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Essential Role of IL-21 in B Cell Activation, Expansion, and Plasma Cell Generation during CD4⁺ T Cell-B Cell Collaboration¹

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During T cell-B cell collaboration, plasma cell (PC) differentiation and Ig production are known to require T cell-derived soluble factors. However, the exact nature of the cytokines produced by activated T cells that costimulate PC differentiation is not clear. Previously, we reported that costimulation of purified human B cells with IL-21 and anti-CD40 resulted in efficient PC differentiation. In this study, we addressed whether de novo production of IL-21 was involved in direct T cell-induced B cell activation, proliferation, and PC differentiation. We found that activated human peripheral blood CD4⁺ T cells expressed mRNA for a number of cytokines, including IL-21, which was confirmed at the protein level. Using a panel of reagents that specifically neutralize cytokine activity, we addressed which cytokines are essential for B cell activation and PC differentiation induced by anti-CD3-activated T cells. Strikingly, neutralization of IL-21 with an IL-21R fusion protein (IL-21R-Fc) significantly inhibited T cell-induced B cell activation, proliferation, PC differentiation, and Ig production. Inhibition of PC differentiation was observed even when the addition of IL-21R-Fc was delayed until after initial B cell activation and expansion had occurred. Importantly, IL-21 was found to be involved in PC differentiation from both naive and memory B cells. Finally, IL-21R-Fc did not inhibit anti-CD3-induced CD4⁺ T cell activation, but rather directly blocked T cell-induced B cell activation and PC differentiation. These data are the first to document that B cell activation, expansion, and PC differentiation induced by direct interaction of B cells with activated T cells requires IL-21. *The Journal of Immunology*, 2007, 179: 5886–5896.

Interactions between Ag-specific T cells and B cells are essential for T cell-dependent humoral immune responses. Both contact-dependent and -independent T cell help is essential for B cell activation and induction of an efficient Ab response (1–4). Supernatants from activated T cells enhance proliferation and differentiation of Ag- or mitogen-stimulated B cells (5–7). Cytokines such as IL-2, IL-4, IL-10, IL-21, as well as type I and type II IFNs have been shown to be critically involved in different aspects of human B cell activation and differentiation (5, 8–15). In addition to cytokines, contact-dependent engagement of CD40 on B cells by CD40L (CD154) expressed by activated CD4⁺ T cells is essential for B cell stimulation, proliferation, and differentiation. This is exemplified by the hyper-IgM syndrome manifested in patients with loss of function mutations in genes encoding CD40 or CD154 (16). Furthermore, the requirement for CD40/CD154 interactions in B cell responsiveness has been

demonstrated by the ability of CD154-expressing T cells (but not T cell clones from hyper-IgM patients) to induce B cells to undergo Ig H chain class switching and differentiation into plasma cells (PC)⁴ (17–20).

Various strategies have been used to mimic T cell-driven B cell activation, expansion, and differentiation in vitro. Activation of human T cells or T cell lines with staphylococcal superantigens as well as pokeweed mitogen has been shown to induce polyclonal PC differentiation from human B cells (6, 21, 22). T cell clones activated with specific Ag have also been shown to induce PC differentiation (23, 24). One of the most efficient means to induce PC differentiation from human peripheral blood B cells in vitro is by activation of CD4⁺ T cells with immobilized anti-CD3 mAb, which is sufficiently potent to induce B cell proliferation, class switching, and PC differentiation in the absence of further B cell costimulation (22, 25). Importantly, in this coculture system, B cells are not required for T cell costimulation, but rather function purely as recipients of T cell signals. Therefore, this model is useful to probe the nature of the T cell-interaction molecules involved in the induction of B cell activation and differentiation.

In previous studies, it was found that this response is contact dependent, in which CD40/CD154 interactions play a major role (17, 22, 26). Moreover, LFA-1 (CD11a/CD18) expressed by resting B cells, interacting with ICAM-1 (CD54) expressed by activated T cells has been shown to play a critical role in T cell-induced PC differentiation (27). Importantly, previous studies suggest that activated T cells not only induce PC differentiation from memory B cells, but are also capable of inducing class switch

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⁴ Abbreviations used in this paper: PC, plasma cell; BAFF/BLyS, B cell-activating factor belonging to the TNF family; ion, ionomycin; PI, propidium iodide; FSC, forward scatter; β_2 M, β_2 microglobulin; ICE, immunoaffinity capillary electrophoresis.

recombination and PC differentiation from peripheral blood naive B cells (28). Many cytokines have been shown to be involved in these processes in humans, including IL-2, IL-4, IL-10, and IL-6 (23–25, 29).

IL-21 is a type I cytokine that signals through a receptor composed of the IL-21R and the common cytokine receptor γ -chain (30). In humans, IL-21 is produced by activated T cells or NK T cells (13, 31), as well as spontaneously by CXCR5⁺ follicular Th cells (32). The IL-21R is closely related to IL-2R β and IL-4R α and is expressed by T, B, and NK cells, and has been shown to be important in T cell, NK cell, and B cell responsiveness (30). Recently, we have reported that IL-21 is much more efficient than IL-10 and IL-2 at inducing PC differentiation and Ig secretion from anti-CD40-stimulated human peripheral blood and naive cord blood B cells (12). Moreover, we have shown that the combination of IL-21 and B cell-activating factor belonging to the TNF family (BAFF/BLyS), in the absence of further costimulation, is capable of inducing PC differentiation from memory B cells that reside in the marginal zone analog region of the human spleen, suggesting a role of IL-21 in the maintenance of serologic memory (33). However, the relative role of IL-21 in direct CD4⁺ T cell-induced PC differentiation has not been addressed.

