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Influenza Virus Infection Causes Global Respiratory Tract B Cell Response Modulation via Innate Immune Signals[1]

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Induction of primary B cell responses requires the presence of Ag and costimulatory signals by T cells. Innate signals further enhance B cell activation. The precise nature and kinetics of such innate immune signals and their functional effects are unknown. This study demonstrates that influenza virus-induced type I IFN is the main innate stimulus affecting local B cells within 48 h of infection. It alters the transcriptional profile of B cells and selectively traps them in the regional lymph nodes, presumably via up-regulation of CD69. Somewhat paradoxically, innate B cell stimulation inhibited the ability of regional lymph node B cells to clonally expand following BCR-mediated stimulation. This inhibition was due to IFNR-signaling independent B cell intrinsic, as well as IFNR-dependent B cell extrinsic, regulation induced following influenza infection. IFNR-mediated signals also reduced B cell migration to various chemotactic agents. Consistent with the lack of responsiveness to CCR7 ligands, unaltered or reduced expression of MHC class II and genes associated with MHC class II Ag processing/presentation and CD40, B cells were unable to induce proliferation of naive CD4 T cells. Instead, they showed increased expression of a subset of nonclassical MHC molecules that facilitate interaction with $\gamma\delta$ T cells and NK T cells. We conclude that type I IFN is the main "third" B cell signal following influenza infection causing early trapping of B cells in regional lymph nodes and, at a time when cognate T cell help is rare, enhancing their propensity to interact with innate immune cells for noncognate stimulation. *The Journal of Immunology,* **2007, 178: 1457–1467.**

B cell responses importantly contribute to survival from both primary as well as secondary infection with influenza virus (1–7). Consistent with their role during primary infection, B cell responses in the local draining lymph nodes of the respiratory tract (mediastinal lymph nodes (MLN)[3]) are induced within 48–72 h after infection (8), thus around the time of peak lung viral loads. After day 3 of infection, the frequency of local virus-specific B cells rapidly expands concomitant with the clearance of the virus by a plethora of cellular and humoral immune mechanisms activated to this virus in the respiratory tract (2, 9–12). Such rapid induction of humoral responses is not only consistent with a local protective role for Abs during a primary infection, it also indicates that local B cell responses are initiated during the acute innate cytokine response. Cytokines induced rapidly to the virus in the respiratory tract include type I IFN, IL-1, IL-6, and TNF-α (13, 14). The potential role and the effects such innate immune stimuli might have on B cell responsiveness to subsequent encounter with Ag and/or Th cells has received little attention in the past. Recently, two studies by others have pointed to innate stimuli such as TLR signals as important "third" signals for B cell regulation (15, 16) and we provided evidence that type I IFN signals positively regulate influenza virus-specific B cell responses (8).

Type I IFN comprises a family of at least 16 cytokines in humans and mice (13 IFN-α subtypes, IFN-β, IFN-κ, and IFN-ζ, also called limitin), all of which use the same type I IFNR expressed on virtually all cells (17–19). These cytokines might shape humoral responses against influenza via direct and indirect mechanisms. Indirect regulation might entail IFN-mediated stimulation of myeloid dendritic cells via induction of IL-6 production. IFN-induced IL-6 production in vitro enhanced the differentiation of B cells to Ab-secreting plasma cells (20). There is a body of earlier, albeit somewhat contradictory literature that also points to potent direct stimulatory and inhibitory effects of IFN on B cells (21–26). More recent in vitro studies showed enhanced anti-IgM-induced calcium flux and B cell proliferation following rIFN stimulation (27). Others showed inhibitory effects of type I IFN on B cell proliferation (22). Differential effects of individual IFN-α subtypes might underlie at least some of the apparently contradictory results obtained (22, 24). In vivo evidence for a direct IFN-mediated role in B cell regulation has come from three recent studies. We provided evidence for direct IFN-mediated B cell stimulation to enhance virus-specific B cell responses to influenza virus infection (8), and similar findings were reported by Fink et al. (28) for vesicular stomatitis virus infections. Tough and colleagues (29) demonstrated a role for direct stimulation of B cells and T cells in supporting maximal Ab responses to protein immunization.

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[3] Abbreviations used in this paper: MLN, mediastinal lymph node; PLN, peripheral lymph node; MHCII, MHC class II; MHCI, MHC class I; PI, propidium iodide; IFIT, IFN-induced proteins with tetratrico peptide repeat.

The study presented here was conducted to more precisely define the nature of the innate signals that drive early local B cell activation during influenza infection and to determine the extent to which these stimuli modulate B cell responsiveness. Our data show that local secretion of type I IFN is the major innate signal stimulating respiratory tract B cells. Local innate B cell stimulation during early influenza infection had potent effects on B cell migratory capacity, proliferation, and propensity to interact with non-CD4 T cells. Direct IFNR signaling accounted for some but not all of the functional alterations observed.

Materials and Methods

Mice and virus

Female 8- to 12-wk-old BALB/c mice were purchased (Harley Sprague Dawley). Age- and sex-matched fully backcrossed IFNR$^{-/-}$ BALB/c mice (provided by J. Durbin, Ohio State University, Columbus, OH) and wild-type and type I IFNR-deficient (IFNR$^{-/-}$) 129 SV/EV mice (30) (both obtained from B&K Universal) were kept in filter top cages under conventional housing conditions. Mice were infected with 1.6×10^6 PFU of the influenza A virus reassortant Mem71 (H3N1) as described (31). Mixed bone marrow irradiation chimeras were generated by lethal irradiation of BALB/c mice (650 rad full-body irradiation) followed by transfer of 1×10^6 mixed bone marrow cells from congenic Igh-6$^{-/-}$ (The Jackson Laboratory) and either wild-type BALB/c or IFNR$^{-/-}$ mice at different ratios. Four to 6 wk after bone marrow transfer, blood was taken from mice by tail vein, stained with fluorescent-labeled anti-CD19, anti-CD4, and anti-CD8 and analyzed by flow cytometry to confirm reconstitution before infection. For BrdU-labeling experiments, mice were injected with 1 mg of BrdU followed by provision of BrdU at 1 mg/ml in the drinking water provided ad libitum. All experiments were performed in accordance with protocols approved by the University of California, Davis, Animal Use and Care Committee.

Cell preparation and flow cytometry

Lymph node cell preparations were generated as previously described (1). Inguinal peripheral lymph nodes (PLN) were used as the source for resting lymph node B cells because MLN are undetectable before infection. Live cell counts were obtained by trypan-blue exclusion using a hemocytometer. For FACS purification of B cells for microarray analysis, lymph nodes from 16 to 22 mice were pooled for each sample and stained as described (32) with CD69-FITC, CD86-PE, and CD19-allophycocyanin (eBioscience). FACS analysis was conducted using the following Abs at previously determined optimal concentrations: IgD-FITC (1126), IgM-Cy7 allophycocyanin (331), T10–22biot (7H9) conjugated in-house (as described at www.drmr.com), CD1-, CXCR4- and CXCR5-biotin (BD Biosciences), CD40-PE, MHC class II (MHCII)-FITC, MHC class I (MHCI)-FITC (eBioscience). T22-specific γδ T cells were stained with a T22 tetramer (33) (gift from Dr. Y.-h. Chien, Stanford University, Stanford, CA) in addition to staining for CD3 (2C11), TCRγδ (GL3) and TCRαβ (H57.597) all in-house conjugated. For all experiments, propidium iodide (PI) was used at 1 μg/ml in final medium to discriminate dead cells. Data acquisition was done using a FACSCalibur or FACSAria (BD Biosciences), the latter equipped with three lasers as described (34). FACS sorting and calcium-flux analysis was done using a MoFlo high-speed cell sorter (DakoCytomation), equipped with water-cooled lasers emitting in the blue (488 nm), red (647 nm), and violet (407 or 350 nm; the latter for calcium-flux analysis) and appropriate dichroics and bandpass filters for 11-color, 13-parameter analysis and sorting. Sorting purities were >94%. Data analysis was conducted with FlowJo software (Tree Star).

