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*J Immunol* 2004; 173:151-156; ;  
doi: 10.4049/jimmunol.173.1.151  
<http://www.jimmunol.org/content/173/1/151>

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The American Association of Immunologists, Inc.,  
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Print ISSN: 0022-1767 Online ISSN: 1550-6606.



# Inducing Experimental Arthritis and Breaking Self-Tolerance to Joint-Specific Antigens with Trackable, Ovalbumin-Specific T Cells<sup>1</sup>

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**The importance of T cell Ag specificity and Th1 vs Th2 phenotype in synovial inflammation remains controversial. Using OVA-specific TCR transgenic T cells from DO11.10 mice, we demonstrate that mice receiving Th1, but not Th2, cells display a transient arthritis following immunization that is characterized by synovial hyperplasia, cellular infiltration, and cartilage erosion. OVA-specific T cells also accumulated in inflamed joints, suggesting that they could exert their inflammatory effect locally in the joint or in the draining lymph node. Importantly, this pathology was accompanied by a breakdown in self-tolerance, as evidenced by the induction of collagen-specific T and B cell responses. This model directly demonstrates a pivotal role for Th1 cells of an irrelevant specificity in the development of inflammatory arthritis. Furthermore, the ability to track these cells in vivo will make feasible studies revealing the dynamic role of T cells in arthritis.** *The Journal of Immunology*, 2004, 173: 151–156.

**R**heumatoid arthritis (RA)<sup>3</sup> is a chronic inflammatory autoimmune disease associated with destruction of cartilage and underlying bone in the joints. The processes that initiate and perpetuate RA are currently unclear, and, in particular, the specificity and phenotype of the T lymphocytes contributing to synovial inflammation remain controversial (1, 2). T cells activated by Ags, by cytokines, or by contact between extracellular matrix and endothelium can induce the production of cytokines, matrix metalloproteases, and PGs by macrophages and fibroblast-like synoviocytes through cell-cell interactions within the synovial membrane. These products may potentially act in positive feedback loops, leading to articular damage (1). In this context, the role of dendritic cells (DCs) may be particularly important because of their unique ability to activate naive T cells (3). Indeed, Leung et al. (4) recently demonstrated that the transfer of collagen-pulsed mature DCs is sufficient to induce arthritis in DBA/1 mice. However, the absence of a suitable system to track the interaction between DC and Ag-specific T cells, which may govern the pathogenesis of RA, has hindered the examination of both of these cell types in vivo and prevented us from addressing the crucial question of whether the Ag specificity of the T cells involved is important. Recently, a number of groups have developed models based upon the adoptive transfer of TCR transgenic (Tg) T cells

specific for model Ags to track such interactions in vivo. These systems have been used to investigate a number of models of disease, such as inflammatory bowel disease (5). However, they have never been applied to RA. This may be particularly important in RA, in which the requirement for joint-specific T cells in the initiation of disease is controversial and the outcome may be related to the Th1 or Th2 phenotype of T cell involved, both of which may be a consequence of the type and location of Ag presentation.

Several authors have recognized distinct roles for Th1 and Th2 responses in several autoimmune models, including collagen-induced arthritis and NOD mice (6–8). Reversal of such T cell polarization confers therapeutic advantage, as immunopathology can be ameliorated by administration of cytokines of reciprocal activity (7). Thus, development of an arthritis model in which the precise development, migration, Ag specificity, and contribution to pathogenesis of Th1 and Th2 cells could be examined would therefore be of considerable value in understanding the disease process and evaluating novel therapeutics. Furthermore, it remains unclear whether the T cell-APC interactions, which probably regulate RA, require a specificity related to the joint, occur locally in the joint, in the draining lymph node (LN), or both. Therefore, in this study, we have transferred Th1- or Th2-polarized, Ag-specific TCR Tg T cells into naive recipients to examine their ability to induce arthritis. We demonstrate for the first time that, following immunization, Th1, but not Th2, cells are able to induce a transient arthritis in BALB/c mice, which is also associated with a loss of self-tolerance to joint-specific Ags. Furthermore, these cells appear to do this in a bystander manner, as their specificity is unrelated to joint Ags.

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Received for publication February 6, 2004. Accepted for publication April 29, 2004.

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<sup>1</sup> This study was supported by grants from the Wellcome Trust and Biotechnology and Biological Sciences Research Council awarded to P.G., J.M.B., and I.B.M.

