The Cellular Source and Target of IL-21 in K/BxN Autoimmune Arthritis

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IL-21 is a pluripotent cytokine that regulates B cell and plasma cell differentiation and is thought to be an autocrine factor for follicular helper T cell (T~FH~) and Th17 differentiation. Although IL-21 has been implicated in autoimmune diseases, its relevant cellular source and target cells have not been well characterized. We investigated this issue in the K/BxN mouse model of autoimmune arthritis. Adoptive transfer of KRN-transgenic CD4+ T cells into appropriate hosts drives germinal center (GC) formation and autoantibody production against glucose-6-phosphate isomerase, leading to joint inflammation and destruction. By comparing transfer of T or B cells deficient in IL-21 or IL-21R, we were able to dissect the contribution of each cell type. T cells deficient in IL-21 did not induce GC formation or autoantibody production, but they went through normal T~FH~ differentiation. However, T cells lacking IL-21R induced Ab titers, GC B cell frequency, and arthritis development similar to wild-type T cells, suggesting that IL-21 is not required for T~FH~ differentiation and function. IL-21 acts on B cells, because IL-21R expression on B cells was required to induce disease. In contrast, Th17 cells, a T cell subset that also produces IL-21 and can provide help to B cells, are not required for the GC response and arthritis. These data have implications in developing effective therapies for rheumatoid arthritis and other Ab-mediated autoimmune diseases. The Journal of Immunology, 2013, 191: 000–000.

Interleukin-21, a member of the common γ-chain–signaling family of cytokines, plays an important role in lymphocyte activation, survival, and differentiation (1). IL-21 production is restricted to activated T cells, such as follicular helper T cells (T~FH~), Th17 cells, and NKT cells. The receptor for IL-21 is widely expressed on a variety of cell types, including B cells, activated T cells, NK cells, and dendritic cells. IL-21 promotes B cell proliferation, Ig class switching and production, and plasma cell differentiation (2). IL-21 also enhances the proliferation of T cells stimulated through their TCRs (3) and was shown to be an autocrine growth factor for T~FH~ and Th17 cell differentiation (4–8).

The T~FH~ subset, a canonical producer of IL-21, is controlled by the transcription factor Bcl6. Changes in chemokine receptor expression allow T~FH~ to migrate from the T cell zone into B cell follicles. Expression of cell surface molecules promote cell–cell contacts with B cells presenting cognate Ag. It is in these intimate interactions that IL-21 from T~FH~ is thought to act on B cells to promote germinal center (GC) and plasma cell differentiation (reviewed in Refs. 9, 10). In addition to T~FH~, the Th17 cell subset produces IL-21. Th17 is a dominant proinflammatory T cell subset, controlled by the transcription factor RORγt, and is involved in a number of autoimmune diseases (reviewed in Ref. 11). Th17 cells were shown to directly interact with and help B cells (12) and promote spontaneous GC formation in autoimmune BXD2 mice (13).

IL-21 is important in a number of animal models of systemic lupus erythematosus and rheumatoid arthritis (14–18). Accordingly, an association of certain IL-21 and IL-21R alleles with a risk for systemic lupus erythematosus in humans was reported (19, 20). In the NOD mouse model, IL-21 is required for the development of type I diabetes (21, 22). Given the complex biological functions of IL-21, it is important to understand the relevant cells producing and responding to the cytokine in the context of B cell–mediated autoimmunity.

We investigated this question of the relevant targets of IL-21 using the K/BxN model of rheumatoid arthritis. K/BxN mice develop arthritis by 4 wk of age (23). The disease is initiated by KRN TCR-transgenic CD4+ T cells that recognize a peptide from the ubiquitously expressed self-protein glucose-6-phosphate isomerase (GPI) presented by the NOD-derived MHC class II molecule I-A~B~ (24). Activated KRN T cells drive B cells to form GCs and to produce anti-GPI IgG autoantibodies, which induce joint pathology (25). K/BxN mice develop spontaneous disease, unlike other models of arthritis that involve Ag vaccination with adjuvants and can affect T~FH~ development (26). The advantage of the model for these studies is that the disease can be induced by transferring naïve KRN T cells into T cell–deficient hosts expressing the MHC class II molecule I-A~B~ (25, 27).