Calcium-flux analysis

For calcium-flux studies, lymph node cells were prepared and stained with anti-CD19 PE and labeled with 1 μg/ml Indo-1 (Invitrogen Life Technologies) in PBS, 0.5% BSA for 45 min at 37°C. Cells were washed and resuspended in PBS, 0.5% BSA, 1 mM $CaCl_2$, and 1 mM $MgCl_2$. Cells were stimulated with 10 μg/ml goat anti-mouse IgM F(ab')$_2$ (The Jackson Laboratory). Data were recorded as the ratio of bound/unbound Indo-1 over time. Calcium flux in B cells was determined after gating on CD19$^+$ cells.

MACS

For B cell enrichment by MACS, spleen single-cell suspensions were stained as for FACS analysis with biotinylated Abs to CD3 (2C11), CD4 (G.K.1.5), CD8 (56.6.8.3), GR-1 (RA3-6C3), F4/80 (all generated in-house), and DX-5 (eBioscience), followed by streptavidin-coupled to magnetic beads (Miltenyi Biotec). B cells were enriched by auto-MACS (Miltenyi Biotec) collecting the nonbound fraction. B cell purities were >93% as determined by FACS via staining with anti-B220 and anti-CD19.

Tissue culture

For microarray analysis on in vitro IFN-β-stimulated B cells, FACS-purified wild-type lymph node B cells were cultured for 16 h at 10^7 cells/ml with 2000 U/ml rIFN-β (R&D Systems) in medium (RPMI 1640, 2 mM L-glutamine, 100 U of penicillin/ml, 100 μg of streptomycin/ml, 10% heat-inactivated FCS, and 50 μM 2-ME) at 37°C with 95% air/5% CO_2 before analysis by RNA extraction.

Chemotaxis assay

Ex vivo transwell migration assays were performed by adding 1×10^6 lymph node cells in 100 μl of RPMI 1640/0.5% BSA to the upper chambers (5-μm pore size) of a 6.5-mm diameter Transwell plate (Costar). In addition, migration assays were conducted with MACS-purified spleen B cells stimulated for 8 h with IFN-β as described above. rCCL19 (MIP-3β; 1000 ng/ml), rCCL21 (1000 ng/ml), rCXCL12 (stromal cell-derived factor 1; 100 ng/ml), and rCXCL13 (B lymphocyte hemoattractant 3000 ng/ml) (R&D Systems) was diluted in 600 μl of RPMI 1640/0.5% BSA and placed in the lower wells. Assembled Transwell plates were incubated at 37°C in 95% air/5% CO_2 for 2 h. Cells migrated to the bottom chamber were harvested to a microcentrifuge tube containing 5 μl of polystyrene microbeads (Polysciences). Cell migration was evaluated by FACS analysis after staining with anti-CD3-PE, anti-B220-allophycocyanin, and PI. For each sample and for the input control, we determined the ratio of B cell numbers to microbeads. The percentage of transmigrated B cells was then calculated as the ratio of tested sample × 100/ratio of input control.

Cell proliferation studies

For T-B cell cocultures, MACS-purified B cells were irradiated with 1200 rad before coculture at a 2:1 ratio with freshly FACS-purified CD4$^+$CD11a^{low}CD44^{low} allotype-mismatched naive T cells at 1×10^6 T cells/ml medium. Seventy-two hours following culture onset, T cell expansion was assessed by MTT assay using the cell proliferation kit I (Roche Diagnostics) according to the manufacturer's instructions. Absorbance at 595 nm was measured on a Spectramax M5 reader (Molecular Devices) using a 650-nm reference wavelength.

In vitro B cell proliferation was assessed following stimulation of total CFSE-labeled (5 μM CFSE in PBS for 10 min at 37°C) lymph node cells with 20 μg anti-mouse IgM F(ab')$_2$/ml medium. Loss of fluorescence intensity by live CD19$^+$ B cells was determined following staining with anti-CD19 (allophycocyanin) (eBioscience) and PI after 72–96 h culture. In vivo B cell proliferation was determined by measuring incorporation of BrdU in conjunction with multicolor flow cytometry as described elsewhere (34).

Microarray analysis

For each sample to be analyzed, RNA was extracted from lymph node B cells isolated to high purity (>96%) by FACS from pooled lymph nodes of 12–16 wild-type 129SV/EV mice before and 44–48 h following influenza virus infection as well as from 16 to 20 infected IFNR$^{-/-}$ mice and from MACS-purified wild-type B cells stimulated at 10^7 cells/ml for 16 h with 2000 U of IFN-β (R&D Systems) in medium. We analyzed 16 RNA samples (four per group, each derived from an independent experiment). A standard protocol for reverse transcription was performed using oligo dT primer containing the T7 promoter sequence (Integrated DNA Technologies) and 100 U of Superscript II (Invitrogen Life Technologies) followed by second strand cDNA synthesis using DNA Polymerase I (Promega). Two rounds of RNA synthesis were then performed using the T7 RNA polymerase (AmpliScribe T7 Transcription kit; Epicentre) and labeled with biotin (Bioarray labeling reaction kit; Enzo Life Sciences). cRNA was fragmented before hybridization to GeneChip Mouse Genome 430, 2.0 Arrays (Affymetrix) for a total of 45,102 measurements per array. Each RNA sample was analyzed on a separate microarray.

Preprocessing of the Affymetrix microarray data was conducted using the affyPLM library within the Bioconductor packages (35). Probe level data were converted to expression values in the following manner: 1) background correction; 2) normalization using quantile normalization (36); 3) summarization of probe-level data into gene expression measurements using Robust Multiarray Average (37). Quality diagnostics were done by plotting weights extracted from the robust linear model fit in Robust Multiarray Average in a pseudo array image. No substantial artifacts were observed in the arrays.

We computed moderated *t* statistics (38) using the limma library within the Bioconductor packages to identify genes that show differential expression between different groups of mice. Transcript expression ratios were estimated by comparing expression means between two groups of interest. To control for family wise type I error rates, adjusted *p* values were obtained using the Holm correction. Two-way hierarchical clustering of genes and samples was performed using Euclidean distance and complete linkage. The full set of the gene array results can be accessed through the GEO database (www.ncbi.nlm.nih.gov/geo/series entry GSE3203).

Real-time RT-PCR analysis

Analysis of IFN-induced proteins with tetratrico peptide repeats (IFIT)-2 and -3 expression levels were conducted on RNA isolated from FACS-purified B cells as described previously (8).

Results

B cells from MLN following influenza virus infection show strong IFNR-mediated changes in their gene expression profile

We recently provided evidence that local lymph node B cells are stimulated by type I IFN within the first 48 h following influenza virus infection (8). This innate stimulus affected all local lymph node but not splenic B cells and was required for maximal B cell responses. To more broadly determine the effects of early influenza virus infection on local B cell responsiveness, we performed a comprehensive, genome-wide transcript expression analysis on highly FACS-purified lymph node B cells from noninfected and influenza virus-infected wild-type mice and from influenza virus-infected IFNR$^{-/-}$ mice. Similar analysis was performed also on FACS-purified lymph node B cells of wild-type mice stimulated for 16 h with IFN-β (Fig. 1). Each group contained four samples obtained from independent experiments. The complete DNA microarray results can be accessed through the GEO database (www.ncbi.nlm.nih.gov/geo/series entry GSE3203).