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<sup>3</sup> Abbreviations used in this paper: RA, rheumatoid arthritis; CII, collagen II; DC, dendritic cell; HAO, heat-aggregated OVA; LN, lymph node; OPGL, osteoprotegerin ligand; Tg, transgenic.

## Materials and Methods

### Animals

Six- to 8-wk-old male BALB/c mice purchased from Harlan Olac (Bicester, U.K.) were used as adoptive transfer recipients. DO11.10 BALB/c TCR Tg mice (9) contain CD4<sup>+</sup> T cells expressing a TCR that recognizes the chicken OVA peptide 323–339 complexed with the MHC class II molecule I-A<sup>d</sup> (detected by the clonotypic mAb KJ1.26). Mice were housed at Glasgow Royal Infirmary, University of Glasgow, and we performed all procedures according to U.K. Home Office regulations.

### Flow cytometry

We pooled peripheral LNs (axillary, inguinal, cervical) and mesenteric LNs from DO11.10 mice and forced through Nitex mesh (Cadisch Precision Meshes, London, U.K.) using a syringe plunger. We stained aliquots of these cells with PE-labeled anti-CD4 mAb CD4 (L3T4) (RM4-5) (BD Pharmingen, San Diego, CA) and biotinylated KJ1.26 mAb, followed by FITC-labeled streptavidin (BD Pharmingen) for the detection of DO11.10 T cells. We analyzed stained cells (20,000 per sample) with flow cytometry using CellQuest software (BD Biosciences, Oxford, U.K.).

### T cell purification and T cell cultures

We purified naive CD4<sup>+</sup> T cells from pooled LN cells from DO11.10 mice by negative selection. We removed B cells, monocytes, and CD8<sup>+</sup> T cells using anti-CD19, anti-CD11c, and anti-CD8 mAbs (all Serotec, Oxford, U.K.), respectively, which were then bound by anti-IgG MACS beads (Miltenyi Biotec, Auburn, CA), according to manufacturer's instructions. We obtained APCs irradiating DO11.10 spleen cells (2000 rad). We induced T cell differentiation culturing  $2 \times 10^5$ /ml CD4<sup>+</sup> T cells with  $2 \times 10^6$ /ml APC and 0.3  $\mu$ M OVA<sub>323-339</sub> (Genosys, Cambridge, U.K.). In addition, Th1 cell cultures included 5 ng/ml IL-12 (PeproTech, Rocky Hill, NJ) and 10  $\mu$ g/ml anti-IL-4 mAb (R&D Systems, Minneapolis, MN), whereas Th2-polarizing cultures incorporated 5 ng/ml IL-4 (R&D Systems) and 1/500 anti-IL-12 (R&D Systems), as described previously (10). After 3 days of culture, we washed and harvested the cells for transfer.

