Gene Therapy of Arthritis with TCR Isolated from the Inflamed Paw

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In recent years, the treatment of autoimmune diseases has been significantly advanced by the use of biological agents. However, some biologies are accompanied with severe side effects, including tuberculosis and other types of infection. There is thus a critical need for nonsystemic and lesion-specific methods of delivering these therapeutic agents. We attempted to treat a mouse model of arthritis by using T cells that expressed a regulatory molecule and were specifically directed to the inflamed paw. To this end, we first identified the TCR αβ genes accumulating in the inflamed paw of mice with collagen-induced arthritis (CIA) by a combination of single-strand chain conformational polymorphism analysis of TCR and single-cell sorting. We identified an expanded clone B47 which is autoreactive but is not specific to type II collagen. In vivo, TCR genes from B47-transduced T cells accumulated in the inflamed paw. Injection of cells cotransduced with the B47 and soluble TNFRIG genes resulted in a significant suppression of CIA. The suppression was correlated with the amount of TNFRIG transcripts in the hind paw, not with the serum concentrations of TNFRIG. Moreover, T cells cotransduced with the B47 and intracellular Foxp3 genes significantly suppressed CIA with reductions in TNF-α, IL-17A, and IL-1β expression and bone destruction. T cells cotransduced with B47 and Foxp3 genes also suppressed the progression of established CIA. Therefore, immunosuppressive therapy with autoreactive TCR is a promising therapeutic strategy for arthritis whether the TCRs are used to deliver either soluble or intracellular suppressive molecules. The Journal of Immunology, 2006, 177: 8140–8147.

Progress in molecular biology reveals many molecular bases for the autoimmune diseases. In recent years, the treatment of autoimmune diseases has been significantly advanced by the use of biological agents. Treatment of rheumatoid arthritis (RA) has long been insufficient to prevent joint destruction. However, anti-TNF therapy has been a breakthrough in the treatment of RA. Anti-TNF therapy significantly ameliorates arthritis symptoms, acute phase reactants, and bone destruction (1–3). In contrast, anti-TNF therapy is accompanied by increased risk of serious infection, including tuberculosis (4). Therefore, it is important to develop an optimal molecular delivery system for anti-TNF drugs and other biological agents.

In addition to the interference of cytokines, there are several other candidate molecules that may suppress autoimmune diseases. For example, Foxp3, a master transcription factor for regulatory T cells (5), is an important candidate for autoimmune suppression. Consequently, specific delivery of intracellular molecules is also important for future molecular therapy.

Because T cells systemically survey specific Ags and migrate to specific organs upon Ag recognition, they are an appropriate candidate vehicle for molecular delivery. T cell therapy has been used for the treatment of several kinds of autoimmune diseases (6–8). However, it is difficult to isolate and culture lesion-specific T cells to realize an amount sufficient for treatment. To date, type II collagen (CII)-specific T cell hybridoma and TCR-transgenic cells have been used for in vivo therapy of arthritis (9, 10), and OVA-specific TCR-transgenic cells have been used to treat OVA-induced arthritis (11). However, tumor cells and transgenic cells are evidently not applicable in human treatment. Moreover, T cells specific for the disease-priming autoantigen have the possibility to exacerbate arthritis inflammation.

We previously established a technique for analyzing T cell clonality by the reverse transcription (RT)-PCR/single-strand conformational polymorphism (SSCP) method (12). This method detects nucleotide changes of the CDR3 regions of clonally expanded T cells in vivo. Using this method, we have demonstrated oligo-clonal expansion of T cells in patients with RA and solid tumors (12, 13). These findings indicate that the knowledge of the specific TCR accumulated at the inflammatory site may make it possible to reconstitute functional and organ-specific T cells. Indeed, we have previously identified the TCR α and β genes of expanded T cell clones infiltrated into p815 tumors (14).

In this study, we isolated a pair of TCR α and β genes, B47 from the paw of a mouse with collagen-induced arthritis (CIA). This TCR was not specific to immunized CII. We reconstituted this clonotype on peripheral CD4+ T cells as a therapeutic vehicle. Cells cotransduced with B47 and TNFRIG suppressed CIA. The suppression was correlated with the amount of TNFRIG transcripts in the hind paw, not with the serum concentrations of TNFRIG. Moreover, T cells cotransduced with B47 and intracellular Foxp3 significantly suppressed CIA with reductions in TNF-α, IL-17A,
and IL-1β expression and bone destruction. Therefore, an in vivo cloned TCR can be considered an efficient tool for molecular therapy.

