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A Nonredundant Role for IL-21 Receptor Signaling in Plasma Cell Differentiation and Protective Type 2 Immunity against Gastrointestinal Helminth Infection

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Pathogen-specific Ab production following infection with the gut-dwelling roundworm Heligmosomoides polygyrus is critical for protective immunity against reinfection. However, the factors required for productive T cell–B cell interactions in the context of a type 2-dominated immune response are not well defined. In the present study, we identify IL-21R signaling as a critical factor in driving pathogen-specific plasma cell differentiation and protective immunity against *H. polygyrus* in mice. We show that B cells require direct IL-21R signals to differentiate into CD138⁺ plasma cells. In contrast, IL-21R signaling is dispensable for germinal center formation, isotype class switching, and Th2 and T follicular helper cell differentiation. Our studies demonstrate a selective role for IL-21 in plasma cell differentiation in the context of protective antiparasitic type 2 immunity. *The Journal of Immunology*, 2010, 185: 6138–6145.

We and others have recently shown that protective immunity against the intestinal-dwelling helminth *Heligmosomoides polygyrus* requires isotype class-switched Ab secretion (1–5). However, the factors that drive pathogen-specific Ab production and subsequent resistance to reinfection are not fully understood and are being intensely investigated.

Recent studies have highlighted a crucial role for IL-21 in T follicular helper (Tfh) differentiation and Ab production (6–9). IL-21 is a monomeric cytokine produced by activated CD4⁺ T cells, NK cells, and NKT cells (10–12). IL-21 signaling is mediated through a cell-surface heterodimeric receptor composed of a cytokine-specific α-chain (IL-21Rα) and the common γ-chain, which it shares with the receptors for IL-2, IL-4, IL-7, IL-9, and IL-15 (10, 13). The IL-21R is constitutively expressed on a number of hematopoietic cell types, including all B and T cell lineages, and its expression may be increased following cellular activation (8, 14). IL-21 signaling was initially shown to promote B cell proliferation and plasma cell differentiation in vitro (15–17). Recent studies have confirmed these observations in vivo using model Ag immunization strategies to mechanistically examine how IL-21 affects B cell differentiation (6, 7). IL-21 has been additionally considered as a cytokine that supports the differentiation of Tfh cells, a subset of CD4⁺ T cells that localize to B cell follicles and germinal centers (GCs) to promote isotype switching and affinity maturation (8, 9). Indeed, we and others have recently found that following *H. polygyrus*, *Schistosoma mansoni*, or *Nippostrongylus brasiliensis* infection, IL-4 producing Tfh cells that accumulate in primary and secondary follicles of the spleen and reactive lymph nodes express high levels of IL-21 compared with conventional Th2 cells (18–20). However, the role of IL-21 in Tfh differentiation itself is somewhat controversial and may depend on the conditions of the immune response examined (6, 7, 21).

In the context of infectious disease, particularly in type 2-mediated responses, our knowledge about the role of IL-21 is rather limited. It has been previously shown that IL-21 promotes alternative activation of macrophages and supports granuloma formation following *S. mansoni* and *H. polygyrus* infection, respectively (22, 23). IL-21 has also been shown to promote pathogen-specific Abs following *Toxoplasma gondii* infection (24). However, none of these studies has assessed the impact of IL-21 on Tfh cell and B effector cell differentiation during a type 2-dominated immune response or its role in protective immunity to helminth infection. To address these issues, we investigated the role of IL-21 in protective immunity and Tfh and B cell differentiation following infection of mice with *H. polygyrus*. Our studies demonstrate that IL-21R signaling is critical for plasma cell differentiation, generation of pathogen-specific IgG1, and for mediating protective immunity against *H. polygyrus* upon reinfection. Unexpectedly, we found that IL-21 signaling was dispensable for Th2 and Thfh differentiation, GC formation, and isotype class switching. Our results demonstrate a nonredundant selective role for IL-21 in type 2 humoral immunity and protection against helminth infection.