### Adoptive transfer

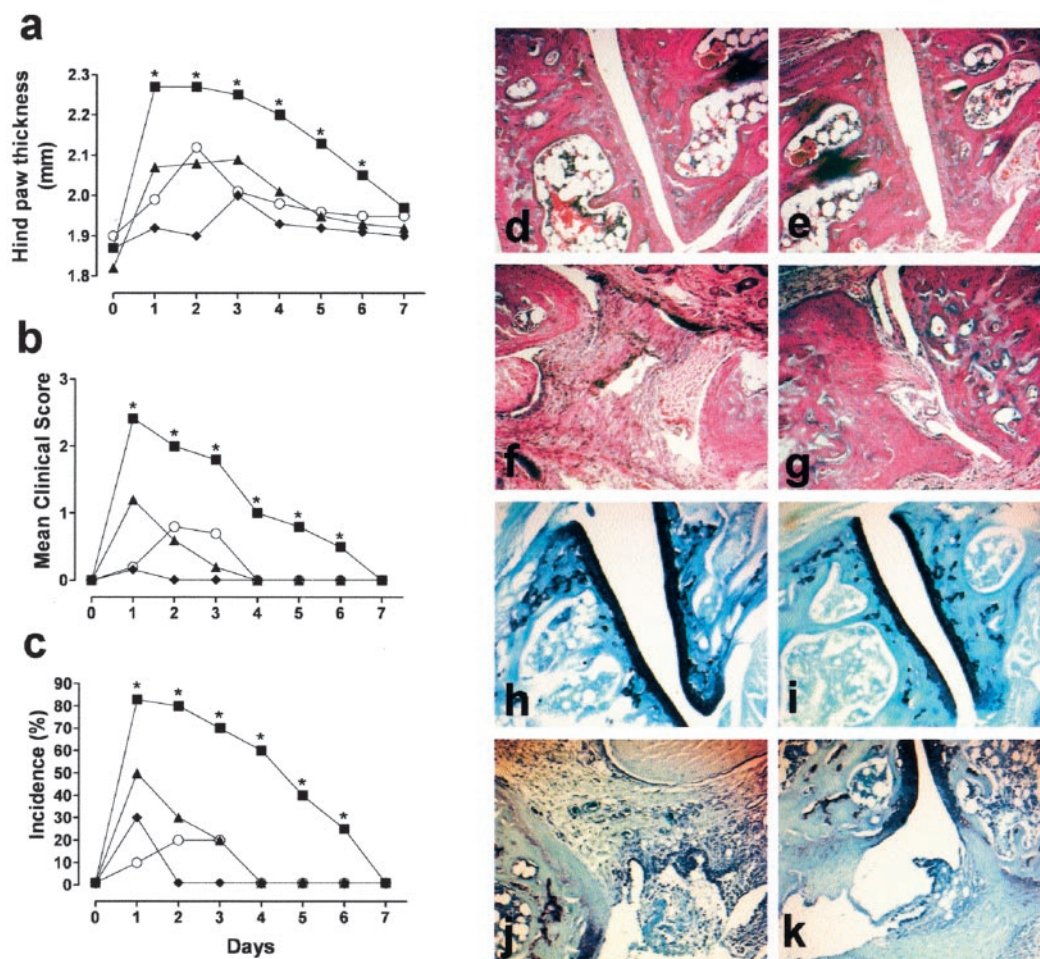
A total of  $2 \times 10^6$  naive, Th1, or Th2 DO11.10 T cells were injected i.v. into BALB/c recipients, as described previously (10). Control mice received PBS only.

### Ag administration

One day following adoptive transfer, recipients were immunized s.c. with 100  $\mu$ g of OVA in CFA (Sigma-Aldrich, Poole, U.K.). We prepared heat-aggregated OVA (HAO), as described previously (11).

### Assessment of arthritis

Ten days after challenge with OVA in CFA, all animals ( $n = 10$  per group) were injected s.c. close to both ankle joints with 100  $\mu$ g of HAO in 50  $\mu$ l of saline. Some control mice received a periarticular injection of PBS instead of HAO (sham controls). We monitored mice subsequently for signs of arthritis, as described previously (12). Disease was scored based on erythema, swelling, or loss of function present in each paw on a scale of 0–3, giving a maximum score of 6 per mouse. We measured paw thickness with a dial caliper (Kroepelin, Munich, Germany). For histological assessment, mice were sacrificed, hind limbs were removed and fixed in 10% neutral-buffered formalin, and 6- $\mu$ m sections were stained with H&E or toluidine blue (both from Sigma-Aldrich).



**FIGURE 1.** Arthritis in recipient mice. Hind paw thickness (a), mean clinical score (b), and incidence (c) of inflammatory arthritis in BALB/c mice receiving PBS (○), unpolarized T cells (naive (◆)), Th1 cells (■), or Th2 cells (▲). Th1 recipient mice developed arthritis of greater severity than PBS, naive, and Th2 recipient mice. The disease was prolonged in Th1-transferred animals. Data represent mean  $\pm$  SEM ( $n = 10$ ). Similar results were obtained in two additional experiments. \*,  $p < 0.05$  (Th1 vs Th2). Histological analysis: staining H&E (d–g); toluidine blue (h–k). Ankle joints of Th1 recipient mice demonstrated extensive synovial hyperplasia, inflammatory cell infiltration (f), and cartilage and bone erosion (j). Th2 recipients developed a less severe inflammation compared with the Th1 recipients (g and k). PBS recipient mice and naive mice revealed no apparent inflammatory cell involvement (d and e) and a normal cartilage integrity (h and i). Original magnification,  $\times 40$ .

### Restimulation of draining LN cells in vitro

At the conclusion of in vivo experiments (day 7), draining LN cells were restimulated with either medium alone, 1 mg/ml OVA, or 50  $\mu$ g/ml collagen II (CII) for proliferation and cytokine production, as described previously (4, 10–13).

### ELISA

Cytokines, anti-OVA, and anti-collagen Ab levels were detected by ELISA, as described previously (4, 10–13).