Materials and Methods

Induction of CIA and scoring of joint swelling and histology

DBA1 mice were purchased from SLC and maintained in our specific pathogen-free facility. Mice were immunized intradermally at the base of the tail with 100 μg of bovine CII (bCII; Chondrex) emulsified with CFA (Chondrex). On day 21, mice were boosted by intradermal injection with 100 μg of bCII emulsified with IFA (Difco). Inflammation of the four paws was graded from 0 to 4 as follows: grade 0, no swelling; grade 1, swelling of the finger joints or focal redness; grade 2, mild swelling of the wrist or ankle joints; grade 3, severe swelling of the entire paw; and grade 4, deformity or ankylosis. Each paw was graded and the four scores were totaled so that the possible maximal score per mouse was 16. All animal experiments were conducted in accordance with the institutional and national guidelines.

Vector construction

We constructed the vectors pMX-CIIT TCR (pMX-CIIT<sub>α</sub>-IRES-CIIT<sub>β</sub>) and pMX-B47<sub>α</sub> (pMX-B47<sub>α</sub>-IRES-B47<sub>β</sub>) to transduce the desired TCR clonotype to activated CD4<sup>+</sup> T cells, bCII-specific TCR, CIIT, α- (V<sub>α</sub>11) and β- (V<sub>β</sub>8.2) chains were constructed based on the published sequences of clone 173 α- and β-chains (15) as previously described (16). A TNFR<sub>g</sub> fragment was constructed by fusing murine TNFR (p75) to the hinge and Fc region of a murine IgG2a H chain. The resulting TNFR<sub>g</sub> fragment was subcloned into a pMX retrovirus vector. We also constructed the vector pMX-Foxp3-IRES-GFP. Retroviral gene transfer was performed as previously described (16).

Single-cell sorting and RT-PCR

The CD4<sup>+</sup> T cells at the inflammation site were stained with FITC-conjugated anti-CD4 and PE-conjugated anti-V<sub>β</sub>8.1/8.2 (BD Pharmingen). The CD4<sup>+</sup>/V<sub>β</sub>8.1/8.2<sup>+</sup> cells were sorted into a ratio of one cell per well using an automatic cell dispensing unit driven by the FACSVantage and Clone-Cyt software (BD Biosciences). Each cell was sorted into a well of 96-well plate containing 20 μl of RT reaction mixture (10 nM C<sub>α</sub>R primer, 10 nM C<sub>β</sub>R primer, 1× RT reaction buffer, 100 μM each dNTP, 2.5 μM RT primer, 0.5% Nonidet P-40 (Boehringer Mannheim), 0.5 U/μl RNasin (Promega) in a 96-well microparticle plate. Immediately, 20 U/μl Superscript II (Invitrogen Life Technologies) reagent was added to each well and the plate was held at 37°C for 90 min. After the reaction mixture received heat inactivation for 10 min at 65°C, an equal volume of DTT solution (2× DTT reaction buffer, 2.5 mM DTT (Amersham Biosciences), 0.5 U/μl TdT (Invitrogen Life Technologies)) was added to each well and the plate was incubated for 15 min at 37°C (17). From the single-cell RT reaction mixtures, 2 μl of cDNA was added to 23 μl of the first PCR premix (1.6 pM/μl each 1st primer, 200 μM each dNTP, and 0.25 U/μl KOD-plus Taq polymerase (Toyobo)) and amplified by a 25-cycle program (95°C for 1 min, 52°C for 1 min, and 72°C for 2 min). Two microliters of first PCR products was used for the second PCR (30 cycles of 95°C for 1 min, 54°C for 1 min, and 72°C for 2 min), using the second PCR premix (1.6 pM/μl each of second primer, 200 μM each of dNTP, and 0.25 U/μl Taq polymerase (Promega)). Then, 2 μl of the second PCR products was used for further amplification reaction (35 cycles of 95°C for 1 min, 54°C for 1 min, and 72°C for 2 min), using the third PCR premix (1.6 pM/μl each third primer, 200 nM each dNTP, 0.25 U/μl Taq polymerase). Single-strand conformational polymorphism