In this study, we used this cell-transfer approach of the K/BxN model to determine the source and action of IL-21 in arthritis. We showed that T cells deficient in IL-21 did not induce GC formation or autoantibody production, but they went through normal T~FH~ differentiation. However, T cells lacking IL-21R induced similar Ab titer, GC B cell frequency, and arthritis development as wild-type (WT) T cells, suggesting that IL-21 is not required for T~FH~ differentiation and function. IL-21 must act on B cells, because IL-21R expression on B cells was required to induce disease. Surprisingly, Th17 cells are not required for arthritis development, stressing the importance of IL-21 production specifically from the...
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T\textsubscript{FH} subset. These results have implications for developing effective therapies for rheumatoid arthritis and other Ab-mediated autoimmune diseases.

Materials and Methods

Mice

KRN TCR-transgenic mice (23), IL-21R\textsuperscript{−/−} mice (28), and ROR\textgamma\textsuperscript{GFP/GFP} mice (29) were maintained on C57/BL6 background. IL-21\textsuperscript{−/−} mice (B6;129S-I-J2)\textsuperscript{mice, obtained from the Mutant Mouse Regional Resource Center, Davis, CA) were maintained on a B6;129S5 mixed background. KRN was crossed to IL-21\textsuperscript{−/−} or IL-21\textsuperscript{−/−} mice to generate K/IL-21R\textsuperscript{−/−} or KRN/IL-21R\textsuperscript{−/−} mice, respectively. TCR C\textalpha\textsuperscript{−/−} BxN mice used as hosts were F1 of C57/BL6\texttimes B6 and C\textalpha\textsuperscript{−/−} NOD (25). IL-21R knockout mice crossed to B6.H2g7-congenic mice (30) were used as donors for purified B cells. All experiments were approved by the Institutional Animal Care and Use Committee of the University of Chicago.

Cell-transfer induction of arthritis

CD4\textsuperscript{+} cells were isolated from splenocytes using AutoMACS positive selection program. A total of 1 \times 10\textsuperscript{6} cells in sterile DMEM was injected into the tail vein of C\textalpha\textsuperscript{−/−} BxN mice. B cells were enriched from splenocytes by depleting T cells with anti-CD90.2 Ab (clone 53-52-1; BioLegend), followed by rabbit complement (Cedarlane).

Abs and flow cytometry

Anti-KRN TCR-specific Ab 3-4G-B7 (J. Perera, B. Stadinski, X. Liu, E. Huseby, and H. Huang, manuscript in preparation) was labeled with Alexa Fluor 647 or biotin. Abs used were against CXCR5, Bcl6, and Fas (BD Biosciences); CD45.1, TCR\alpha, GL-7, CD19, IL-17A, and B220 (eBioscience); and CD4 and PD-1 (BioLegend). Intracellular staining for Abs and flow cytometry

Immunohistochemistry staining of splenic sections

Sections of frozen spleen (5 \mu m) were thawed, rehydrated, and then stained. Peanut agglutinin (PNA; Alexa Fluor 488), anti-mouse IgD (PE), and anti-mouse V\textbeta\textsix (Alexa Fluor 647) or anti-KRN TCR\alpha (Alexa Fluor 647) were used. Images were taken on an Axiovert200m microscope (Zeiss) and visualized with ImageJ.

