IL-17-producing T cells can augment autoantibody-induced arthritis

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Rheumatoid arthritis is a T lymphocyte-mediated disorder, but the precise nature of T cell involvement remains unclear. In the K/BxN mouse model of inflammatory arthritis, T cells initiate disease by providing help to B cells to produce arthritogenic autoantibodies. Here, we have characterized an additional, nonhumoral role for T cells in promoting autoantibody-induced arthritis. Autoreactive KRN T cells introduced either by direct transfer or bone marrow transplantation into B-cell-deficient hosts enhanced K/BxN serum-transferred arthritis, an effect that was dependent on expression of the cognate MHC-molecule/peptide complex. The T cell influence was dependent on interleukin (IL)-17; in contrast, standard serum-transferred arthritis, unenhanced by the addition of T cells, was unaffected by IL-17 neutralization. An IL-17-producing population of transferred KRN T cells was identified and found to be supported by the cotransfer of arthritogenic autoantibodies. IL-17-producing KRN T cells were enriched in inflamed joints of K/BxN mice, suggesting either selective recruitment or preferential differentiation. These results demonstrate the potential for autoreactive T cells to play two roles in the development of arthritis, both driving the production of pathogenic autoantibodies and bolstering the subsequent inflammatory cascade dependent on the innate immune system.

arthritis model | inflammation | Th17 cell

T lymphocytes are critical players in the pathogenesis of rheumatoid arthritis (RA) (1, 2). This notion is supported by the genetic association of RA with specific HLA-DR alleles, suggesting that arthritis requires presentation of a restricted set of antigens to T cells, and by the efficacy of therapies presumably targeting T cells, such as CTLA4-Ig. In animal models, a dependence on T cells has been demonstrated for arthritis induced by adjuvants (e.g., complete Freund's adjuvant, pristane), by immunization with joint antigens [type II collagen, glucose-6-phosphate isomerase (GPI), proteoglycan], and by transgenes or gene mutation (the K/BxN, human T cell lymphotropic virus type 1 env-px, IL-1ra−/−, gp130 F759, and SKG models) (3). Indeed, joint-reactive T lymphocytes, usually $CD4^+$ T cells expressing the $\alpha\beta$ T cell receptor (TCR), are sufficient in many settings to confer arthritis when transferred into naïve recipients that express the cognate MHC molecule and antigen (3–6).

On the other hand, the mechanism of action of $CD4^+$ T cells remains conjectural. Two basic models have been proposed to explain their importance in arthritogenesis. The first posits a direct local role in the arthritic joint, akin to the likely mechanism of other autoimmune diseases, such as type 1 diabetes. Autoreactive $CD4^+$ T cells in the arthritic synovium would recognize antigens presented by synovial antigen-presenting cells (APCs) and respond by orchestrating myeloid cells, synoviocytes, and osteoclasts to engender synovitis (1, 2). According to this scenario, cytokines produced by T cells would be the key drivers of the local effector phase. This local inflammatory response has been thought to involve cells of the Th1 phenotype, although recent observations have argued for a protective role for IFN-γ and suggested a potential role for T cells expressing IL-17A (hereafter referred to as IL-17) (1, 2, 7). The second model contends that the role of T cells is to trigger B cells to produce pathogenic autoantibodies, which then initiate an inflammatory cascade via immune complex formation, complement fixation, and Fc receptors. With autoreactive T cells fueling the continuous production of these autoantibodies, a chronic inflammatory response develops, with progressive joint destruction mediated by neutrophils, synoviocytes, and osteoclasts (3, 8). According to this scenario, autoantibodies would be the key drivers of the local effector phase. Note that the two models are not mutually exclusive, allowing for a spectrum of scenarios in which T cells or autoantibodies would be the primary, but not exclusive, driver of synovitis.

A central role for humoral immunity in the pathogenesis of arthritis has been argued by the B-cell dependence of many animal models and successes in treating RA with antibodies against the B cell molecule, CD20 (3, 9). In two mouse models of arthritis, K/BxN and collagen-induced arthritis (CIA), passive administration of pathogenic immunoglobulins is sufficient to confer disease (10, 11). The effector phenomena induced by this transfer do not require T or B lymphocytes, demonstrating that adaptive immunity is not required for arthritis after the development of arthritogenic autoantibodies. However, these results do not rule out the possibility that autoreactive T cells may contribute, via nonhumoral effector mechanisms, to the progression of pathology provoked by arthritogenic autoantibodies.

We addressed this issue using the K/BxN model of spontaneous arthritis. These mice carry the KRN transgene, which encodes a TCR reactive against a peptide from GPI presented by the A^{g7} MHC class II molecule (4, 12). When the KRN transgene is crossed into an A^{g7}-positive genetic background such as NOD, the autoreactive T cells promote the production of vast quantities of anti-GPI antibodies, which are sufficient to induce arthritis after transfer into normal recipients (11). T cells are dispensable at this stage, as arthritis can be induced effectively by transfer of K/BxN serum into T cell-deficient or alymphoid recipients. On the other hand, comparison of B-cell-deficient hosts with and without the KRN transgenes suggested that T cells, although unable to orchestrate arthritis without B cells, might have an enhancing effect on autoantibody-induced arthritis (11).

