locally inflamed joints, we used an Ag-induced arthritis (AIA) model that is frequently employed as a model of RA. BALB/c mice were immunized with OVA, followed by intra-articular injection of OVA. The immune response against OVA causes inflammation in the joints, leading to destruction of the joints. Adoptively transferred OVA-reactive DO11.10 mouse splenocytes retrovirally transfected with IL-10 migrated selectively to arthritic joints and reduced disease severity without impairing the systemic immune response to the Ag. Although highly efficient transduction and selection of the transfected cells are both rather difficult to achieve for splenocytes, compared with transformed cell lines that can be selected by drug resistance, we overcame these problems by employing green fluorescent protein (GFP) selection. Our results suggest that Ag-specific T cells have the potential to serve as a therapeutic vehicle in autoimmune diseases.

**Materials and Methods**

**Mice**

Female BALB/c mice were purchased from JSLC (Shizuoka, Japan), and DO11.10 transgenic mice, whose T cells express receptors specific for OVA (21), were bred in our animal facility under specific pathogen-free conditions. All mice were used at the age of 8–10 wk.

**Antibodies**

Anti-CD4 Ab (L3T4) was purchased from PharMingen (San Diego, CA). A DO11.10 TCR-specific mAb, KJ1-26 (21), was purified from the supernatant of a hybridoma culture and labeled with biotin (Immunoprobe Bio- Technology Kit; Sigma, St. Louis, MO) according to the manufacturer’s protocol.

**Induction of AIA**

Female BALB/c mice were immunized with 100 μg of OVA (Sigma) in CFA (Difco, Detroit, MI) by injection into the base of the tail and were boosted 2 wk later with 100 μg of OVA in IFA. Before the immunization, the mice were injected i.p. with 200 ng of pertussis toxin (Wako, Tokyo, Japan). Two weeks after the booster, 100 μg of OVA dissolved in 20 μl of PBS was intra-articularly (i.a.) injected into the left ankle joint. The right ankle joint was injected with 20 μl of PBS as a negative control. The joint thickness was measured with a dial gauge caliper calibrated with 0.01-mm graduations (Mitsutoyo, Tokyo, Japan). The net increase in joint thickness attributable to the antigenic challenge was calculated by subtracting the increase in thickness of the right ankle from the increase in thickness of the left ankle. There was no net joint swelling after i.a. injection of OVA in nonimmunized mice. Induction of arthritis with methylated BSA (mBSA; Sigma) was performed in almost the same manner as OVA-induced arthritis. TCR RT-PCR/single-strand conformation polymorphism (SSCP) analysis

**Production of replication-defective retrovirus**

pMFGmIL-10, a retroviral plasmid containing the mIL-10 gene, was obtained from Riken Gene Bank (Tsukuba, Japan) with the approval of Dr. H. Hamada (23, 24). The production of retrovirus was performed as described previously (25). Briefly, an ecotropic retrovirus was produced in the BOSC23 packaging cell line. BOSC23 cells were seeded onto 100-mm dishes at 5 × 10^5 cells/plate 1 day before transfection. Transfection using Lipofectamine reagent (Life Technology, Gaithersburg, MD) was performed according to the manufacturer’s protocol using 10 μg/plate of the pMFGmIL-10 plasmid. Cells were cultured for 48 h, with fresh medium replacement at 24 h. The supernatant was harvested as a replication-defective retrovirus source and centrifuged twice at 1000 × g for 5 min to remove nonadherent producer cells. The replication-defective retrovirus was concentrated to 1 ml by centrifugation at 6000 × g at 4°C for 16 h and then stored at −80°C until use.

**Infection of the retrovirus**

Splenocytes from the DO11.10 transgenic mice were cultured in the presence of 10 nM OVA peptide with RPMI 1640 medium supplemented with 2 mM l-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, 10% heat-inactivated FCS, and 5 × 10^-3 M 2-ME. After 48-h culture, 4 × 10^6 cells were resuspended in 1 ml of the concentrated retrovirus stock in the presence of 4 μg/ml Polybrene (Sigma) and incubated for 2 h. After replacement of the medium, the cells were cultured for 48 h in the presence of 10 μg/ml Con A (Sigma). Infection of Ag nonspecific splenocytes was performed using PHA (5 μg/ml; Difco) instead of OVA peptide.