### Immunohistochemistry

Draining LNs were snap frozen in liquid nitrogen in OCT embedding medium (Miles Diagnostic Division, Elkart, IN) and stored at  $-70^{\circ}\text{C}$ . Six- to 10- $\mu\text{m}$  joint sections were cut from hind limbs decalcified in a 5.5% EDTA solution in a phosphate buffer, pH 7.4, for 2 wk.

### Single staining for the DO11.10 TCR

DO11.10 T cells were detected in tissue sections with the clonotypic mAb KJ1.26, as described previously (13).

### Statistics

Statistical analysis was done using Minitab software (Minitab, State College, PA), and results were compared using Student's unpaired *t* test. We applied Bonferroni's correction for multiple comparisons, as appropriate. A *p* value of  $<0.05$  was regarded as significant.

## Results

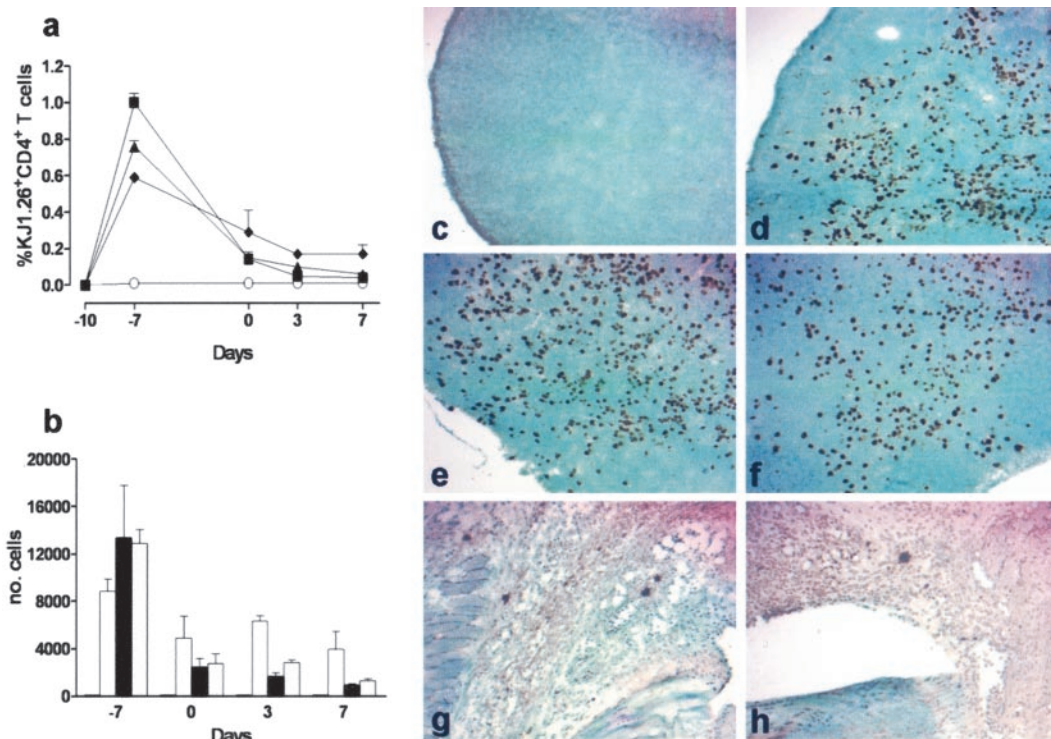
### Phenotype of polarized cells

The cytokine profiles of aliquots of the in vitro polarized T cells were determined by ELISA. Th1 cells produced high levels of IFN- $\gamma$  ( $9213 \pm 1040$  pg/ml;  $p < 0.001$ , Th1 vs Th2) and little IL-5 ( $66 \pm 8$  pg/ml), whereas Th2-polarized cells produced significant amounts of IL-5 ( $5000 \pm 100$  pg/ml;  $p < 0.001$ , Th2 vs Th1) and

low levels of IFN- $\gamma$  ( $145 \pm 7.8$  pg/ml). Therefore, after polarization under the appropriate conditions in vitro, DO11.10 T cells adopted the expected Th1 and Th2 phenotypes, as described previously (10).