Results

$CD4^+$ KRN T Cells Augmented Serum-Transferred Arthritis. We set out to evaluate T cell effector functions in isolation by modifying the K/BxN serum-transfer system to incorporate KRN T cells activated by their cognate MHC-molecule/peptide target (A^{g7}/$GPI_{282–294}$) in the absence of A^{g7}-positive B cells that would produce arthritogenic antibodies. Activation of KRN T cells has been previously shown not to require B cells or anti-GPI (11). Irradiated BxN Rag−/− mice were reconstituted with a 1:1 mix of bone marrow (BM) from KRN TCR transgenic mice on the C57BL/6 (B6) background and BxN Rag−/− mice. The former are a source of KRN^+ T cells (and A^{g7}-negative B cells), while the latter provide stimulatory A^{g7}-positive APCs but no A^{g7}-positive B cells. Control groups received BM from either OTII

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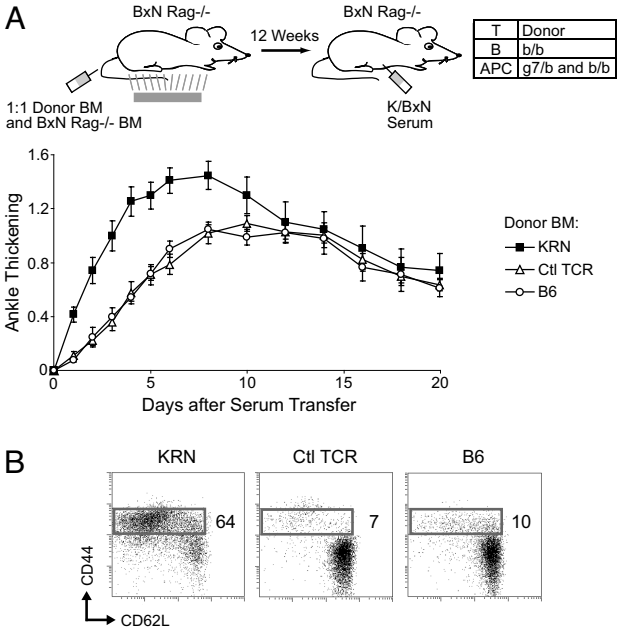

Fig. 1. KRN T cells augment serum-transferred arthritis. Irradiated BxN Rag−/− mice were reconstituted with a 1:1 mix of KRN ($n = 6$), OTII (Ctl TCR, $n = 6$), or B6 ($n = 3$) BM and BxN Rag−/− BM. (*A*) Serum-transferred arthritis was induced after 12 weeks and assessed by ankle thickening (in millimeters), represented as mean change from day 0 ± SEM. P = 0.002 for KRN vs. OTII using area under the curve analysis. A side table summarizes the source of T cells (T) and the MHC genotypes (b/b for B6, g7/b for BxN) of B cells (B) and APCs in recipient mice. (*B*) Donor-derived CD4$^+$ T cells from cervical lymph nodes surgically removed at week 11 were stained for CD44 and CD62L.

TCR transgenic mice, which express an irrelevant TCR, or nontransgenic B6 animals. Arthritis was induced in all three groups by transfer of K/BxN serum 12 weeks after BM reconstitution. Recipients that had received KRN BM showed more severe arthritis than did either of the control groups, demonstrating that KRN T cells can augment arthritis provoked by arthritogenic antibodies (Fig. 1*A*). The engrafted KRN cells were in an animated state (CD44^{hi}62L^{lo}), unlike the grafted control cells (Fig. 1*B*).

We then tested whether KRN T cells could potentiate arthritis in the context of a short-term assay. These experiments used as recipients BxN μMT−/− mice, which are devoid of B cells but have a largely normal T cell compartment, thereby avoiding the potential confounding effects of homeostatic proliferation (13). KRN T cells were transferred into BxN μMT−/− mice via i.v. injection of splenocytes from KRN mice. Recipients of KRN splenocytes had augmented serum-transferred arthritis relative to recipients of OTII or B6 splenocytes (Fig. 2*A*). Equivalent enhancement was observed with purified CD4$^+$ T cells from KRN mice, confirming that other transferred splenocyte populations were not required. Histological analysis of ankle tissue obtained from KRN recipients revealed the characteristic features of K/BxN arthritis including synovial hyperplasia, leukocyte infiltration, neutrophil-rich synovial effusions, and invasive pannus formation (Fig. 2*B*) (4, 11). Again, the engrafted KRN T cells showed an activated phenotype (Fig. 2*C*).

To test whether activation of KRN T cells by their cognate MHC-molecule/peptide target was necessary for their proarthritic function, we compared their influence upon transfer into μMT−/− recipients expressing or lacking A^{g7}, via introduction of a congenic interval on the B6 background. KRN splenocytes augmented arthritis only when the host expressed the stimulatory A^{g7} allele (Fig. 2*D*).