Differential gene expression analysis between B cells from noninfected and infected wild-type mice showed significant differences in expression of transcripts recognized by 414 oligonucleotide probe sets (0.9% of the probe sets on the array, $p < 0.05$ for each probe set after adjustment for multiple comparisons). In the wild-type mice, 278 (67%) of the 414 probe sets indicated increased expression in B cells following infection and 136 showed reduced expression. After exclusion of redundant probe sets and uncharacterized genes, we identified 171 (62%) unique genes that were increased by influenza virus infection in wild-type mice. Twenty-seven percent of those are known type I IFN-regulated genes (Table I) according to the published literature. Among the 20 most strongly induced genes all but 1 (macrophage activation 2 like, National Center for Biotechnology Information (NCBI) gene ID 100702) are known to be regulated by type I IFN (Table I). Only 12% of the 414 probe sets that detected differential transcript expression in wild-type mice also indicated differential expression in the infected IFNR$^{-/-}$ mice (Table I), indicating that the large majority of B cell gene transcript expression changes during early influenza virus infection were dependent upon IFNR signaling.

The gene showing the greatest induction (92-fold) in MLN B cells following influenza virus infection was IFIT1 (Table I and Fig. 1*B*). We had previously shown that two other members of the same gene family (IFIT2 and IFIT3) are induced strongly in MLN B cells using a cDNA subtraction library approach (8). IFIT2 and IFIT3 were also among the 10 most strongly induced genes by microarray analysis (Table I and Fig. 1*B*). The fold inductions measured by microarray analysis for those genes were in line with those assessed by quantitative RT-PCR: 52- vs 40-fold for IFIT2 and 40- vs 28-fold for IFIT3, respectively. The function(s) of these genes is currently unknown.

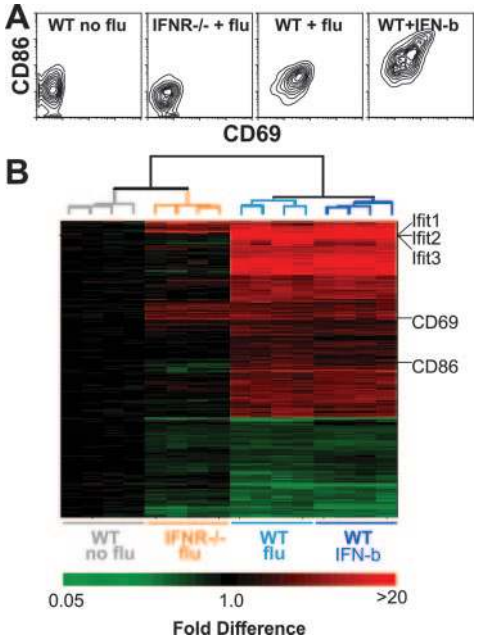

FIGURE 1. Influenza virus-induced gene expression changes in lymph node B cells are similar to those induced by type I IFN. *A*, FACS contour plots of CD69 and CD86 expression by FACS-purified PLN B cells from wild-type mice before (wt no flu) and 2 days following influenza virus infection (MLN, wt flu), as well as from 2 day influenza virus-infected congenic IFNR$^{-/-}$ mice (IFNR$^{-/-}$ flu) and from PLN B cells from wild-type mice, stimulated for 16 h with 2000 U of IFN-β (wt IFN-β). RNA from these cells was isolated for genome-wide gene expression analysis. *B*, A clustering dendrogram obtained from the microarray analysis of 414 measurements that showed significant ($p < 0.05$) changes between lymph node B cells from noninfected and 48 h influenza virus-infected wild-type mice. A total of 16 samples from four groups of highly FACS-purified B cells described under *A* were analyzed. Each of the 16 samples was obtained from independent experiments. Note the clustering of infected IFNR$^{-/-}$ mouse samples with the samples from noninfected wild-type mice and the clustering of influenza infected wild-type samples with that of IFN-β-stimulated cells. A complete listing of all results can be found online (www.ncbi.nlm.nih.gov/geo/, series entry GSE3203).

Next, we performed hierarchical clustering analysis with the set of transcripts that were differentially expressed between noninfected and influenza virus infected wild-type mice. The data showed that the samples from each group were more similar to each other than to samples from any other group studied (Fig. 1*B*). Influenza virus infection and in vitro IFN-β treatment induced marked changes in the gene expression profile of lymph node B cells when compared with B cells from noninfected mice. Importantly, the samples from the MLN B cells of infected wild-type mice clustered more closely with the samples from the in vitro IFN-stimulated B cells, than with the MLN B cells from influenza virus infected IFNR$^{-/-}$ mice (Fig. 1*B*). In contrast, B cells isolated from day 2 influenza virus-infected IFNR$^{-/-}$ mice showed a gene expression profile that most closely resembled that of B cells from noninfected wild-type mice (Fig. 1*B*).

Thus, influenza virus infection causes significant gene expression changes in MLN B cells within the first 48 h, which are markedly attenuated by the absence of IFNR signaling and are reproduced in vitro by direct stimulation of wild-type B cells with IFN-β (Fig. 1*B*). This genome-wide gene expression study identifies type I IFNR-mediated signals as the main "third signal" of B cell activation following influenza virus infection in agreement with our previous study (8).

Table I. *Induction of type I IFN-regulated genes in MLN B cells following influenza virus infection*

Affymetrix Probe Set ID	Gene Name	NCBI Gene ID	Fold Induction (infected wild type/noninfected)[a]	Fold Induction (infected IFNR$^{-/-}$/noninfected)[a]
1450783_at	IFN-induced protein with tetratricopeptide repeats (IFIT) 1	15957	93**	1.5
1423555_a_at	IFN-induced protein 44	99899	73**	0.8
1425917_at	Histocompatibility 28	15061	73**	0.8
1451905_a_at	Myxovirus (influenza virus) resistance (MX) 1	17857	69**	0.7
1418191_at	Ubiquitin-specific protease 18	24110	56**	1.2
1418293_at	IFIT 2	15958	52**	1.5
1423754_at	IFN-induced transmembrane protein 3 (IFITM3)	66141	43**	0.8
1449025_at	IFIT 3	15959	41**	0.8
1426276_at	IFN induced with helicase C domain 1	71586	28**	1.4
1436058_at	Radical *S*-adenosyl methionine domain containing 2	58185	26**	1.2
1431591_s_at	IFN-α-inducible protein	53606	22**	1.1
1422782_s_at	TLR3	142980	22**	1.5
1450484_a_at	Thymidylate kinase family LPS-inducible member	22169	15**	0.8
1440866_at	IFN-inducible double-stranded RNA-dependent protein kinase	19106	19**	1.2
1424339_at	2′-5′ oligoadenylate synthetase (OAS)-like 1	231655	19**	0.9
1417244_a_at	IFN regulatory factor 7	54123	18**	0.8
1419676_at	MX2	17858	14**	0.8
1419569_a_at	IFN-stimulated protein	57444	14**	0.6
1425065_at	2′-5′ OAS 2	246728	14**	0.7
1418930_at	CXCL10	15945	13*	1.2
1424775_at	2′-5′ OAS 1A	246730	12**	0.7
1418825_at	IFN-inducible protein 1	15944	9.0**	1.7
1421998_at	ATP-dependent IFN responsive	30935	7.2**	0.9
1450033_a_at	Signal transducer and activator of transcription 1 (Stat1)	20846	8.8**	1.4
1417793_at	IFN-inducible GTPase 2	54396	6.9**	1.6
1427511_at	β_2-Microglobulin	12010	6.9*	6.9*
1422010_at	TLR7	170743	6.8**	0.7
1419026_at	Fas death domain-associated protein (Daxx)	13163	6.0**	1.4
1417141_at	IFN-γ-induced GTPase	16145	5.8**	1.1
1453196_a_at	2′-5′ OAS-like 2	23962	5.4**	0.9
1417292_at	IFN-γ-inducible protein 47	15953	5.3**	1.4
1428735_at	CD69	12515	4.4*	2.3
1429184_at	IFN-inducible GTPase 1	74558	3.0**	1.5
1417172_at	Ubiquitin-conjugating enzyme E2L 6	56791	3.8*	0.9
1425374_at	2′-5′ OAS 3	246727	3.6**	0.8
1448775_at	IFN-activated gene 203	15950	3.6*	0.7
1421322_a_at	IFN-dependent positive acting transcription factor 3 γ (IRF-9)	16391	3.4**	1.3
1425119_at	2′-5′ oligoadenylate synthetase 1B	23961	3.1**	0.9
1448436_a_at	IFN regulatory factor 1	16362	2.6*	1.5
1420404_at	CD86	12524	2.5**	0.8
1445897_s_at	IFN-induced protein 35	70110	2.2*	0.7
1443837_x_at	B-cell leukemia/lymphoma (BCL) 2	12043	2.2*	2.0
1452349_x_at	IFN-activated gene 205	226695	2.2*	1.1
1418115_s_at	IFN-α-responsive gene	64164	2.1*	1.1
1420412_at	Tumor necrosis factor (ligand) superfamily, member 10 (TRAIL)	22035	2.0**	0.9

[a] **, $p < 0.001$; *, $p < 0.05$; no asterisk = not significant.

