Chapter 4

Naïve transgenic T cells expressing cartilage proteoglycan-specific TCR induce arthritis upon in vivo activation

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Abstract
Proteoglycan (PG)-induced arthritis (PGIA), a murine model for rheumatoid arthritis (RA), is driven by antigen (PG)-specific T and B cell activation. In order to analyze the pathogenic role of antigen-specific T cells in the development of autoimmune arthritis, we have generated a transgenic (Tg) mouse. The CD4+ T cells of this TCR-5/4E8-Tg line express a functional T cell receptor (TCR) composed of the Vα1.1 and Vβ4 chains with specificity for the dominant arthritogenic T cell epitope of human cartilage PG. Adoptive transfer of naïve TCR-5/4E8-Tg cells induced arthritis with severe clinical symptoms in syngeneic immunodeficient BALB/c.RAG2−/− mice. In vivo activation of TCR-5/4E8-Tg CD4+Vβ4+ cells with cartilage PG seemed to be critical for arthritis induction. Arthritis never developed after transfer of naïve wild-type cells. The arthritis was characterized as a chronic progressive disease with intermittent spontaneous exacerbations and remissions. Inflamed joints showed extensive cartilage damage and bone erosions leading to massive ankylosis in peripheral joints. These PG epitope-specific TCR-5/4E8-Tg mice can be valuable research tools for studying antigen-driven T cell regulation in arthritis, and migration of T cells to the joints. In addition the model may be used for the development of immune modulating strategies in T cell-mediated autoimmune diseases.
Introduction

Rheumatoid arthritis (RA) is a systemic autoimmune inflammatory disease that predominantly manifests in synovial joints and affects approximately 1-2% of the human population worldwide. Over time, RA results in significant disability due to persistent inflammation in diarthrodial joints leading to cartilage and bone erosions, and joint deformities. Despite many years of intensive investigation, the etiology and pathogenesis of this multifactorial polygenic autoimmune disease have remained unclear. Accumulating evidence indicates that RA is a T cell-dependent antibody-mediated autoimmune disease in which genetic and environmental factors play crucial roles (1-3). Antigen-specific T cells may extravasate and migrate into the joints. These T cells in the synovium may be reactivated by potentially cross-reactive antigens of joint tissues and/or cytokines, which then may lead to their clonal expansion. Although the presence of T cells in the joint seems to be detrimental and contributes to the destructive joint process (4-7), there is evidence that CD4⁺CD25⁺ T cells of a more benign character can also be found in the joint (8-12). Knowledge about the pathogenic role of T cells in RA, or RA-like diseases, has been derived from corresponding animal models of arthritis (13). In many of these models, T cell responses are central to the initiation and maintenance of the disease, and the arthritis can be transferred via autoreactive T cells to naïve recipients (14,15). Although no animal model appears to be a perfect replica of human RA, they clearly show how T cell involvement in the immune-mediated mechanisms can lead to joint destruction.

In this study, we have used the proteoglycan (PG) aggrecan-induced arthritis (PGIA) model which bears several similarities to the clinical expression, histopathology, and genetics of RA (16). Immunization of genetically susceptible BALB/c mice with human cartilage PG (hPG) induces chronic progressive polyarthritis and ankylosing spondylitis. Immunization with hPG elicits strong T cell responses and specific antibodies to the immunizing antigen, and these PG-specific T cells and antibodies cross-react with the mouse (host) cartilage PG (17,18). Depletion of CD4⁺ T cells was shown to prevent disease development (19,20) and adoptively transferred PG-specific T cell hybridoma 5/4E8 cells were able to induce arthritis (21). This CD4⁺ 5/4E8 T cell hybridoma was T cell receptor (TCR)-α⁺β⁺, and belonged to the Th1 subset, as the cells secreted IL-2 and IFN-γ, but not IL-4, upon hPG stimulation. The antigen-specific response was MHC class II (I-A⁺) restricted (21,22). The 5/4E8 hybridoma recognized an epitope \(^{70}\text{ATEGVRVNSAYQDK}_{84}\) (consensus sequence underlined) in the G1 domain of the core protein of hPG (22,23). Later, this particular sequence was found to be the most dominant arthritogenic epitope in BALB/c (21-23), in HLA-DR4 and -DQ8 transgenic mice (24), and that this epitope could specifically stimulate T cells in a subset of RA patients (25-28). It has also been shown that this 5/4E8 hybridoma was able to cross-react with the mouse homologue of the epitope (\text{ATEGQVRVNSIYQDK}) (29). Since the hPG 5/4E8-specific T cells are able to recognize the homologous mouse sequence, despite a two-amino acid difference, we hypothesized that sufficient amounts of mouse epitope from damaged articular cartilage could be released and elicit a T cell response, which would then lead to intra-articular inflammation via a molecular mimicry mechanism (22,30,31).