In this study, we evaluated the role of IL-21 in human CD4⁺ T cell-dependent PC differentiation of peripheral blood B cells. We show that T cells activated by immobilized anti-CD3 secrete substantial levels of IL-21 protein. Strikingly, blockade of de novo produced IL-21 significantly inhibited the ability of anti-CD3-activated T cells to induce B cell activation, proliferation, PC differentiation, and Ig secretion. These data are the first to demonstrate that IL-21 is essential for generation of B cell responses induced directly by activated T cells.

Materials and Methods

Isolation of human B cells and T cells

All human studies have been approved by the Warren G. Magnuson Clinical Center Institutional Review Board and informed consent was obtained according to the Declaration of Helsinki. Human peripheral B cells and T cells were isolated from Buffy coats of anonymous healthy donors drawn at the National Institutes of Health Division of Transfusion Medicine. Tonsil samples were obtained from children and young adults undergoing routine tonsillectomy. For most studies, peripheral CD19⁺ B cells and CD4⁺ T cells were isolated by negative selection using the RosetteSep technique following the manufacturer's instructions (StemCell Technologies). Preparations were typically >96% pure. In some experiments, B cells positively selected with anti-CD19 magnetic beads (Miltenyi Biotec) were used. These were typically >95% pure. Importantly, we noted similar results in experiments in which B cells were isolated by negative vs positive selection. To isolate B cell subpopulations, B cells were isolated from multiple donors by negative selection using RosetteSep. The cells were then stained with the combination of anti-CD27-PE, anti-CD19-PerCP-Cy5.5, and anti-CD38-allophycocyanin (clone HB7) (all BD Biosciences). Naive B cells were identified as CD19⁺CD27⁻CD38^{int} and memory B cells were CD19⁺CD27⁺CD38^{low/int}. The CD38^{high}CD27^{high} PC were excluded from the gate.

Cell culture

Ninety-six-well flat-bottom plates were coated with 10 μ g/ml (0.5 μ g/well in 50 μ l) anti-CD3 (clone 64.1) (22, 34) or anti-CD3 clone UCHT1 (BD Biosciences) overnight at 37°C in Tris buffer (50 mM (pH 7.4)). Similar results were found with both anti-CD3 mAbs. The plates were washed twice with PBS before addition of cells. T cells were purified as described above and treated with a final concentration of 30 μ g/ml mitomycin C (Sigma-Aldrich) for 30 min at 37°C. The cells were washed extensively before culture. A total of 1×10^5 mitomycin C-treated CD4⁺ T cells and 0.5×10^5 purified CD19⁺ B cells were cultured alone or together in duplicate or triplicate in 200 μ l on the anti-CD3-precoated microtiter wells. In most experiments, autologous T cells and B cells were used, but in some experiments, T cells or B cells from different or multiple donors were used. No functional differences related to the origin of the T cells and B cells

were noted, as previously reported (28). The cells were incubated at the start of culture with 10 μ g/ml IL-21R- γ 1Fc, BAFF/BLyS receptor (BAFFR)- γ 1Fc, or 10 μ g/ml neutralizing Abs to human IL-2, IL-4, and IL-10 (all R&D Systems) or 10 μ g/ml anti-human CD154 (clone TRAP-1; BD Biosciences). We found that in cultures of purified B cells, between 2.5 and 20 μ g/ml of either anti-IL-4, or anti-IL-2 and anti-IL-10 was sufficient to inhibit IL-4 (50 ng/ml) and anti-CD40-induced CD23 and CD27 up-regulation or IL-2 (100 U/ml) and IL-10 (25 ng/ml)-induced PC differentiation, respectively. Moreover, IL-21R-Fc had no effect on IL-4-, IL-2-, or IL-10-mediated responses demonstrating that the IL-21R-Fc is specific and does not neutralize these cytokines.

In some experiments, 1×10^5 purified B cells were cultured with increasing concentrations of recombinant human IL-21 (BioSource International) or 60 ng/ml IL-10 (R&D Systems) and 1 μ g/ml anti-CD40 (R&D Systems) with 5 μ g/ml anti-IgM (Jackson ImmunoResearch Laboratories), in the presence or absence of 10 μ g/ml neutralizing human IL-21R-Fc.

IL-21 analysis

Purified CD4⁺ T cells were cultured at 1×10^6 cells/ml in either 200 μ l in flat-bottom 96-well plates or 1 ml in 24-well plates. T cells were stimulated with either anti-CD3 (precoated-microtiter wells with 10 μ g/ml clone 64.1), or 10 ng/ml PMA and 1.3 μ M ionomycin (ion; Sigma-Aldrich) with or without 10 U/ml IL-2 (Biological Research Branch, National Cancer Institute, National Institutes of Health, Frederick, MD) to increase the viability of the control cultures without stimulation. Following 24 or 48 h, cell supernatants were removed and frozen for cytokine analysis (below) and mRNA was purified from harvested T cells for RT-PCR analysis (below).