The SSCP study was performed as described previously (14, 18). In brief, the synthesized cDNA was amplified by PCR with a pair of V<sub>β</sub>11 to V<sub>β</sub>19 primers and a β<sub>β</sub> common primer. The amplified DNA was electrophoresed on a nondenaturing 4% polyacrylamide gel. After transfer onto a nylon membrane, the cDNA was hybridized with a biotinylated internal common β<sub>β</sub> oligonucleotide probe and visualized by subsequent incubations with streptavidin, biotinylated alkaline phosphatase, and a chemiluminescent substrate system (Phototope-Star Chemiluminescent Detection kit, New England Biolabs).

Cell purification

A CD4<sup>+</sup> T cell population was prepared by negative selection with MACS (Miltenyi Biotec) using anti-CD19 mAb, anti-CD11c mAb, and anti-CD8α mAb. CD11c<sup>+</sup> DCs were prepared as previously described (19, 20).

Brieﬂy, spleen cells or lymph node cells were digested with collagenase type IV (Sigma-Aldrich) and DNase I, and the CD11c<sup>+</sup> cells were selected twice by positive selection using MACS CD11c microbeads and magnetic separation columns. The purity (85% in average) was determined by visualization with anti-CD11c-biotin followed by streptavidin-PE. A CD19<sup>+</sup> B cell population was prepared by positive selection with MACS using anti-CD19 mAb. For CFSE-labeling (Molecular Probes), cells were resuspended in PBS at 1×10<sup>6</sup>/ml and incubated with CFSE at a final concentration of 5 mM for 30 min at 37°C, followed by two washes in PBS. An Anti-FITC MultSort kit (Miltenyi Biotec) was used in the negative selection experiment in the CD11c<sup>+</sup> population.

Paw tissues were prepared by removing the skin and separating the limb below the ankle joint. Finely minced tissues were incubated in complete RPMI 1640 medium with 1 mg/ml type IV collagenase (Sigma-Aldrich) for 60 min. The cell suspension was strained through nylon mesh and washed with PBS. In the single-cell sorting experiment, anesthetized mice were sacrificed by cardiac perfusion with PBS before the paw preparation.

Proliferation assay

At 24 h postinfection, purified CD4<sup>+</sup> T cells were cultured at 0.5–1×10<sup>5</sup> cells/well, with 1×10<sup>6</sup> cells/well of irradiated splenocytes or 1×10<sup>5</sup> cells/well of irradiated CD11c<sub>α</sub> DCs in 96-well, flat-bottom microtiter plates in volumes of 100 μl of complete medium with or without 100 μg/ml heat denatured bCII or murine CII (mCII) (Chondrex). After 24 h of culture, the cells were pulse-labeled with 1 Ci of [3H]thymidine/well (NEN Life Science Products) for 15 h and the [3H]thymidine incorporation was determined.

Flow cytometry

The percentage of TCR gene transduced cells in each organ was determined by FACS analysis. Cell suspensions were first incubated with anti-CD16/CD32 (BD Pharmingen) to block FeRs. The cells were then stained with anti-CD4-allophycocyanin-Cy7, anti-V<sub>β</sub>8.1/8.2-PE, anti-CD2-biotin followed by streptavidin-allophycocyanin (BD Pharmingen). Flow cytometry was performed using FACSVantage.
Real-time PCR

The skin was stripped from the mouse paws and the paws were frozen in isogen (Nippon Gene). mRNA extraction and cDNA preparation were performed according to the manufacturer’s (Nippon Gene) instructions. Real-time quantitative PCR was performed using CyberGreen master-mix (Quagen) and an iCycler (Bio-Rad). Primer pairs were selected as previously described for β-actin, GAPDH, TNF-α, IFN-γ, IL-1β, and IL-10 (21). IL-17 primer pairs were as follows: IL-17 forward 5′-GCTCCAGAAGG and IL-17 reverse 5′-AGCTTTCCCTCCGCATTGA-3′. The PCR parameters were 95°C for 15 min, followed by 50 cycles of 95°C for 30 s, 52°C for 30 s, and 72°C for 60 s.