Influenza virus infection causes strong changes with potential effects on B cell responsiveness

GOstat analysis (39) was conducted with genes significantly altered in MLN B cells from wild-type mice following influenza virus infection to identify the likely physiological processes affected by the gene expression changes. The analysis showed that the most significant enrichment of differentially expressed genes occurred in two clusters of genes: "immune response" (GO:0006955) and "defense response" (GO:0006952) genes ($p = 5.06e-21$). A list of genes with known effects on the immune system and their grouping by predicted function is provided in Table II. Those functional groups are: cell growth, survival, and differentiation; response modulation via surface molecules and chemokines/cytokines; Ag processing and presentation; and non-BCR-specific Ag recognition.

Type I IFNR-dependent stimulation is required for the preferential accumulation of B cells in regional lymph nodes following influenza virus infection

Consistently, MLN of IFNR$^{-/-}$ mice were less cellular compared with wild-type controls (2.9 ± 0.3 and $5.2 \pm 2.4 \times 10^6$ total cells/mouse $p < 0.05$). In addition, MLN from IFNR$^{-/-}$ mice failed to show the usual preferential albeit small increases ($5.0 \pm 2.0\%$, $p < 0.05$) in B cell frequencies seen in wild-type mice at day 2 after infection (Fig. 2*A*). Thus, less than half the B cells accumulated in the draining lymph nodes in IFNR$^{-/-}$ mice compared

Table II. *Alterations in transcriptional profile of immune response genes in B cells from MLN of day 2 influenza virus-infected wild-type mice*

Affymetrix Probe Set ID	Gene Function/Name	NCBI Gene ID	Fold Change (infected/ noninfected wild type)[a]
	Antigen recognition		
1422782_s_at	TLR3	142980	22***
1422010_at	TLR7	170743	6.8***
	Antigen processing and presentation		
1422160_at	H2-T24	15042	7.6***
1427511_at	β_2-Microglobulin	12010	6.9*
1449875_s_at	H2-T10/T17/T22/T9	15024/15032/15039/15051	2.4*
1449556_at	H2-T23 (Qa1)	15040/319828	1.7***
1419589_at	CD93 (AA4)	17064	0.3***
1460180_at	Hexosaminidase B	15212	0.4**
1417491_at	Cathepsin B	13030	0.5***
1443814_x_at	Cathepsin H	13036	0.6***
	B cell response modulators		
1450033_a_at	Stat1	20846	8.8***
1419714_at	CD274 (B7-H1)	60533	4.0***
1417185_at	Ly6A	110454	3.0***
1455886_at	Cbl	12402	2.8*
1420404_at	CD86	12524	2.5***
	Complement		
1460242_at	DAF1 (CD55)	13136	0.3**
	Cytokines/chemokine response		
1417244_a_at	IRF7	54123	18***
1418930_at	CXCL10	15945	14*
1448380_at	Cyclophilin C association protein	19039	9.8***
1427736_a_at	CCRL2	54199	3.7***
1449591_at	Caspase-11	12363	3.3***
1448436_a_at	IRF1	16362	2.6**
1460220_a_at	CSF-1	12977	2.4*
	Actin remodeling/motility		
1428735_at	CD69	12515	4.4***
1419204_at	δ1	13388	2.6***
1449507_a_at	CD47	16423	1.8***
1437171_x_at	Gelsolin	227753	0.4***
1456085_x_at	CD151?	12476	0.5***
	Cell cycling/differentiation		
1423754_at	IFITM3	66141	43***
1434745_at	Cyclin D2	12444	5.1***
1421963_a_at	Cdc25b	12531	0.3***
1456174_x_at	*N*-myc downstream-regulated gene 1	17988	0.3*
	Cell growth/apoptosis		
1440866_at	PKR	19106	19***
1419026_at	Daxx	13163	6.0***
1442130_at	Hemopoietic SH2 domain containing	209488	4.7***
1421322_a_at	ISGRF3 (IRF-9)	16391	3.4***
1443837_x_at	Bcl2	12043	2.2*
1420412_at	TRAIL	22035	2.0***
1449353_at	Wild-type p53-induced gene (Wig)1	22401	0.4***
1425567_a_at	Annexin 5	11747	0.4*
1451112_s_at	Death-association protein	223453	0.4*

[a] Adjusted p value: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.0001$.

with controls. CD69 was recently shown to act downstream of IFN-$\alpha\beta$ to inhibit cell egress from lymphoid organs (40). Consistent with our previous report (8), phenotypic (Figs. 1*A* and 2*B*) and gene expression analyses (Fig. 1*B*, Tables I and II) identified CD69 up-regulation on B cells as a prominent feature of IFNR-dependent B cell stimulation in regional lymph nodes early following infection. In contrast, most but not all (see below) $CD3^+$ T cells failed to up-regulate CD69 (Fig. 2*B*). Given the importance of CD69 for blocking cell egress from the lymph nodes, our data suggest that virus-induced IFN induction causes the early preferential accumulation of B cells over T cells via differential induction of CD69 on B but not T cells in MLN early following infection.

The results of our gene expression analysis indicated few changes in chemokine receptor expression itself with the exception of increases in expression of the putative orphan chemokine receptor CCRL2 (Table II). Protein expression analysis for CXCR4 and CXCR5 showed slightly reduced levels of CXCR4 and little change in CXCR5 expression when comparing B cells from MLN of wild-type mice with that of control B cells (Fig. 2*C*). The reduction in CXCR4 expression following infection was IFNR-dependent, as it was not seen in $IFNR^{-/-}$ mice (Fig. 2*C*). Somewhat surprisingly, functional studies demonstrated significant infection-induced inhibition of local B migration toward various chemoattractants in vitro (Fig. 2*D*). B cells from MLN of day 2 influenza virus-infected wild-type mice showed reduced ex vivo migration toward a number of ligands compared with B cells from noninfected controls (Fig. 2*D* and data not shown). Significant reductions in migration were seen for CCL19 (MIP-3β) and CCL21 (secondary lymphoid-tissue chemokine), CCR7 ligands responsible for migration of cells toward the T cell areas, CXCL12 (stromal

