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Autoreactive Th1 Cells Activate Monocytes To Support Regional Th17 Responses in Inflammatory Arthritis

Donald M. Simons,1 Soyoung Oh, Elizabeth Kropf, Malinda Aitken, Victoria Garcia, Alissa Basehoar, and Andrew J. Caton

We have examined mechanisms underlying the formation of pathologic Th17 cells using a transgenic mouse model in which autoreactive CD4+ T cells recognize influenza virus hemagglutinin (HA) as a ubiquitously expressed self-Ag and induce inflammatory arthritis. The lymph nodes of arthritic mice contain elevated numbers of inflammatory monocytes (iMO) with an enhanced capacity to promote CD4+ Th17 cell differentiation, and a regional inflammatory response develops in the paw-draining lymph nodes by an IL-17–dependent mechanism. The activation of these Th17-trophic iMO precedes arthritis development and occurs in the context of an autoreactive CD4+ Th1 cell response. Adoptive transfer of HA-specific CD4+ T cells into nonarthritic mice expressing HA as a self-Ag similarly led to the formation of Th1 cells and of iMO that could support Th17 cell formation, and, notably, the accumulation of these iMO in the lymph nodes was blocked by IFN-γ neutralization. These studies show that autoreactive CD4+ Th1 cells directed to a systematically distributed self-Ag can promote the development of a regional Th17 cell inflammatory response by driving the recruitment of Th17-trophic iMO to the lymph nodes. The Journal of Immunology, 2013, 190: 3134–3141.

The processes that drive Th17 cell formation are not fully understood, particularly in the setting of autoimmune disease, where Th17 cell induction is pathological and presumably reflects a dysregulation of processes that are normally protective to the host.

Both in vitro and in vivo studies have shown that cytokines such as TGFβ1, IL-6, and IL-23 can play important roles in promoting the differentiation of naive CD4+ T cells into Th17 cells (3–5). More recently, an important role for gut-residing bacteria in Th17 cell formation in vivo has become apparent, such that colonization of mice with particular commensal microbes (such as segmented filamentous bacteria) can profoundly influence the magnitude of Th17 cell responses that can be induced (6). Notably, these effects on Th17 cell formation were found to influence the development of autoimmune arthritis in K/BxN mice, indicating that commensal bacteria can play a role in promoting the formation of pathologic autoreactive Th17 cells in vivo (7). However, it is clear that additional factors must contribute to autoimmune disease development, in addition to those received from commensal bacteria, because most strains of mice carrying these bacteria do not develop autoimmunity. In particular, these findings do not explain why many autoimmune diseases show strong genetic linkages with MHC class II (MHC II) alleles, which imply an important role for CD4+ T cell recognition of self-peptides in the disease process (8). Indeed, mechanisms by which CD4+ T cell recognition of self-peptides might participate in and/or promote the formation of pathologic Th17 cells in autoimmune settings remain poorly understood.

We have examined this question in a mouse model of inflammatory arthritis, in which autoreactive CD4+ T cells responding to a self-peptide expressed by APCs induce arthritis by an IL-17–dependent mechanism (9). We show that autoimmune disease is initiated by a systemic autoreactive CD4+ Th1 response, which drives the formation of Th17-trophic inflammatory monocytes (iMO) that mobilize to the lymph nodes (LN). These studies provide a basis by which autoreactive CD4+ T cells responding to a systematically distributed Ag can promote a regional IL-17–mediated inflammatory response.

Materials and Methods

Mice

TS1, HACII, and TS1xHACII mice were previously described (10, 11). HACII.CΔ1−/− mice were generated by crossing HACII mice with TCR. C57BL/6 mice that were bred to homozygosity for the H-2k haplotype and backcrossed onto the BALB/c background for at least seven generations (12). TS1.CD45.1+/+ mice were generated by breeding TS1 mice with CD45.1+/+ mice on the BALB/c background that were purchased from The Jackson Laboratory. Mice were housed under specific pathogen-free conditions at The Wistar Institute Animal Facility. All experiments were performed according to protocols approved by The Wistar Institutional Animal Care and Use Committee.