Results

Reconstitution of paw specificity by transfer of the CII-specific TCR

Our aim was to generate an inflamed paw-directed T cell that expresses a regulatory molecule using TCR cloned from an arthritic paw. To this end, we first examined whether TCR reconstituted specificity on CD4+ T cells of DBA1 mice in vitro.

Table 1. Major clones in Vβ8.1/8.2+ CD4+ T cells from seven arthritic mice

<table>
<thead>
<tr>
<th>Mouse</th>
<th>Vβ8.2</th>
<th>CDR3</th>
<th>Jβ</th>
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<tbody>
<tr>
<td>Mouse 1 major</td>
<td>YFC</td>
<td>D R G NSD</td>
<td>Y F G S G</td>
</tr>
<tr>
<td>Mouse 2 major</td>
<td>YFC</td>
<td>D GF N R L</td>
<td>F F G H G</td>
</tr>
<tr>
<td>Mouse 3 major</td>
<td>YFC</td>
<td>D L G</td>
<td>V F G P G</td>
</tr>
<tr>
<td>Mouse 4 major</td>
<td>YFC</td>
<td>D S G G</td>
<td>F F G H G</td>
</tr>
<tr>
<td>Mouse 5 major</td>
<td>YFC</td>
<td>D A G D T</td>
<td>Y F G P G</td>
</tr>
<tr>
<td>Mouse 6 major</td>
<td>YFC</td>
<td>V P G Q G N E R</td>
<td>F F G H G</td>
</tr>
<tr>
<td>Mouse 7 major</td>
<td>YFC</td>
<td>D P G G Q D T Q</td>
<td>Y F G P G</td>
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In the gene transduction experiment, control cells that were transduced with an empty vector (pMX) were designated as mock-transduced cells. In a representative experiment, the percentage of Vβ8.1/8.2+ T cells in the CD4+ population was increased from 36% (100 × 13.0/(13.0 + 23.3)) to 68% (100 × 30.4/(30.4 + 14.1)) after infection of CIIT-α (Vα11) and β- (Vβ8.2) chains into DBA1 splenocytes (Fig. 1B). The calculated efficiency of β-chain transduction into initially Vβ8.1/8.2-negative cells was ~50% (100 × (68 − 36))/(100 − 36). We speculated that the transduction efficiency of the α-chain was equal to that of the β-chain. Therefore, the clonotypic transduction efficiency was estimated to be ~25%.

We next examined the specific reactivity of CIIT-transduced cells. Though CIIT-transduced cells showed only marginal proliferation in the presence of autologous irradiated splenocytes alone, these cells proliferated strongly in the presence of mCII and bCII (Fig. 1C). Moreover, this proliferation was blocked by anti-I-Aβ Ab (data not shown). The reactivity of CIIT-transduced cells to mCII is consistent with a previous report that a T cell hybridoma expressing this TCR was accumulated in the inflamed joints of mice (9). There was no significant difference in proliferation between mock- and DO11.10 TCR (I-Aβ-restricted, OVA323–339-specific TCR) (22) transduced cells in the presence of DCs with or without CII. Thus, CIIT gene transfer can reconstitute Ag specificity on CD4+ T cells of DBA1 mice in vitro.