Materials and Methods

**Mice**

C57BL/6 (CD45.1⁺ and CD45.2⁺) were bred and kept under specific pathogen-free conditions at the Trudeau Institute and used at 8–16 wk of age. IL-21R−/− mice were obtained from Dr. Mercedes Rincon (University of Vermont, Burlington, VT) (24). All experiments were performed under Trudeau Institute Institutional Animal Care and Use Committee-approved protocols.

**H. polygyrus stocks, Ag, and infection**

The life cycle of *H. polygyrus* was maintained by routine passage through BALB/c mice. Soluble Ag was prepared from adult parasites as previously described (25). Mice were infected by gavage with 200 L3 *H. polygyrus* larvae. In some experiments, adult *H. polygyrus* parasites were eliminated by two oral administrations of 100 mg/kg pyrantel pamoate (Effcon Labo-
ratories, Marietta, GA) delivered 2 d apart. Mice were challenged 10 d after the last drug treatment with 200 L3 *H. polygyrus*. Adult *H. polygyrus* parasites in the intestinal lumen were enumerated by visual inspection under a dissecting microscope 14 d after challenge infection.

**Real-time RT-PCR**

cDNA was prepared as described (25). TaqMan reagents for GAPDH have been described (25), and BCL-6 primers and probes were Assays on Demand purchased from Applied Biosystems (Foster City, CA). Quantitative real-time RT-PCR was performed by using an Applied Biosystems Taq-Man 7500 Fast system and software. Fold expression was calculated using the \( \Delta \Delta C_T \) method and GAPDH as a reference gene.

**Flow cytometry**

Single-cell suspensions were prepared from the mesLNs, stained and analyzed as described (25). The following mAbs were used for flow cytometry: CD4 (RM4-5), CD62L (MEL-14), ICOS (C398.4A), CXCR5 (2G8), CD19 (281-2), IgD (11-26c.2a) (BD Biosciences, San Jose, CA), peanut agglutinin (PNA; Invitrogen, Carlsbad, CA), PD-1 (RMP1-30), CD138 (281-2), IgG1 (SouthernBiotech, Birmingham, AL), CD45.1 (A20), and CD45.2 (104) (eBioscience, San Diego, CA). A “dump” gate in the FL1 channel was included in some experiments, and FITC-conjugated Abs from BD Biosciences were used. Propidium iodide was used to eliminate dead cells from analysis. Samples were acquired on a FACS-Canto II (BD Biosciences) and analyzed with FlowJo software (Tree Star, Ashland, OR).

**Immunohistochemistry**

mesLNs were harvested from *H. polygyrus*-infected animals and immediately frozen in optimal cutting temperature embedding compound (Sakura Finetek, Torrance, CA) over liquid nitrogen for detection of surface Ags. Frozen lymph nodes were cut into 6-μm sections on a Leica cryostat and fixed in a mixture of ice cold 75% acetone/25% ethanol for 5 min. Sections were then blocked in PBS plus 2% BSA and 5% normal mouse serum for 60 min. Sections were then stained with rat anti-mouse IgD-Alexa Fluor 647 and anti-mouse GL7-FITC. All images were captured using a Zeiss Axiosvert 200M microscope and analyzed with AxioVision software.

**Generation of mixed bone marrow chimeras**

CD45.1^+^CD45.2^+^ wild-type (WT) and CD45.1^-^CD45.2^-^ IL-21R^α/−/−^ bone marrow cells were mixed at a 1:1 ratio (2.5 × 10^7^/donor population) and injected into the tail veins of lethally irradiated (1000 rad split into two doses) CD45.1^-^CD45.2^-^ congenic hosts. In all cases, the reconstitution efficiency of lymph node CD19^+^ B cells was >95%. Mice were infected with *H. polygyrus* after allowing at least 6 wk for hematopoietic reconstitution.