B cell proliferation and CFSE dilution

To assess proliferative responses of cultured cells, 1×10^5 purified B cells were cultured as described above in triplicate in 96-well round-bottom microtiter wells. After 4 days of culture, [³H]thymidine (37 kbq/well) was added to the cultures for an additional 16 h. Thymidine uptake was measured using a liquid scintillation counter. In some experiments, B cells were isolated via negative selection as described above and then labeled with CFSE. In brief, purified B cells were washed extensively in PBS to remove all FCS, and CFSE/PBS (Molecular Probes) was added at a final concentration of 2.5 μ M to $2\text{--}5 \times 10^7$ cells/ml for 8 min. Labeling was quenched by addition of FCS, and cells were washed four times in medium containing 10% FCS before culture. The CFSE-labeled B cells and mitomycin C-treated T cells were cocultured on precoated anti-CD3-treated microtiter wells as described above with and without IL-21R-Fc. Following various days of culture, the cells were then stained with CD19-allophycocyanin (BD Biosciences) and propidium iodide (PI; Molecular Probes) to determine the percentage of dead (PI⁺) cells in CFSE⁺ B cells in each culture condition.

Flow cytometry

Four-color flow cytometry was performed using a FACSCalibur (BD Biosciences). Briefly, supernatants were collected and then all cells were harvested from 96-well cultures at the end of the incubation period and stained for 30 min on ice with a combination of mAb. The combination of anti-IgD-FITC, anti-CD4-PE, anti-CD19-PerCP-Cy5.5, and anti-CD38-allophycocyanin (all BD Biosciences) was routinely used. PC phenotype was further investigated with anti-CD27-PE (BD Biosciences) and anti-CD138-PE (syndecan-1) clone B-A38 (Diaclone Research). To determine the activation states of CD4⁺ T cells and CD19⁺ B cells, cells were stained with a combination of anti-CD4-FITC and anti-CD19-PerCP-Cy5.5 (BD Biosciences) with anti-CD25-PE (Immunotech/Beckman Coulter), anti-CD69-PE (Caltag Laboratories/Invitrogen Life Technologies), or anti-CD154-PE (clone 24-31; BioLegend). Viable cells were identified by gating based on scatter characteristics. In selected experiments, unfixed cells were stained with PI documenting that the forward scatter (FSC) vs side scatter gate used contained >99% PI⁻ viable cells. Cells were analyzed immediately. All samples were collected for 1 min, and as a result the density of the dot plots reveals relative cell numbers. AccuCount particles (Spherotech) were added before analyzing samples by flow cytometry, and total cell numbers were determined as per the manufacturer's instructions. Total B cells numbers were determined based on CD4⁻CD19⁺ expression and PC numbers were determined based on CD4⁻CD19^{low/+}IgD⁻CD38^{high} expression.

Determination of Ig levels

Secreted Ig in the culture supernatant was quantitated by ELISA. Briefly, to detect IgM, 96-well flat-bottom Nunc-Brand Immuno plates (Nalgen

Nunc International) were coated with 5 $\mu\text{g}/\text{ml}$ affinity purified goat anti-human IgM (Bethyl Laboratories) overnight at 4°C. Wells were then washed and blocked with a 0.2% BSA/PBS, and then the culture supernatants were titrated onto the treated plates and incubated overnight at 4°C. Bound IgM was detected with 0.2 $\mu\text{g}/\text{ml}$ alkaline phosphatase-conjugated goat anti-human IgM (Bethyl Laboratories) and developed using Sigma-Fast *p*-nitrophenyl phosphate tablets (Sigma-Aldrich). Specific absorbance was measured and OD was quantified at 410 nm by a Powerwave X 96-well plate reader (Bio-Tek Instruments). To detect IgG, 96-well plates were treated as above with 2 $\mu\text{g}/\text{ml}$ each of goat anti-human κ and λ L chain (Bethyl Laboratories) and bound IgG was detected with 0.2 $\mu\text{g}/\text{ml}$ AP-conjugated goat anti-human IgG (Bethyl Laboratories). To detect IgA, 96-well plates were treated as above and coated with 1 $\mu\text{g}/\text{ml}$ goat anti-human IgA (Bethyl Laboratories) and bound IgA was detected with 0.5 $\mu\text{g}/\text{ml}$ AP-conjugated goat anti-human IgA (Bethyl Laboratories).

Real-time quantitative PCR

Purified CD4⁺ T cells were stimulated as described above. After 24 or 48 h in culture, RNA was isolated using the RNeasy mini kit (Qiagen) according to the manufacturer's directions. Reverse transcription reactions were prepared using the Superscript One-Step PCR System with Platinum Taq Polymerase and ROX reference dye (Invitrogen Life Technologies). Fifty nanograms of isolated RNA was added per reaction with 1.2 mM MgSO₄. TaqMan Assays-on Demand Gene expression primer/probe sets (Applied Biosystems) were used for IL-21 (Hs0022327_m1), IL-2 (Hs00174114_m1), and β_2 microglobulin ($\beta_2\text{M}$; Hs9999907_m1). Final concentrations were 1.8 μM for primers and 0.5 μM for probes. RT-PCR was performed using the ABI Prism 7700 Sequence Detection System (Applied Biosystems) and cycle conditions and relative quantification were completed as described by the manufacturer's instructions (Applied Biosystems). mRNA expression for each gene was calculated using the comparative cycle threshold method with efficiency calculations and with all mRNA levels normalized to $\beta_2\text{M}$.