**Adoptive transfer**

Before adoptive transfer, CD4+ T cells were enriched using MACS (Miltenyi Biotech, Bergisch Gladbach, Germany) as previously described (26). Analysis by flow cytometry revealed that the purity of CD4+ T cells was >96%. Various numbers (5 × 10^5, 1 × 10^6, 3 × 10^6) of CD4+ DO11.10 splenocytes were transferred i.v. into nonirradiated OVA-primed BALB/c mice the day before i.a. antigenic challenge.

**Quantitation of IL-10 by ELISA**

IL-10 in the supernatant of transduced T cells or the sera from AIA mice was quantitated using a sandwich ELISA kit (Endogen, Woburn, MA).

**GFP selection**

An internal ribosome entry site (IRES) and the GFP gene were inserted downstream of the mouse IL-10 gene, designated pMFGmIL-10-IRES-GFP. GFP-positive CD4+ cells were sorted with FACSVantage (Becton Dickinson, San Jose, CA).

**Ab assay**

The level of anti-OVA Ab was measured by ELISA as described previously (26), except that OVA was employed as a coated Ag instead of human U1snRNP-A.

**T cell proliferation assay**

Spleen cells and LN cells were cultured at 1 × 10^6 cells/well with various concentrations of OVA in RPMI 1640 medium supplemented with 2 mM l-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, 10% heat-inactivated FCS, and 5 × 10^-3 M 2-ME for 5 days, followed by a final 16 h of culture in the presence of 1 μg/ml of [3H]thymidine. The incorporated radioactivity was counted with a gamma scintillation counter. The proliferative response was expressed as the stimulation index (the mean cpm of test cultures/the mean cpm of control cultures without Ag) ≥ 2 SD.

**Histology**

Excised ankles and knees were fixed in 10% phosphate-buffered formalin and decalcified. The tissues were then dehydrated in a gradient of alcohols, paraffin-embedded, sectioned, mounted on glass slides, and stained with hematoxylin and eosin.
Flow cytometric analysis of infiltrating cells into arthritic lesions

Both arthritic and control knee joints were excised and minced, followed by digestion with 1 mg/ml of collagenase (Sigma) and 1 mg/ml of DNase (Sigma) for 2 h. After passing through a nylon mesh, cells were collected by centrifugation. GFP-transduced CD4-positive DO11.10 T cells were detected by staining with either anti-CD4-PE Ab or KJ1-26-biotin in combination with streptavidin-PE (PharMingen).

Statistical analysis

Statistical evaluation was performed with one-way ANOVA or Mann-Whitney’s U test.

Results

Establishment of Ag-induced arthritis in mice

Although OVA is frequently used in rabbits to induce AIA (29–31), to date there have been no reports of use of OVA as an induction Ag in mice. The BALB/c mice we immunized with OVA developed severe arthritis after intra-articular challenge (see the positive control in Figs. 1 and 2, left). Neither the joints challenged with PBS nor the joints of mice immunized with PBS exhibited obvious inflammation. Thus, we successfully established an OVA-induced murine AIA model.

Transduction of mIL-10 into DO11.10 splenocytes using a retroviral vector

To generate functionally modulated Ag-specific T cells, we transduced murine IL-10 into splenocytes from DO11.10 transgenic mice, which bear TCRs specific for OVA. Almost 70–80% of CD4+ T cells from the DO11.10 transgenic splenocytes were positive for KJ1-26, which is specific for clonotype DO11.10 (data not shown). After 48 h of infection by pMFGmIL-10, the culture supernatant of 10^6 CD4+ T cells from DO11.10 splenocytes was found to contain IL-10 at 5.2 ± 0.5 ng/ml, whereas the supernatant of both nontransfected and mock-transfected DO11.10 splenocytes did not contain a detectable level of IL-10. The transduction efficiency into splenocytes of our retrovirus vector, examined using a GFP-expressing construct, was ~5%.

DO11.10 splenocytes transduced with IL-10 reduce the severity of joint swelling in AIA

We next examined the effect of IL-10-transduced DO11.10 splenocytes on AIA. One day after the adoptive transfer of DO11.10 CD4+ T cells (3 × 10^6) transduced with either the IL-10 or control vector, the ankle joints of mice primed with OVA were challenged with OVA. Mice that underwent i.v. transfer of CD4+ T cells producing IL-10 exhibited less joint swelling than the nontransferred positive control mice (Fig. 1). When we transferred three different numbers (5 × 10^5, 1 × 10^6, and 3 × 10^6) of CD4+ DO11.10 splenocytes infected with pMFGmIL-10, the percent inhibition of hind paw thickness increased as the number of transferred cells increased. The transfer of 3 × 10^6 cells exhibited the greatest inhibition, i.e., as much as 96.0 ± 1.92% on day 4. These results indicate that mIL-10-transduced OVA-specific T cells reduce the disease severity and that the disease amelioration depends on the number of IL-10-transduced cells transferred.