**T cell purification and restimulation**

CD4^+^ T cells were purified (~90% purity) from mesLNs by negative selection over CD4 Immunocolumns (Cedarlane Laboratories, Burlington, NC). Purified CD4^+^ T cells (2 × 10^5^/well) were stimulated with plate-bound anti-CD3 (1 μg/ml; clone 145-2C11) and anti-CD28 (1 μg/ml; clone 37.51) in round-bottom 96-well plates. In some experiments where indicated, total lymph node cells were restimulated as described above. Supernatants were removed for analysis after 48 h.

**FIGURE 1.** IL-21 signaling is dispensable for Tfh and Th2 differentiation following helminth infection. A, WT and IL-21R^α/−/−^ mice were orally infected with L3 larvae from *H. polygyrus*. Two weeks postinfection, mesLNs were harvested and the frequency (left) and number (right) of CXCR5^+^PD-1^+^ were assessed by flow cytometry. Zebra plots shown are gated on CD4^+^ T cells. B, mesLN cells were harvested as in A and the number of activated CD62L^−^ICOS^+^ CD4^+^ T cells was enumerated based on flow cytometry analysis. Data shown are representative of five independent experiments with four mice per group. C and D, Purified CD4^+^ T cells from mesLNs of 2 wk-infected mice were cultured with or without anti-CD3 and anti-CD28. Supernatants were assessed by ELISA for IL-4 (C) and IFN-γ (D) after 48 h of culture. Data shown are representative of two independent experiments with three mice per group. Error bars represent SD from the mean. *p ≤ 0.05; **p ≤ 0.005. ND, not detectable.
ELISAs and ELISPOTs
Cytokines were measured by ELISA with Ab pairs from BD Biosciences. H. polygyrus-specific Abs were assayed by ELISA with the use of plates coated with 2 μg/ml H. polygyrus Ag. Serum samples were serially diluted, and bound Abs were detected with anti-mouse IgM or anti-mouse IgG1 (SouthernBiotech). Total IgE was measured by the use of anti-mouse IgG Ab pairs from BD Biosciences in ELISAs. For ELISPOTs, single-cell suspensions of splenocytes or bone marrow cells from 3× H. polygyrus infected WT or IL-21Rα−/− mice were cultured in RPMI 1640 supplemented with FCS for 24 h in MultiScreen-HA plates (Millipore, Billerica, MA) coated with 2 μg/ml anti-mouse IgG1 (BD Biosciences). Bound Abs were detected with noncompeting anti-mouse IgG1 (SouthernBiotech).

Statistical analyses
Data were analyzed with the unpaired Student t test via Prism 4.0 (GraphPad Software). All data shown represent mean ± SD, and p values ≤0.05 were considered statistically significant.

Results
IL-21 is dispensable for Th2 and Tfh differentiation following helminth infection
We have previously shown that IL-4-producing CXCR5+PD-1+ CD4+ T follicular helper cells localize to the B cell follicles and GCs following H. polygyrus infection and express high levels of IL-21 mRNA (18, 19). As IL-21 has been implicated in the differentiation of Tfh cells using model Ags and adjuvant (8, 9), we examined whether this cytokine is also required for Tfh differentiation in a Th2-dominated immune response to infection with an intestinal parasite. To this end, we orally infected IL-21Rα−/− mice and C57BL/6 controls with H. polygyrus larvae. Animals were sacrificed at 2 wk postinfection, a time when the primary Th2 response is at its peak (25), and we examined for the frequency and number of CD4+CXCR5+PD-1+ Tfh cells by flow cytometry. Contrary to our expectations, we found no decrease in the Tfh response in IL-21Rα−/− mice compared with WT controls; in fact, the frequency and number of Tfh cells was increased in IL-21Rα−/− mice (Fig. 1A). Correspondingly, IL-21Rα−/− mice had more activated ICOS+CD62L− CD4+ T cells (Fig. 1B), a population we have previously shown to include all Th2 cells in vivo using 4get IL-4-eGFP reporter mice (19). Consistent with this finding, purified CD4+ T cells from H. polygyrus-infected IL-21Rα−/− mice produced more IL-4 than did their WT counterparts following restimulation in vitro (Fig. 1C), but displayed no difference in IFN-γ production (Fig. 1D). These results indicate that IL-21R is dispensable for a vigorous Tfh and Th2 response following primary infection with H. polygyrus.

