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CUTTING EDGE

Cutting Edge: Enhancement of Antibody Responses Through Direct Stimulation of B and T Cells by Type I IFN[1]

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Type I IFN (IFN-αβ) is induced rapidly by infection and plays a key role in innate antiviral defense. IFN-αβ also exerts stimulatory effects on the adaptive immune system and has been shown to enhance Ab and T cell responses. We have investigated the importance of B and T cells as direct targets of IFN-αβ during IFN-α-mediated augmentation of the Ab response against a soluble protein Ag. Strikingly, the ability of IFN-α to stimulate the Ab response and induce isotype switching was markedly reduced in mice in which B cells were selectively deficient for the IFN-αβR. Moreover, IFN-α-mediated enhancement of the Ab response was also greatly impaired in mice in which T cells were selectively IFN-αβR-deficient. These results indicate that IFN-αβR signaling in both B and T cells plays an important role in the stimulation of Ab responses by IFN-αβ. *The Journal of Immunology,* **2006, 176: 2074–2078.**

Signals associated with infection play an important role in regulating adaptive immunity, helping to ensure that appropriate responses are generated after infection with pathogens. Cells of the immune system can detect pathogens directly by virtue of receptors that recognize components of infectious agents, and can also sense indirect consequences of infection, e.g., cytokine production by infected cells or release of molecules from damaged/dying cells. One example of an indirect marker of infection with immunoregulatory activity is type I IFN (IFN-$\alpha\beta$). Expression of IFN-$\alpha\beta$ is triggered rapidly in response to infection and by contact with components of infectious agents (1, 2). Although best characterized for its key role in innate resistance to viral replication, IFN-$\alpha\beta$ has also been recognized for several decades as a cytokine able to influence the adaptive immune response (3).

Injection of IFN-$\alpha\beta$ has been shown to enhance both Ab and T cell responses against soluble protein Ags in vivo (4, 5). In addition, there is evidence that host production of IFN-$\alpha\beta$ contributes to the induction of immune responses by adjuvants and during infections, as indicated by reduced responses to these challenges in IFN-$\alpha\beta$R-deficient (IFN-$\alpha\beta R^{-/-}$) mice (4–9). At least part of the immunostimulatory activity of IFN-$\alpha\beta$ is linked to direct stimulation of dendritic cells (DCs),[3] in keeping with the crucial role of DCs in translating signals arising from innate recognition of infection into adaptive immune responses. Treatment with IFN-$\alpha\beta$ causes DCs to up-regulate expression of MHC and costimulatory molecules and to acquire an increased capacity for T cell stimulation (2). IFN-$\alpha\beta$ also elicits the production B cell stimulatory cytokines by DCs, including B cell-activating factor of the TNF family (BAFF) and a proliferation-inducing ligand (APRIL); these cytokines were shown to mediate enhancement of Ig class switching by IFN-α-treated DC in vitro (10). Moreover, exposure of DCs to IFN-$\alpha\beta$ can enhance both Ab responses and cross-priming (4, 5).

Whether DCs represent the only important direct targets of IFN-$\alpha\beta$ during the stimulation of immune responses is unknown. Although we have observed that adoptive transfer of IFN-$\alpha\beta R^{+}$ DCs into IFN-$\alpha\beta R^{-/-}$ mice is sufficient to allow for IFN-$\alpha\beta$-mediated enhancement of Ab production and cross-priming of $CD8^{+}$ T cells, these responses were of relatively low magnitude compared with those in intact wild-type (WT) mice, suggesting that other IFN-$\alpha\beta R^{+}$ cells might be required for optimal adjuvant activity (4, 5). In this respect, B and T cells are worth considering as IFN-$\alpha\beta$ targets, because there are numerous reports of IFN-$\alpha\beta$ affecting the function of these cells in vitro. For example, IFN-$\alpha\beta$ has been shown to promote the development of T cell effector activity in vitro, and a recent study of $CD8^{+}$ T cells suggests that these effects are mediated through a STAT4-dependent pathway initiated by direct triggering of the IFN-$\alpha\beta$R on T cells (11, 12). In addition, both stimulatory and inhibitory effects of IFN-$\alpha\beta$ on in vitro B cell proliferation and Ig production have been reported (13–17), and IFN-$\alpha\beta$ has been shown to protect activated T cells and resting B cells from apoptosis in vitro (18, 19). However, despite the in vitro data showing that IFN-$\alpha\beta$ can act on T and B cells, there is currently no information on how IFN-$\alpha\beta$R-mediated signaling affects the function of these cells in vivo.