Histopathologic analysis of AIA

To confirm the effect of DO11.10 T cells transduced with mIL-10, we examined ankle sections from animals sacrificed 21 days after disease induction and transfer of the cells. As shown in Fig. 2, left, severe arthritis was observed in the ankle joints of arthritic mice to which mock-infected T cells were transferred, because the periarticular tissues were accompanied by synovial hyperplasia and massive cell infiltration. In contrast, the ankle joints of mice transferred with mIL-10-transduced T cells demonstrated almost the same histology as ankles derived from healthy mice (Fig. 2, right).

Number of transduced cells sufficient to ameliorate the disease after GFP selection

Interestingly, mice transferred with mock-transduced DO11.10 CD4+ T cells demonstrated more severe swelling, indicating that functionally nonaltered Ag-specific T cells contribute to exacerbation of the arthritis (see Fig. 1). Considering that only a small fraction of the transferred DO11.10 splenocytes was successfully transduced with the vector and that the larger population remained nontransduced, the reduction of the disease severity by this composite population indicates that the IL-10 produced by the small population of transduced T cells is sufficient to ameliorate the disease despite the presence of a larger number of nontransduced
The number of transferred cells was 1 in each group is shown. The maximum hind paw thickness usually occurred on day 1. The results from mice transferred with the same number of mock-transfected DO11.10 CD4+ T cells are also shown (1 × 10^5 [], 5 × 10^4 [], and 1 × 10^5 []). As a control, the results for mice given only PBS are shown at the center of the panel (Ⅲ). Bars show the mean ± SD (n = 10/group). The difference between the control group and the groups of mice transferred with GFP-selected cells was statistically significant (+, p < 0.01; †, p < 0.05), whereas the difference between the control group and the groups of mice transfected with mock-transfected cells was not significant.

Amelioration of the disease severity requires Ag-specificity

Although it seems that the Ag specificity of the transferred CD4+ T cells is required for efficient recruitment to the inflamed joints and that locally released IL-10 might be involved in the reduction of disease severity, it might also be possible that IL-10-producing T cells migrate into the joint in response to the inflammation regardless of their Ag specificity. To examine these points, we transferred wild-type BALB/c splenocytes transfected with IL-10. The mice developed AIA as severe as that in the positive control, whereas splenocytes from DO11.10 mice resulted in amelioration (Fig. 4). This result indicates that the Ag specificity of the CD4+ T cells is indispensable for efficient reduction of disease severity, supporting the concept that the T cells should recognize the Ag in the joints and migrate to such joints.

Transferred DO11.10 T cells infiltrated into the arthritic ankle joint

To confirm that transferred DO11.10 T cells migrate into arthritic joints preferentially, we employed the TCR RT-PCR/SSCP method (27, 28). Because each CDR3 sequence demonstrates unique mobility on nondenatured gel due to its unique single-strand conformation, the identical mobility of the amplified Vβ CDR3 region on a gel indicates that the clones are identical. This rule has always been confirmed by sequencing (32).

Whereas splenocytes from BALB/c mice immunized with OVA exhibited a smear pattern that indicates a heterogeneous T cell population, splenocytes from mice which were transferred with DO11.10 cells shared a distinct band with the positive control in addition to a heterogeneous smear pattern (Fig. 5). The arthritic left ankle joint exhibited the unique distinct band, indicating that the transferred DO11.10 cells selectively migrated to this site. Other noninflamed joints also exhibited a faint band corresponding to DO11.10 T cell, probably due to contamination by peripheral blood.

Because the cells were transfected with pMFGmIL10-IRES-GFP, to detect transfected cells in arthritic lesions we used the GFP
transferred with PBS ( ), DO11.10 CD4 T cells transduced with mIL-10 and selected by GFP expression. Because DO11.10 clonotype-positive cells are exclusively CD4 positive, the results of analysis using KJ1-23 are similar to those of analysis using CD4. In one experiment, three arthritic mice were sacrificed, and their cells were mixed before staining. A representative result is shown. Three separate experiments gave similar results.

 marker in flow cytometry. Although only about 5% of the isolated cells from the arthritic joints were positive for CD4, 4.57% of the isolated cells joint were positive for both GFP and KJ1-26, which accounts for 88% of the infiltrating CD4 cells in the arthritic lesion. However, only 0.16% of splenocytes were positive for GFP and KJ1-26 in the spleen (Fig. 6). GFP-positive cells were not detected either in joints from the negative control or in arthritic joints of mice transferred with BALB/c mice splenocytes transduced with GFP. Thus, we confirmed the selective accumulation of the transduced DO11.10 cells in the arthritic joints by both clonotype analysis and flow cytometric analysis.