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Plasma cell differentiation and IgG1 production, but not GC formation or isotype class switching, are compromised in the absence of IL-21 signaling

Similar to the common γ-chain subunit (26), the α-chain of the IL-21R is constitutively expressed by B and T lymphocytes (8). Therefore, we examined whether the primary B cell response to infection with *H. polygyrus* was altered in the absence of IL-21R signaling. Consistent with an exaggerated Tfh response in the absence of IL-21R (Fig. 1), the frequency and number of isotype-switched CD19⁺IgD⁻IgG1⁺ cells as well as PNA⁺Fas⁺ GC cells were increased in IL-21R⁻deficient animals relative to WT controls (Fig. 2A–C). Of note, the vast majority of surface IgG1⁺ B cells were contained within the GC population (data not shown). Furthermore, the architecture of the GC was grossly similar in mesLN from WT and IL-21R⁻ mice at 2 wk after *H. polygyrus* infection based on IgD and GL7 staining (Fig. 2D), and GC cells from WT and IL-21R⁻⁻ mice expressed similar levels of BCL-6 (Fig. 2E). These data indicate that IL-21 signals are not required for B cell isotype class switching or GC formation.

As IL-21 has been implicated in promoting plasma cell differentiation and Ab production (15–17), we compared the number of CD138⁺ cells that did not express IgD or the lineage markers CD4, CD8, and CD11b (shown as dump gate) in the reactive mesLN of WT and IL-21R⁻⁻ following *H. polygyrus* infection. In contrast to other measures of B cell activation and differentiation described above, the frequency and number of dump CD138⁺ cells was significantly decreased compared with WT controls (Fig. 3A). Consistent with this finding, the amount of total serum IgG1 was decreased in *H. polygyrus*-infected IL-21R⁻⁻ mice compared with WT controls (Fig. 3B). In contrast, serum IgM was comparable between groups and, consistent with prior studies (22, 24), IgE levels were increased in the absence of IL-21R signals.

**IL-21 acts directly on B cells to mediate PC differentiation**

IL-21 has been shown to promote the activation of both B and T cells in a cell-intrinsic manner (6–9). However, which cells require direct IL-21 signaling in the context of a type 2 immune response to infection has not been investigated. To address this issue, we generated mixed bone marrow chimeras in which bone marrow from WT mice heterozygote for the CD45 allele (CD45.1⁺CD45.2⁺) was mixed at a 1:1 ratio with CD45.1⁻CD45.2⁺ IL-21R⁻ deficient bone marrow and injected into lethally irradiated CD45.1⁺CD45.2⁻ WT hosts. This strategy allowed us to identify both donor populations from residual host lymphocytes. Upon reconstitution, chimeric mice were infected with *H. polygyrus* as described above and the mesLNs were analyzed by flow cytometry at 2 wk postinfection. As shown in Fig. 4, IL-21R⁺–sufficient CD4⁺ T cells had a slight competitive advantage during Tfh differentiation.