To gain more insight into the role of antigen-specific T cells in the development of autoimmune arthritis, we generated transgenic (Tg) mice expressing the TCR of hybridoma 5/4E8 specific for hPG. In this study, we have also shown that the adoptive transfer of splenocytes from naïve TCR-5/4E8-Tg donor mice to syngeneic BALB/c. RAG2⁻/⁻ recipient mice induced autoimmune arthritis. These arthritogenic PG-specific TCR-5/4E8-Tg mice are valuable tools
for further analysis of T cell effector mechanisms in autoimmune arthritis, and potentially developing T cell-directed immune interventions.
Generation of cartilage PG-specific TCR transgenic mouse

Material and Methods

5/4E8 T cell hybridoma
hPG-reactive T cell hybridoma 5/4E8 was generated by the fusion of T cells of mice with acute PGIA and BW5147 thymoma cells as described earlier (21). The 5/4E8 T cell hybridoma specifically recognized a peptide sequence 70ATEGRVRVNSAYQDK84 (henceforth: hPG P70-5/4E8 peptide; core sequence underlined) in the G1 domain of hPG and, although weakly, cross-reacted with the mouse homologue sequence (ATEGQVRVNSIYQDK; mPG P70).

RNA isolation, oligonucleotides, first strand synthesis, amplification and cloning of the αβ TCR
Total RNA was isolated from 5/4E8 hybridoma cells by TRIzol (Invitrogen B.V., Breda, the Netherlands) extraction, reverse transcribed with the oligo(dT)12-18 primer using Superscript Reverse Transcription kit (Invitrogen), and then amplified by polymerase chain reactions (PCR) using either degenerate Vα and Cα primers, or the Vβ4 and the Cβ primers. Oligonucleotide primers and the conditions of the corresponding PCR are listed in Table 1. Both the Vα1- and Vβ4-carrying PCR products were cloned into pGEM-T Easy Vector (Promega, Madison, WI), introduced in E. coli DH5α cells (Invitrogen) and sequenced to determine the TCR usage of the 5/4E8 hybridoma. The TCR of 5/4E8 hybridoma was compressed by Vα1.1 and Vβ4 chains (GeneBank accession nos. AY823583 and U19234, respectively).

Amplification and cloning of the αβ genomic TCR genes
To express the TCR-5/4E8 genes in transgenic mice, we made use of the plasmid pTα and pTβ cassettes obtained from Dr. C. Benoist and Dr. D. Mathis (32). Genomic DNA was isolated from 5/4E8 hybridoma cells to obtain full length rearranged TCRα and β DNA, including leader and intron sequences. 5/4E8-TCR was amplified by PCR using Vα1-Xmal and JαTA31-NotI primers for Vα1 chain, and the Vβ4-XhoI and the Jβ2.5-SacII primers for the Vβ4 chain (Table 1). The two PCR fragments were cloned into the pGEM-T Easy Vector (Promega), introduced in E. coli DH5α cells, and sequenced. The Xmal- and NotI-released DNA fragment containing the 5/4E8-TCRα chain was recloned into the pTα cassette, and the Xhol- and SacII-digested and purified 5/4E8-TCRβ fragment inserted into the pTβ cassette (32). Subsequently, XL10-Gold cells (Stratagene, La Jolla, CA) were transfected with the constructs, and sequences were confirmed.