### Arthritis in recipient mice

We immunized control and cell-recipient mice, 24 h after the transfer, with OVA/CFA, and subsequently challenged them by periarticular injection in right and left hind ankle joints with HAO 10 days later. The subsequent development of inflammatory arthritis was monitored by paw swelling (Fig. 1*a*), severity (mean clinical score; Fig. 1*b*), and incidence (Fig. 1*c*). Transfer of Th1 cells into recipient mice caused the induction of arthritis, first evident at day 1 postimmunization with HAO. In contrast, control mice, mice receiving unpolarized or Th2 cells, developed only low levels of inflammation. In addition, the disease was prolonged in Th1-transferred animals. Finally, Th1 mice displayed a significantly greater clinical score and paw swelling compared with Th2 mice. Mice that received a periarticular injection of PBS rather than HAO (sham controls) failed to develop inflammation (data not shown). We performed sequential histological analyses (Fig. 1, *d–k*). Control mice and mice receiving unpolarized cells exhibited no apparent local inflammatory reaction (Fig. 1, *d* and *e*) and normal cartilage integrity (Fig. 1, *h* and *i*). Th1-transferred mice exhibited extensive synovial hyperplasia and inflammatory cell infiltration (Fig. 1*f*) in the involved joint. We observed partial or complete erosion of the cartilage matrix in both central and peripheral regions of joint surfaces, as assessed by loss of toluidine blue staining (Fig. 1*j*). Appearances resembled those of CFA-induced collagen-induced arthritis (14) and RA synovial tissue (15). Th2-transferred mice exhibited a less severe inflammation than the



**FIGURE 2.** Ag-specific T cells in vivo. *a*, Percentage of CD4<sup>+</sup>KJ1.26<sup>+</sup> T cells in the draining LNs of mice receiving PBS (○), unpolarized T cells (◆), Th1 (■), or Th2 cells (▲) assessed by flow cytometry. *b*, Number of TCR Tg T cells in popliteal LNs. Mice receiving PBS, unpolarized T cells (□), Th1 (■), or Th2 cells (▨). Each time point represents the mean  $\pm$  SEM for at least three mice per group. Similar results were obtained in two additional experiments. *c–h*, Immunohistochemistry. *c–f*, Draining LNs at day 7 from mice receiving PBS (*c*), unpolarized T cells (*d*), Th1 (*e*), or Th2 cells (*f*). KJ1.26<sup>+</sup> T cells (brown) had expanded above control level in all the transferred groups. Transferred T cells observed in the inflamed joint of Th1 recipient mice from the beginning of the pathology (*g*, day 1; *h*, day 7). Original magnification,  $\times 40$ .

Th1-transferred group, with a modest reduction of toluidine blue staining only in peripheral cartilage regions that were in direct contact with inflamed synovial tissue (Fig. 1, g and k).

#### Clonal expansion of Tg T cells in vivo

We injected BALB/c mice with  $2 \times 10^6$  CD4<sup>+</sup>KJ1.26<sup>+</sup> unpolarized (naive), Th1, Th2 T cells or PBS on day -11. On day -10, mice were immunized s.c. with 100  $\mu$ g of OVA/CFA. On day 0, mice were challenged with 100  $\mu$ g of HAO close to both ankle joints. Draining LNs were removed on days -7, 0, 3, and 7 for flow cytometric analysis. Th1, Th2, and unpolarized Tg T cells were detected immediately ex vivo, indicating that TCR Tg T cells can survive and respond to Ag in vivo (Fig. 2a). In agreement with our previous studies (10), we observed peak clonal expansion on day 3 after immunization, which declined thereafter. Th1 and Th2 cells expanded to similar levels, whereas unpolarized cells proliferated to a lesser extent. The number of TCR Tg T cells in the popliteal LN at different time points is shown in Fig. 2b.

#### Localization of Tg T cells by immunohistochemistry

Immunohistochemical analysis of draining LNs confirmed that the population of KJ1.26<sup>+</sup> T cells had expanded above control levels in all transferred groups (Fig. 2, c-f). This expansion was highest at day -7 and declined thereafter.

Using immunohistochemistry, we directly examined the localization of KJ1.26<sup>+</sup> T cells in the inflamed paw. We have been unable to find Tg T cells in the joints of mice transferred with unpolarized T cells. Moreover, we only ever observed Tg T cells in the joint of one Th2-transferred mice at day 1 (data not shown). Interestingly, we observed transferred T cells in the inflamed joint of Th1-transferred mice from the beginning (Fig. 2g, day 1) until the end (Fig. 2h, day 7) of the pathology. These results suggest that the transferred Th1 cells could exert their inflammatory effect in the joint.