Assessment of arthritis and anti–IL-17 treatment

Mice were assessed weekly for the number of arthritic limbs beginning at 4 wk of age and continuing for at least 12 wk. For anti–IL-17 treatment, TS1xHACII mice received three i.p. injections per wk of 150 μg IL-17A–neutralizing Ab (M210; provided by Amgen) or of an isotype control Ab (2A3; BioXCell) beginning at 4 wk of age.
Abs and flow cytometry

Single-cell suspensions from spleens or lymph node were stained with the Live/Dead Fixable Aqua Dead Cell stain kit from Invitrogen (except when sorting) and then with fluorochrome-labeled Abs purchased from eBio-science or BD Biosciences unless stated otherwise. The following clones were used in all experiments. For analysis and sorting of APCs: F4/80 (BM8), plasmacytoid dendritic cell Ag-1 (PDCA-1) (eBio927), CD19 (M1/70), CD11b (N418), Ly6G (1A8), Ly6C (HK1.4), MHC II (M5/114.15.2), B220 (RA3-6B2), CD115 (AF598), CD49b (DX5), and CCR2 (475301; R&D Systems); and for analysis and sorting of T cells: CD4 (RM4-5), CD25 (PC61.6), IL-17 (eBio17B7), IFN-γ (XMG1.2), CD45.1 (A20), and CD8 (53-6.7). Biotinylated 6.5 was prepared in-house and detected with APC (BD Biosciences) or Qdot655-conjugated (Invitrogen) streptavidin.

Cell sorting

For flow cytometric analysis and sorting of APC subsets, spleens and LN were minced and digested with 400 U/ml collagenase D (Roche) at 37˚C prior to use. Spleen and/or LN cells were sorted with a MoFlow (DakoCytomation) or FACSAria (BD Biosciences) cell sorter into the following subsets: inflammatory monocytes (B220−CD11c−Ly6G−CD11b+Ly6Chigh), conventional dendritic cells (cDC) (B220−Ly6G−Ly6C−CD11b+/−CD11chigh), and B cells (CD11b−CD11c−Ly6C−B220high).

For coculture experiments, hemagglutinin (HA)-specific CD4+CD25−6.5+ cells were sorted from the pooled spleens and LN of TS1.CD45.1+/− or TS1.Thy1.1+/− mice.

In vitro cocultures

FACS-purified APCs and HA-specific T cells were combined at T cell/APC ratios ranging from ∼1:2 (LN APCs) to 1:10 (spleen APCs) and cultured at ∼1.5 × 10⁶ total cells/ml in cell-culture media supplemented with 50 ng/ml IL-23 (R&D Systems). After 7 d, PMA, ionomycin, and brefeldin A were added to the cultures, and IL-17 and IFN-γ production were assessed 5 h later by intracellular cytokine staining and flow cytometry.

Serum cytokine analysis

Serum was collected from whole blood, and inflammatory cytokine expression was determined by multiplex ELISA using the Luminex platform (Millipore) at the University of Pennsylvania Human Immunology Core.

Adoptive transfer

CD4+ T cells were purified from the spleens and LN of TS1.CD45−/− mice by MACS depletion of unwanted cells with Abs against B220, I-Aδ, CD11b, F4/80, and CD8. A total of 10⁶ cells/mouse were adoptively transferred into HACII.Ca−/− recipients by retro-orbital injection, and recipient mice were sacrificed 7 d later for analysis. In some experiments, recipients were injected i.p. with 0.5 mg anti–IFN-γ (XMG1.2; BioXCell) or 0.5 mg isotype control Ab (HRPN; BioXCell) on days 0 and 3. For analysis of in vivo proliferation, CD4+ T cells purified as described above were labeled with 5 μM CFSE (Sigma-Aldrich) prior to adoptive transfer into BALB/c or HACII recipients, which were sacrificed 3 d later for analysis.