ELISA for anti-GPI total IgG

Ninety-six-well plates were coated with 5 \mu g/ml recombinant GPI in PBS overnight at 4°C and blocked with 1% BSA 0.05% Tween-20 in PBS at room temperature. A serial dilution of the samples added to the plate was detected with a biotinylated goat anti-mouse IgG (subclasses 1+2a+2b+3) Fc\gamma or goat anti-mouse IgM Fc\mu fragment–specific Ab, followed by alkaline phosphatase–conjugated streptavidin (all from Jackson ImmunoResearch). Samples were developed with phosphatase substrate (Sigma) and were read at 405 nm. A four-parameter variable slope was fitted to the data points, and the EC\textsubscript{50} (infection point) for a standard sample was calculated from this nonlinear regression. Serum titers were calculated as the serum dilution (x value) that gave the calculated EC\textsubscript{50} (y value) based on the fitted nonlinear regression for each sample. Samples where the curve could not be fitted because of low signal (low Ab binding) are indicated as ND (not detectable), and a titer of 1 was assigned for statistical comparisons. All analyses were conducted using Prism 5.0b software (GraphPad).

Statistical analysis

Normally distributed data were analyzed by the unpaired t test using Prism 5.0b software (GraphPad).

Results

T\textsubscript{FH} differentiation in the cell-transfer model of autoimmune arthritis

To investigate the role of IL-21 in a cell-specific manner, we took advantage of the cell-transfer model of the K/BxN mouse. Naive CD4\textsuperscript{+} KRN T cells are isolated from healthy KRN/B6 mice (KRN maintained on C57/BL6 background) and transferred into C\textalpha\textsuperscript{−/−} BxN hosts (TCR C\textalpha\textsuperscript{−/−} on B6xNOD F1 background) (25, 27). These hosts lack αβ T cells and express the MHC class II allele I-A\textsuperscript{E7}, which is required for the KRN TCR to recognize a peptide from the self-Ag GPI. Transferred KRN T cells are activated and induce high titers of anti-GPI IgG Abs, resulting in ankle swelling and joint remodeling. To follow autoreactive T\textsubscript{FH} differentiation and GC response, T\textsubscript{FH} and GC B cells were characterized after T cell transfer. For comparison, naive KRN/B6 T cells were transferred into C\textalpha\textsuperscript{−/−} B6 hosts. Because C\textalpha\textsuperscript{−/−} B6 mice do not carry the MHC class II allele I-A\textsuperscript{E7}, KRN T cells do not precipitate disease upon transfer. This allowed us to verify that T\textsubscript{FH} differentiation is dependent on Ag recognition rather than lymphopenia-induced homeostatic proliferation. PD-1\textsuperscript{+} CXCR5\textsuperscript{+} or Bcl6\textsuperscript{+} CXCR5\textsuperscript{+} T\textsubscript{FH} were identified in C\textalpha\textsuperscript{−/−} BxN hosts but not in C\textalpha\textsuperscript{−/−} B6 hosts or KRN/B6 mice (Fig. 1A). In the following experiments, we used CXCR5 and intracellular Bcl6 to mark T\textsubscript{FH}, because these markers showed a more distinct pattern. Consistent with the T\textsubscript{FH} staining, GC B cells (identified as GL-7\textsuperscript{+} Fas\textsuperscript{+}) were induced in C\textalpha\textsuperscript{−/−} BxN hosts, whereas C\textalpha\textsuperscript{−/−} B6 hosts had a small GC population, similar to what was observed in naive KRN/B6 mice (Fig. 1B).

IL-21 production by T cells is required to induce arthritis

To determine whether IL-21 production by T cells acts on T cells in an autocrine manner to induce arthritis, we compared naive CD4\textsuperscript{+} KRN T cells purified from WT K/B6 mice (denoted as “K” in the figures) with those purified from KRN mice deficient in IL-21 (referred to as K/IL-21\textsuperscript{−/−}) or IL-21R (referred to as K/IL-21R\textsuperscript{−/−}) after transfer into C\textalpha\textsuperscript{−/−} BxN hosts. In the K/IL-21\textsuperscript{−/−} transfer, KRN T cells could not produce IL-21; in the K/IL-21R\textsuperscript{−/−} transfer, KRN T cells were able to produce IL-21 but unable to receive IL-21R signaling. These two groups allowed us to test whether IL-21 production by autoreactive T cells is required, as