#### Cytokine profile and cellular proliferation of OVA-specific cells ex vivo

LNs were removed from control and transferred mice 7 days post-challenge with HAO and cultured in vitro with or without 1 mg/ml OVA or with 50  $\mu$ g/ml CII, and their cytokine production profile and proliferation were determined. OVA<sub>323-339</sub> peptide-specific T cells from animals receiving Th1-polarized cells and OVA/CFA continued to produce IFN- $\gamma$  (Fig. 3a), but little IL-5 (Fig. 3b) after stimulation with OVA or CII, whereas peptide-specific T cells from animals transferred with Th2-polarized cells and immunized with OVA/CFA produced IL-5 (Fig. 3b), but little IFN- $\gamma$  (Fig. 3a). Cells from control mice and T cells from animals receiving unpolarized cells produced little cytokine of any description when restimulated in vitro with CII (Fig. 3, a and b). Little or no cytokine production was apparent in the absence of stimulation (Fig. 3, a and b). TNF- $\alpha$  and IL-6 were not detectable in the culture supernatant of all of the groups of mice.

OVA<sub>323-339</sub> peptide-specific T cells from animals receiving Th1- or Th2-polarized cells and OVA/CFA proliferated to similar levels after in vitro OVA stimulation, whereas cells from control mice and T cells from animals receiving unpolarized cells proliferated to a lesser extent (Fig. 3c). Little proliferation was apparent in the absence of stimulation in all the groups, except Th2 recipient group (Fig. 3c). Interestingly, only draining LN cells from OVA-specific Th1 and Th2 recipient mice proliferated when cultured in vitro with CII (Fig. 3c).

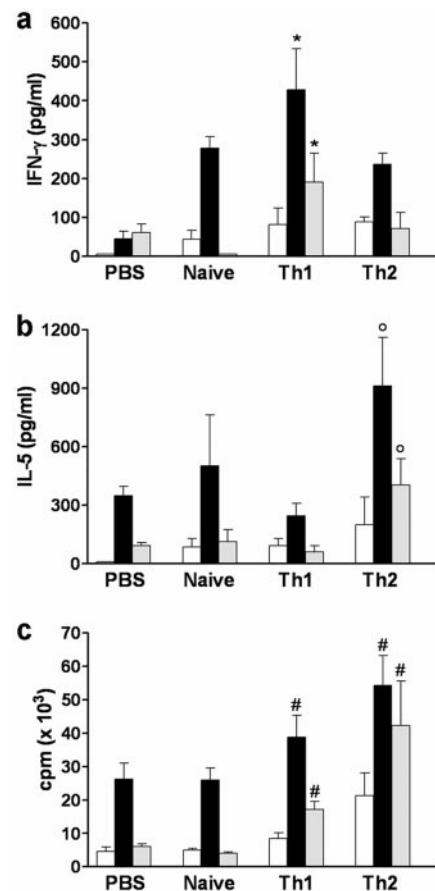
#### Anti-OVA and anti-collagen Ab production in vivo

We took serum from animals transferred with unpolarized, Th1, Th2 cells or PBS at day 7. As expected, all of the animals were able to produce high levels of anti-OVA Abs. IgG1 (Fig. 4a) and IgG2a (Fig. 4b) Abs were higher in the Th1 recipient group compared with the Th2 group. Lower level of anti-OVA IgG2a Abs was detectable in serum from mice receiving unpolarized Tg T cells or PBS.

Finally, we sought evidence for anti-collagen Ab production at day 7. IgG1 Abs (Fig. 4c) were higher in the Th2 recipient group compared with the Th1 recipient group. Collagen-specific IgG2a Abs (Fig. 4d), which are typically produced during a Th1 response, were higher in the Th1 recipient group compared with the Th2 group. We detected little anti-collagen IgG1 Abs and no IgG2a Abs in serum from mice receiving unpolarized Tg T cells or PBS.