Statistical analysis

All statistical analyses were performed using GraphPad Prism software package (GraphPad). Unpaired, two-tailed t tests were used for data analysis and the generation of p values.

Results

Arthritis in TS1xHACII mice is accompanied by a regional Th17 response to a systemic self-Ag

TS1 mice express a transgenic I-Eδ-restricted TCR that recognizes the S1 determinant of influenza PR8 HA and can be detected with the clonotypic mAb 6.5 (10). HACII mice express HA as a neo–self-Ag under the control of a MHC II promoter, and we previously reported that the majority of TS1xHACII mice (generated by mating TS1 mice with HACII mice) spontaneously develop arthritis in adulthood (10). In this study, we determined that arthritis in TS1xHACII mice is accompanied by a regional Th17 response to this systemic self-Ag. To study arthritis in TS1xHACII mice, we used well-characterized strain-specific markers for TS1 and HACII mice, and we performed comprehensive analyses involving the identification of the 6.5+CD4+ self-reactive T cell subset, the production of Th17 cytokines, the development of arthritis, and the effects of Th17 depletion with anti–IFN-γ Ab.

FIGURE 1. Arthritis in TS1xHACII mice is accompanied by a regional Th17 response to a systemic self-Ag. (A) Photographs of age- and sex-matched TS1 and arthritis TS1xHACII mice. Middle and bottom panels show front and rear paws. Graph (right panel) shows arthritis incidence in a cohort of TS1xHACII mice. (B) CFSE dilution by CD4+ T cells from TS1 mice 3 d posttransfer into HACII or BALB/c recipients. (C) Histograms showing 6.5 staining by CD4+ T cells in pdLN and spleens of TS1 and arthritic TS1xHACII mice. (D) Numbers of 6.5+CD4+ T cells in pdLN and spleens of TS1 and arthritic TS1xHACII mice. (E) Ex vivo production of IFN-γ and IL-17 by 6.5+CD4+ T cells from pdLN and spleens of TS1 and arthritic TS1xHACII mice. (F) Arthritis incidence in 15-wk-old TS1xHACII mice given weekly injections of anti–IL-17 beginning at 4 wk of age. All data are means ± SEM of ≥5 independent determinations. *p < 0.05, **p < 0.01.
Inflammatory arthritis as they reach adulthood (Fig. 1A) (11). Arthritis depends on coexpression of the TS1 and HACII transgenes (because neither TS1 nor HACII mice develop the disease) and is driven by CD4⁺ T cells without a requirement for B cells, because TS1xHACII mice that congenitally lack B cells also develop arthritis (11). It is noteworthy that TS1xHACII mice develop arthritis as a prominent autoimmune manifestation, because the targeting of HA to MHC II⁺ cells results in its expression as a self-Ag by APCs throughout the body. Indeed, CD4⁺ T cells from TS1 mice underwent extensive division in both the spleens and LN following transfer into HACII mice (Fig. 1B), and HA-specific 6.5⁺CD4⁺ T cells are extensively deleted in TS1xHACII mice (Fig. 1C, 1D), indicating that HA is a systemically expressed self-Ag in TS1xHACII mice.

To examine how autoreactive 6.5⁺CD4⁺ T cells that evade deletion can promote arthritis development, we first analyzed their representation and phenotype in the spleens and paw draining LN (pDLN) of arthritic TS1xHACII mice. There were increased frequencies of both IFN-γ- and IL-17-producing 6.5⁺CD4⁺ T cells (Th1 and Th17 cells, respectively) relative to TS1 mice (Fig. 1E). Notably, however, the percentage of 6.5⁺CD4⁺ T cells that were Th17 cells was significantly higher in the pDLN of arthritic TS1xHACII mice than in the spleens, whereas the percentages of 6.5⁺CD4⁺ T cells that were Th1 cells at these sites did not differ. Moreover, administration of an anti–IL-17A mAb led to a significant decrease in the numbers of arthritic paws that developed in TS1xHACII mice, consistent with other studies demonstrating a role for Th17 cells in inflammatory arthritis (Fig. 1F) (7, 9, 13).