In vitro expression of the αβ TCR
pTα 5/4E8- and pTβ 5/4E8-TCR constructs were tested for their functionality in vitro. The pTα 5/4E8 and pTβ 5/4E8-TCR constructs together with plasmid pcDNA3 (Invitrogen) containing the neomycin resistance gene for positive selection were electroporated into a mouse 58αβ- T cell hybridoma deprived of endogenous functional TCR chains (33). Transfected cells after Geneticin (Invitrogen) treatment were cloned and positive cell lines were selected.
Generation of TCRαβ-5/4E8-Tg (TCR-5/4E8-Tg) mice, and screening of TCR expression

pTα 5/4E8- and pTβ 5/4E8-TCR DNA fragments were linearized with SalI (pTα) and KpnI (pTβ), and then separated from prokaryotic DNA by electroelution. DNA was further purified by phenol extraction followed by ethanol precipitation, and both TCR fragments were injected in equal amounts into the pro-nuclei of fertilized eggs of F1(CBAxC57BL/6) mice (Charles River Laboratories, Sulzfeld, Germany). TCR-5/4E8-Tg founders were identified by PCR analysis of tail genomic DNA (Table 1), and the transgenic expression of the TCR-Vβ4 was confirmed by flow cytometric analysis on peripheral blood lymphocytes.

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*Vα is a degenerate consensus primer (N=A, C, G or T; D=A, T or G; V=A or C; Y=C or T). *Restriction sites for cloning in the TCR cassettes are underlined.

**PCR conditions:**

A: 94°C for 2 min, 35x (94°C for 15 sec, 62°C for 15 sec, 72°C for 25 sec) + final extension at 72°C for 5 min;

B: 94°C for 2 min, 35x (94°C for 15 sec, 55°C for 15 sec, 72°C for 25 sec) + final extension at 72°C for 5 min;

C: 94°C for 2 min, 35x (94°C for 15 sec, 58°C for 15 sec, 72°C for 30 sec) + final extension at 72°C for 5 min;

**Measurement of antigen-specific T cell responses**

Iscove's Modified Dulbecco's Medium (IMDM) supplemented with 10% fetal bovine serum (Bodinco B.V., Alkmaar, the Netherlands), 2mM L-glutamine, 100 units/ml penicillin, 100 µg/ml streptomycin, and 5 x 10⁻⁵ M 2-mercaptoethanol was used as culture medium. Cultures were performed in triplicates in 96-well flat bottom plates
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Corning B.V. Life Sciences, Schiphol-Rijk, the Netherlands) in a humidified atmosphere of 5% CO₂ at 37°C. Functionality of the 5/4E8-TCR-transfected and cloned 58αβ cells was confirmed by IL-2 secretion in the presence or absence of hPG P70-5/4E8 and control peptides (20 µg/ml). Peptide-specific IL-2 secretion was determined by bioassay using CTLL-2 cells pulsed with supernatants from 24 h-cultured cells. Peripheral blood mononuclear cells (PBMC; 2 x 10⁴ cells per well) of the TCR-5/4E8-Tg founder were tested in a proliferation assay. Assays were done in the presence or absence of hPG P70-5/4E8 (20 µg/ml) and A20 (H-2³) cells as antigen presenting cells (APCs). An irrelevant peptide (GRVRVNSAYDKVSL; 20 µg/ml) was used as a negative control peptide for T cell proliferation. Proliferation was measured by adding 0.4 µCi of [³H]-thymidine (Amersham Biosciences Europe GmbH, Roosendaal, the Netherlands) to each well for the last 18 h. [³H]-thymidine uptake was measured using a liquid scintillation counter (Microbeta, Perkin-Elmer Inc., Boston, MA).

hPG isolation
Proteoglycan (aggrecan) was purified from human articular cartilage, removed during knee joint replacement surgery, by 4 M guanidinium chloride extraction as described (34,35). The use of human cartilage was approved by the Institutional Review Board (Rush University Medical Center, Chicago, USA), and animal protocols were approved by the Animal Experiment Committee of Utrecht University (Utrecht, the Netherlands).