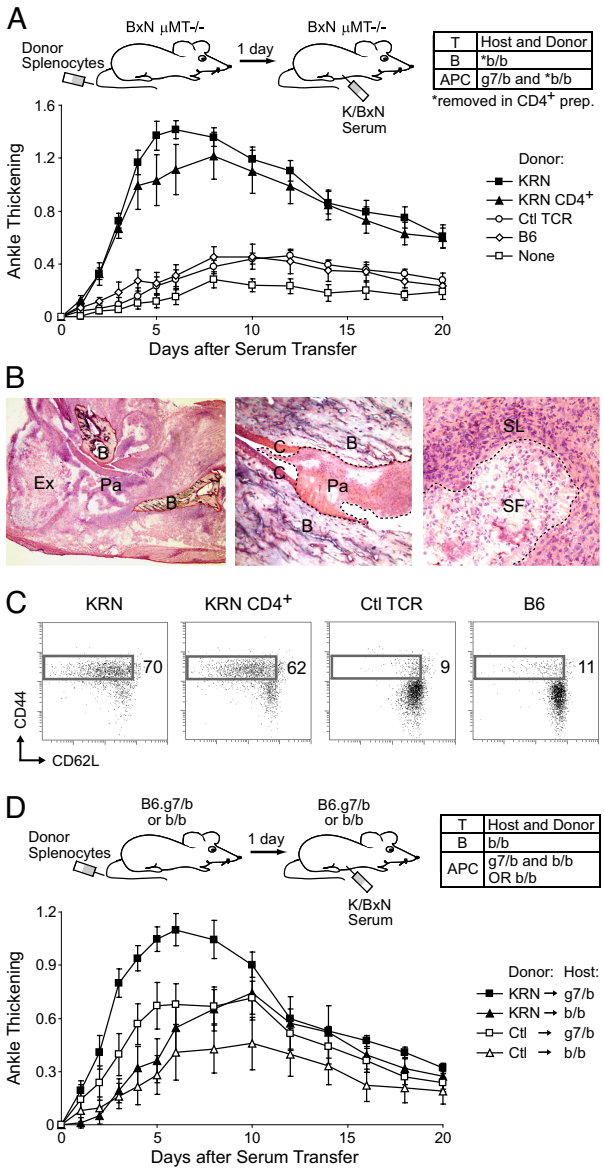

Fig. 2. CD4$^+$ KRN T cells short-term activated by their cognate MHC-molecule/peptide complex boost serum-transferred arthritis. (*A–C*) Twenty million splenocytes from KRN, OTII (Ctl TCR), or B6 mice were transferred into BxN μMT−/− recipients ($n = 5$). An additional group received KRN CD4$^+$ T cells prepared from 2×10^7 KRN splenocytes enriched by magnetic separation to 80–85% purity. One control group received medium alone (None). (*A*) Arthritis was induced in recipient mice 1 day after cell transfer and measured by ankle thickening. $P = 3 \times 10^{-5}$ for KRN vs. Ctl TCR, $P = 0.008$ for KRN CD4$^+$ vs. Ctl TCR, and $P = 0.43$ for KRN vs. KRN CD4$^+$. (*B*) Additional BxN μMT−/− mice receiving KRN splenocytes and K/BxN serum were killed on day 5 of arthritis. H&E staining demonstrated extensive leukocyte infiltration, synovial hyperplasia with pannus (Pa) formation, and fibrinous exudates (Ex) (*Left* magnification, ×40); pannus invasion into cartilage (C) and superficial bone (B) (*Middle* magnification, ×200); and neutrophil accumulation in synovial lining (SL) and synovial fluid (SF) (*Right* magnification, ×400). (*C*) Transferred CD4$^+$ T cells (identified by the absence of NOD-derived CD45.1) were isolated from cervical lymph nodes on day 5 and stained for CD44 and CD62L. (*D*) Twenty million splenocytes from KRN or OTII (Ctl) mice were transferred into B6.H2g7/b μMT−/− or B6.H2b/b μMT−/− littermates ($n = 6$). Arthritis was induced 1 day after cell transfer with a limiting dose of K/BxN serum. $P = 0.02$ for KRN→g7/b vs. KRN→b/b.

A Unique Ability of KRN T Cells to Enhance Arthritis. One possible interpretation of these findings was that the augmentation of arthritis by KRN T cells was merely a function of generic activation