Together, these data show that TS1xHACII mice spontaneously develop an IL-17–dependent inflammatory arthritis that is accompanied by a regional increase in the frequency of HA-specific CD4⁺ Th17 cells in the pDLN.

**FIGURE 2.** Th17-trophic inflammatory monocytes accumulate in arthritic TS1xHACII mice. (A) Representative flow data showing APC subsets in the pDLN. The percent of total live cells falling within each gate is indicated. MHC II expression by iMO and the total numbers of each APC are shown in the accompanying panels. (B) Percentage of 6.5⁺CD4⁺ T cells that produced IL-17 or IFN-γ following 7 d of coculture with APC subsets purified from the LN of arthritic TS1xHACII mice. (C) As in (A), except for spleen. (D) As in (B), except coculture was with APC subsets purified from the spleens of HACII mice or arthritic TS1xHACII mice. All data are means ± SEM of ≥5 independent determinations. *p < 0.05, **p < 0.01, ***p < 0.005. pDC, Plasmacytoid DC.
Notably, iMO purified from the pdLN of arthritic TS1xHACII mice induced a significantly higher proportion of 6.5+CD4+ cells from TS1 mice to become IL-17 secretors than were induced by either B cells or cDC that had also been isolated from the pdLN (Fig. 2B). Inflammatory monocyte and cDC numbers were also elevated in the spleens of arthritic TS1xHACII mice, and, although lower than was the case for iMO in the pdLN, the splenic iMO from arthritic TS1xHACII mice expressed higher levels of MHC II than iMO from the spleens of HACII mice (Fig. 2C). Splenic iMO from arthritic TS1xHACII mice again induced the formation of IL-17–secreting 6.5+CD4+ T cells more efficiently than was the case for cDC isolated from arthritic TS1xHACII mice, and they were also more efficient at inducing the formation of IL-17-secreting 6.5+CD4+ T cells than were either iMO or cDC obtained from the spleens of control HACII mice (Fig. 2D). Thus, the accumulation of IL-17–secreting 6.5+CD4+ T cells in the pdLN of arthritic TS1xHACII mice is associated with a sizable increase in the representation of an iMO population that is a more potent inducer of Th17 cells than is the case for other APC populations in the pdLN. Together, these data strongly suggest that these Th17-trophic iMO are important in promoting the regional Th17 cell response that develops in the pdLN of arthritic TS1xHACII mice.

**FIGURE 3.** A systemic CD4+ Th1 cytokine response precedes arthritis in TS1xHACII mice. (A) Number of CD4+ and 6.5+CD4+ T cells in the pdLN and spleens of TS1, prearthritic TS1xHACII, and arthritic TS1xHACII mice. (B) Percentage of 6.5+CD4+ splenocytes that produced IL-17 or IFN-γ. (C) Heat map showing the concentrations of Th17 and Th1 cytokines in the serum of prearthritic and arthritic TS1xHACII mice. Average values from seven control (CTL) mice are shown in the leftmost column. The concentration of each cytokine is indicated within the tiles. The \( p \) values refer to comparisons between prearthritic and arthritic TS1xHACII mice. Number of APCs in the pdLN (D) and spleen (E) and percentages of cells in the blood (F) of 5-wk-old TS1xHACII and TS1 mice. (G) MHC II expression by iMO in the spleens and blood of 5-wk-old TS1xHACII and TS1 mice. (H) Percentage of 6.5+CD4+ T cells from TS1 mice that produced IL-17 or IFN-γ following 7 d of coculture with APC subsets purified from the spleens of 5-wk-old TS1xHACII or HACII mice. All data are means ± SEM of \( n = 5 \) independent determinations. *\( p < 0.05 \), **\( p < 0.01 \), ***\( p < 0.005 \). N/A, Equivalent subset not present; pDC, plasmacytoid DC.
A systemic CD4+ Th1 cytokine response precedes arthritis in TS1xHACII mice