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[3] Abbreviations used in this paper: DC, dendritic cell; BAFF, B cell-activating factor of the TNF family; WT, wild type; BM, Bone marrow; CGG, chicken gamma globulin; GC, germinal center; LN, lymph node.

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We have investigated the mechanisms by which IFN-α enhances Ab responses in vivo. The results show that the stimulatory effects of IFN-α are markedly reduced when either B cells or T cells are unable to respond directly to IFN-$\alpha\beta$, demonstrating important functional consequences of IFN-$\alpha\beta$R-mediated signaling in T and B cells in vivo.

Materials and Methods

Mice

C57BL/6 mice were purchased from Charles River or from the specific pathogen-free unit at the Institute for Animal Health (Compton, U.K.). 129S6 (129), 129-*Ifnar1*^{tm1Agt} (*Ifnar1*$^{-/-}$), B6.129S2-*Igh-6*^{tm1Cgn}/J (μMT), and B6; 129P-*Tcrb*^{tm1Mom} *Tcrd*^{tm1Mom}/J (*Tcrb*$^{-/-}$ *Tcrd*$^{-/-}$) mice were purchased from the Institute for Animal Health. Bone marrow (BM) chimeras were produced by injecting 5×10^6 total BM cells into irradiated (900 cGy) recipients as indicated in the text.

To generate mice with a conditional IFN-$\alpha\beta$R α-chain, IB10 embryonic stem cells were gene targeted so that exon 10 of the *Ifnar1* gene was loxP flanked (*Ifnar1*$^{F/F}$). Upon Cre-mediated deletion of exon 10, a frameshift results in an open reading frame devoid of the transmembrane region and the cytoplasmic signaling domain. Control experiments revealed complete IFN-$\alpha\beta$R inactivation upon exon 10 deletion (E. Kamphuis and U. Kalinke, manuscript in preparation). 129/Sv-Ifnar1^{tm1Uka} (*Ifnar1*$^{F/F}$) mice generated from targeted embryonic stem cells and C.129P2-*Cd19*$^{tm1(cre)Cgn}$/J (CD19-Cre mice) (20) were backcrossed 10 times with C57BL/6 mice before both strains were intercrossed. Mice homozygous for *Ifnar1*$^{F/F}$ and carrying one CD19-Cre allele showed B cell-specific *Ifnar1* deletion that was >97% efficient, as indicated by genetic and functional analysis (data not shown). Mice with a conditional *Ifnar1* gene were bred under specific pathogen-free conditions.

All animal experimentation was done with the approval of the Home Office and the Ethical Review Committee of the Institute for Animal Health.

Immunizations

One hundred micrograms of chicken gamma globulin (CGG) (Stratech Scientific) was injected s.c. either in PBS alone or in PBS containing 10^5 U IFN-α. Recombinant mouse IFN-α4 was produced by NSo mouse myeloma cells in serum-free medium as described previously (5). In IFN-α-treated mice, IFN-α was also injected 1 and 2 days after administration of Ag at the site of the primary injection (4).

Assay of serum Ab by ELISA

CGG-specific Abs were detected by ELISA as described previously (4). For measurement of IgM and IgG subclasses, rat anti-mouse-IgM (R6-60.2), IgG1 (A85-1), -IgG2a (R19-15), -IgG2b (12-3), and -IgG3 (R40-82) Abs (all from obtained from BD Biosciences Pharmingen) were used. To distinguish IgG2a^a and IgG2a^b Abs, allotype-specific mouse anti-mouse IgG2a^a (8.3) and IgG2a^b (5.7) Abs (both from BD Biosciences Pharmingen) were used for detection.

Visualization of germinal centers (GC)

Draining lymph nodes (LN) were obtained from mice 12 days after immunization. Sections (5–6 μm) were cut from frozen tissues using a CM1900 cryostat (Leica Microsystems). Acetone-fixed sections were labeled with biotinylated peanut agglutinin (Vector Laboratories) at 50 μg/ml for 30 min, and, after washes, with HRP-avidin-biotin-complex (Vector Laboratories). Sections were then treated for 4 min with a peroxidase diaminobenzidine solution (Vector Laboratories), after which the reaction was stopped with water. Sections were counterstained with Meyer's hematoxylin. The average number of GC per section was determined by counting 3–9 different sections per LN, analyzing 9 LN each for mice receiving injections with CGG and CGG + IFN-α. No GC were detected in LN sections from control unimmunized mice. Sections were visualized and photographed using a DMLS microscope (Leica Microsystems) and a Polaroid DMC.