Adoptive transfer of cells
C.129S6(B6)-Rag2tm1Fwa N12 mice backcrossed into BALB/c background (BALB/c.RAG2⁻/⁻; H-2³) were purchased from Taconic Farm (Doussard, France). All mice were 12-16 weeks old at the beginning of experiments. Cells for transfer were isolated from spleens of TCR-5/4E8-Tg, wild-type (littermate) BALB/c, or arthritic BALB/c mice immunized with hPG. Spleen cell suspensions were prepared, erythrocytes lysed, and cells were washed and suspended in phosphate buffered saline (PBS; 0.14 M NaCl in 0.01 M phosphate buffer, pH: 7.4), and then injected intraperitoneally (IP) into BALB/c.RAG2⁻/⁻ mice (2 x 10⁷ cells per mouse) together with 100 µg of hPG on days 0 and 43 as described for adoptive transfer of PGIA into BALB/c.SCID mice (36,37). Paws of mice were examined 3 times a week to record arthritic changes of the joints. The onset and severity of arthritis were determined using a visual scoring system based on swelling and redness of paws as described (35).

Histology
Joints of BALB/c.RAG2⁻/⁻ mice with adoptively transferred arthritis (received naïve TCR-5/4E8-Tg cells) and non-arthritic BALB/c.RAG2⁻/⁻ mice (received naïve wild-type cells) were fixed in 10% buffered formalin, decalcified in 0.5 M neutralized EDTA, embedded in paraffin and 6 µm sections were stained with hematoxylin and eosin.

Flow cytometric analysis
PBMCs, after erythrocyte lysis using FACS lysing solution (BD Biosciences Pharmingen, San Diego, CA), or popliteal lymph nodes, were washed with PBS containing 0.5% BSA and 0.01% sodium azide (FACS buffer), and then stained
with phycoerythrin (PE)-conjugated anti-V\(\beta4\), anti-CD8 or anti-CD25; FITC-conjugated anti-CD19; anti-CD44; or allophycocyanin (APC)-conjugated anti-CD4 antibodies; or relevant isotype control monoclonal antibodies (BD Biosciences Pharmingen, San Diego, CA) for 30 min on ice. Cells were analyzed using a flow cytometer (FACSCalibur) and Cell Quest software (BD Biosciences Immunocytometry, San Jose, CA).

**Statistical analysis**

Unless stated otherwise, data are expressed as mean ± standard deviation (SD). Statistical analyses were carried out using Student's t test or the Kruskal-Wallis test using Prism software and \(p<0.05\) value was considered statistically significant.
Results

Identification and cloning of the 5/4E8-TCR, and in vitro functional testing of the pTα 5/4E8-TCR and pTβ 5/4E8-TCR transgenic expression vectors

Using mouse Vα and Vβ primers, we first cloned and sequenced the 5/4E8-TCR variable (V), diversity (D) and joining (J) regions out of the mRNA. The 5/4E8-TCR α chain was closely homologous to mouse Vα1.1 and the Jα gene showed highest identity with a mouse TA31 gene. The 5/4E8 β chain was composed of Vβ4, Dβ2 and Jβ2.5 (21). These TCR sequences allowed us to generate new primers to obtain full length TCR variable regions, including the leader sequence and the intron for cloning into the TCR cassettes (32) (Fig. 1).