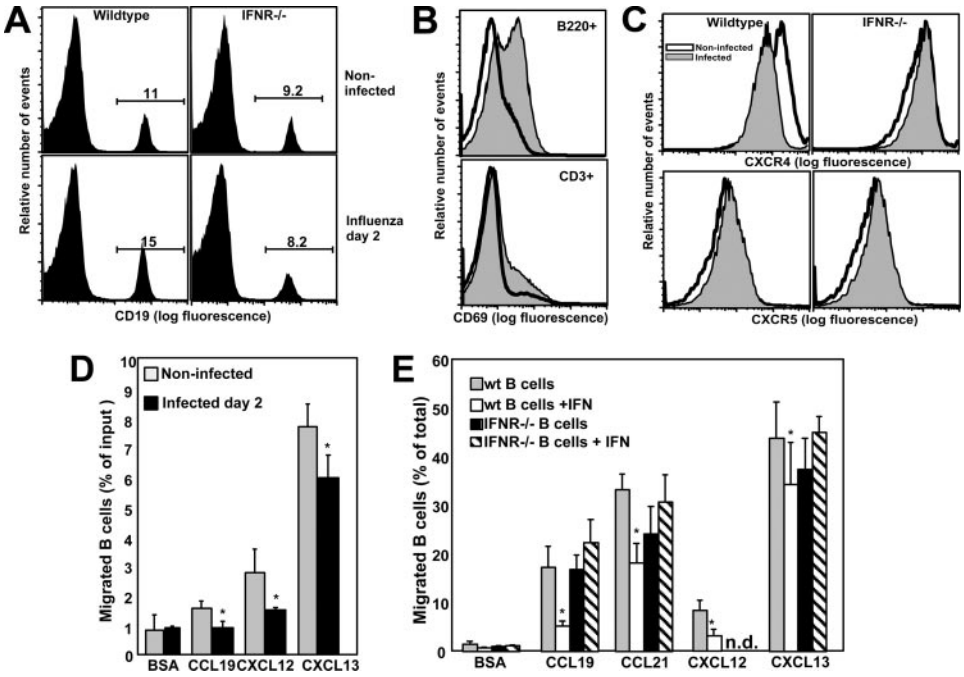

FIGURE 2. Influenza virus infection induces IFN-mediated alterations in B cell accumulation and migration. *A*, FACS analysis of lymph node B cells from wild-type (*left panels*) and IFNR$^{-/-}$ mice (*right panels*) before (PLN, *top*) and 2 days following infection with influenza A/Mem71 (MLN, *bottom*) showed the early increase in relative B cell numbers in lymph nodes of wild-type but not IFNR$^{-/-}$ mice. Numbers indicate frequencies of CD19^{+} B cells. Shown are data from a representative sample of at least four independent experiments. *B*, Histogram profiles of CD69 expression showed the strong up-regulation of this molecule on a large fraction of B220^{+} B cells. In contrast, only a small fraction of CD3 T cells showed up-regulated CD69 expression. *C*, FACS analysis on MLN lymphocytes 2 days after influenza virus infection shows the down-regulation of CXCR4 in B cells from wild-type (*left panels*) but not IFNR$^{-/-}$ (*right*) mice compared with those from PLN of noninfected controls. In contrast, CXCR5 expression levels were unaffected. Shown are histogram profiles on cells gated for CD19 expression. Data are representative from three independent experiments. *D*, Ex vivo Transwell migration experiments with PLN cells from noninfected (▨) and day-2 influenza virus infected wild-type mice (■) showed a strong reduction in B cell migration to the indicated chemokines. Data are expressed as mean percent of migrated B cells ± SD from cultures set up in quadruplicate and are a representative of three independent experiments. *E*, Transwell migration experiments conducted with MACS-purified spleen B cells from either wild-type or IFNR$^{-/-}$ mice following 8 h in vitro culture in the presence/absence of 2000 U/ml IFN-β. *, Statistical significant differences $p < 0.05$.

cell-derived factor 1), regulating migration toward splenic red pulp and lymph node medullary region and CXCL13 (B lymphocyte chemoattractant), the follicular B cell-homing chemokine. Similarly, short-term stimulation of purified resting spleen B cells from wild-type but not IFNR$^{-/-}$ mice with IFN-β or IFN-α reduced their ability to migrate to the same ligands (Fig. 2*E* and data not shown).

The data indicate that influenza infection-induced type I IFN affects B cell migration directly in a number of ways, one of which is via induction of CD69 expression and another might be a more global reduction in their migratory capacity. This is supported by the fact that MLN B cells from infected mice showed increased expression of a number of genes associated with actin remodeling/cell motility (Table II). Thus, we conclude that IFNR-mediated stimulation following influenza infection strongly alters the migratory capacity of local B cells, retaining them in higher numbers in lymphoid tissues at the site of infection and reducing their responsiveness to migratory signals within the tissue.

Influenza virus infection-induced stimulation reduced the ability of B cells to clonally expand following BCR stimulation

Because we have shown previously that IFNR signaling on B cells following influenza virus infection enhanced the local virus-specific response (8), we tested first whether innate influenza virus infection-induced B cell stimulation could drive clonal B cell expansion in vitro. The results suggest a complex regulatory process that is in part regulated by type I IFN. First, anti-IgM stimulation of total lymph node cells from day 2-infected wild-type mice showed a greatly reduced capacity of lymph node B cells to proliferate compared with cultures with lymph node cells from noninfected mice (Fig. 3*A*, *upper panels*). This infection-induced inhibition of B cell proliferation was not observed in when total lymph node cells from infected IFNR$^{-/-}$ mice were stimulated (Fig. 3*A*, *lower panels*). B cell proliferation analysis of MLN from day 2-infected wild-type and IFNR$^{-/-}$ mice therefore showed strong hyperproliferation of IFNR$^{-/-}$ B cells (Fig. 3*A*, *right panels*).

These experiments did not distinguish whether IFNR-mediated signals following infection caused the hypoproliferation of lymph node B cells directly or indirectly via stimulation of other lymph node cells. Therefore, we conducted these studies also with negatively MACS-enriched B cells isolated from the same lymph node cell pool as shown in Fig. 3*A*. First, in comparison to cultures with total lymph node cells, purified B cells showed reduced entry into cell cycle and reduced numbers of cell division over the 4-day cultures compared with their nonseparated counterparts (Fig. 3, *A* and *B*), suggesting the presence of noncognate help, likely in form of cytokine secretion. In addition, purified B cells from noninfected IFNR$^{-/-}$ mice showed enhanced proliferation compared with B cells isolated from wild-type mice (Fig. 3*B*, *left panels*). This difference in B cell proliferation was noted also in three of the five experiments done with total lymph node cultures (data not shown). In stark contrast to stimulation of B cells in the context of other lymph node cells, purified MLN B cells from 2 day-infected IFNR$^{-/-}$ mice also showed reduced proliferation in response to 20 μg/ml anti-IgM stimulation, similar to purified or nonpurified