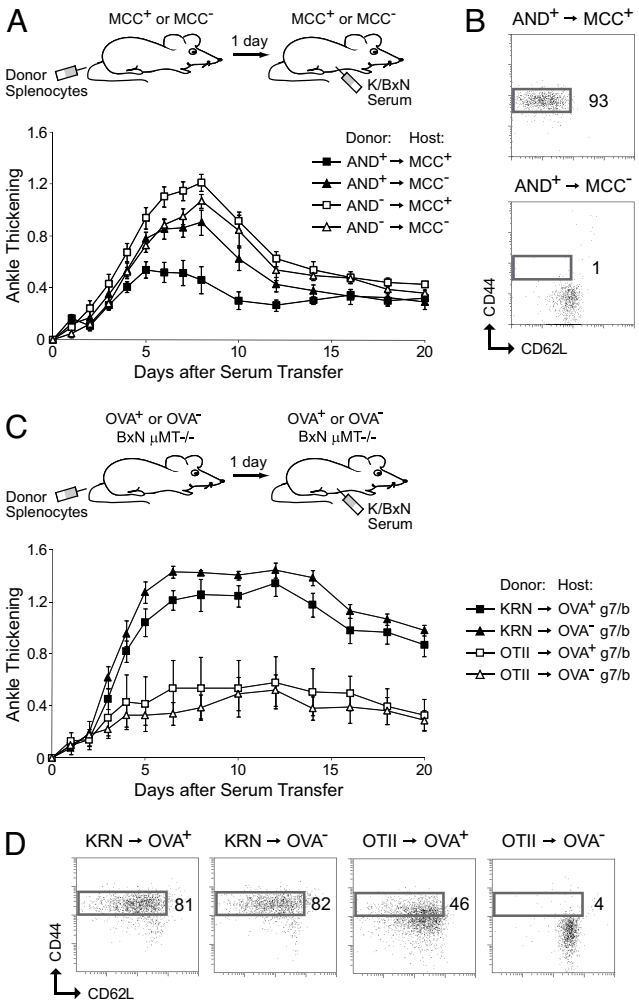

Fig. 3. Monoclonal T cell autoreactivity is not sufficient to augment serum-transferred arthritis. (*A* and *B*) Twenty million splenocytes from AND/CD45.1 B10.BR (AND$^+$) or B10.BR (AND$^-$) mice were transferred into Eα-MCC (MCC$^+$) or transgene-negative B10.BR littermates (MCC$^-$) ($n = 7$ AND$^+ \rightarrow$MCC$^+$, $n = 5$ for other groups). (*A*) Arthritis was induced in recipient mice 1 day after cell transfer with a limiting dose of K/BxN serum and measured by ankle thickening. $P = 0.008$ for AND$\rightarrow$MCC$^+$ vs. AND$\rightarrow$MCC$^-$. (*B*) Transferred CD4$^+$ T cells (CD45.1$^+$) obtained from cervical lymph nodes surgically removed on day 5 of arthritis were stained for CD44 and CD62L. (*C* and *D*) Twenty million splenocytes from KRN or OTII were transferred into Act-mOva/BxN μMT$-/-$ (OVA$^+$) or BxN μMT$-/-$ littermates (OVA$^-$) ($n = 5$). (*C*) Arthritis was induced in recipient mice 1 day after cell transfer and measured by ankle thickening. (*D*) Transferred CD4$^+$ T cells (CD45.1$^-$) obtained from cervical lymph nodes surgically removed on day 5 of arthritis were stained for CD44 and CD62L.

of CD4$^+$ T cells. To assess this possibility, we introduced TCR transgenic T cells of two other specificities into mice that ubiquitously expressed the corresponding MHC/peptide agonist. The first system used the AND TCR transgene, which recognizes the 88–103 peptide from moth cytochrome *c* (MCC_{88-103}) (14). AND splenocytes were transferred, together with a limiting amount of K/BxN serum, into Eα-MCC mice, which carry a transgene encoding a fusion protein of MCC_{88-103} and the invariant chain expressed under the dictates of the MHC class II Eα promoter (15). Rather than augmenting arthritis, the transferred AND T cells actually suppressed disease relative to transgene-negative littermate donor and recipient controls (Fig. 3*A*). Yet, the transferred AND T cells transferred into Eα-MCC mice were strongly activated, as evidenced by up-regulation of CD44 and down-regulation of CD62L (Fig. 3*B*).

These AND/Eα-MCC transfers used a genetic background (B10.BR) different from that of the KRN transfer experiments, for which the recipients were BxN F1 hybrids. We therefore designed a system in which the effects of KRN or other autoreactive T cells could be compared directly in identical hosts. BxN μMT$-/-$ mice were bred to express the Act-mOva transgene, which encodes ovalbumin (OVA) expressed ubiquitously under the control of a β-actin promoter (16). The resulting Act-mOva BxN μMT$-/-$ mice or their transgene-negative littermates were used as recipients for parallel transfers of KRN (A^{g7}/GPI-reactive) or OTII (A^b/OVA-reactive) splenocytes. OTII cells had no effect on arthritis despite showing an activated phenotype (CD44^{hi}CD62L^{lo}), while KRN cells enhanced arthritis irrespective of the Act-mOva transgene (Fig. 3 *C* and *D*). Thus, enhancement of antibody-induced arthritis appears to be a particular feature of KRN T cells, one that is not shared by just any monoclonal T cell population reactive against a ubiquitous self-antigen.

Augmentation of Arthritis Was IL-17 Dependent. We next investigated the mechanism by which KRN T cells, when activated by their cognate MHC-molecule/peptide target, enhanced antibody-induced arthritis. A reasonable hypothesis was that the transferred KRN T cells acted via cytokine mediators. The classic proinflammatory cytokines, IFN-γ, and tumor necrosis factor (TNF)-α were considered, as was the Th2 cytokine, IL-4, which is produced at high levels by K/BxN T cells and is essential for the production of high titers of anti-GPI IgG (17). The KRN transgene was bred onto IFN-γ-, TNF-α-, and IL-4-deficient backgrounds, and the resulting mice were used as donors for transfers into BxN μMT$-/-$ mice. Deficiency of these cytokines had no effect on KRN T cell augmentation of arthritis, demonstrating that none of these mediators were critically involved (Fig. 4 *A*–*C*).