Results and Discussion

Impaired ability of IFN-α to enhance Ab response when B cells lack expression of the IFN-$\alpha\beta$R

Previously, we reported that injection of IFN-$\alpha\beta$ greatly enhances the Ab response against soluble protein Ags (4). The augmenting effects of IFN-$\alpha\beta$ included stimulating production of IgM and all subclasses of IgG and were found to increase when IFN-$\alpha\beta$ was injected not only at the same time as the Ag but also 1 and 2 days after administering the Ag. To investigate further the mechanisms of IFN-$\alpha\beta$ adjuvant activity, we used the same immunization protocol, i.e., s.c. injection of soluble Ag (CGG) plus IFN-α on day 0 followed by injections of IFN-α alone on days 1 and 2 (given at the same site as the day 0 injection). As shown in Fig. 1, injection of IFN-α caused a significant increase in the number of GC generated after immunization with CGG.

As an initial approach to determine whether B cells serve as direct targets of IFN-α adjuvant activity, we generated mixed BM chimeras in which B cells were deficient in expression of the IFN-$\alpha\beta$ receptor. B cell-deficient μMT mice (21) were irradiated and reconstituted with a 1:1 mixture of BM from syngeneic (μMT) mice and *Ifnar1*$^{-/-}$ mice. In the resulting chimeras (termed IFN-$\alpha\beta$R$^{-/-}$ B cell chimeras), all B cells were derived from *Ifnar1*$^{-/-}$ progenitors, whereas other BM-derived cells arose from both *Ifnar1*$^{-/-}$ and *Ifnar1*$^{+/+}$ (μMT-derived) precursors. Control chimeras were produced by reconstituting irradiated μMT mice with μMT plus 129 (WT) BM; in these mice, all cells were IFN-$\alpha\beta$R$^{+/+}$.

Groups of both types of chimeras were immunized by injection of CGG alone or CGG + IFN-α, and CGG-specific serum Ab titers were measured 10 days later. IFN-α markedly enhanced the anti-CGG response, including production of IgM and all subclasses of IgG, in control chimeras (Fig. 2). In striking contrast, injection of IFN-α engendered little or no increase in the anti-CGG Ab response in IFN-$\alpha\beta$R$^{-/-}$ B cell chimeras. Because B cells represented the only uniformly IFN-$\alpha\beta$R$^{-/-}$ population in these mice, the results suggested that direct stimulation of B cells was required for optimal enhancement of Ab responses by IFN-α.

In IFN-$\alpha\beta$R$^{-/-}$ B cell chimeras, non-B hemopoietic cells included a mixture of IFN-$\alpha\beta$R$^{-/-}$ and IFN-$\alpha\beta$R$^{+/+}$ cells. To exclude the possible influence of IFN-$\alpha\beta$R$^{-/-}$ non-B cells on the adjuvant activity of IFN-α, a second approach was taken to target IFN-$\alpha\beta$R-deficiency selectively to B cells, using the Cre-loxP system (22). Mice were generated in which the *Ifnar1* gene, which encodes one of the two chains of the IFN-$\alpha\beta$R, was flanked by loxP sites (*Ifnar1*$^{F/F}$). These mice were then crossed to mice expressing the Cre recombinase under control of the CD19 promoter (CD19-Cre$^+$), leading to deletion of the IFN-$\alpha\beta$R selectively in B cells (20). The number of B cells in these

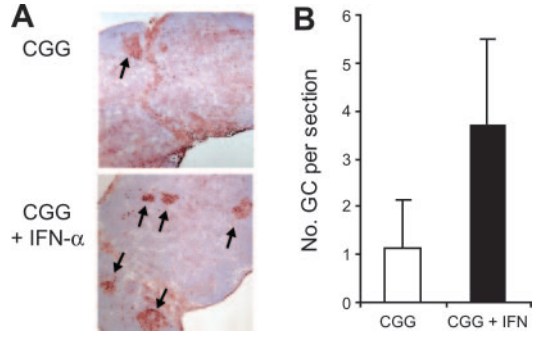

FIGURE 1. Stimulation of GC formation by IFN-$\alpha\beta$. *A*, Representative sections of LN from mice receiving injections with CGG (*top*) or CGG + IFN-α (*bottom*), showing GC (indicated by arrows) at 12 days after immunization. *B*, Average number of GC per section for LN from CGG- or CGG + IFN-α-injected mice. Data are mean counts for 3–9 sections per LN, 9 LN per treatment (±SD). Differences are statistically significant ($p < 0.005$ by two-sample *t* test).