Immunoaffinity capillary electrophoresis (ICE)

ICE analysis was performed as previously described (35, 36). Fab Ab fragments were prepared from Abs specific to the cytokines of interest (R&D Systems) and immobilized onto 4 cm of the inner surfaces of a 100- μm i.d.-fused silica capillary via a disulphide linkage. The Ab-coated capillary was then mounted into a Crystal 660 Capillary Electrophoresis System (Prince Technologies) equipped with a Zetlif laser-induced fluorescence detector and a 12 mW 633 helium-neon laser (Picometrics). A 50- μl sample of culture supernatant was vacuum injected into the capillary and allowed to incubate for 5 min. During this phase, the immobilized Abs interact with and bind their respective analytes, leaving the unbound materials free to be flushed out of the capillary. The bound analytes were labeled in situ with Alexa Fluor 633 laser dye, flushed to remove nonreactive material, and the bound analytes were electroeluted at pH 1.5. The individual analytes were then separated by electrophoresis at 175 mA constant current and the individual peaks analyzed with a fluorescence detector using the instruments DAX Peak Area Analytical Program (Prince Technologies). All peak areas were compared with those obtained by analyzing sets of known standards, run under identical conditions.

Statistics

Statistics were performed using a one-tailed, two-sample (assuming unequal variances) *t* test with significant differences noted by *p* value—*, *p* < 0.05; **, *p* < 0.01; ***, *p* < 0.005—compared with cultures of anti-CD3-activated T cells and B cells without inhibitors (nil) for each group.

Results

Anti-CD3-activated CD4⁺ T cells induce PC differentiation and secrete IL-21

Human CD4⁺ T cells activated by immobilized anti-CD3 have been shown to induce Ig production from peripheral blood B cells (22). We now extend these results to demonstrate that anti-CD3-activated human CD4⁺ T cells also induce large numbers of CD19⁺IgD⁻CD38^{high} PCs (Fig. 1). In contrast, B cells in the absence of T cells, or B cells and T cells in the absence of anti-CD3 did not undergo efficient PC differentiation (Fig. 1A). The CD19⁺IgD⁻CD38^{high} cells generated in these cultures had further phenotypic characteristics of PC, including bright expression of CD27 (Fig. 1B). Moreover, a portion (6–64% mean 28%, *n* = 7)

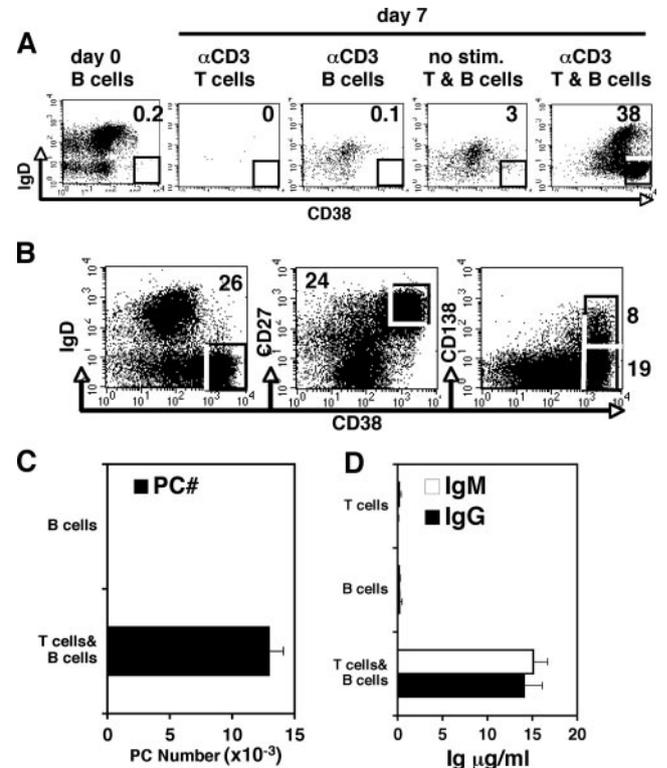


FIGURE 1. Anti-CD3-activated T cells induce PC differentiation. *A*, Negatively selected B cells and mitomycin C-treated autologous CD4⁺ T cells were cultured alone or together in anti-CD3-precoated microtiter wells and assessed for CD4, CD19, and IgD and CD38 expression following 7 days of culture. All plots shown are gated on CD19⁺CD4⁻ cells. PC are IgD⁻CD38^{high} in the boxed region, and numbers represent percent PC. The B cell subset distribution as defined by CD38 and IgD expression at the initiation of culture is shown (day 0 B cells). Data are from one representative of seven independent experiments. *B*, Negatively selected B cells and mitomycin C-treated CD4⁺ T cells were cultured in anti-CD3-precoated microtiter wells and assessed for expression of CD38 and either IgD, CD27, or CD138 following 7 days of culture. All plots shown are gated on CD19⁺CD4⁻ cells. PC are in the boxed region(s), and numbers represent percent PC. Data are from one representative of five experiments. *C*, Negatively selected B cells and mitomycin C-treated autologous CD4⁺ T cells were cultured alone or together on anti-CD3-precoated microtiter wells. PC number was determined using AccuCount particles and assessment of CD19⁺CD4⁻IgD⁻CD38^{high} cells following 7 days of culture. Data indicate the mean \pm SEM number of PC from seven independent experiments. *D*, IgM and IgG concentrations were determined after 8–10 days of culture and data are the mean \pm SEM from six independent experiments.

of these PC also expressed CD138 (syndecan-1) (Fig. 1B), which has been shown to be expressed predominately by bone marrow PC, but also by a portion of blood PC (37). Notably, in vitro activation of peripheral blood B cells with activated CD4⁺ T cells not only induced PC (Fig. 1C), but also induced secretion of large amounts of IgG and IgM (Fig. 1D).