There has been considerable interest in the role of IL-17 in a variety of inflammatory responses, including several murine models of arthritis (1). To determine whether IL-17 was involved in KRN T cell augmentation of serum-transferred arthritis, we performed antibody-inhibition experiments using an anti-IL-17 monoclonal antibody (mAb) with demonstrated efficacy in experimental autoimmune encephalomyelitis (18). Anti-IL-17 strongly suppressed the enhancement of arthritis by KRN T cells, such that disease severity was close to that of the nonenhanced control (Fig. 4*D*). IL-17 blockade operated at the level of enhancement by KRN T cells, rather than through other elements of the arthritic response, as anti-IL-17 treatment had no effect on arthritis elicited by anti-GPI antibodies in the absence of transferred T cells (Fig. 4*E*).

A growing body of literature has suggested that cytokines produced in inflammatory responses, particularly IL-6 and IL-23, are capable of promoting differentiation of CD4$^+$ T cells toward an IL-17-producing phenotype (Th17) (19). We assessed the contribution of IL-6 and IL-12p40, the shared subunit of IL-12 and IL-23, to KRN T cell augmentation of serum-transferred arthritis using mAbs with demonstrated in vivo activity (20, 21). Neutralization of IL-6 had no effect whereas inhibition of IL-12p40 increased the augmentation of disease by transferred T cells (Fig. 4*F*). IL-12 deficiency was previously shown to have no effect on straight serum-transferred arthritis (17).

IL-17-Producing KRN T Cells Were Supported by Arthritogenic Autoantibodies and Enriched in Arthritic Joints of K/BxN Mice. We hypothesized that the inflammatory response elicited by arthritogenic antibodies promoted IL-17 production by transferred KRN T cells. To test this notion, we isolated KRN and OTII T cells 6 days after cotransfer with K/BxN serum or PBS and performed intracellular staining for IL-17 and IFN-γ (Fig. 5 *A* and *B*). IFN-γ was clearly expressed in the introduced KRN (but not control OTII) T cells, in a proportion that was unaffected by cotransfer of K/BxN serum. IL-17 was minimally expressed in KRN T cells transferred in the absence of serum, but the fraction of positive cells was very

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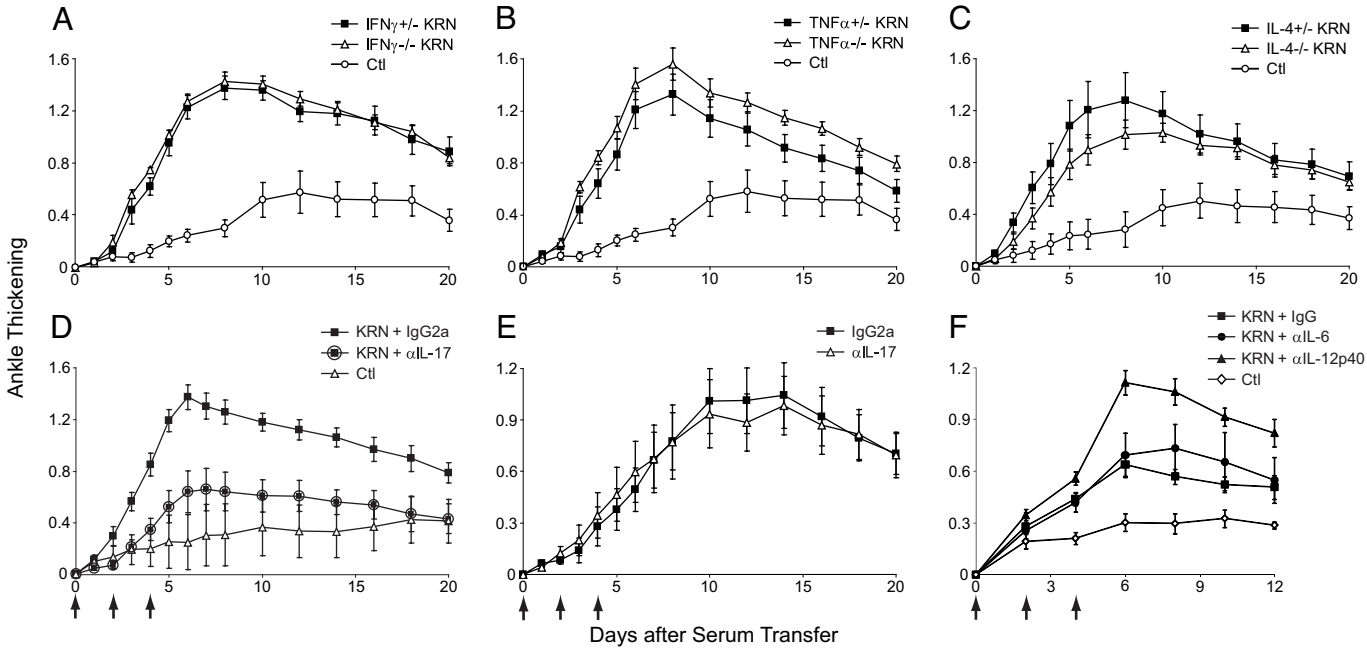