The pTα 5/4E8-TCR and pTβ 5/4E8-TCR constructs were first tested for the in vitro expression of the TCR chains using TCR-deficient (58αβ) T cell hybridoma (33). 5/4E8-TCR transfectant expression of the Vβ4 chain was detected by flow cytometry, and the expression of the functionally active TCR was demonstrated by using hPG P70-5/4E8 peptide-specific IL-2 production. Note, flow cytometric analysis of Vα1 chain was impossible due to lack of a specific monoclonal Vα1 antibody. Antigen-specific IL-2 secretion in response to specific (hPG P70-5/4E8) and not to non-specific peptides presented by A20 (H-2k) APCs was observed by a bioassay using CTLL-2 cells (Fig. 2).
Figure 2. In vitro functional analysis of the transgenic constructs (pTα 5/4E8-TCR and pTβ 5/4E8-TCR). TCR-negative 58αβ T cell hybridoma was co-transfected with both transgenic constructs and with pcDNA3 vector for positive selection as described in Materials and Methods. The peptide-specific T cell response (IL-2 production) was measured by CTLL-2 bioassay. Viable 58αβ T cells (1 x 10^5), transfected only with pTα 5/4E8-TCR construct and pcDNA3 vector (left-side columns), or simultaneously with both pTα and pTβ constructs (right-side columns), were co-cultured with 2 x 10^5 irradiated A20 APCs in the presence or absence of 20 µg/ml positive hPG P70-5/4E8 peptide (ATEGRVRVNSAYQDK) or negative control peptide (GRVRVNSAYGDKVSL). IL-2 was measured in 24 h-culture supernatants. The results are expressed as the mean (cpm) of triplicate wells ± SD, and significant difference (p < 0.0001) is indicated by an asterisk.

Generation of 5/4E8 αβ TCR+ transgenic (TCR-5/4E8-Tg) mice

Linearized α and β 5/4E8-TCR fragments were co-injected into the germline of cell-stage fertilized eggs of CBaxC57BL/6 F1 hybrids (H-2b) by microinjection. Transgenic founders were identified by PCR using genomic DNA (Table 1), and by flow cytometric analysis of the Vβ4 chain transgene expression of blood lymphocytes. Two αβ-positive founders and two β-positive founders were identified. A proliferation assay using PBMCs of αβ-positive TCR-5/4E8-Tg founder showed that the cells were functionally active and able to respond to the specific hPG P70-5/4E8 peptide (Fig. 3). As CD4+ hybridoma 5/4E8 was of BALB/c (H-2d) origin and MHC class II restricted, T cells of the TCR-5/4E8-Tg founder (H-2b) could respond to the specific hPG P70-5/4E8 peptide only in the presence of H-2d APCs (Fig. 3). Besides the alloreactive reaction, however, there was a significantly increased response to the hPG P70-5/4E8 peptide, which was antigen-specific because the TCR-5/4E8-Tg T cells failed to proliferate in response to a negative control peptide (Fig. 3). In contrast, hPG P70-5/4E8 peptide-specific response was not detected in cultures of PBMCs from naïve wild-type (littermate) mice. As soon as the TCR-5/4E8-Tg founders were identified, they were backcrossed into BALB/c background. Normal mating of transgenic males with BALB/c females was used for the first five backcrosses, and 5/4E8-TCR+ transgenic mice selected by PCR. Later, after selecting TCR+ Tg males, a marker assisted approach (18) was used to identify males with the most advanced BALB/c background, and these males were used for the next backcross. After 5 additional backcrosses, all TCR-5/4E8-Tg mice have a BALB/c background (>97%) as confirmed by 140 microsatellite markers (24,38). Throughout these backcrosses of TCR-5/4E8-Tg mice, co-expression of Vα1.1 and Vβ4 chains was always detected. Therefore, it is very likely that either both of the TCR transgenic constructs underwent homologous integrations in one of the transgenic lines or were incorporated together into the mouse genome and without segregation in the offspring.
To study whether PG-specific T cells were able to induce arthritis in syngeneic immunodeficient RAG2° mice, we have adoptively transferred TCR-5/4E8-Tg cells to BALB/c.RAG2-/- mice. Splenocytes of naïve TCR-5/4E8-Tg donor mice or wild-type littermate mice were injected IP together with hPG (without adjuvant) into syngeneic BALB/c.RAG2-/- recipient mice. Splenocytes from acutely arthritic BALB/c mice (PGIA), known to be capable of inducing arthritis in BALB/c.SCID mice, were used as positive control cells (36,39). A mild but progressive, eventually chronic arthritis with extensive ankylosis in peripheral joints developed in mice injected with spleen cells of naïve TCR-5/4E8-Tg cells. The clinical symptoms (redness and swelling) were comparable to arthritic joints of mice injected with cells from acutely arthritic BALB/c mice with PGIA. However, the onset of arthritis was delayed 2-3 weeks in BALB/c.RAG2-/- mice injected with naïve TCR-5/4E8-Tg cells, when compared to those that received spleen cells from arthritic BALB/c mice. None of the BALB/c.RAG2° mice injected with wild-type cells developed arthritis. Figure 4 summarizes these results showing the maximum arthritis scores in BALB/c.RAG2° mice with transferred spleen cells. The mean arthritis score was identical in BALB/c.RAG2° mice that received naïve (non-immunized) TCR-5/4E8-Tg spleen cells and those that received spleen cells from arthritic BALB/c mice (Fig. 4).