To determine the cytokine profile of the anti-CD3-activated T cells, IL-21 protein levels from activated peripheral blood CD4⁺ T cells were determined by ICE analysis from culture supernatant following 24 h stimulation with anti-CD3. Substantial amounts of IL-21 were produced by anti-CD3-activated T cells (Fig. 2A). mRNA was also isolated from peripheral blood CD4⁺ T cells stimulated for 24 h with either plate-bound anti-CD3 or soluble PMA/ion with or without IL-2. As shown in Fig. 2B and reported previously (38, 39), stimulation with anti-CD3 or PMA/ion induced

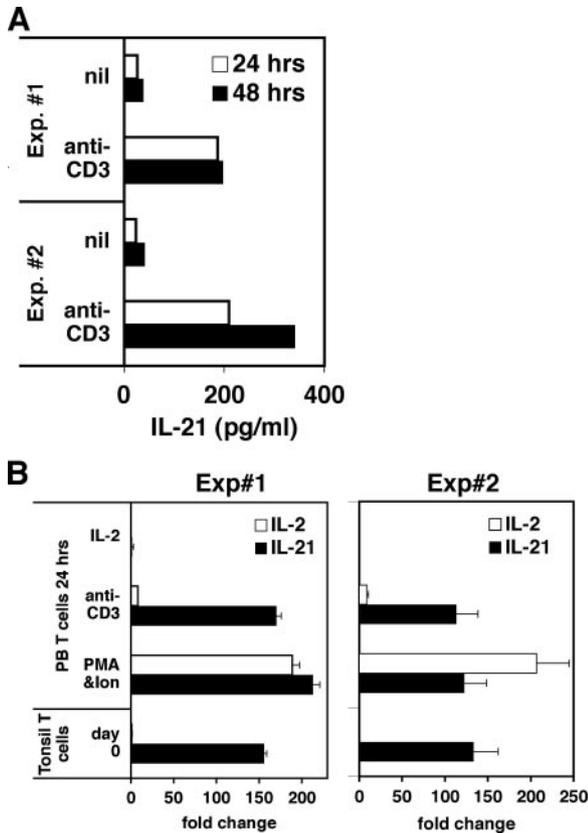


FIGURE 2. Activated T cells produce IL-21. *A*, IL-21 protein was determined in culture supernatants from peripheral blood CD4⁺ T cells activated for 24 and 48 h by immobilized anti-CD3. Data shown are from ICE analysis of two independent experiments (T cells in Expt. 1 were mitomycin-C treated and in Expt. 2 were not). *B*, IL-21 and IL-2 mRNA were quantified from purified peripheral blood CD4⁺ T cells activated with immobilized anti-CD3 or PMA/ion for 24 h or from freshly obtained tonsillar T cells. All values were first normalized to β₂M mRNA and data shown represent fold change of either cultured blood T cells or freshly isolated tonsillar T cells compared with IL-21 or IL-2 mRNA levels of freshly isolated blood CD4⁺ T cells. Means ± SEM determined from triplicate wells of two independent experiments are shown.

CD4⁺ T cells to up-regulate IL-21 mRNA expression. IL-21 mRNA was not increased in cultured T cells that had not been activated compared with freshly obtained T cells. Supplemental IL-2 did not have an impact on IL-21 mRNA levels. Notably, a different pattern of response was noted in purified tonsillar CD4⁺ T cells. Unlike peripheral blood T cells, freshly isolated tonsillar CD4⁺ T cells expressed IL-21 mRNA at similar levels to activated peripheral blood T cells (Fig. 2*B*). However, IL-2 mRNA was not expressed by these cells.

Table I. Cytokines produced by anti-CD3-activated CD4⁺ T cells^a

	IL-1β	IL-2	IL-3	IL-4	IL-5	IL-6	IL-9	IL-10	IL-12	IL-13	IL-16	IL-17	IL-18	IL-21	IL-22	IL-23	TNF	IFN-γ
B cells nil	9	<0.1	<0.1	7	<0.1	10	<0.1	<0.1	10	<0.1	3	2	4	16	2	1	1	<0.1
B cells anti-CD3	7	<0.1	<0.1	15	<0.1	7	<0.1	<0.1	7	<0.1	3	1	6	10	1	1	2	<0.1
T cells nil	13	21	10	52	5	10	10	12	3	12	14	6	4	26	4	13	16	17
T cells anti-CD3	148	753	110	512	173	170	45	315	23	195	11	17	67	187	41	168	186	211
T and B cells nil	15	23	8	50	5	16	15	18	8	12	16	22	5	45	5	10	16	23
T and B cells anti-CD3	42	683	134	482	134	148	111	222	86	152	69	16	75	170	11	88	93	158

^a All cells cultured for 24 h. All values expressed in picograms per milliliter. Under 1 pg/ml found for IL-7, IL-8, IL-15, IL-19, and IL-20. Values represent one of two similar experiments.