**The immune response to OVA was not impaired in the spleen**

IL-10 could not be detected in sera from mice transferred with IL-10-transduced DO11.10 splenocytes (data not shown). Considering this together with the selective accumulation of the transferred cells and the restricted space of the joint, it is conceivable that the suppressive effect of IL-10 could be observed preferentially in the joint. To examine whether suppression of the disease was induced locally or systemically, we examined both the Ab titer to OVA and the T cell proliferative response to OVA after adoptive transfer of the IL-10-transduced T cells selected by GFP. Neither the T nor the B cell response of the splenocytes from mice transferred with 10 T cells demonstrated a significant difference compared with that of the splenocytes from control mice (Fig. 7). This result indicates that the transferred T cells express their suppressive activity locally.

**Methylated BSA-induced arthritis can be ameliorated by IL-10-transduced DO11.10 splenocytes**

Finally, we examined whether IL-10-transduced DO11.10 T cells are able to ameliorate arthritis induced by another Ag, using mBSA as an arthritis-inducing Ag (33, 34). Arthritis induced by mBSA in BALB/c mice showed severer joint swelling than that induced by OVA. DO11.10 splenocytes (5 10) that were transduced with mIL-10 and selected by GFP were transferred to BALB/c mice immunized with mBSA. We observed a reduction of the swelling in the joint that was coinjected intra-articularly with OVA, but not in the joint that was not coinjected with OVA (Fig. 8). Mock-transfected DO11.10 T cells exacerbated the swelling of joints that were coinjected with OVA. These results indicate that DO11.10 T cells transduced with mIL-10 migrate into the joint in response to the intra-articularly injected OVA and that they are

**FIGURE 7.** Neither the T cell nor the B cell response against OVA was impaired by transfer of 5 10 DO11.10 CD4 T cells transduced with mIL-10. A, Proliferation assay upon OVA challenge. The proliferation of splenocytes from OVA-primed BALB/c mice transferred with PBS ( ), DO11.10 CD4 T cells mock-transfected ( ), and DO11.10 CD4 T cells transected with mIL-10 ( ), respectively, were measured by [H]thymidine incorporation in the presence of 10 g/ml OVA. Bars show the mean ± SD (n = 10/group). The difference between each group was not significant. B, Anti-OVA Ab level. Mice were bled 30 days after intra-articular challenge with OVA, and blood was assayed for the anti-OVA Ab level by ELISA. The mean Ab titer (±SD) of each group of 10 mice is shown. The difference between each group was not significant.

**FIGURE 8.** IL-10-transduced CD4 DO11.10 splenocytes ameliorated mBSA-induced arthritis in mice that were coinjected intra-articularly with OVA. CD4 DO11.10 splenocytes (5 10) transduced with mIL-10 and selected by GFP marker (■) were transferred the day before arthritis induction. The induction of arthritis was achieved by intra-articular injection of 20 g of mBSA and 100 g of OVA into the left ankle joint. Mice transferred only with PBS (○) were included as a control. The severity was evaluated by hind paw thickness. Bars show the mean ± SD (n = 10/group). *, Significant difference (p < 0.01) between the control group and the transfer group.

**FIGURE 6.** The selective accumulation of the transferred T cells in the arthritic joint was confirmed by flow cytometry. Cells were recovered from spleens (A) as well as joints (B) from mice that had been transferred with DO11.10 CD4 T cells transduced with pMFGmIL10-IRES-GFP and selected by GFP marker. Because DO11.10 clonotype-positive cells are exclusively CD4 positive, the results of analysis using KJ1-23 are similar to those of analysis using CD4. In one experiment, three arthritic mice were sacrificed, and their cells were mixed before staining. A representative result is shown. Three separate experiments gave similar results.
able to exhibit bystander suppression of mBSA-induced arthritis. Taken together, the IL-10-transduced DO11.10 splenic T cells preferentially migrated into the joint, responding to the Ag, and reduced disease severity quite efficiently without impairing the systemic Ag-specific immune response. These results suggest that Ag-specific T cells could serve as a therapeutic vehicle for arthritis.