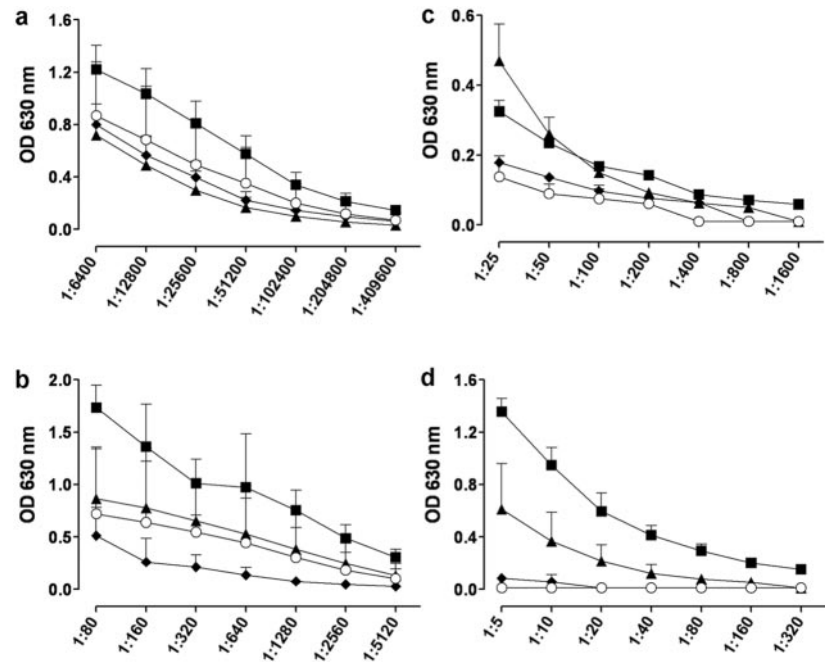
#### Discussion

A number of animal models of RA have been developed to characterize the cell types and mechanisms involved in the pathogenesis of RA, with the ultimate objective of therapeutic intervention in the disease process (16). The system described in this study, based upon the adoptive transfer of TCR Tg T cells, has allowed us to track, in detail, the ability of Th1 and Th2 cells of known Ag



**FIGURE 3.** Cytokine profile and cellular proliferation of OVA-specific cells ex vivo. LN cells (three mice per group) were removed at day 7 and cultured with medium alone (□), OVA (■), or CII (▨). a and b, Cytokine levels in the culture supernatant at 72 h were measured by ELISA. c, [<sup>3</sup>H]Thymidine incorporation measured at 96 h. Data are the mean  $\pm$  SEM of triplicate cultures. \*,  $p < 0.05$  (Th1 vs Th2); <sup>o</sup>,  $p < 0.05$  (Th2 vs Th1); #,  $p < 0.05$  (Th1/2 vs PBS/naive). Similar results were obtained in two additional experiments.

**FIGURE 4.** Serum anti-OVA and anti-CII Ab titration. Anti-OVA IgG1 (a), IgG2a (b), and anti-collagen IgG1 (c) and IgG2a (d) Ab levels were measured on day 7 by ELISA. Mice receiving PBS (○), unpolarized T cells (◇), Th1 (■), or Th2 cells (▲). Results represent three animals per group and are presented as the mean OD  $\pm$  SEM. Similar results were obtained in two additional experiments.



specificity to induce arthritis. Not only could this have implications for a variety of therapeutic approaches, but it also provides an ideal system for studying the mechanisms and cellular interactions underlying RA induction in vivo.

We believe that tissue-infiltrating T lymphocytes play a pivotal role in the inflammatory response in RA (17, 18). The abundance of T cells, in RA synovium, expressing differentiation and activation markers supports this concept (18). This evidence suggests that Ag-specific T lymphocytes act as initiators of the inflammatory process and that T cell-derived cytokines play a major role in maintaining inflammation and in mediating matrix destruction of the synovium (17–19). Moreover, T cells can also directly trigger osteoclastogenesis through the TNF family molecule osteoprotegerin ligand (OPGL), which regulates bone loss, LN organogenesis, lymphocyte development, and interaction between T cells and DCs in the immune system (20, 21). Thus, in a model of rat adjuvant arthritis, systemic activation of T cells in vivo leads to an OPGL-mediated increase in osteoclastogenesis and bone loss, and blocking OPGL through treatment with its decoy receptor osteoprotegerin prevents bone and cartilage destruction (22). However, the Ag specificity of the T cells that initiate and maintain this pathology remains unclear.

Murine and human T lymphocytes may be identified as Th1 or Th2 cells depending on their cytokine production profile. The balance between these subsets strongly influences many inflammatory responses, although this may also be influenced by regulatory subsets (23). The Th1/Th2 imbalance in RA joints is associated with high numbers of activated macrophages, leading to an aggressive form of arthritis with rapidly occurring joint destruction (24). Moreover, the demonstration that RA synovial T cells produce cytokines with anti-inflammatory properties confirms the hypothesis that the course of RA synovitis may be the expression of a balance between pro-(Th1) and anti-(Th2) inflammatory cytokines (2).