![Image](https://example.com/image1.png)

**FIGURE 1.** KRN T cell transfer leads to T\textsubscript{FH} and GC B cell differentiation upon Ag recognition. (A) CD4\textsuperscript{+} cells from KRN/B6 (K) mice were transferred into C\textalpha\textsuperscript{−/−} BxN or C\textalpha\textsuperscript{−/−} B6 hosts, and splenocytes were analyzed 8 d later. Transferred cells, identified as CD4\textsuperscript{+} TCR\alpha\textsuperscript{+} BxN hosts (TCK C\textalpha\textsuperscript{−/−} on B6xNOD F1 background) (25, 27). These hosts lack αβ T cells and express the MHC class II allele I-A\textsuperscript{E7}, which is required for the KRN TCR to recognize a peptide from the self-Ag GPI. Transferred KRN T cells are activated and induce high titers of anti-GPI IgG Abs, resulting in ankle swelling and joint remodeling. To follow autoreactive T\textsubscript{FH} differentiation and GC response, T\textsubscript{FH} and GC B cells were characterized after T cell transfer. For comparison, naive KRN/B6 T cells were transferred into C\textalpha\textsuperscript{−/−} B6 hosts. Because C\textalpha\textsuperscript{−/−} B6 mice do not carry the MHC class II allele I-A\textsuperscript{E7}, KRN T cells do not precipitate disease upon transfer. This allowed us to verify that T\textsubscript{FH} differentiation is dependent on Ag recognition rather than lymphopenia-induced homeostatic proliferation. PD-1\textsuperscript{+} CXCR5\textsuperscript{+} or Bcl6\textsuperscript{+} CXCR5\textsuperscript{+} T\textsubscript{FH} were identified in C\textalpha\textsuperscript{−/−} BxN hosts but not in C\textalpha\textsuperscript{−/−} B6 hosts or KRN/B6 mice (Fig. 1A). In the following experiments, we used CXCR5 and intracellular Bcl6 to mark T\textsubscript{FH}, because these markers showed a more distinct pattern. Consistent with the T\textsubscript{FH} staining, GC B cells (identified as GL-7\textsuperscript{+} Fas\textsuperscript{+}) were induced in C\textalpha\textsuperscript{−/−} BxN hosts, whereas C\textalpha\textsuperscript{−/−} B6 hosts had a small GC population, similar to what was observed in naive KRN/B6 mice (Fig. 1B).
well as whether autoreactive T cells require IL-21 as an autocrine factor for disease. As shown in Fig. 2A, K/IL-21−/− CD4+ T cells transferred into C57/B16 hosts did not induce arthritis. In contrast, K/IL-21−/− CD4+ T cells induced severe arthritis with the same kinetics as the WT KRN T cells. We determined the anti-GPI IgG titers both early (8 d) and late (29–31 d) in disease. WT KRN T cells and K/IL-21−/− T cells induced high titers of anti-GPI IgG at both time points. In contrast, anti-GPI IgG titers were two to three orders of magnitude lower in the K/IL-21−/− T cell–transfer model (Fig. 2B). These data demonstrate that IL-21 production by T cells is crucial for IgG Ab response and arthritis but that IL-21 is not required on T cells.

To test the role of IL-21 in extrafollicular response in this model, we determined anti-GPI IgM and IgG at earlier time points after transferring WT KRN or K/IL-21−/− splenocytes. In the WT KRN transfer, anti-GPI IgM titers were elevated by day 4 and continued to increase over time; this occurred before IgG titers increased. In contrast, both anti-GPI IgM and IgG titers remained low in K/IL-21−/− transfers (Fig. 2C). These data demonstrate that IL-21 is required in the early extrafollicular response in this model.