Fig. 4. Augmentation of serum-transferred arthritis requires IL-17 but not IL-6, IL-12p40, or KRN-T cell-derived IFNγ, TNFα, and IL-4. (*A–C*) Serum-transferred arthritis was induced in BxN μMT−/− mice receiving splenocytes from (*A*) IFNγ+/− or IFNγ−/− ($n = 5$) KRN littermates or OTII mice (Ctl). Similar comparisons were made of (*B*) TNFα+/− vs. TNFα−/− ($n = 5$) and (*C*) IL-4+/− vs. IL-4−/− ($n = 9$) KRN donors. (*D*) 100 μg anti-IL-17 (αIL-17) or isotype control (IgG2a) antibodies were given i.p. on days 0, 2, and 4 (indicated by arrows) of serum-transferred arthritis in BxN μMT−/− mice receiving KRN splenocytes ($n = 5$). OTII recipients were used as a control. $P = 0.004$ for KRN αIL-17 vs. KRN IgG2a; $P = 0.35$ for KRN αIL-17 vs. Ctl. (*E*) B6 mice with serum-transferred arthritis were treated with 100 μg αIL-17 or isotype control IgG2a antibodies on days 0, 2, and 4 ($n = 6$). (*F*) As in (*D*) except that KRN recipients were treated with 500 μg anti-IL-12p40 (αIL-12p40), 2.66 mg/kg anti-IL-6 (αIL-6), or 500 μg Rat IgG control (IgG) antibodies. A representative experiment ($n = 4$) of three is shown. $P = 0.003$ for KRN αIL-12p40 vs. KRN IgG.

significantly ($P < 0.007$) increased by cotransfer of K/BxN serum. Donor KRN T cells expressed IL-17 at a frequency similar to that of KRN T cells isolated from mice receiving K/BxN serum (Fig. 5*C*). Minimal IL-17 was detected in transferred OTII cells irrespective of K/BxN serum transfer or in T cells from mice with straight serum-transferred arthritis (Fig. 5 *A–C*). These findings suggested that the Th17 phenotype was maintained or expanded during serum-transferred arthritis whereas transferred Th17 cells were largely lost in the absence of arthritogenic autoantibodies.

It proved technically impossible to recover enough transferred KRN T cells from arthritic joints of boosted mice to address whether there was local enrichment of the Th17 phenotype. However, we could examine this point in the straight K/BxN model. Lymphoid organs and synovial fluid were collected from arthritic K/BxN mice after approximately 4 weeks of disease (i.e., at 8 weeks of age). KRN T cells isolated from the spleen and lymph nodes showed an increased frequency of IL-17 expression relative to that of T cells from BxN littermate controls (Fig. 6). Synovial fluid was highly enriched in IL-17-producing T cells, suggesting that cells of this phenotype were preferentially elicited in or recruited to the environment of the arthritic joint.

Discussion

Animal models such as K/BxN arthritis have permitted dissection of the mechanisms by which T cell autoreactivity can lead to joint-specific inflammatory disease. Previously, we reported a critical role for autoreactive KRN T cells in the initiation phase of K/BxN arthritis, eliciting a humoral response that generates arthritogenic autoantibodies directed against GPI (11). In this study, we have demonstrated that this antibody-centric view may not fully capture the role of T cells in the K/BxN arthritis model, as T cells can augment antibody-induced arthritis independently of their influence on antibody production. This enhancement was mediated by IL-17-producing $CD4^+$ KRN T cells activated by their cognate MHC-molecule/peptide complex ($A^{g7}/GPI_{282–294}$).

Intriguingly, IL-17-producing KRN T cells that arose in donor transgenic mice were selectively maintained in adoptive hosts in the setting of serum-transferred arthritis. This effect may be mediated by cytokines that promote the Th17 phenotype. While IL-6 and IL-23 were found to not be critical for T cell augmentation of arthritis, others including transforming growth factor (TGF)-β, IL-1, IL-21, and TNF-α remain possibilities (19, 22–25). A similar lack of dependence on IL-23 has been described in other contexts (26). A role for TGF-β is supported by the finding in SKG lymphocyte-transferred arthritis that anti-TGFβ treatment halved Th17 frequency (27). IL-1 and TNF-α, which are produced in abundance early in antibody-mediated inflammation, are also attractive candidate mediators but their contribution would be difficult to separate from their critical role in the inflammatory cascade in unaugmented serum-transferred arthritis (23, 24, 28). The Th17 phenotype may be supported by down-regulation of certain cytokines. Deficiency of IL-12 was shown in CIA to result in worsened disease and increased IL-17 production (29). The enhanced T cell augmentation of arthritis with anti-IL-12p40 treatment may have reflected a shift toward increased IL-17 production by transferred KRN T cells.

IL-17 has been reported to be critical for other arthritis models including CIA, antigen-induced arthritis (AIA), IL1ra−/− arthritis, streptococcal-cell-wall-induced arthritis, and SKG-lymphocyte-transferred arthritis (27, 30–33). It is not known precisely how it promotes arthritis in these contexts. The IL-17 receptor is widely expressed by cell types residing in the synovium, including fibroblast-like synoviocytes, chondrocytes, monocytes/macrophages, and osteoclasts (1, 34). In vitro, IL-17 has been shown to induce synthesis by these cells of numerous proinflammatory mediators, including cytokines (IL-1, IL-6, TNF-α), chemokines (C-X-C family