FIGURE 2. Identification and reconstitution of B47 TCR, which was autoreactive and not specific to CII. A, An example of identification of an expanded TCR in CIA by the TCR-SSCP method. RT-PCR was performed with Vβ8.1/8.2-specific and Cβ primers for cDNA of sorted single cells and the total paw. PCR products were subjected to electrophoresis. Lane Sc: Single-cell-sorted T cells. Lane P, Total paw T cells. A few TCR β-chains from sorted single cells exhibited the same mobility as that from the total paw (arrow). B, Amino acid sequences of identified B47 TCR α- and β-chains expanded in CIA. C, Schematic representation of the bicistronic retrovirus construct of B47 TCR. D, Representative result of retroviral transduction of B47 TCR in DBA1 splenocytes. The cells were triple stained for Vα2, Vβ8.1/8.2, and CD4. CD4 gated dot plots are shown. E, Representative result of autoreactivity of B47-transduced CD4+ T cells. Indicated numbers of mock-, DO11.10-, or B47-transduced CD4+ T cells and 1 × 105 of CD4+ dendritic cells were cultured in 96-well plates. F, A total of 1 × 105 mock- or B47-transduced cells were cultured with no APC, 1 × 106 splenic CD11c+ cells, splenic CD11c+ cells plus 100 μg/ml mCII, or splenic CD11c+ cells plus 100 μg/ml bCII.
To investigate in vivo migration capacity of CIIT-transduced cells, CFSE-labeled CIIT-transduced cells were transferred to arthritic mice via the tail vein. The spleen, inguinal lymph nodes (ILN), paws, and lungs were analyzed for Vα2⁺Vβ8.1/8.2⁺CFSE⁺CD4⁺ T cells by FACS. The average percentages of Vα2⁺Vβ8.1/8.2⁺CFSE⁺CD4⁺ T cells in the indicated organs from three independent experiments. *, A significant difference (p < 0.05) compared with mock group. C, CFSE analysis of Vα2⁺Vβ8.1/8.2⁺CD4⁺ T cells in the indicated organs. Vα2⁺CD4⁺ gated profiles are shown. D, IFN-γ expressions were quantified with real-time PCR in Vα2⁺Vβ8.1/8.2⁺CFSE⁺CD4⁺ T cells from the indicated organs.

Identification of a Vβ8.1/8.2⁺CD4⁺ T cell clone expanded in the arthritic paw using the TCR-SSCP method

To identify the TCR clone expanded in the arthritic paw, we focused on the TCR Vβ8.1/8.2 subfamily, which is one of the largest TCR Vβ subfamilies. We first examined sequences of the CDR3 motif of Vβ8.1/8.2⁺ T cells in the inflamed paw of CIA mice. When we analyzed major clones in Vβ8.1/8.2⁺CD4⁺ T cells from seven arthritic mice, five of the seven mice had major clones with a similar motif containing aspartic acid and glycine in their CDR3, DXGX, and DXGX (Table I).

To obtain a pair of TCR α- and β-chains from a cell expanded in the arthritic paws, we performed single-cell sorting of Vβ8.1/8.2⁺CD4⁺ T cells. cDNA was synthesized and the sequence of the TCRβ chain was determined by three-step nested PCR. The sequence of the TCRα chain was determined by three-step seminested PCR using a series of Vβ1–22 primers. The TCRα chain belonged to the Vβ2 subfamily. B47-transduced cells showed strong autoreactive response to CD11c⁺ DCs from arthritic mice

We subcloned cDNA of the B47 α- and β-chain into a bicistronic retrovirus vector (Fig. 2C). The transduction efficiency of the B47 clonotype was determined by anti-Vα2 and Vβ8.1/8.2 Abs, and the clonotypic transduction efficiency was 30–40% on average.
ILN whole CD11c that is presented more efficiently in arthritic mice. Therefore, B47 TCR was found to recognize an autoantigen from naive and arthritic mice, B47-transduced CD4+ cells proliferated in the presence of autologous CD11c+ DCs of the spleen and draining lymph nodes from naive mice (Fig. 2D). Though mock- and DO11.10-transduced CD4+ cells were stimulated weakly by CD11c+ DCs from naive and arthritic mice, B47-transduced CD4+ cells proliferated more strongly in the presence of CD11c+ DCs from arthritic mice (Fig. 2E). In addition, B47-transduced CD4+ cells did not show increased proliferation in response to mCII and bCII (Fig. 2F). Therefore, B47 TCR was found to recognize an autoantigen that is presented more efficiently in arthritic mice.

We next examined the kinetics of B47-transduced CD4+ cells in the arthritic mice. Mock- or B47-transduced CD4+ cells were labeled for CFSE and transferred to arthritic mice via the tail vein. These mice groups were designated as the mock group and the B47 group, respectively. Five days after transfer, the accumulation of Vα2+ Vβ8.1/8.2+ CFSE+ CD4+ T cells was similar in the ILN and paws of the B47 group and in the ILN and paws of the mock group (Fig. 3, A and B). In contrast, the accumulation of Vα2+ Vβ8.1/8.2+ CFSE+ CD4+ T cells in the ILN and paws of B47 group was significantly greater than that in mock group (Fig. 3, A and B). Moreover, Vα2+ Vβ8.1/8.2+ CFSE+ CD4+ T cells in the ILN and paws showed lower CFSE fluorescence than those in the spleen of the B47 group and in the ILN and paws of the mock group (Fig. 3C). Vα2+ Vβ8.1/8.2+ CFSE+ CD4+ T cells in the ILN of the B47 group showed higher expression of IFN-γ than those in the spleen of the B47 group or in the spleen and ILN of the mock group (Fig. 3D). This result indicated that transfer of B47 allowed CD4+ T cells to accumulate in the draining lymph nodes and arthritic paws.