We have developed an adoptive transfer system in the mouse that has allowed us to further characterize and localize the Th cells involved in the induction of arthritis. In our current study, we stably polarized naive TCR Tg T cells toward a Th1 or Th2 phenotype in vitro before adoptive transfer. Our transferred T cells

secreted the expected pattern of cytokines (IFN- $\gamma$  for Th1 and IL-5 for Th2) before adoptive transfer and maintained their phenotypes, over the course of the experiment, following restimulation after recovery from immunized animals, as we have described previously (10).

We have demonstrated directly in vivo that transfer of Th1, and not Th2, cells into recipient mice caused the induction of a transient arthritis, first evident at day 1 postchallenge with HAO. Th1-transferred mice exhibited extensive synovial hyperplasia, inflammatory cell infiltration, and cartilage and bone erosion in the involved joint.

FACS analysis revealed Ag-specific T cell expansion in the draining LN, which peaked 3 days after initial Ag exposure, with naive, Th1, and Th2 cells showing similar kinetics of clonal expansion. Immunohistochemistry confirmed that Tg T cell accumulation peaked in the draining LN 3 days after initial immunization with OVA/CFA and declined thereafter, returning to a very low level even after challenge with HAO. Despite this, we could observe accumulation of the transferred OVA-specific T cells in the inflamed joint from the beginning of the pathology, suggesting that the transferred T cells, which expanded in the LN, could exert their inflammatory effect directly or indirectly in the joints where the Ag is present, or possibly in the draining LNs where Ag presentation occurs. Interestingly, Setoguchi et al. (25) recently demonstrated that adoptive transfer of IL-10-transduced DO11.10 splenocytes ameliorated OVA-induced arthritis, with a similar accumulation of the transferred Tg T cells in the inflamed joint.

As expected at the end of the experimental procedure, serum from all of the immunized animals contained high levels of anti-OVA IgG1 and IgG2a Abs. Surprisingly and importantly, only Th1 and Th2 recipient groups were able to produce anti-CII Abs, with the Th1 recipients producing higher levels of anti-CII IgG2a Abs at the end of the transient arthritis. Moreover, only draining LN cells from Th1 and Th2 recipient mice proliferated and produced cytokines in response to CII. Thus, we have been able to initiate arthritis and break self-tolerance to a joint-specific Ag with T cells of a specificity entirely unrelated to the joint. This finding has considerable implications for the way we think about the

pathogenesis and therapy of RA and autoimmunity. We hypothesize that by providing an overwhelming number of activated Th1 cells of an irrelevant specificity and a local inflammatory stimulus in the joint, we have been able to overwhelm the usual regulatory circuits and break self-tolerance. We propose that the inflammation initiated by OVA-specific T cells causes normally quiescent, tolerogenic DC carrying self Ag (collagen) from the joint to the local LN to present this in an immunogenic fashion to self-reactive T cells, thereby breaking tolerance and initiating autoimmunity.

A wide spectrum of autoantibodies can be found in RA and related arthritides, not only the rheumatoid factor typical of RA patients, but also a variety of other reactivities. However, the true role of autoantibodies in the pathogenesis of these diseases has been a matter of substantial controversy (26). Several authors consider arthritis due to the loss of tolerance against a cartilage-specific autoantigen. Models have been developed that have proved the arthritogenic potential of cartilage autoantigens such as collagen type II and proteoglycan (27, 28). Moreover, arthritogenic potential has been demonstrated for novel cartilage components such as collagen types IX and XI, cartilage-derived oligomeric protein, and hyaline cartilage gp39 (29, 30). Although both Th1 and Th2 recipient groups were able to produce anti-CII Abs, we saw a fulminant pathology only in the Th1 group. Abs may be sufficient to cause RA in other models (31), and it will now be important to determine why this is not the case in the system we describe.

In summary, we have shown, for the first time, in a TCR Tg trackable Ag-specific system, that following immunization, Th1, but not Th2, cells are able to induce a transient arthritis in BALB/c mice, which is characterized by synovial hyperplasia, cellular infiltration, and cartilage erosion, paralleled by a significant production of anti-CII Abs. This condition mimics some of the clinical and pathological features of human RA. The transferred T cells may exert their inflammatory effect locally in the joint or perhaps more interestingly in the draining LN. This model directly demonstrates a pivotal role of Th1 cells in the development of inflammatory arthritis. Moreover, this will allow us to track the nature and location (e.g., joints vs LN) of the cellular and molecular interactions underlying RA. These results clarify some features of pathogenesis of RA and may have implications for the design of new therapeutic strategies.

## Acknowledgments

We thank Peter Kerr (Department of Pathology, Western Infirmary, Glasgow, U.K.) for providing technical assistance with histology.

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