**FIGURE 3.** Plasma cell differentiation and IgG1 Ab production is compromised in helminth-infected IL-21R⁻⁻ mice. *A*, mesLN cells from 2 wk *H. polygyrus*-infected WT and IL-21R⁻⁻ mice were assessed by flow cytometry for the frequency (left) and number (right) of CD4⁺CD8⁺CD11b⁺IgD⁻ (shown as dump) CD138⁺ cells. Data shown are representative of four independent experiments with four mice per group. *B*, Total serum Ab levels at 2 wk postinfection with *H. polygyrus*. Representative data from one of four experiments with at least four mice per group are shown. Error bars represent SD from the mean. *p* ≤ 0.05; **p** ≤ 0.005. ND, not detectable.
tion. Specifically, ~60% of CXCR5+PD-1+ CD4+ T cells from H. polygyrus-infected chimeras were of WT origin. Although somewhat variable, both IgD⁺PNA⁺ GC cells and IgD⁺IgG1⁺ cells from H. polygyrus-infected chimeras were equally represented by WT and IL-21Rα⁻/⁻ cells (Fig. 4). Strikingly, however, >90% of dump CD138⁺ mesLN cells from infected chimeras were of WT origin (Fig. 4). These results indicate that IL-21 signaling acts directly to promote plasma cell differentiation downstream of GC formation while playing a minimal role in Tfh differentiation following helminth infection.

**FIGURE 4.** Plasma cell differentiation, but not Tfh generation or B cell activation, requires direct IL-21 signaling. Mixed bone marrow chimeras were generated as described in Materials and Methods. Following reconstitution, mice remained naive (left column) or were infected with H. polygyrus (middle and right columns) and mesLNs were harvested 2 wk later for analysis by flow cytometry. A, Zebra plots are gated on CD4⁺ T cells and middle two rows are gated on CD19⁺ cells. Bottom row is gated on total live lymphocytes. Dot plots delineating the frequency of WT (CD45.1⁺CD45.2⁺) and IL-21Rα⁻/⁻ (CD45.1⁺CD45.2⁻) donor populations from host cells (CD45.1⁺CD45.2⁻) are based on the gated population from each zebra plot on its immediate left. The left two columns show mesLN cells from a representative uninfected and H. polygyrus-infected chimeric animal.

B, The bar graph indicates data reproducibility across multiple mice. Data shown are representative of four independent experiments with at least three uninfected and three H. polygyrus-infected chimeras per experiment. Error bars represent SD from the mean.

IL-21 is required for pathogen-specific IgG1 production and protective immunity against H. polygyrus

We and others have shown that isotype class-switched Ab production is critical for mediating protective immunity against H. polygyrus (2, 3). Based on the compromised ability of IL-21Rα⁻/⁻ mice to generate high titters of total serum IgG1 relative to WT controls, we hypothesized that IL-21 signaling would be critical for the production of helminth-specific IgG1, thus compromising protection against reinfection. To test this hypothesis, WT or IL-21Rα⁻/⁻ mice were infected with H. polygyrus and
cured with pyrantel pamoate 3 wk later. This infection and cure regimen was repeated, and 2 wk following a third round of infection, pathogen-specific serum IgM and IgG1 was measured (3). This cycle of repeated infection was carried out to generate detectable levels of *H. polygyrus*-specific Abs that are either not produced or remain undetectable 2 wk after primary infection (data not shown and Ref. 2). In support of our hypothesis, IL-21Ra−/− mice generated much fewer dump− CD138+ plasma cells in their mesLNs following tertiary infection with *H. polygyrus* (Fig. 5A) and mounted (even after the third challenge) a compromised total and *H. polygyrus*-specific IgG1 response compared with WT controls (Fig. 5B, 5C). Additionally, the frequency of IgG1-secreting cells in the spleen and bone marrow from *H. polygyrus*-infected IL-21Ra−/− mice was significantly decreased relative to controls, confirming a defect in plasma cell differentiation (Fig. 5D). In contrast, IL-21 signaling was not required for efficient generation of CXCR5+PD-1+ CD4+ T cells (Fig. 5E) or IL-4-producing cells (Fig. 5F) even after multiple rounds of infection, further supporting a specific role for IL-21 in B cell differentiation and effector function in type 2 antihelminth immunity.