In Fig. 3A, the Vα2+ Vβ8.1/8.2+ population also increased in these mice. In vitro experiments using GFP-reported TCR α and β expression vectors (pMIG-TCRα and pMIG-TCRβ), the expression of the transduced TCR β-chain was rather unstable compared with that of the transduced TCR α-chain (K. Fujio, unpublished data). We suppose that this phenomenon was related to phenotypic allelic exclusion of the TCR β protein, because internal ribosomal entry site (IRES)-driven GFP expression was sustained despite a decrease of TCR β-chain expression. We think that at least a part of the Vα2+ Vβ8.1/8.2+ population may have come from B47-transduced cells that lost TCR β expression.

We next explored the subpopulation of CD11c+ DCs that can present arthritis-associated autoantigens. CD11c+ DCs in ILN can be classified into three groups, CD11c+ CD11b– CD8– cells, CD11c+ CD11b+ CD8– cells, and CD11c+ CD11b+ CD8+ cells (Fig. 4A). We compared the Ag presentation of total ILN CD11c+ DCs, MACS-depleted CD8– CD11c+ DCs, and CD11b– CD11c+ DCs to
B47-transduced CD4+ cells. As shown in Fig. 4B, CD11b-depleted CD11c+ DCs lost their autoantigen presentation to B47-transduced CD4+ cells. We next examined the autoantigen presentation to CII-transduced CD4+ cells. CD11b-depleted CD11c+ DCs from ILN cells of CIA mice also lost their autoantigen presentation to CII-specific T cells (Fig. 4C). These results indicated that CD11b+CD11c+ DCs are important APCs in arthritis.

**B47 plus TNFRlg-transduced cells suppressed CIA**

We next attempted to use paw-directed B47-transduced CD4+ cells as a vehicle for therapeutic molecules. We constructed a TNFRlg-expressing vector by fusing the murine p75 TNFR and Fc domain of IgG2a. TNFRlg-producing paw-directed cells were generated by triple gene transfer of B47 TCR and TNFRlg. We prepared three groups receiving controlled gene transfer of either mock vector, B47 alone, or TNFRlg alone. The clonotypic transduction efficiency was ~30% on average (Fig. 5A). Though we could not directly detect the transduction efficiency of TNFRlg, the TNFRlg protein concentrations in the culture supernatant of B47 plus TNFRlg-transduced CD4+ cells were equivalent to those of TNFRlg (Fig. 5B). Therefore, the transduction efficiency of the TNFRlg gene was considered to be almost equal in these two groups.

These mock, B47, TNFRlg, or B47 plus TNFRlg-transduced cells were i.v. transferred to CII-immunized mice via the tail vein just before the onset of arthritis at day 28. These mice groups were designated as mock group, B47 group, TNFRlg group, and B47 plus TNFRlg group, respectively. The arthritis score of B47 plus TNFRlg group was evidently suppressed compared with those of the mock and B47 groups (Fig. 5C). The arthritis score of the TNFRlg group was slightly suppressed. In terms of the incidence of severe arthritis, the B47 plus TNFRlg group clearly showed the lowest rate.

**Accumulation of TNFRlg transcript in the paws was important for arthritis suppression**

We next examined the kinetics of the transduced TNFRlg gene. Because the titers of anti-CII IgG at day 38 were equivalent in all experimental groups, TNFRlg did not directly affect the humoral immune response (Fig. 5D). The serum concentrations of TNFRlg protein in the B47 plus TNFRlg group were equivalent to those in the TNFRlg group at day 38 (Fig. 5E). This result indicated that the serum concentration of TNFRlg was not the main determinant of arthritis suppression in the B47 plus TNFRlg group.