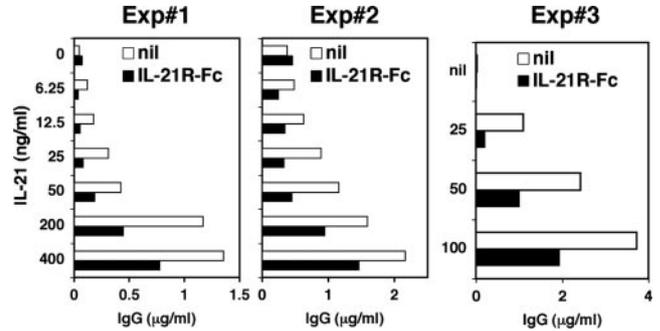


FIGURE 3. IL-21 induces IgG production from activated B cells in a concentration-dependent manner and the effect is inhibited by an IL-21R-Fc construct. Negatively selected peripheral blood B cells were cultured with anti-CD40 and anti-IgM with increasing concentrations of IL-21 in the presence or absence of 10 µg/ml IL-21R-Fc. IgG was measured following 7 days of culture. Data from three of four independent experiments are shown.

To analyze the cytokines produced by activated peripheral blood CD4⁺ T cells in greater detail, and determine whether the presence of B cells affected the cytokine profile of T cells, a complete panel of cytokines was assessed by ICE. As shown in Table I, following stimulation of T cells with anti-CD3, IL-21 was induced by 3.5- to 7-fold, whereas IL-2 was induced >20-fold, and IL-4 by ~9-fold. In addition to IL-2, IL-4, and IL-21, the other cytokines that bind the common receptor γ-chain were also assessed. IL-9 was produced in small amounts and little or no IL-7 and IL-15 were detected. IL-2, IL-4, and IL-21 production was not influenced by the presence of B cells in the cultures (Table I). IL-6 and IL-10 have been shown to be important in human B cell responsiveness (40–43), and were both induced in these cultures of activated T cells.

Blockade of IL-21 inhibits T cell-induced B cell proliferation, PC differentiation, and Ig secretion

Previously, we have reported that rIL-21 induces PC differentiation and Ig production from purified human peripheral blood B cells costimulated with anti-IgM and/or anti-CD40 (12). Importantly, a human IL-21R-Fc fusion protein neutralized this response in a concentration-dependent manner (Fig. 3). IL-21R-Fc inhibited IgG production supported by 6.25–200 ng/ml IL-21 by over 50% (Fig. 3). To address whether blockade of de novo-expressed IL-21 would inhibit T cell-dependent B cell activation and differentiation, the IL-21R-Fc fusion protein was added to cultures of B cells and anti-CD3-activated T cells. As shown in Fig. 4*A*, very few CD19⁺IgD⁻CD38^{high} PC (boxed region) were present at the initiation of culture. Culture of these B cells with activated CD4⁺ T cells, as before, induced PC differentiation (nil) as noted by the presence of CD19⁺IgD⁻CD38^{high}CD27^{high} PC (Fig. 4*A*), a portion of which expressed CD138 as in Fig. 1 (Fig. 4*B*). Notably,

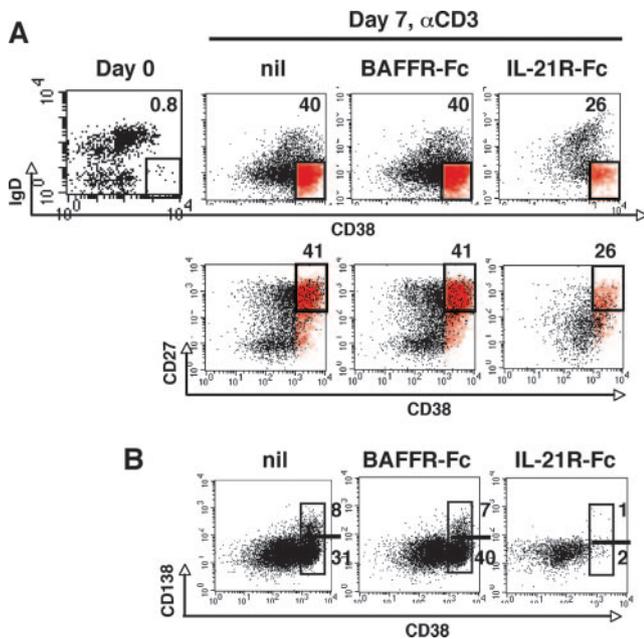


FIGURE 4. IL-21R-Fc inhibits PC differentiation. *A*, Anti-CD3-activated CD4⁺ T cells and negatively selected autologous B cells were cultured as described in Fig. 1 with or without 10 μ g/ml IL-21R-Fc or BAFFR-Fc and assessed for CD4, CD19, IgD, CD27, and CD38 expression following 7 days of culture. Plots shown are gated on CD19⁺CD4⁻ cells. The phenotype of the B cells before culture (day 0) is shown for comparison. PC are shown in red in the IgD⁻CD38^{high} boxed region. CD27 expression of IgD⁻CD38^{high} cells is shown in row 2 in red. Of IgD⁻CD38^{high} cells, 86, 85, and 71% express a high density of CD27 in nil, BAFFR-Fc- and IL-21R-Fc-containing cultures, respectively. Numbers shown represent the percent of cells in the boxed area. Data are from one of seven independent experiments with similar results. *B*, T cells and B cells were cultured as in *A* and after 8 days in culture, CD38 and CD138 expression by CD19⁺ B cells was assessed. Plots shown are gated on CD19⁺CD4⁻ cells. Data are from one of three experiments with similar results.