To examine whether the observed defect in pathogen-specific Ab production in IL-21Ra−/− mice corresponded with compromised protective immunity to *H. polygyrus*, adult worm burden was assessed in the small intestine of 3× *H. polygyrus*-infected WT or

**FIGURE 5.** Plasma cell differentiation and pathogen-specific IgG1 production are compromised in the absence of IL-21 signaling after multiple rounds of *H. polygyrus* infection. A–F, WT and IL-21Ra−/− mice were subjected to two rounds of *H. polygyrus* infection and cure. Two weeks after a third round of infection, the frequency and number of dump− CD138+ cells were determined by flow cytometry as shown in A. In B, the amounts of total serum IgG1, IgM, and IgE are shown. C, Serum *H. polygyrus*-specific IgG1 and IgM titers are shown. D, The frequency of IgG1-secreting cells per thousand cells in the bone marrow and spleen of WT and IL-21Ra−/− mice following three rounds of *H. polygyrus* infection. E, The absolute number of CXCR5+ PD-1+ CD4+ T cells in the mesLNs as determined by flow cytometry. F, IL-4 production by whole mesLN cells isolated from either uninfected or infected WT and IL-21Ra−/− mice following restimulation in vitro. All data shown are representative of two individual experiments with four mice per group. Error bars represent SD from the mean. *p ≤ 0.05; **p ≤ 0.005; ***p ≤ 0.0005.
IL-21Ra−/− mice. Indeed, mice incapable of IL-21R signaling carried a significantly higher worm burden compared with WT mice that had undergone a similar infection and cure regimen (Fig. 6).

**Discussion**

The present study identifies IL-21R signaling as a critical pathway for protective immunity against *H. polygyrus*. Consistent with an indispensable role for Ab in resistance to reinfection (2–5), B cells from mice deficient in the IL-21Ra subunit exhibited compromised plasma cell differentiation despite efficient GC formation and isotype class switching following *H. polygyrus* infection. Concomitantly, IL-21Ra−/− mice were unable to mount helminth-specific IgG1 responses comparable to WT mice. IL-21 appears to unequivocally affect B cell differentiation across a range of immune responses. For example, mice immunized with nitrophenylykeyhole limpet hemocyanin in alum mounted compromised Ag-specific Ab responses compared with WT controls (7). Similar results were found following infection with *T. gondii*, a protozoa that elicits a systemic type 1 response, as well as with *S. mansoni* and *N. brasiliensis*, other type 2-inducing helminths (22–24). Until now, however, the impact of IL-21 on protective immunity and the point at which B cell differentiation is affected during type 2 immunity were unknown.

Although our studies do not identify the exact molecular event by which IL-21 impacts B cell differentiation, we found that despite enhanced GC formation and isotype class switching by IL-21Ra–deficient B cells, the number of dump CD138+ plasma cells was severely reduced. Although this result appears contradictory at first, it may indicate that a hyperactivated GC response skews B cell differentiation toward a memory pathway rather than an Ab-secreting plasma cell pathway. Indeed, a recent study by Zotos et al. (7) found that the absence of IL-21 resulted in premature GC dissolution following systemic immunization of mice with nitrophenylykeyhole limpet hemocyanin in adjuvant and an increased propensity of B cells to differentiate into CD38+ memory cells. These studies and others attributed this compromised GC response to an inability to express WT levels of BCL-6, a transcription factor critical for GC formation (6, 7). Although we cannot exclude this mechanism is at play in our system, we were unable to detect a difference in BCL-6 mRNA expression in GC cells from 2 wk *H. polygyrus*-infected IL-21Ra−/− and WT animals. Furthermore, our studies indicate that B cells from IL-21Ra−/− mice exhibit a defect in CD138+ plasma cell differentiation even at a time when an enhanced GC reaction is occurring. The decrease in mesLN CD138+ cells that we observed is unlikely to be a result of early or rapid egress from lymph nodes to secondary sites, as we found a similar decrease in the number of Ab-secreting cells in the spleen and bone marrow. An alternative mechanism operating in response to helminth infection may be that GC cells are proliferating to a greater extent in the absence of IL-21, resulting in an increased rate of apoptosis that precludes their ability to differentiate into Ab-secreting cells. Although prior studies have indicated a proapoptotic role for IL-21 following B cell activation (27), cell death predominated in the absence of T helper signals (e.g., anti-CD40). Conversely, IL-21 serves an antiapoptotic role in BCR-stimulated B cells receiving CD40+ help signals such as anti-CD40 (14, 17). As we found that the Th population increases in parallel with the GC response in *H. polygyrus*-infected IL-21Ra−/− mice, Tfh-mediated stimulation of GC B cells may paradoxically induce apoptosis in the late stages of the GC reaction in the absence of IL-21. Studies are underway to examine the proliferation rate and survival of B cells following helminth infection in the absence of IL-21 signaling.