IL-21 is not required for T<sub>FH</sub> differentiation in vivo

We next compared the fate of transferred T cells and T<sub>FH</sub> differentiation in all three transfer settings. Congenic markers on transferred cells (CD45.2<sup>+</sup>) and C57/B16 host cells (CD45.1<sup>−</sup>/CD45.2<sup>+</sup>) allowed us to identify the transferred T cells as the CD45.1<sup>+</sup>CD45.2<sup>−</sup> population (Fig. 3A). Eight days after cell transfer, just after disease onset, there was a small but significant increase in the percentage and number of K/IL-21−/− T cells compared with WT KRN T cells in the spleen. The percentage and number of K/IL-21−/− T cells were comparable to WT KRN T cells. At 29–31 d, when disease was fully established in WT KRN and K/IL-21−/− T cell–transfer mice, there was no significant difference in the percentage and numbers of transferred T cells among the three transfer groups. These data suggest that the survival of transferred CD4<sup>+</sup> KRN T cells was not affected by their ability to produce or respond to IL-21.

To determine how T<sub>FH</sub> differentiation was affected, CXCR5 and intracellular Bcl6 staining were used to identify T<sub>FH</sub>. WT KRN, K/IL-21−/−, and K/IL-21−/− T cells differentiated into T<sub>FH</sub> at similar frequencies in the spleen 8 d after transfer (Fig. 3B). The absolute number of T<sub>FH</sub> from K/IL-21−/− animals was transiently higher than that from WT donors as a result of the higher total number of CD4<sup>+</sup> T cells (Fig. 3A). However, by 29–31 d, K/IL-21−/− T<sub>FH</sub> percentage and numbers decreased to half of those in WT KRN-transfer mice. This presumably reflects the defects in the maintenance phase of T<sub>FH</sub> differentiation (31), given that there were no GCs formed in these mice (see later discussion). However, there were comparable numbers of WT and K/IL-21−/− T<sub>FH</sub> 8 d after transfer, and a small, but not statistically significant, decrease in T<sub>FH</sub> numbers in K/IL-21−/− cells compared with WT at days 29–31 (Fig. 3B). These data suggest that IL-21 is not a requisite autocrine factor for KRN T<sub>FH</sub> differentiation.

IL-21 is required for GC formation

Although T<sub>FH</sub> differentiation was normal in all transfers, anti-GPI IgG Ab production was severely impaired following the transfer of K/IL-21−/− T cells (Fig. 2B). Therefore, we investigated GC formation and T cell migration by immunofluorescence on spleen sections. As shown in Fig. 4A, there were abundant GCs in both WT KRN and K/IL-21−/− T cell–transfer mice. In contrast, GCs in K/IL-21−/− T cell–transfer mice were rarely observed, and the few GCs were amorphous and dimly labeled with PNA. The number of GCs was counted from multiple spleen sections from multiple mice (Fig. 4B). KRN WT and K/IL-21−/− T cell transfer induced equivalent numbers of GCs/section. GC size was measured; KRN WT mice had slightly larger GCs on average compared with K/IL-21−/− T cell–transfer mice. We also assessed
were shown to drive the formation of spontaneous GCs in autoimmune responses of the Bx22 mice (13), and they can provide effective help to B cells (12, 32). Therefore, we tested the contribution of Th17 cells in initiating GC responses and arthritis. K/B6 mice were crossed to RORγtGFP/GFP mice (33) to generate K/RORγtGFP/GFP mice. In these mice, GFP insertion inactivates RORγt expression, and Th17 differentiation is defective. CD4+ T cells were purified from either K/RORγtGFP/GFP or K/RORγtGFP/GFP mice and transferred into Cxα−/−BxN hosts. As shown in Fig. 5A, K/RORγtGFP/GFP CD4+ T cells induced arthritis with a similar kinetics and severity. There also was no difference in the serum anti-GPI IgG titers (Fig. 5B). The transferred K/RORγtGFP/GFP cells were indeed deficient in generating Th17 cells as determined by IL-17 intracellular staining after in vitro stimulation (Fig. 5C). However, there was no difference in the differentiation of Th17 (Fig. 5D). These results demonstrate that Th17 cells and their production of IL-21 are not essential for GC responses and arthritis development, supporting the conclusion that Tfh are the major source of IL-21 production.