**Figure 3.** In vitro proliferation of TCR-5/4E8-Tg (Tg) founder cells. PBMCs (4 x 10⁴ cells/well) of TCR-5/4E8-Tg founder and wild-type (Wt) littermates were cultured in the presence or absence of 1 x 10⁶ irradiated A20 APCs/well without or with 20 µg/ml positive (hPG P70-5/4E8) or 20 µg/ml negative control peptides as indicated. The sequences of negative and positive peptides are described in Figure 2 legend. As the PBMCs were isolated from TCR-5/4E8-Tg founder and wild-type littermate mice, both carrying the H-2b allele, a relatively high background (alloreactive reaction) can be detected in the presence of A20 APCs carrying H-2b allele. The results are expressed as the mean (cpm) of triplicate wells ± SD, and the level of significance (p < 0.01) is indicated by an asterisk.

**Figure 4.** The maximum (highest) arthritis scores after adoptive transfer. Spleen cells from naïve (non-immunized) TCR-5/4E8-Tg mice, wild-type (Wt) littermates, or BALB/c mice with PGIA (positive control) were transferred into BALB/c.RAG2° mice. Freshly isolated 2 x 10⁷ donor spleen cells/recipient with 100 µg of hPG protein were injected intraperitoneally as described earlier for adoptive transfer into BALB/c.SCID mice (36,37). BALB/c.RAG2° mice received cells and hPG on days 0 and 43. Arthritis is depicted as the highest score per mouse at any time during disease. Statistically significant differences (p < 0.05) are indicated (Kruskal-Wallis Test).
The clinical appearance and histopathological features of arthritis were similar in both groups, and identical with the primary form of PGIA in BALB/c mice (16,34). Joint inflammation was progressive and characterized by intermittent spontaneous exacerbations and remissions resulting in extensive cartilage damage and bone erosions, leading to ankylosis and deformities of the peripheral joints (Fig. 5). The synovium was hyperplastic with massive cellular infiltration. In summary, in vivo activation of arthritogenic epitope-specific 5/4E8-TCR expressed by CD4+ cells seems to be sufficient to induce arthritis in syngeneic immunodeficient animals.

**Figure 5.** Histology of tarso-metatarsal joints of BALB/c.RAG2−/− mice transferred with Wt cells (A) or 5/4E8-TCR Tg cells (B). BALB/c.RAG2−/− recipient mice received twice 2 x 10⁷ spleen cells (on days 0 and 43) from naïve (non-immunized) Wt or naïve TCR-5/4E8-Tg mice. BALB/c.RAG2−/− mice were sacrificed on day 70 after the first cell transfer. Massive synovial hyperplasia with infiltrating mononuclear cells, and cartilage (arrow heads) and bone erosions (arrows) can be seen in mice that received naïve T cells from TCR-5/4E8-Tg mice. Sections were stained with hematoxylin and eosin.