addition of the neutralizing IL-21R-Fc fusion protein to the cultures markedly inhibited the ability of activated CD4⁺ T cells to induce B cell expansion and PC differentiation (Fig. 4). Moreover, blockade of IL-21 inhibited B cell proliferation and PC generation as noted by significantly less incorporation of [³H]thymidine,

fewer total CD19⁺ B cells as well as CD19⁺IgD⁻CD38^{high} PC (Fig. 5). In contrast, BAFF/BLYS receptor (BAFFR)-Fc had a small, but insignificant, effect (increase) in these cultures (Figs. 4 and 5), ruling out any influence of FcRs or a role of BAFF/BLYS. Importantly, the IL-21R-Fc fusion protein also significantly inhibited IgM production (Fig. 5*B*). The ability of IL-21R-Fc to inhibit B cell expansion, PC differentiation and Ig production was observed over a range of 5–10 days (data not shown), demonstrating that neutralization of IL-21 is effective early in the response, and continues throughout the culture period.

Blockade of IL-21 does not induce cell death

To examine the effect of IL-21R-Fc on B cell proliferation in greater detail, purified B cells were labeled with CFSE and cultured with anti-CD3-activated T cells. As shown in Fig. 6*A*, anti-CD3-activated T cells induced ongoing B cell proliferation as determined by CFSE dilution. IL-21R-Fc inhibited initial B cell expansion and also ongoing B cell proliferation. However, some degree of B cell proliferation was noted in the presence of IL-21R-Fc. The effect of the IL-21R-Fc fusion protein on B cell death was determined by assessing the viability of B cells by staining cocultures of T and CFSE-labeled B cells with PI. As shown in Fig. 6*B*, in the presence of IL-21R-Fc there was less B cell death which was associated with less B cell proliferation, compared with cultures with no inhibitors.

Late addition of IL-21R-Fc inhibits PC differentiation after initial expansion of B cells

Because IL-21R-Fc inhibited both B cell proliferation and PC differentiation, experiments were conducted to determine whether the loss of PC differentiation was a direct result of reduced proliferation, or rather if there was an independent inhibitory effect on PC differentiation. To examine this question, the addition of IL-21R-Fc was delayed until day 4 of culture (Fig. 7), after substantial B cell proliferation had already occurred (see Fig. 6*A*), but before the differentiation of PC (data not shown). Delayed addition of IL-21R-Fc resulted in significant inhibition of PC differentiation as measured by IgG production, whereas BAFFR-Fc resulted in greater Ab production in one of two experiments (Fig. 7). These data indicate that IL-21 is required for both B cell division and PC differentiation and that blockade of IL-21 does not inhibit differentiation merely as an indirect effect mediated by inhibition of proliferation or induction of cell death.

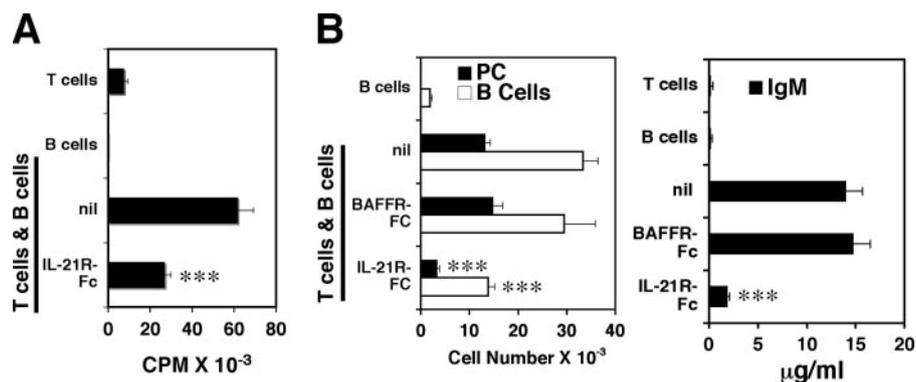


FIGURE 5. Neutralization of IL-21 results in significantly reduced B cell proliferation, PC differentiation, and IgM production. *A*, B cells and mitomycin C-treated CD4⁺ T cells were cultured in anti-CD3-coated microtiter wells with or without 10 μ g/ml IL-21R-Fc and proliferation was measured following 4 days of culture. Data are the mean \pm SEM cpm from three independent experiments. ***, Significant difference between cultures with nil and IL-21R-Fc ($p < 0.005$). *B*, B cells and mitomycin C-treated autologous CD4⁺ T cells were cultured as described in *A*. Mean \pm SEM number of B cells and PC on day 7 was determined from seven experiments and IgM secretion was determined after 8–10 days of culture from six independent experiments. ***, Significant differences between cultures with nil and inhibitors ($p < 0.005$).