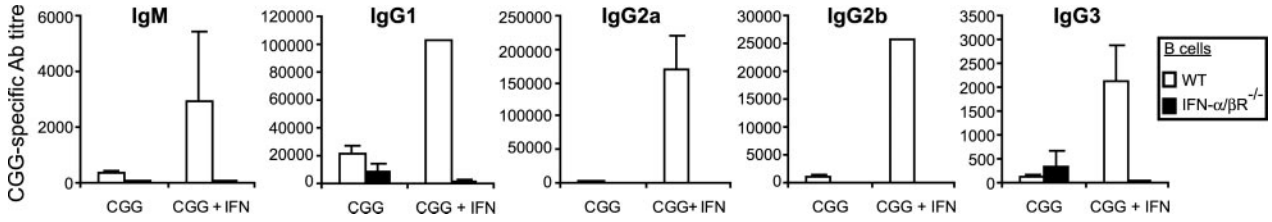

FIGURE 2. Defective stimulation of Ab response by IFN-α in chimeras in which B cells lack IFN-$\alpha\beta$R expression. IFN-$\alpha\beta R^{-/-}$ B cell chimeras (■) or control chimeras (□) mice were immunized by injection of CGG alone or CGG + IFN-α, and serum Ab levels were measured 10 days later. Data show mean endpoint titers ± SD for IgM and the indicated IgG subclasses (2–3 mice/group), and are representative of two separate experiments.

mice was equivalent to that in control mice, indicating that there is no requirement for IFN-$\alpha\beta$R signaling in B cell development, consistent with normal lymphocyte production in IFN-$\alpha\beta R^{-/-}$ mice (data not shown).

CD19-Cre$^+$ *Ifnar1*$^{F/F}$ mice and Cre-negative *Ifnar1*$^{F/F}$ mice were immunized with CGG or CGG + IFN-α, and serum Abs were measured 10 or 35 days later (Fig. 3). Consistent with the results from the mixed BM chimeras, the ability of IFN-α to augment the anti-CGG Ab response was greatly reduced when B cells were unable to respond directly to IFN-$\alpha\beta$. Thus, injection of IFN-α strongly enhanced the production of anti-CGG Abs in Cre$^-$ *Ifnar1*$^{F/F}$ mice but stimulated minimal augmentation of the response in CD19-Cre$^+$ *Ifnar1*$^{F/F}$ mice. Of note, IFN-α showed equivalent adjuvant activity in control CD19-Cre$^+$ and Cre-negative mice, indicating that expression of Cre alone in B cells did not alter Ab response (data not shown). Therefore, these data provide strong evidence in an independent model that direct stimulation of B cells by IFN-α is important in IFN-α-mediated enhancement of the Ab response.

Impaired ability of IFN-α to enhance Ab response when T cells lack expression of the IFN-$\alpha\beta$R

Because the Ab response to CGG is completely dependent on T cell help, it was of interest to determine whether T cells also represent direct targets of IFN-α in the enhancement of Ab responses. For this purpose, mixed BM chimeras were generated to produce mice in which T cells were IFN-$\alpha\beta$R-deficient. T cell-deficient *Tcrb*$^{-/-}$*Tcrd*$^{-/-}$ mice were irradiated and reconstituted with a mixture of BM from *Tcrb*$^{-/-}$*Tcrd*$^{-/-}$ and *Ifnar1*$^{-/-}$ mice, so that all T cells were derived from *Ifnar1*$^{-/-}$ progenitors. In these mice (termed IFN-$\alpha\beta R^{-/-}$ T cell chimeras), B cells (and other BM-derived cells) were both IFN-$\alpha\beta R^{-/-}$ and IFN-$\alpha\beta R^{+/+}$. Usefully, however, Abs produced by the two types of B cells could be distinguished, because *Ifnar1*$^{-/-}$- and *Tcrb*$^{-/-}$*Tcrd*$^{-/-}$-derived B cells expressed h chains of a and b allotypes, respectively.