It was shown previously that Th17 cell development is correlated with disease induction in K/BxN mice (34). We investigated whether there were differences in Th17 cell development and distribution in K/BxN mice versus the transfer model. Cells isolated from the spleen, draining lymph nodes (pooled popliteal and inguinal lymph nodes), and mesenteric lymph nodes stimulated in vitro revealed a similar profile of IL-17A production from K/BxN mice and Cxα−/−BxN hosts after KRN T cell transfer (Fig. 5E). IL-17A production was detected from KRN T cells identified with the anti-KRN Vα7/8 and anti-KRN/VaaMHC class II alleles were transferred along with naive K/B6 CD4+ T cells into Rag1−/−B6xNOD F1 hosts. Most mice that received IL-21R+/+ T cells developed arthritis, whereas mice that received IL-21R−/− B cells never showed signs of arthritis (Fig. 6A). The disease states were reflected in the dramatic difference in anti-GPI IgG titers between these two groups of mice (Fig. 6B). There was a moderate decrease in Tfh in hosts receiving IL-21R−/− B cells compared with those receiving IL-21R+/+ B cells (Fig. 6C). This result is consistent with what was observed in the K/IIL-21−/−T cell transfer; Tfh numbers decreased after 29–30 d, likely due to a lack of interaction with GC B cells to promote Tfh maintenance. There was a more dramatic decrease in GC B cells in hosts receiving IL-21R−/− B cells compared with those receiving IL-21R+/+ B cells (Fig. 6D). Immuno-histological analysis of GCs on spleen section confirmed the results obtained by flow cytometry, although there were few B cell follicles and smaller GCs in general compared with T cell transfer into Cxα−/−BxN hosts (data not shown). This is not surprising considering that Rag1−/− mice have defective follicular structures and a much smaller population of transferred B cells. These data support the conclusion that IL-21 signaling in B cells is essential for GC formation, Ab production, and arthritis development, and GC B cells are required for Tfh maintenance.

**Discussion**

IL-21 is a pleiotropic cytokine affecting a diverse array of cell types (2). Without conditional deletion of IL-21 or IL-21R, it has been
difficult to evaluate the specific roles of IL-21 in autoimmunity. We used a cell-transfer system based on the K/BxN mouse model of autoimmune arthritis to address these roles in T<sub>FH</sub> differentiation and B cell activation. Our results demonstrate that IL-21 production by T cells is important in disease induction. However, IL-21 is not required for T<sub>FH</sub> differentiation, maintenance, or function. There was no defect in T cell survival when KRN T cells were deficient in either IL-21 or IL-21R; in fact, survival was increased 1 wk after transfer in IL-21<sup>−/−</sup> T cells (Fig. 3A). A similar increase was observed in IL-21<sup>−/−</sup> mice after immunization with NP-KLH (35), although the mechanism for enhanced survival is unclear. Both IL-21<sup>−/−</sup> and IL-21R<sup>−/−</sup> KRN T cells proliferated, differentiated into T<sub>FH</sub>, and were able to migrate into the B cell follicles in an Ag-specific manner, because this process did not take place when KRN T cells were transferred into C<sub>α<sup>−/−</sup></sub>B6 hosts that do not express the self-peptide–MHC complex.

In an earlier study using IL-21R<sup>−/−</sup> K/BxN mice, it was shown that there were fewer CD4+ T cells in the spleen and joint-draining lymph nodes compared with normal K/BxN mice (36). IL-21R<sup>−/−</sup> K/BxN mice did not develop arthritis, and it was attributed to a requirement of IL-21 by KRN T cells for homeostatic proliferation. The different conclusions from our study and the earlier results suggest that the role of IL-21 in autoimmune arthritis may be more complex than previously thought.
study highlight the complex biological function of IL-21 and the problem of understanding the direct versus indirect mechanisms in total knockout animals.