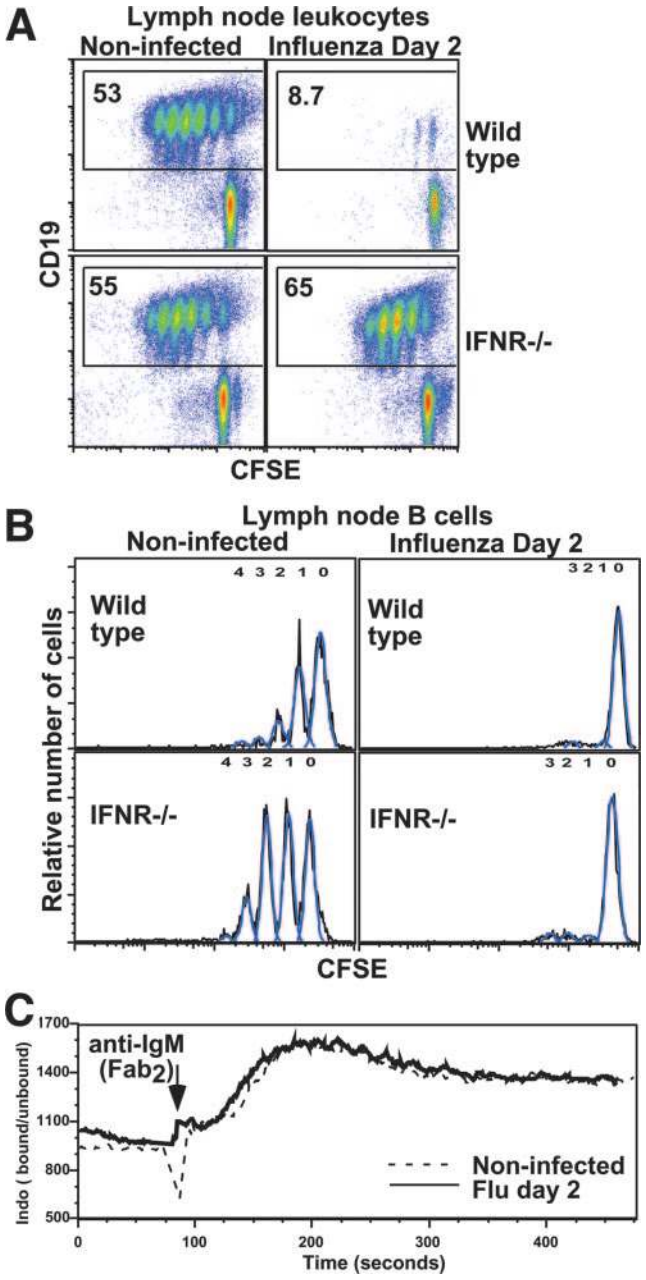

FIGURE 3. Influenza virus infection induces IFNR-dependent inhibition of B cell proliferation. *A*, Lymph node cells from wild-type and IFNR$^{-/-}$ mice before (PLN) and 2 days after influenza virus A/Mem71 infection (MLN) were stained with CFSE and stimulated with 20 μg/ml anti-IgM for 4 days. Shown are FACS pseudocolor profiles of live cells gated for lymphocyte forward scatter (FSC)/side scatter (SSC) and lack of PI inclusion. Numbers indicate the frequencies of CD19^{+} cells in the cultures. *B*, CFSE histogram profiles of negatively MACS-enriched B cells from the same cell pools as shown in *A* and then stimulated with anti-IgM as for *A* in the absence of other lymph node cells. Numbers indicate the number of proliferation cycles identified by CFSE. *C*, Calcium-flux measurements were conduced on single-cell suspensions from lymph node cells of noninfected (PLN) and day 2 influenza virus A/Mem71-infected wild-type mice (MLN) using indo-1 analysis by FACS. Shown are histogram profiles of the ratios of bound/nonbound indo-1 in live B cells gated for expression of CD19 and lack of PI incorporation before and after stimulation with anti-IgM F(ab')$_2$ at 10 μg/ml.

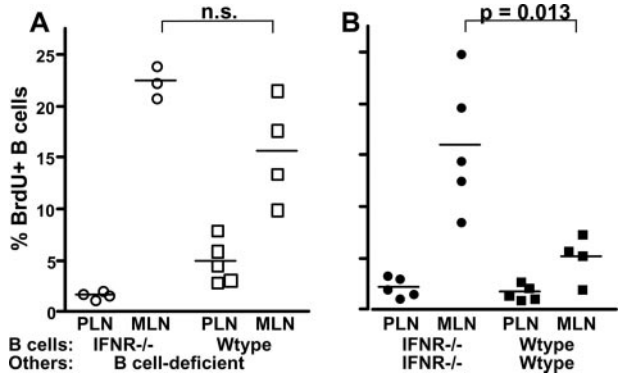

FIGURE 4. Lack of IFNR expression on non-B cells enhances B cell proliferation following influenza virus infection in vivo. Two sets of mixed bone marrow irradiation chimeras were created. *A*, In the first set two groups of mice were created with 75% bone marrow from B cell-deficient mice and 25% from either IFNR$^{-/-}$ or wild-type mice. Thus, only the B cells lacked expression of IFNR (○) or all cells, including B cells, expressed the IFNR (□). *B*, A second set was generated with 25% bone marrow from B-deficient mice and 75% from either IFNR$^{-/-}$ (●) or wild-type mice (■). Thus, most cells either lacked or expressed the IFNR. The chimeras were infected with influenza A/Mem71 for 7 days. On day 4, mice were injected i.p. with 1 mg of BrdU and also received BrdU via drinking water for another 3 days. MLN and PLN were harvested on day 7 of infection and FACS analysis conducted for BrdU incorporation on CD19^{+} B cells as described (34). Shown are results from three to five individual mice per group. n.s., not significant, $p = 0.08$.

wild-type B cells. Thus, these data suggest that influenza virus infection-induced inhibition of B cell proliferation was due at least in part to direct signals other than type I IFN. This is supported by in vitro studies with purified B cells stimulated with 2000 U of IFN-β overnight before anti-IgM stimulation, in which we failed to see any difference in B cell proliferation (data not shown). IFNR signals did not affect the dose-response curve of B cells to anti-IgM, as similar doses of anti-IgM (5 μg/ml) were required to induce measurable B cell proliferation in wild-type and IFNR$^{-/-}$ mice (data not shown). However, type I IFN appears to modulate noncognate helper activity that can drive B cell proliferation, because the lack of IFNR signaling enhanced the ability of cells other than B cells to support B cell proliferation in the total MLN cell cultures from day 2-infected IFNR$^{-/-}$ mice.

Consistent with a major indirect effect of IFN on B cell proliferation, the lack of B cell proliferation following anti-IgM stimulation in vitro was not due to altered calcium mobilization following BCR-mediated stimulation. Calcium-flux measurements on B cells from infected mice repeatedly showed unaltered kinetics in induction and maintenance compared with B cells from noninfected controls (Fig. 3*C*). Consistent with a published study by others (27), calcium-flux experiments conducted with in vitro IFN-β-stimulated B cells showed a somewhat more pronounced reduction in the magnitude of their responses following anti-IgM cross-linking compared with non-IFN-treated controls (data not shown). That might be due to differences in the IFN concentrations used in vitro compared with B cells exposure levels in vivo. B cells from mice at day 2 after infection or B cells stimulated in vitro with IFN displayed a homogenous calcium-flux response. Thus, it is unlikely that the difference in calcium flux following in vivo and in vitro stimulation is due to prior activation of subsets of B cells in vivo. Consistent with the unaltered gene expression levels of BCR-associated genes measured by microarray analysis, the reduction in proliferation following BCR-cross-linking was not due to reduced levels of surface IgM and IgD (see Fig. 5*B*).

The suppressive effects of IFNR signaling on local B cell expansion was confirmed in vivo using mixed bone marrow irradiation chimeras (Fig. 4). One set of chimeras was created by re-

constituting lethally irradiated wild-type BALB/c mice with bone marrow from B cell-deficient and either IFNR$^{-/-}$ or wild-type congenic mice (75:25%). In these mice all B cells either lack IFNR or express it whereas most other cells express the receptor. Another set of chimeras was created with bone marrow from B-deficient and either IFNR$^{-/-}$ or wild-type mice at a ratio of 25:75%. Thus in those mice most cells either lacked or expressed the IFNR. BrdU uptake was measured on day 7 following infection in MLN B cells. Compared with PLNs (inguinal and axillaries) that do not drain the site of infection, higher frequencies of B cells within MLN of all chimeras showed BrdU incorporation (Fig. 4). Chimeras in which only B cells lacked the IFNR (Fig. 4*A*) showed enhanced BrdU uptake 7 days after infection with influenza virus compared with the control chimeras with wild-type B cells. However, the levels did not reach statistical significance ($p = 0.08$). Comparison of a separate set of chimeras in which most cells either expressed or lacked expression of the IFNR, the lack of IFNR expression on all cells caused a significant increase in BrdU uptake by B cells in vivo ($p = 0.013$; Fig. 4*B*). Thus, the lack of IFNR expression on all hemopoietic cells resulted in more vigorous B cell proliferation in the draining lymph nodes in vivo compared with those reconstituted with wild-type cells.

Together, the data suggest that influenza virus-infection-induced innate B cell stimulation reduces their capacity to respond to Ag alone (even a strong BCR stimulus such as anti-IgM) via direct and indirect mechanisms and that IFN might modulates B cell proliferation predominantly (but not exclusively) indirectly via suppressing noncognate help.