Arthritis arises spontaneously in adult TS1xHACII mice, and we were interested to evaluate processes occurring in younger mice that might play a predisposing role in arthritis development. The pdLN of 4- to 5-wk-old prearthritic TS1xHACII mice exhibited a marked hypocellularity, with total CD4+ T cells and 6.54CD4+ T cells substantially underrepresented relative to both TS1 and arthritic TS1xHACII mice (Fig. 3A). The spleens of prearthritic TS1xHACII mice contained CD4+ and 6.54CD4+ cells in numbers that were more comparable to the spleens of TS1 and arthritic TS1xHACII mice. Notably, >50% of 6.54CD4+ splenocytes in prearthritic TS1xHACII mice were IFN-γ-secretting Th1 cells, compared with 25 and 5% of 6.54CD4+ splenocytes in arthritic TS1xHACII and TS1 mice, respectively (Fig. 3B). Moreover, serum obtained from prearthritic TS1xHACII mice contained significantly higher levels of Th1-associated cytokines and chemokines (IFN-γ, CXCL9, and CXCL10) than serum from arthritic TS1xHACII and TS1 mice; by contrast, the serum from arthritic TS1xHACII mice expressed significantly higher levels of Th17-associated cytokines (IL-17, IL-6, and IL-1β) than were found in serum from prearthritic TS1xHACII mice (Fig. 3C).

When we examined the APC subsets present in prearthritic TS1xHACII mice, we again found very low numbers of cells in the pdLN (Fig. 3D). By contrast, the spleens of prearthritic TS1xHACII mice contained similar numbers of APCs (including iMO) as were found in age-matched TS1 mice, and there was a significant increase in the frequency of circulating iMO in the blood of prearthritic TS1xHACII mice relative to their TS1 counterparts (Fig. 3E, 3F). Notably, iMO in the spleens and blood of prearthritic TS1xHACII mice expressed MHC II at levels that were comparable to those found on iMO in the pdLN of arthritic TS1xHACII mice (compare Figs. 3G and 2A, respectively). Moreover, splenic iMO from prearthritic TS1xHACII mice were significantly better at inducing 6.54CD4+ T cells from TS1 mice to become Th17 cells than was the case for either cDC from prearthritic TS1xHACII mice or cDC or iMO obtained from the spleens of age-matched HACII mice (Fig. 3H). Together, these data indicate that the autoreactive CD4+ T cell response in prearthritic TS1xHACII mice differs from that observed in arthritic TS1xHACII mice in that it is characterized by IFN-γ production and occurs predominantly in the spleen rather than the pdLN. Notably, the formation of Th17-trophic iMO precedes the onset of an inflammatory response in the pdLN and arthritis.

Autoreactive CD4+ T cells promote activation of Th17-trophic inflammatory monocytes via IFN-γ production

To examine whether the autoreactive CD4+ Th1 cells that arise in prearthritic TS1xHACII mice can play a direct role in the formation of Th17-trophic iMO, we used an adoptive transfer approach in which CD4+ T cells from TS1 mice were introduced into T cell–deficient mice that express the HACII self-Ag (HACII.Ca2/2 mice). By 7 d posttransfer, 80% of the donor CD4+ T cells in the spleens and LN of recipient mice were IFN-γ–secreting cells, with a smaller subset coproducing IL-17 or producing IL-17 alone (Fig. 4A). The serum of the recipient mice also contained elevated concentrations of proinflammatory cytokines relative to mice that had not received T cells, with IFN-γ showing the greatest increase (>100-fold), consistent with the high frequency of IFN-γ–secreting cells detected by intracellular cytokine stain-
The introduction of HA-specific CD4+ T cells also led to increased numbers of MHC II+ iMO in the pdLN and spleens of recipient mice (Fig. 4C, 4D), and as had been observed in intact TS1xHACII mice, these iMO were significantly more capable of inducing Th17 cell formation than were cDC from HACII.Cα−/− mice that had received CD4+ T cells or iMO or cDC from unmanipulated HACII mice (Fig. 4E).