We then checked the accumulation of TNFRlg transcript in the lymphoid organs and paws. The amount of TNFRlg transcript was
In response to the treatment with TNFRIg, T cells coexpressing B47 and TNFRIg exhibited suppressive activity associated with local accumulation. This result suggested that the main determinant of therapeutic efficacy in anti-TNF therapy is local accumulation, not serum concentration. Therefore, the conventional systemic administration of an anti-TNF drug that depends on serum concentration may not be a reasonable therapy. An elevated serum concentration is associated with systemic immunosuppression and high cost of treatment. Local injection of an anti-TNF drug is another approach to avoid a systemic suppressive effect (23, 24). However, this approach is not ideal due to the polyarthritic nature of RA. In contrast, T cells that produce TNFRIg and accumulate in the paws at the arthritic sites can reach multiple paws with reduced systemic effect.

The TCR transfer was also effective in the treatment with intracellular Foxp3 expression. Though suppression of murine arthritis with polyclonal regulatory T cells have been reported (25), the importance of T cell specificity has not been addressed. In the Foxp3 transfer experiment, Foxp3-expressing T cells with arthritis-associated TCR were effective. Once activated, regulatory T cells exhibit suppression in an Ag nonspecific manner (26). However, Ag specificity is important in the migration and expansion of regulatory T cells (27, 28). Indeed, Ag-specific regulatory T cells are efficient in suppressing various autoimmune diseases. The problem is how to obtain a sufficient amount of organ-Ag-specific regulatory T cells for therapeutic transfer. TCR and Foxp3 gene transfer is one possible approach to overcome this problem. Many mice spleens may be required to obtain 0.5–1.0 × 10⁹ of CD4⁺CD25⁺ regulatory T cells, which is required to treat one mouse in the prior CIA treatment (25). In contrast, in vitro-expanded cells derived from a quarter of spleen were sufficient to treat one mouse in our experiment.

Several groups have reported that regulatory T cells are accumulated in the joints of arthritis patients (29, 30). These joint-accumulating CD4⁺CD25⁺ T cells display a greater ability to suppress arthritis than blood CD4⁺CD25⁺ T cells. However, the precise role that these accumulating regulatory T cells play in the pathology of arthritis has not been clarified. Our experiments suggest that regulatory T cells in arthritic joints have the capacity to suppress pathogenic cytokine expression and bone destruction. Moreover, it is noteworthy that reconstituted regulatory T cells suppressed ongoing arthritis (Fig. 6E). There are several evidences that blocking of a specific inflammatory cascade ameliorates CIA after the onset. IL-10 and anti-IL-17A have been reported to inhibit ongoing CIA (31, 32). Our results suggested that regulatory T cells suppress arthritis by blocking the continuous inflammatory process. Therefore, regulatory T cells or Foxp3 therapy may be a feasible approach for established RA patients.

In therapeutic experiments for autoimmune diseases, use of a TCR without specificity to the disease-priming Ag can be an advantage. In our experiment, transfer of B47-transduced cells did not exacerbate arthritis. If CII-specific TCR is used for treatment, there is a possibility that the arthritis will be exacerbated due to enhancement of anti-CII immunity. This potential risk is important for the priming Ag-specific T cell-based treatment of other autoimmune diseases or human diseases that last for a significantly longer period than the diseases in mouse models. Indeed, it is necessary to clarify the specificity of these TCRs associated with arthritis or other autoimmune disorders before clinical application.

Despite epitope screening with synthetic combinatorial peptide libraries in a positional scanning format (PS-SCL) (33), the precise autoantigen for B47 has not been determined.

We confirmed the clonal expansion of autoreactive CD4⁺ T cells that were not specific to the priming Ag in the arthritic paws.
of this mouse model. This result may have important implications for the treatment of autoimmune inflammation. Because CD11c<sup>+</sup>CD11b<sup>+</sup> DCs present both CD11c<sup>+</sup> and an Ag recognized by B47, this DC population may be associated with coping of B47 upon CD11c<sup>+</sup> immunization.

In summary, we identified a TCR that is expanded in arthritic paws by a combination of TCR-SSCP and single-cell sorting. This arthritis-associated TCR that was not specific to the disease-primed CD11c<sup>+</sup> T cells by retrotransfer of the TCR αβ-chain genes isolated from a clonally expanded P815-infiltrating lymphocyte. J. Immunol. 171: 2154–2160.