Type I IFN-induced B cell stimulation is insufficient to enable T cell priming

Humoral responses to influenza virus are strongly enhanced by cognate interaction of B cells with CD4$^+$ T cells (2, 6). This interaction is facilitated in part through binding of peptide/MHCII complexes by the TCR as well as interaction of a number of costimulatory molecules such as CD86 and CD28. Although naive follicular B cells are unable to induce T cell priming, Ag-stimulated B cells can prime T cell responses (41), presumably due to increased ability of the B cell to provide necessary costimulatory signals. Because influenza virus infection strongly up-regulated CD86 expression on local lymph node B cells (Fig. 1 and Ref. 8), we determined next whether the infection-induced early local B cell stimulation enabled these cells to prime naive T cells. Such mechanism could explain the overall better virus-specific Ab responses seen in mice expressing B cells that carry the IFNR compared with those that do not (8). However, cocultures of naive FACS-purified CD11a^{low}CD44^{low}CD4$^+$ T cells with purified lymph node B cells from noninfected or day 2-infected allotype-mismatched mice showed that innate-stimulated B cells remained unable to prime naive T cells (Fig. 5*A*), despite their strong increased expression of CD86 (Fig. 1*A*). Furthermore, infection-induced B cell stimulation did not enhance T cell proliferation induced by further anti-IgM-stimulated B cells (Fig. 5*A*). The results are consistent with the fact that neither MHCII nor CD40 surface expression were increased in the infection-stimulated B cells (Fig. 5*B*), nor are any genes induced that are associated with peptide processing for MHCII expression. In fact, some of these genes were significantly reduced in B cells from infected compared with noninfected mice (Table II).

Enhanced expression of nonclassical MHC surface molecules on regional lymph node B cells after infection

Interestingly, influenza virus infection significantly increased gene expression levels for MHCI and five nonclassical MHC molecules

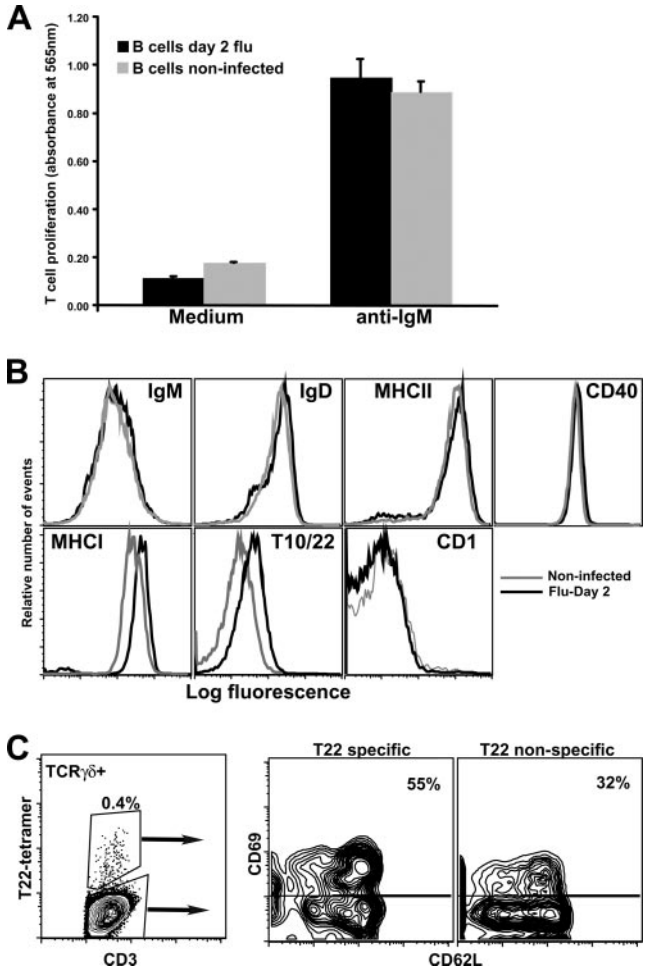

FIGURE 5. IFNR-mediated B cell activation insufficient to enable T cell priming. *A*, MACS-purified irradiated PLN B cells from noninfected (▨) and day-2 influenza virus A/Mem71 infected (MLN, ■) mice are unable to induce proliferation of FACS-purified CD4$^+$ CD44^{low} CD11a^{low} T cells (*left bars*). Following overnight B cell stimulation with 10 μg/ml anti-IgM F(ab')$_2$ B cells from noninfected and infected mice showed similar levels of naive T cell activation as assessed by MTT assay (*right bars*). Data are expressed as mean OD ± SD calculated from cultures set-up in triplicate. Similar results were observed following in vitro stimulation of B cells from noninfected mice with 2000 U/ml IFN-β (data not shown). *B*, FACS histograms for expression of indicated cell surface markers on live B cells from PLN of noninfected and MLN of day 2 influenza A/Mem71-infected wild-type mice gated for expression of CD19 and exclusion of PI. *C*, Contour plots with outliers from multicolor FACS analysis of MLN from day 2 influenza virus A/Mem71-infected wild-type mice. γδ T cells were identified by gating for expression of γδ TCR and lack of expression for TCR αβ, CD4, CD8, and CD19 and exclusion of PI. Staining with a biotinylated T22-tetramer (gift from Dr. Y.-h. Chien) identified T22-specific and nonspecific γδ T cells (*left box*). *Right boxes* indicate expression of CD69 and C62L on T22-specific and nonspecific γδ T cells. Note the strong expression of CD69 on T22-specific γδ T cells.

(T9, T10, T22, T23, Qa) in an IFNR-dependent manner (Table I). Increased expression at the protein level was confirmed for MHCI as well as for T10 and/or T22 (Fig. 5*B*). T10 and T22 are two closely homologous nonclassical MHC molecules that act as ligands for roughly 0.5% of all γδ T cells in many inbred mouse strains (33). Increased expression of these nonclassical MHC molecules was not due simply to an up-regulation of all MHCI like molecules caused by increased β_2-microglobulin expression (Table I). For example, CD1 protein expression levels were unaffected

(Fig. 5*B*). Together, these data indicate that influenza virus infection-induced stimulation of B cells cannot overcome the need for BCR-mediated activation to enable cognate T-B interaction. Instead, it seems to enable B cells to interact with cells such as γδ T cells (via T10/22) and possibly NK T cells via Qa (42, 43), which might provide noncognate "help" at a time when Ag-specific CD4 T cells are rare.

To show further whether γδ T cells could be involved in the regulation of B cells early during influenza virus infection, we determined the presence and activation status of T22-specific γδ T cells. Multicolor flow cytometric evaluation showed that T22-tetramer-binding γδ T cells were present in regional lymph nodes on day 2 after influenza virus infection and the majority of these expressed CD69 and CD62L (Fig. 5*C*). Thus, in contrast to most αβ T cells (Fig. 2*B*), γδ T cells quickly up-regulate CD69 following infection and thus presumably are retained in the regional lymph nodes for possible interaction with B cells via IFNR-mediated induction of T10/22 and possibly other ligands.

Discussion

This study demonstrates that type I IFN is the dominant early innate immune signal during infection with influenza virus that acts on all regional lymph node B cells within 48 h of infection where it modulates B cell immune responsiveness by altering their migratory capacity and their ability to clonally expand and differentiate. Our data provide credence to accumulating evidence that innate immune signals act as "third" signals for B cells that shape their responses (8, 15, 16) and identify a physiological context, regional immune responses to influenza virus infection, in which such signals are provided.