To identify signals that the CD4+ Th1 cells were providing to induce the formation of Th17-trophic iMO, we first generated mixed bone marrow chimeras in which 75% of marrow-derived cells expressed the HACII transgene and were CD45.1+, and the remaining 25% were CD45.1− and lacked expression of HA. When CD4+ T cells from a TS1 mouse were transferred into these chimeras, there was a significant increase in the number of iMO in their pdLN compared with control HA-chimeric mice that had not received T cells (Fig. 5A). However, the frequencies of HA+ to HA− iMO (based on CD45.1 expression) in the LNs of recipient mice were no different from in chimeras that had been left unmanipulated as controls and were similar to that of PMN, which were uniformly MHC II− and would therefore not be expected to interact with the transferred T cells in an Ag-specific manner (Fig. 5B). Moreover, MHC II was upregulated by iMO in the spleens of recipient mice independent of HA expression (Fig. 5C).

We then repeated the approach of transferring CD4+ T cells into HACII.Cα−/− mice and in this case treated the recipients either with an anti–IFN-γ mAb or an isotype control Ab. The number of iMO was significantly reduced in the pdLN of mice that received the anti–IFN-γ mAb (Fig. 5D). Moreover, there was little or no expression of MHC II on the iMO that were present in either the pdLN or the spleens of mice that were treated with anti–IFN-γ mAb, whereas MHC II expression by cDC in the spleens of recipient mice was unaffected by anti–IFN-γ treatment (Fig. 5E). Together, these data indicate that IFN-γ produced by CD4+ Th1 cells responding to a self-Ag can play a direct role in the activation of Th17-trophic iMO and that CD4+ T cell recognition of self-Ag expressed by the iMO themselves is not required for this activation.

**Discussion**

Inflammatory arthritis is a prominent disease manifestation in a number of systemic autoimmune disorders including rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE). Because MHC II alleles display by far the strongest genetic linkages with diseases such as SLE and RA, it is likely that CD4+ T cell recognition of self-peptides drives these disease processes, but how autoreactive CD4+ T cells can cause both systemic and joint-targeted autoimmune manifestations remains poorly understood, at least partly because the Ags that are recognized by autoreactive CD4+ T cells in human inflammatory arthritis are mostly undefined (18, 19). In TS1xHACII mice, the target autoantigen rec-
ognized by autoreactive CD4+ T cells (i.e., HA) is expressed selectively by APCs, which is notable in light of findings indicating that PBls from RA patients exhibit an autologous MLR suggestive of an autoreactive CD4+ T cell response to APCs (and APC-derived peptides) (19, 20). Moreover, in both SLE and RA, tissues other than the joints can also be affected by inflammatory processes. Indeed, these autoimmune diseases are typically classified as systemic because they involve multiple tissues and organ systems, and it is noteworthy in this regard that TS1xHACII mice develop inflammatory processes affecting the lungs and exhibit additional evidence of systemic immune activation resembling the processes that can occur in patients who develop autoimmune inflammatory arthritis (11). The studies in this paper describe an autoimmune process in which an initial systemic Th1-dominated autoreactive CD4+ T cell response against an APC-derived peptide leads to the activation of iMO that are biased toward inducing Th17 cell responses. Because iMO have an intrinsic capacity to mobilize from sites such as the blood and the spleen to the LNs and tissues, their activation by autoreactive CD4+ T cells provides a basis by which an autoimmune response that is systemic in nature can exhibit regional manifestations.