IFN-$\alpha\beta R^{-/-}$ T cell chimeras and control chimeras (*Tcrb*$^{-/-}$*Tcrd*$^{-/-}$ recipients reconstituted with 129 (WT) and *Tcrb*$^{-/-}$*Tcrd*$^{-/-}$ BM) were immunized as described above and Ab responses measured 10 days later (Fig. 4). As expected, when IgH^a Abs (produced by *Ifnar1*$^{-/-}$ B cells in IFN-$\alpha\beta R^{-/-}$ T cell chimeras, and by 129 (WT)-derived B cells in control chimeras) were measured, it was apparent that IFN-α strongly enhanced the response in control chimeras but not in IFN-$\alpha\beta R^{-/-}$ T cell chimeras (Fig. 4*A*). This result was in accordance with the requirement for direct stimulation of B cells by IFN-α demonstrated above. Significantly, a similarly reduced response was apparent in IFN-$\alpha\beta R^{-/-}$ T cell chimeras when IgH^b Abs (produced by *Tcrb*$^{-/-}$*Tcrd*$^{-/-}$-derived B cells in both types of chimeras) were measured (Fig. 4*B*). Thus, when T cells were uniformly IFN-$\alpha\beta R^{-/-}$, expression of the

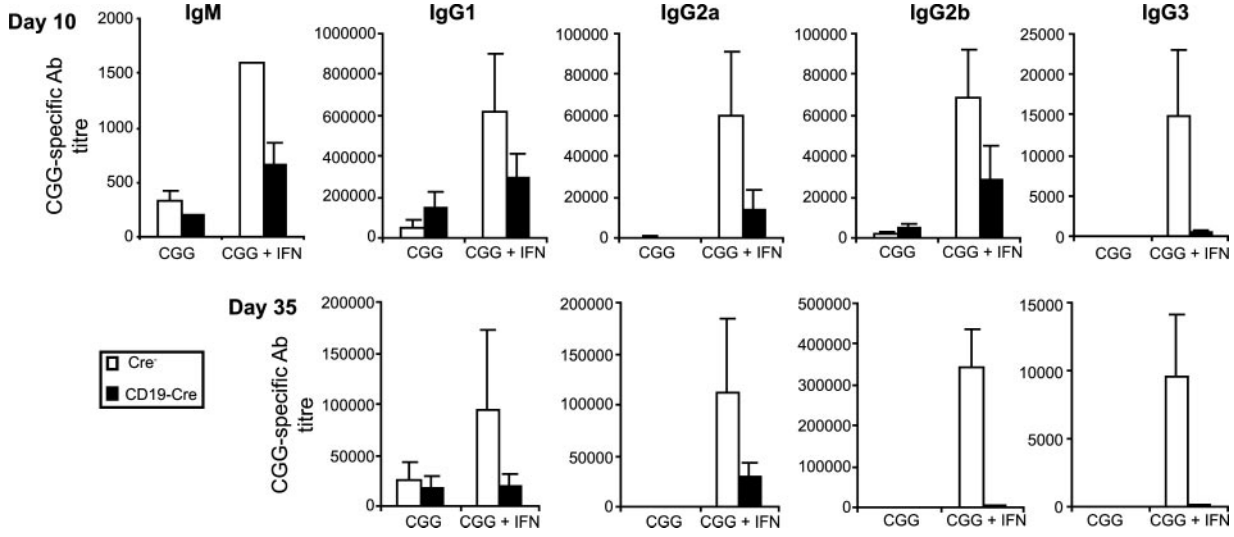

FIGURE 3. Defective stimulation of Ab response by IFN-α in mice selectively lacking the IFN-$\alpha\beta$R on B cells. Cre$^-$ *Ifnar1*$^{F/F}$ (□) and CD19-Cre$^+$ *Ifnar1*$^{F/F}$ mice (■) were immunized by injection of CGG alone or CGG + IFN-α, and serum Ab levels were measured 10 (*upper graphs*) or 35 days (*lower graphs*) later. Data show mean endpoint titers ± SD for IgM and the indicated IgG subclasses (3 mice/group), and are representative of two separate experiments. IgM titers were very low in all groups on day 35 (data not shown).