**Characterization of transferred cells in BALB/c.RAG2−/− mice**

The cell recovery and reconstitution of (auto)immune homeostasis in BALB/c.RAG2−/− mice after adoptive transfer using naïve TCR-5/4E8-Tg or wild-type spleen cells was analyzed by flow cytometry. The ratios of CD4+Vβ4+ donor T cells from wild-type and TCR-5/4E8-Tg mice are shown in Figure 6A. On days 41 and 70, cells were isolated from peripheral blood from BALB/c.RAG2−/− mice and analyzed for CD4/CD19 and CD4/TCR Vβ4 surface expression (Figs. 6B and 6C). On day 70, popliteal lymph node (PLN) cells were also analyzed for T and B cell reconstitution, and T cell activation (Fig. 6D). In BALB/c.RAG2−/− mice, which received TCR-5/4E8-Tg cells, the CD4+Vβ4+ cells were detectable and expanded significantly accordingly to the progression of arthritis. The number of CD19+ B cells in peripheral blood was undetectable (Fig. 6B) after the first transfer with TCR-5/4E8-Tg T cells, but increased after the second transfer (Fig. 6C), and transferred cells migrated to the PLN. The T/B cell ratio in the joint-draining PLNs was comparable in BALB/c.RAG2−/− mice having either transferred TCR-5/4E8-Tg or wild-type cells, but the number of CD4+Vβ4+ T cells was significantly higher in arthritic animals (Fig. 6D). Moreover, these CD4+Vβ4+ T cells seemed to be activated in the PLNs of arthritic animals as shown by the expression of high levels of CD25 and CD44, whereas the expression of these activation markers were low on the CD4+ PLN cells of animals that received wild-type cells. In summary, PG-specific TCR-5/4E8-Tg cells expanded and became activated in joint-draining PLNs of BALB/c.RAG2−/− mice with arthritis.
Figure 6. Flow cytometric analysis of lymphocytes repopulating BALB/c.RAG2−/− mice after adoptive transfer. Freshly isolated spleen cells from naïve TCR-5/4E8-Tg mice (2 x 10⁷ cells/recipient), their wild-type (Wt) littermates, and from BALB/c mice immunized with cartilage PG for PGIA (positive control group) along with 100 µg of hPG protein were injected IP into BALB/c.RAG2−/− mice on days 0 and 43. Donor spleen cells were analyzed prior to the transfer (A), and then cells were isolated from peripheral blood on day 41 (B) and day 70 (C). The bottom panel shows the flow cytometry results of popliteal lymph node cells on day 70 (D). The percentage of cells per quadrant is indicated, and the numbers within brackets the percentage of CD4+ T cells.
Discussion

We have generated TCR $\alpha\beta$ Tg mice with $V_{\alpha}1.1$ and $V_{\beta}4$ TCR chains obtained from T cell hybridoma 5/4E8 with MHC restricted specificity for hPG (consensus sequence of $^{73}$GRVRVNSAY). In these TCR-5/4E8-Tg mice the transgenic T cells appear to have been positively selected in the thymus and exported to the periphery. Several observations provide compelling evidence that “arthritogenic” transgene-encoded $\alpha$ and $\beta$ chains were co-expressed on the surface of a significant number of transgenic peripheral CD4$^+$ T cells. First, PCR of genomic DNA revealed only a product when both the $V_{\alpha}1.1$ transgene and $V_{\beta}4$ transgene were in the genome. Second, specific in vitro T cell proliferation to hPG P70-5/4E8 peptide in the presence of H-2d APCs without priming provided further evidence that functional 5/4E8 TCR had been generated in the transgenic mice (Fig. 3). This also showed that, despite the fact that hPG-specific TCR-5/4E8-Tg T cells also recognize the homologous mouse sequence and could therefore cross react with self PG, TCR-5/4E8-Tg T cells in the transgenic mice were not tolerized in the periphery by either clonal deletion or anergy. This is consistent with other reports, showing that potentially self-reactive T cells exist in the peripheral T cell pool. Osman et al. showed in type II collagen (CII)-TCR-Tg mice that peripheral T cells were not tolerant to CII, and responded to CII stimulation in vitro (40). Finally, adoptive transfer of naive TCR-5/4E8-Tg donor cells, given together with hPG, could induce arthritis in syngeneic BALB/c.RAG2-/- recipient mice (Fig. 4), which never occurred after the transfer of wild-type cells.