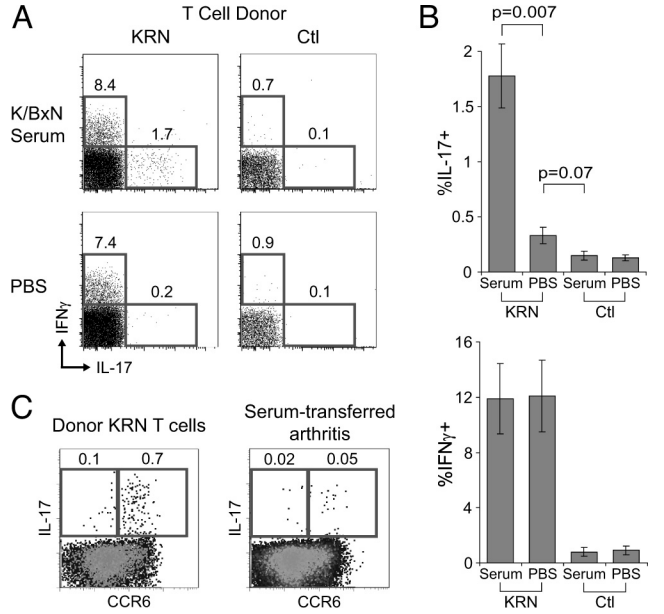

Fig. 5. Transferred IL-17-producing CD4$^+$ KRN T cells are supported by arthritogenic antibodies. (*A* and *B*) K/BxN serum or PBS was injected i.p. into BxN μMT$-/-$ mice 1 and 3 days after transfer of KRN or OTII (Ctl) splenocytes. Six days after cell transfer, cervical and inguinal lymph nodes were harvested for flow cytometry. (*A*) Intracellular staining for IL-17 and IFNγ is shown for transferred CD4$^+$ T cells. (*B*) The bar graphs depict the mean percentage $\pm$ SEM of transferred CD4$^+$ T cells in each group expressing IL-17 or IFNγ (n = 5 for KRN groups, n = 4 for Ctl groups). (*C*) Intracellular staining for IL-17 and surface staining for CCR6, a chemokine receptor expressed on Th17 cells, is shown for CD4$^+$ T cells from donor KRN B6 splenocytes and splenocytes obtained from B6 mice on day 5 of serum-transferred arthritis.

members, monocyte chemoattractant protein-1), granulocyte colony-stimulating factor, vascular endothelial growth factor, cyclooxygenase, receptor activator of NFκB ligand, and matrix-metalloproteases (1, 34). In vivo, IL-17 induces granulopoiesis, neutrophil recruitment, cartilage destruction, and bone resorption (34–37). The documented activities of IL-17 suggest that it must be present locally to promote an inflammatory response. This notion is consistent with the finding that in K/BxN serum-transferred arthritis, local administration of IL-17 via intraarticular injection of an adenovirus vector increases the histologic severity of arthritis (38).

We speculate that IL-17-producing KRN T cells amplify the inflammatory process in the joints of transgenic K/BxN mice. Their contribution may be subtle as anti-CD4 antibody depletion of helper T cells after the onset of full disease in K/BxN mice did not reduce ankle swelling (4). It may be that in the setting of sufficient titers of arthritogenic antibody for full expression of ankle disease, as in K/BxN mice, the true role of Th17 cells is to potentiate disease in joints—such as elbows, knees, and the spine—that are involved in K/BxN mice but spared in mice with serum-transferred arthritis (4, 39). The enrichment of Th17 cells in the synovial fluid of K/BxN mice is a striking finding given that the majority of synovial T cells have a Foxp3$^+$ phenotype consistent with their being regulatory T cells (Tregs) (39). Th17 and Treg cell differentiation has been thought to be antagonistic, with both sharing a dependence on TGF-β, but with cytokines such as IL-6 promoting differentiation of one at the expense of the other (22, 25, 40). Simultaneous enhanced differentiation of both lineages in the same environment would run counter to this paradigm, suggesting that one if not both of these populations is recruited to inflamed joints and that only one population, if any, differentiates in situ. The interplay of Th17 cells and Tregs in the transgenic model will be an intriguing area of further study. In K/BxN mice, the absence of Foxp3-dependent Tregs resulted in an accelerated onset of arthritis and spread of disease to joints normally unaffected even in mice with longstanding arthritis (39). This effect could not be explained simply by increased autoantibody titers, indicating that Tregs modulate the activity of effector cells such as neutrophils, mast cells, monocytes/macrophages, synoviocytes, and now Th17 cells. It is tempting to speculate that Th17 cells and Tregs exist in a balance such that in the absence of Tregs, unopposed Th17 activity amplifies inflammatory cascades initiated by GPI/anti-GPI immune complexes in joints that are typically spared.

We have demonstrated that autoreactive T cells can promote arthritis through both the generation of arthritogenic autoantibodies and an IL-17-mediated effector response that amplifies inflammation. Studying each of these pathways in isolation poses a challenge in light of recent evidence that IL-17 is critically involved in germinal center development (41). T cell-augmented serum-transferred arthritis separates the contributions of T cells as effector cells from their role in humoral immunity, allowing for dissection of the mechanisms by which IL-17-producing T cells can contribute to antibody-mediated arthritis. We anticipate that the relative importance of IL-17-producing cells and autoantibodies will vary greatly among arthritis models. For those that require B cells, including K/BxN arthritis, CIA and proteoglycan-induced arthritis, the T cell-dependent humoral response would be the dominating element and IL-17 would serve as an adjunct amplifier (11, 42, 43). Others that are unaffected by B-cell deficiency, such as AIA and gp130 F759 arthritis, would be primarily driven by Th17 effector activity (5, 6). It is likely that the balance of the humoral and effector arms of T cell involvement span a similar continuum in human arthritides, and perhaps even among patients sharing the diagnosis of RA. This point will be critical to elucidate to direct therapeutic targeting of T cell-mediated pathways of inflammation.