We found no difference in the GC B cell response between KRN WT and K/IL-21R−/− T cell–transfer mice by Ab titers or GC formation led to different conclusions in mice immunized with Ags. GC formation was relatively unaffected in IL-21−/− mice in some studies (35, 38, 39), whereas a more profound effect on T FH and GCs was found in other studies (5, 8). It was suggested that the different results obtained in these studies might be explained by the different types of Ag or adjuvant, the avidity of TCR involved for peptide–MHC, or the timing of analysis (35, 39). Our conclusion that IL-21 is not required for T FH development is not dependent on IL-21 in autoimmunity raises the possibility that an efficient inhibition of ongoing GC B cell proliferation may be difficult to achieve in practice with partial effectiveness of IL-21 blockade. It is tempting to speculate that IL-21 on IL-21R signaling may be related to the difference in frequency or affinity of alloreactive T cells, because it was shown that naive Ag-specific Th cells with TCRs of higher affinity preferentially differentiate into the CXCR5+ “resident” T FH compartment (37).

Studies on the role of IL-21 in T FH differentiation and GC formation led to different conclusions in mice immunized with protein Ags. GC formation was relatively unaffected in IL-21−/− or IL-21R−/− mice in some studies (35, 38, 39), whereas a more profound effect on T FH and GCs was found in other studies (5, 8). This variation could be attributed to the partial effectiveness of IL-21R–Fc in blocking IL-21 signaling. The evidence that T FH development is not dependent on IL-21 in autoimmunity raises the possibility that an efficient inhibition of ongoing GC B cell response may be difficult to achieve in practice with partial effectiveness of IL-21 blockade. It is tempting to speculate that IL-21 blockade is more effective in cases in which T FH is more dependent on the cytokine. IL-21 blockade together with a therapy targeting T cells might be most beneficial for treating certain Ab-mediated autoimmune diseases.
Arthritis development in K/BxN mice is dependent on gut microbiota, particularly the colonization of segmented filamentous bacteria. Because the KRN-transfer model involves different strains of mice as the source of donor cells and hosts, the potential difference in their gut microbiota might be a confounding factor in the interpretation of our experiments. However, we think this is not likely to be the case. In our experiments, we always used littermates for our cell-transfer hosts, dividing the hosts housed in the same cage for different KRN-genotype transfers. We used donors of different genotypes housed in the same cage when possible. Furthermore, the gut microbiota of K/IL-21−/− and K/IL-21R−/− donor mice should be very similar because they are defective in the same pathway.

Th17 cells are now recognized to interact with Ag-specific B cells as potential B cell helpers (12, 13). Therefore, it was important to investigate whether IL-21 required for disease induction was produced by TGFβ or from Th17 cells that enter the B cell follicle to initiate GC. By eliminating the Th17 subset through RORγtEGFP/GFP, K/RORγtEGFP/GFP T cells did not dramatically alter disease kinetics or severity, suggesting that IL-21 is derived from TGFβ but not Th17 cells.

At first glance, the result that Th17 cells are not essential for disease induction seems unexpected, because Th17 cells were suggested to play an important role in this disease model. Th17 cell induction correlated with disease onset, and neutralizing Ab against IL-17A prevented disease in K/BxN mice (34). IL-17R−/− B cells were defective in differentiating into GC B cells, suggesting that they are the targets of IL-17. However, other lymphocyte and innate-like cell populations, including γδ T cells and some recently characterized innate lymphoid cells, are major producers of IL-17 (44, 45). The intriguing possibility that IL-17 produced constitutively by innate lymphoid cells plays an important role is currently being investigated.

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Disclosures

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