In contrast to its major role in plasma cell differentiation, our data suggest that IL-21 signaling is not only dispensable, but may actually negatively regulate Th2 and Tfh differentiation in the context of type 2 immune responses. Indeed, we found that CD4+ T cells from *H. polygyrus*-infected mice lacking the IL-21Rα-chain produced more IL-4 as a population compared with T cells from WT controls. Although previous studies have not indicated an increase in IL-4 production in the absence of IL-21 signaling (23, 24), we found more CXCR5+PD-1+ T follicular helper cells in *H. polygyrus*-infected IL-21Ra−/− mice, a population we have previously shown to be the dominant IL-4-producing cell in the reactive lymph nodes of helminth-infected animals (19). Our data also challenge previous studies using model T cell Ags in adjuvant that demonstrated a cell-intrinsic role for IL-21 in promoting Tfh differentiation (8, 9). However, more recent studies have failed to find a significant role for this cytokine in Tfh generation (7, 21). Taking our results and prior studies together, a role for IL-21 signaling in T cell differentiation may be more nuanced and highly context-dependent.

Protective immunity to *H. polygyrus* has been previously shown to require T-dependent isotype class-switched Ab production, but not IgE or IgA, suggesting a critical role for the IgG isotype. Indeed, transfer of IgG purified from serum of immune mice (in which the IgG1 isotype predominates) can confer protection against *H. polygyrus* reinfection in normally susceptible strains (2, 5). Interestingly, Ab responses to other infectious agents appear to also depend on IL-21, indicating that the role of this cytokine in B cell differentiation may be an indispensable pathway regardless of the type of infectious pathogen (24, 28–30).

The present study identifies a role for IL-21 in B cell differentiation, focusing on its action in the secondary lymphoid organs. However, these results do not rule out a relevant role for IL-21 at the target site of infection, namely the small intestine. As mentioned above, Wynn and colleagues (22) found an important role for IL-21 in promoting alternatively activated (AA) macrophages in the lungs of *S. mansoni*-infected mice despite the presence of IL-4, the cytokine most associated with this differentiation pathway (31). Indeed, Guse and colleagues (32) previously found a high density of AA macrophages within lamina propria granulomas of *H. polygyrus*-infected animals, which promoted clearance of larvae following reinfection. Although they found IL-4 to be the driving force of this process, IL-21 may also promote the full effector functions of AA macrophages in this context. These findings dovetail with the results of Kopf and colleagues (23) showing fewer intestinal granulomas in IL-21Ra−/− mice infected with *H. polygyrus*. Future work should collectively consider the above studies and the results described in this study to determine

![Graph](image-url)
whether pathogen-specific Ab production directly relates to AA macrophage effector functions in the context of helminth infection.

In conclusion, we have identified IL-21R signaling as an obligatory component of plasma cell differentiation in the context of type 2 immunity and protective immunity to an intestinal parasitic infection. Pairing the functional implications of this study in the context of infectious disease with recent mechanistic data using model Ag systems may now provide the impetus for investigating the role of IL-21 signaling in clinical settings of infectious disease.

Acknowledgments
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Disclosures
The authors have no financial conflicts of interest.

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