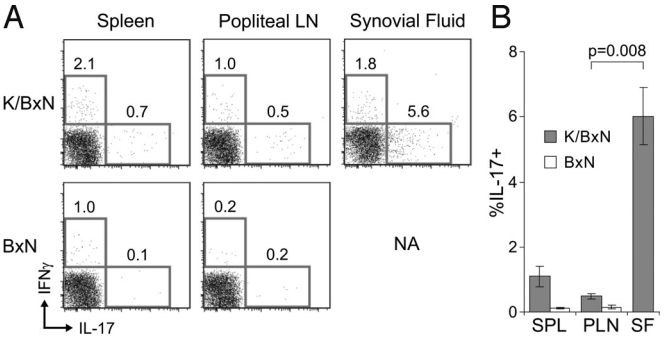

Fig. 6. IL-17-producing CD4$^+$ KRN T cells are enriched in K/BxN transgenic mice, with greatest concentration in arthritic joints. (*A*) Intracellular staining for IL-17 and IFNγ is shown for CD4$^+$ T cells from the spleen (SPL), popliteal lymph nodes (PLN), and ankle synovial fluid (SF) (K/BxN only) of 8-week-old K/BxN mice and transgene negative littermates. (*B*) The bar graphs depict the mean percentage $\pm$ SEM of CD4$^+$ T cells that express IL-17 in each group (n = 4).

Materials and Methods

Mice. OTII, Act-mOva, B6.H2g7, Rag$-/-$, μMT$-/-$, IFN$\gamma-/-$, IL-4$-/-$, and TNF$\alpha-/-$ mice were obtained from Jackson Laboratory. KRN, AND, and Eα-MCC mice have been described in refs. 4, 14, and 15. The lines used were of the C57BL/6 background, except AND and Eα-MCC, which were of the B10.BR background. K/BxN mice were generated by crossing KRN TCR transgenic C57BL/6 mice with NOD mice. All experiments used matched littermate controls generated from heterozygote or heterozygote/homozygote crosses. Genotypes were assessed by genomic PCR. Experiments were reviewed by the Institutional Animal Care and Use Committee, protocols 02956 and 03024.

Serum-Transferred Arthritis. K/BxN serum was collected from 8-week-old K/BxN mice and pooled for each experiment, which involved its own serum batch, potentially accounting for disease variability between experiments using genetically equivalent mice. Arthritis was induced by i.p. injection of 150 μL on days 0 and 2; where noted a limiting dose of 37.5 μL was used instead. Ankle thickness

was measured with a caliper (J15 Blet micrometer), and was reported as ankle thickening: the change in ankle thickness from day 0. Disease severity was quantified using the area under the curve of ankle thickening over the first 14 days. Two-tailed Student's *t* tests were used to generate *p* values. Inhibition experiments used monoclonal antibodies against IL-17 (R&D Systems), IL-6 (BD Biosciences), and IL-12p40 (C17.8, a kind gift from Giorgio Trinchieri, National Cancer Institute, Frederick, MD). Control antibodies included a monoclonal isotype control antibody (R&D Systems) and purified polyclonal rat IgG (Jackson Immunoresearch). For histological analysis, ankles were dissected, snap-frozen in O.C.T. medium (Sakura Finetek), cryosectioned to a thickness of 5 μm, and stained with hematoxylin and eosin.

Cell Transfers. Spleens were dissected from donor mice and squeezed against a nylon mesh filter to yield a single-cell suspension. Red blood cells were lysed with ACK buffer and washed in DMEM. Twenty million cells were transferred in DMEM into recipient mice by tail-vein injection. $CD4^+$ T cell purification was performed using a commercial kit (Miltenyi Biotec).

BM Transfers. BM was collected from donor mice by flushing dissected tibias and femurs with PBS. Red blood cells were lysed with ACK buffer, and a single-cell suspension prepared by passing the BM flush through a nylon mesh filter. BM cells were stained with biotin-labeled antibodies against CD3, CD4, and CD8α; treated with magnetic streptavidin-linked beads (Miltenyi Biotec); and passed through magnetic separation columns. Recipient mice were irradiated with 600 Rads and reconstituted with 3×10^6 BM cells in DMEM transferred by tail-vein injection.

Flow Cytometry. Cells were collected for flow cytometry by filtering crushed cervical lymph nodes obtained by lymphectomy under ketamine/xylazine anesthesia or crushed spleens and lymph nodes harvested from mice. Synovial fluid was collected by flushing dissected ankles with 1 mM EDTA in PBS. For intracellular staining, cells were incubated for 5 h at 37 °C in the presence of 50 ng/mL phorbol 12-myristate 13-acetate (Sigma), 1 μM ionomycin (Calbiochem), and Golgiplug (BD PharMingen) in RPMI medium 1640 + 10% FCS. Staining was done using Cytofix/Cytoperm (BD PharMingen) per the manufacturer's instructions. Cells were run on either the Beckman Coulter or BD LSRII.

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