The clinical appearance of arthritis in BALB/c.RAG2-/- mice was similar to the primary form of PGIA in BALB/c mice, and to the arthritis in BALB/c.SCID mice adoptively transferred with acutely arthritic BALB/c spleen cells (36,37,39). Inflamed joints of BALB/c.RAG2-/- mice transferred with TCR-5/4E8-Tg cells showed extensive cartilage degradation and bone erosions (Fig. 5) similar to that seen in arthritic joints of human patients with RA. Because this arthritis transfer model makes use of naive TCR-5/4E8-Tg cells with one defined specificity, it provides a straightforward arthritis model for studying the role of joint antigen-specific T cells in arthritis.

Our analysis of the recovered TCR-5/4E8-Tg cells after the adoptive transfer in BALB/c.RAG2-/- mice showed a repopulation and a marked increase of CD4$^+$ transgenic T cells (Fig. 6B and C). Repopulation and T cell activation (Fig. 6D) coincided with arthritis induction. The process responsible for focusing autoimmune destruction to the joints in BALB/c.RAG2-/- mice that received TCR-5/4E8-Tg cells seems to be the presence of the large number of arthritogenic joint-specific T cells with a high expression level of joint (cartilage PG)-specific TCRs. These data clearly underline the critical function of CD4$^+$ T cells in arthritis (41). However, previously it has been shown that there is also a role for antibodies to mouse (self) PG, and/or B cells in the pathogenesis of PGIA (19,29). PG-specific B cells appeared to play a major role in antigen presentation (29). Nevertheless, neither anti-PG Abs nor PG-specific B cells alone were able to induce arthritis (19,42). An antigen-specific T cell population seems to play a central role in the mechanism of murine PGIA. Most likely, highly specific cooperation between antigen primed CD4$^+$ Th1 and B cells is needed for the development of the disease.

As the PG-specific T cell response is rather confined to the joint-draining lymph nodes (43), it is very likely that an autoantigen-driven mechanism of joint inflammation becomes local and self-sustaining by PG (cartilage) degradation. Autoreactive T cells can migrate to, and proliferate in, the synovium and joint draining lymph nodes (42), where the self-peptides are present in relatively high concentrations as a result of the normal turnover of the cartilage matrix.
and, even more, as a consequence of increased PG degradation in inflammatory joint diseases. There is growing
evidence that at least a subset of patients with RA exhibits antigen-specific T cell responses to hPG (25-28,44-47).
Moreover, the core protein of human/mouse cartilage PG has been mapped in HLA-DR4- and HLA-DQ8-humanized
transgenic mice (24)
This is the first report of a TCR transgenic mouse generated to a hPG epitope having a dominant/arthritogenic
function in BALB/c mice. CD4+ T cells harboring TCR αβ transgenes comprised the vast majority of peripheral CD4+
T cells. The finding that naïve TCR-5/4E8-Tg mice enabled the transfer of arthritis to BALB/c.RAG2-/- recipient mice
is direct evidence that circulating PG-specific T cells can be activated in vivo, and these cells may migrate to joint-
draining lymph nodes and synovial joints. These TCR-5/4E8-Tg mice offer a valuable tool for investigating the role of
antigen specific T cells in the development of autoimmune arthritis and studying immunopathogenic and modulatory
mechanisms of RA.

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