Discussion

The present results show that OVA-specific T cells transduced with mIL-10 ameliorated experimental arthritis induced by either OVA or mBSA as long as OVA was present in the joint. This amelioration was not accompanied by significant reduction of the systemic immune response of splenocytes to OVA, suggesting that OVA-specific transferred T cells exert their role at sites where the Ag exists. Because in our experiment OVA is a substitute for a joint-specific autoantigen, we could expect that joint-Ag-specific T cells have a similar potentiality.

IL-10 has already been demonstrated to exert a therapeutic effect on arthritis in a collagen-induced arthritis (CIA) model (17–20). To date, the effect of IL-10 seems to be mediated by suppression of the systemic immune response to CII, although contradictory results has been reported. Walmsley et al. demonstrated that the disease severity of CIA was reduced in mice treated with i.p. injection of mIL-10 (35). No difference in the amount of anti-CII IgG1 was observed between the treatment and control groups. Similarly, the ratio of anti-ChIgG1 to anti-ChIgG2a level revealed no significant differences. Ma et al. demonstrated successful inhibition of CIA by systemic administration of a viral IL-10-encoding adenovirus (Av(vIL-10)) (19). They found that T cells from mice treated with Av(vIL-10) showed a decreased proliferative response to CII, whereas the serum level of Ab against CIA was not affected. Apparailly et al. also demonstrated that viral IL-10 gene transfer mediated by adenovirus inhibits CIA (18). They did not examine the T and B cell responses against CIA, except for the ratio of IgG1/IgG2a of anti-CIA Abs, which increased in adenovirus-vIL-10-treated mice, suggesting that the Th2 response is dominant. Therefore, although IL-10 also possesses some immunostimulatory activities, including enhanced expression of class II MHC molecules on B cells and induction of CTL differentiation, in these experiments IL-10 seems to mediate immunosuppression of the T cell response. This immunosuppression, however, appeared to be Ag nonspecific, because they could detect viral IL-10 in the sera from adenovirus-vIL-10-treated mice. By suppressing the immune responses to a wide variety of foreign Ags, Ag nonspecific immune suppression might increase the risk of immunocompromised infections.

Interestingly, quite recently it was demonstrated that viral IL-10-expressing adenovirus injected periarticularly into mouse paws suppressed the development of CIA in both the injected and the uninjected contralateral paws (36, 37). Such a distal anti-arthritis effect has also been reported using OVA-induced arthritis in rabbits to which IL-1 and TNF-α soluble receptors were delivered by adenovirus-mediated gene transfer (9). It was demonstrated that certain cells infected with the delivered viral vectors moved to other joints (37). It would be interesting to know whether they are T cells.

Ex vivo gene delivery using T cells in experimentally induced arthritis has been reported by Chernajovsky et al. (38). Splenocytes from DBA/1 mice immunized with CII that were transduced with either soluble TNF receptor or TGF-β were able to inhibit development of disease in SCID mice transduced with anti-CII-responding splenocytes. In their experiments the significance of Ag-specific receptors on T cells remained ambiguous. Although they demonstrated that T cells infected with TGF-β were able to reduce the anti-CII Ab level, they did not mention whether the transduced T cells ameliorated the disease. The inability of their T cells to reduce disease severity might be due to the low infection efficiency, because in their system only between 0.2 and 0.3% of the transferred lymphocyte population was infected.

Adoptive transfer of IL-10-transduced T cells has been reported in nonobese diabetic (NOD) mice. Islet-specific Th1 cells that were transduced with IL-10 and selected by G418 abrogated the diabetes in NOD mice (39). The number of transferred cells needed to ameliorate the disease was 2–6 × 10⁶. Although the affected organ is different, considering that the number of transferred cells needed to reduce disease activity was smaller in our experiments (1 × 10⁶ cells were needed), GFP selection would be one of the best strategies for selecting transduced peripheral blood cells that could be frequently used in ex vivo gene therapy for patients.

Joint Ag-specific T cells transduced with the IL-10 gene might fit the idea of regulatory T cells (40), which were demonstrated to suppress experimentally induced colitis. Ag specificity of the engineered regulatory T cells is indispensable for Ag-specific immunomodulation as well as for cell migration into arthritic lesions, where the regulatory T cells exhibit bystander suppression (41). We are now planning an experimental system in which the authentic joint-specific Ag serves as the target of the transporter.

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Ag-SPECIFIC T CELLS AS A VEHICLE FOR IL-10 IN ARTHRITIS

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