It has long been appreciated that the nature and type of pathogen encountered by dendritic cells and recognized via pattern recognition receptors will shape the quality of the T cell response, particularly their cytokine profile. It has been widely assumed that differences in the quality of B cell responses induced to differing pathogens are mainly a reflection of the quality of the T cell response. For example, measurement of IgG1 and IgG2a (or IgG2c levels in C57BL/6 mice) is used often to deduce the nature of the T cell response (TH1 vs TH2). Our data, consistent with recent studies by us and others (8, 29), demonstrate that differential induction of type I IFN also directly regulate B cell responses, by strongly altering their transcriptional profile (Fig. 1 and Tables I and II), affecting the degree of B cell accumulation and migration (Fig. 2), modulating B cell proliferation (Figs. 3 and 4) and regulating the isotype profile of the ensuing response (8, 29). Because the IFN-induced changes were observed only in the respiratory tract but not the spleen or PLNs (Ref. 8 and data not shown), direct innate stimulation of B cells might at least in part underlie the qualitatively different B cell responses induced at the site of infection vs those induced systemically.

Our previous study identified type I IFN as a necessary direct B cell signal for induction of maximal B cell responses following influenza virus infection. In this study, we show that type I IFNR signaling is the main signal that stimulates local B cells. Global gene expression analysis revealed the dramatic effects of influenza virus infection on local B cells, causing changes in expression of over 400 genes, including a number of immune response regulators (Table II) that were similar to those seen after in vitro stimulation with IFN-β. These changes clearly shape the local B cell response. The earliest preferential accumulation of B cells in the regional lymph nodes observed following influenza virus infection does not occur unless IFNR signals are provided. This is most likely due to the selective up-regulation of CD69 on B cells but not αβ T cells. CD69 has recently been shown to act upstream of S1P1 to block lymphocyte egress from lymph nodes by inhibiting S1P1 signaling (40). Availability of larger numbers of B cells increase the chance of selecting an Ag-specific B cell for BCR-mediated activation.

We did not observe significantly reduced B cell frequencies in the MLN of IFNR$^{-/-}$ mice compared with wild-type controls in our previous study conducted on days 5 and 7 after influenza virus infection (8). MLN B cell frequencies in IFNR$^{-/-}$ mice, however, often show higher mouse-to-mouse variation (W. L. W. Chang, E. S. Coro, and N. Baumgarth unpublished results). It is possible that the lack of early B cell accumulation observed on day 2 of infection in IFNR$^{-/-}$ mice is offset by the increased B cell proliferation in these mice observed after day 4 (Figs. 3 and 4) and/or differences in cell death. Further studies are required to fully identify all of the mechanisms through which innate stimuli such as type I IFN regulate lymphocytic accumulation in regional lymph nodes.

B cell migration was affected by type I IFN also in other ways. Our ex vivo studies showed strong inhibition of B cell migration to a number of chemokines, including chemokines that direct B cell migration toward lymph node follicles (CXCR5), T cell zones (CCL19 and CCL21), and medullary cords (CXCL13) (Fig. 2) (44). Migration toward chemokines expressed at inflammatory sites, such as CXCL9, was similarly reduced (data not shown), indicating that these B cells are not directed away from lymph nodes. Rather B cells might be trapped at the source of the IFNR signal. Ongoing studies in our laboratory are directed toward identification of this source. Evidence in the literature would suggest that dendritic cells are the most likely source in lymph nodes (45). In fact, dendritic cells are not only strong producers of type I IFN, they are also able to present Ag to B cells (46). Increased interaction of local B cells with dendritic cells facilitated by directed secretion of type I IFN might facilitate increased Ag presentation to the B cells, resulting in stronger Ag-specific responses. Alternatively, changes in the migration pattern of B cells might enhance interactions with cells other than dendritic cells.

Our gene and protein expression data show the distinct induction of a subset of nonclassical MHC molecules (Table II and Fig. 4*B*) that indicate potential enhanced interaction of B cells with cells such as γδ T cells (via T10/22) (33) or NKT cells (via Qa) (42, 43) at a time of infection when cognate CD4-T cell-mediated help is rare. Interestingly, in contrast to the lack of CD69 expression on most MLN T cells on day 2 after infection (Fig. 2), γδ T cells showed strong surface expression of CD69 (Fig. 5*C*) indicating a trapping of B cells and γδ T cells (but not αβ T cells) in regional lymph nodes and thus the potential for local γδ T cell-B cell interaction during early influenza virus infection. Although detailed information on γδ T cell or NK T cell-mediated B cell help during influenza virus infection is lacking, the importance of noncognate help for the induction of early local virus-specific IgA responses to influenza virus infection in the MLN has been demonstrated (47). Moreover, the presence of neutralizing IgG Abs and B cell memory formation following infection of TCR αβ gene-targeted mice with vesicular stomatitis virus was shown to dependent on γδ T cells (48). Thus, activated γδ T cells can provide the necessary signals for Ab formation. Our data indicate that such interaction might be regulated at least in part by type I IFN. In this context, it is interesting to note that Maloy et al. (48) reported γδ T cell-mediated Ab formation only following live virus infection but not following immunization with inactivated virus. Presumably, only live virus infection would have stimulated high levels of type I IFN. Consistent with the conclusion that IFN stimulation might be chiefly for the benefit of noncognate interactions, our studies have failed to show any positive effect of IFNR-mediated B cell stimulation on naive CD4 T cell proliferation (Fig. 5*A*).

Our studies further identified the presence of a complex regulatory circuit controlling B cell expansion following infection. Our data show a strong infection-induced reduction in the ability of B cells from regional lymph nodes to proliferate (but not calcium flux) in response to a strong Ag stimulus (anti-IgM). This was a B cell intrinsic change that does not appear to be regulated by type I IFN, or at least not by type I IFN alone, as it was seen in cultures of purified B cells from wild-type and IFNR$^{-/-}$ mice. However, a role for type I IFNR-mediated signals in inhibiting B cell proliferation was evident from the strong B cell hyperproliferation seen in total lymph node cultures from infected IFNR$^{-/-}$ mice (Fig. 3*A*) and in vivo following influenza virus infection in chimeras in which most cells lacked the IFNR (Fig. 4). Given the fact that purified B cells proliferated less vigorously to anti-IgM and fewer B cells proliferated following their isolation (via negative selection), lymph node cell suspensions contained important noncognate helper activity. It thus appears that influenza virus infection-induced type I IFN signals inhibit these noncognate nonspecific helper activities, possibly to prevent clonal expansion of nonspecific responses. This might facilitate protection from potentially harmful B cell responses and/or simply ensure availability of critical cytokines and other support factors for Ag-specific B cells that receive appropriate Th signals in addition to BCR-mediated signals. It is intriguing that infection-induced innate signals so fundamentally alter the ability of B cells to respond to Ag. Our results are in agreement with the body of literature on IFN that has demonstrated the dichotomous nature of signals submitted via IFNR to lymphocytes. In the case of influenza virus infection, we show that IFNR signals were required to achieve maximal antiviral Ab titers (8), while these signals also inhibited polyclonal B cell expansion (Figs. 3 and 4).

Our data show that B cells can respond more vigorously in the absence of signs of infection (as mediated via type I IFN). Innate signals that alert the host's immune system to the presence of an infection seem to increase the threshold of stimuli needed for B cells to be fully activated. This change in susceptibility to activation signals, such as provided here experimentally in the form of BCR signaling alone in the absence of further costimulatory signals, could suppress potential harmful responses directed against self-Ags such as those released by the infection- and immune response-induced tissue damage. Type I IFN has been linked to autoimmune diseases, particularly systemic lupus erythematosus (19, 49). One side-effect of type I IFN treatment of patients with hepatitis C virus infection is the appearance of Ab-mediated autoimmune disease (50). Although speculative at this time, mechanisms that cause enhanced rather than suppressed Ab production and clonal B cell expansion in the face of IFN-mediated signals might underlie the B cell-mediated autoimmunity seen in such patients and would suggest a mechanism by which repeated viral infections could enhance or trigger autoimmune-mediated disease in susceptible patients.

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Disclosures

The authors have no financial conflict of interest.

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