The reason that inflammatory arthritis is a prominent manifestation of the autoimmune response in TS1xHACII mice most likely reflects the sensitivity of the joints to the activities of IL-17 (21). We have shown that blocking IL-17A inhibits arthritis development in TS1xHACII mice, and IL-17 has been shown to promote joint pathology in other mouse models of inflammatory arthritis. In SKG mice (which harbor a mutation in the ZAP-70 gene that causes increased self-reactivity and autoreactive Th17 cell formation), synoviocytes produce CCL20, which attracts CCR6-bearing Th17 cells to the joints (22). In the K/BxN arthritis model, IL-17–producing T cells can augment autoantibody-induced arthritis and were enriched in inflamed joints of arthritic mice (23). Increased frequencies of IL-17–secreting CD4+ T cells were similarly found to promote arthritis development in another mouse model, even when these T cells lacked specificity for joint Ags (24). Our data provide evidence that the mobilization of iMO by autoreactive CD4+ T cells can also contribute to the development of a regional autoimmune response in inflammatory arthritis and resembles studies showing that the inflamed synovium and synovial fluid of patients with active RA contain activated monocytes that specifically promote Th17 responses (25). Increased iMO activity may also promote arthritis development through activation of CD4+ Th17 cells that react with joint-derived Ags because recent studies in this system suggested that the CD4+ Th17 cells that cause joint inflammation include cells using nonclonotypic TCRs, suggesting the process of epitope-spreading may contribute to the development of arthritis in TS1xHACII mice (9).

As is also the case in RA, TS1xHACII mice develop inflammatory arthritis as adults, and we have shown in this study that the autoimmune environment is distinct in young prearthritic and older arthritic TS1xHACII mice. Thus, the accumulation of autoreactive Th17 cells in the pdLN of arthritic TS1xHACII mice was accompanied by a shift in serum cytokines toward elevated expression of Th17-related cytokines and away from the elevated levels of Th1-related cytokines found in younger mice. It was also notable that the LNs of prearthritic mice exhibited a marked hypocellularity relative to age-matched TS1 controls. This hypocellularity in the LNs, but not in the spleens, could be an indication that the inflammatory environment of young TS1xHACII mice interferes with the formation and/or activity of lymphoid tissue inducer cells, which play a crucial role in LN formation (26). Additional studies will be necessary to determine why LN formation is impaired in young TS1xHACII mice, but at this stage, it is notable that impaired LN formation in young TS1xHACII mice may contribute to the delay in arthritis development until TS1xHACII mice become adults.

The biphasic cytokine response in TS1xHACII mice (Th1-dominated in prearthritic mice, Th17-dominated in arthritic mice) is also likely a reflection of an autoimmune environment in which various cytokines exert counterregulatory effects. For example, IFN-γ can be a negative regulator of IL-17 production and may tend to inhibit IL-17 production at early stages of the autoimmune response (27, 28). The decreased levels of IFN-γ in older mice may in turn be a consequence of increased IL-6 production (which was elevated in the serum of arthritic TS1xHACII mice), because IL-6 has been found to inhibit IFN-γ production and promote Th17 cell formation (4, 29). However, it is also possible that the IFN-γ–induced activation of iMO contributes to this effect, because monocytes secrete a number of inflammatory cytokines that support Th17 responses (including IL-6), and monocyte production of IL-6 is enhanced by TNF-α (30, 31). Similarly, activated monocytes and other myeloid cells can produce IL-23, which is likely to play an important role in supporting Th17 cell formation, and IL-23 can itself directly and indirectly induce myeloid cell activation, as indicated by studies in which elevated levels of IL-23 were found to lead to arthritis development (32–34). In these respects then, the activation of iMO by autoreactive CD4+ Th1 cells during the initial phases of the autoimmune response may be viewed as both a consequence of and a contributor to an evolving autoimmune environment.

Disclosures
The authors have no financial conflicts of interest.

References


Figure S1. iMO from TS1xHACII mice express characteristic cell surface molecules. Histograms show the expression of the indicated markers by iMO from TS1xHACII mice. Plasmacytoid DC and CD11b<sub>low</sub>Ly6C<sup>+</sup> cells (which include NK cells) are shown on the B220 and CD49b histograms as positive controls. Data are representative of 5 independent determinations.