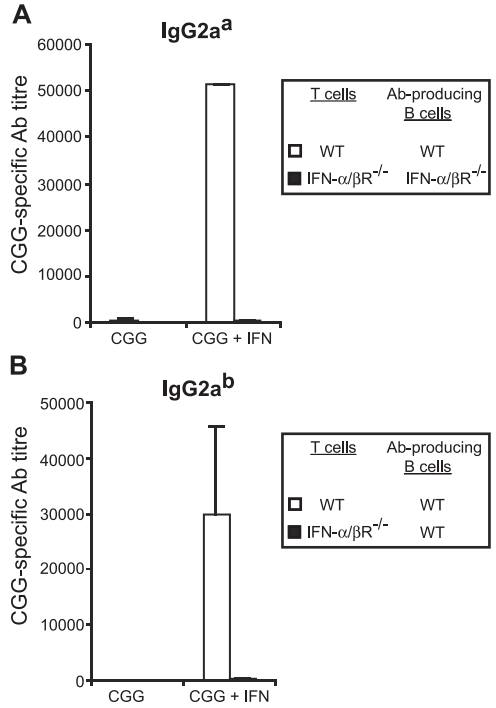

FIGURE 4. Defective stimulation of Ab response by IFN-α in chimeras in which T cells lack IFN-$\alpha\beta$R expression. IFN-$\alpha\beta R^{-/-}$ T cell chimeras (■) and control chimeras (□) were immunized by injection of CGG alone or CGG + IFN-α, and serum Ab levels were measured 10 days later. *A*, Endpoint titers (±SD) of IgG2a^a Abs, which were produced by 129-derived B cells in control chimeras and by *Ifnar1*$^{-/-}$-derived B cells in IFN-$\alpha\beta R^{-/-}$ T cell chimeras. *B*, Endpoint titers (±SD) of IgG2a^b Abs, which were produced by *Tcrb*$^{-/-}$ *Tcrd*$^{-/-}$-derived B cells in both types of chimeras. Data are means from 3 mice/group.

IFN-$\alpha\beta$R on B cells was insufficient to allow for IFN-α-mediated enhancement of the Ab response. These results indicated that IFN-$\alpha\beta$R-mediated stimulation of T cells also contributes to the enhancement of Ab responses by IFN-α.

Overall, these results demonstrate a clear stimulatory effect of IFN-$\alpha\beta$R-mediated signaling in B and T cells relevant to the generation of Ab responses in vivo. In considering the potential mechanism(s) of action, it should be noted that the present data do not establish that IFN-$\alpha\beta$ acts directly on those cells that are responding specifically to Ag. For example, it is conceivable that IFN-α triggers B or T cells globally to release cytokines that act indirectly to stimulate Ag-specific cells. Moreover, although it seems most likely that the requirement for T cell responsiveness to IFN-$\alpha\beta$ relates to the priming and/or function of CD4$^+$ Th cells, we cannot rule out the possibility that triggering of other T cells (including CD8$^+$ T cells) might lead to signals that indirectly stimulate the Ab response.

Notwithstanding these caveats, in vitro studies have indicated a number of stimulatory effects of IFN-$\alpha\beta$ on B and T cells that could be relevant to our in vivo observations. For B cells, these include protection from apoptosis, augmentation of Ag receptor-triggered proliferation, and promotion of differentiation into Ab-forming cells (15–17, 19). Similarly, protection of activated T cells from apoptosis could lead to increased and prolonged availability of T cell help (18). However, given the sometimes conflicting results stemming from in vitro investigation of IFN-$\alpha\beta$ function, elucidation of how IFN-$\alpha\beta$ enhances Ab responses will require further examination of the effects of IFN-$\alpha\beta$R signaling in T and B cells in vivo. It will be of particular interest to investigate whether IFN-$\alpha\beta$ can enhance one or more stimulus essential for T cell-B cell collaboration and the GC reaction, such as those mediated by CD28-B7, CD40-CD40L, OX40-OX40L, or BAFF-BAFF-R interactions (23–25), perhaps through up-regulation of the relevant receptor.

The results of this study provide further insight into the way that infection-associated signals can impact on the adaptive immune response. The demonstration that exposure of B and T cells to the innate cytokine IFN-$\alpha\beta$ enhances the in vivo Ag-specific Ab response adds to previous observations that these cells express certain Toll-like receptors and can respond to pathogen components (26, 27). Hence, lymphocytes are sensitive to direct and indirect signs of infection and can modify their function in response to these signals. Therefore, optimal generation of immune responses is likely to rely upon innate triggering of multiple cell types, including DCs, B cells, and T cells.

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Disclosures

The authors have no financial conflict of interest.

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