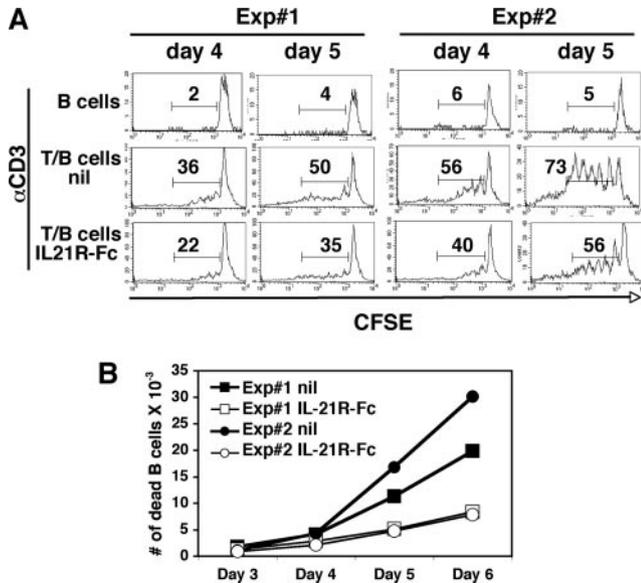


FIGURE 6. IL-21R-Fc reduces CFSE dilution but does not induce cell death of B cells. B cells from two different donors were separately purified by negative selection, stained with CFSE and cocultured with mitomycin C-treated T cells in anti-CD3-precoated microtiter wells with and without IL-21R-Fc. *A*, Histograms show CFSE dilution of CD19⁺PI⁻ B cells cultured with anti-CD3-stimulated CD4⁺ T cells with and without IL-21R-Fc following 4 and 5 days of culture. *B*, After various lengths of culture, cells were stained with anti-CD19-allophycocyanin and PI. Plots show total numbers of dead CD19⁺CFSE⁺PI⁺ cells at each time point. Data shown are from two independent experiments.

To document that the effect of IL-21R-Fc was caused by neutralization of IL-21, proliferation of CFSE-labeled B cells was examined. As before, the addition of IL-21R-Fc greatly suppressed the ability of the B cells to dilute CFSE (Fig. 8). Addition of high concentrations of IL-21 to these cultures overcame the inhibitory effect of the blocking fusion protein, but had no effect on control

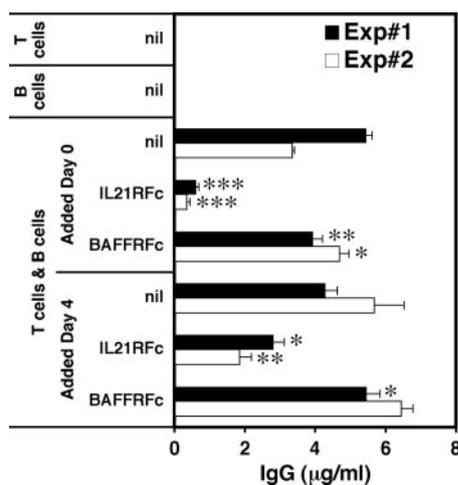


FIGURE 7. IL-21R-Fc inhibits PC differentiation after initial B cell activation. Positively selected B cells and mitomycin C-treated autologous T cells were either cultured in anti-CD3-precoated microtiter wells as above with the Fc constructs added on day 0, or cultured for 4 days before Fc constructs (or medium as control) were added to the wells. All samples were then assessed for IgG content on day 12 of culture. Data shown are mean \pm SEM from triplicate cultures for each of two independent experiments (cells were stained on day 4 and shown not to contain CD19⁺IgD⁻CD38^{high} PC before addition of inhibitors).

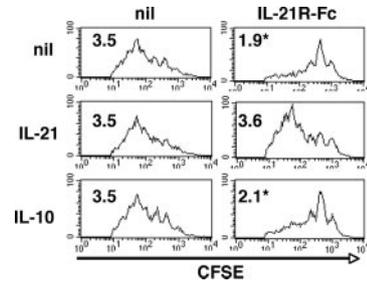


FIGURE 8. IL-21R-Fc inhibition of B cell expansion is reversed by addition of IL-21. B cells were purified by negative selection, labeled with CFSE, and cocultured with mitomycin C-treated T cells in anti-CD3-precoated microtiter wells with and without IL-21R-Fc (10 μ g/ml). Cultures were supplemented with either 400 ng/ml IL-21 or 60 ng/ml IL-10 or with no additional cytokines (nil). Following 5 days of culture, cells were gated on CD3⁻CFSE⁺ cells in the lymphocyte gate and assessed for CFSE dilution. Data shown are from one of two independent experiments. IL-21 reversal of the inhibition of IL-21R-Fc was concentration dependent with substantial reversal of inhibition noted between 10 and 100 ng/ml IL-21. Numbers represent the median numbers of divisions of divided cells based on CFSE dilution; *, $p < 0.05$, compared with parallel cultures lacking IL-21R-Fc (nil).

(nil) cultures. In contrast, addition of IL-10 had no effect on the inhibition of proliferation by IL-21R-Fc.

IL-21 blockade inhibits B cell, but not anti-CD3-induced, T cell activation

We assumed that inhibition of PC differentiation mediated by IL-21R-Fc resulted from a direct effect on B cells. However, because anti-CD3-activated human CD4⁺ T cells up-regulate the IL-21R (38, 39), it was possible that IL-21 blockade was affecting T cell activation. To address this issue, we assessed the impact of IL-21R-Fc on T cell activation. As shown in Fig. 9A, freshly isolated blood CD4⁺ T cells were small and expressed very low densities of CD25, CD69, and CD154, demonstrating that the T cells were not activated at the initiation of culture. Forty-eight hours following culture on anti-CD3-coated plates, the mitomycin C-treated T cells had increased in size and up-regulated expression of CD25, CD69, and CD154. The addition of B cells to these cultures (nil) did not affect cell size nor CD25 or CD69 expression, but did result in decreased expression of CD154 as previously reported (26, 44) (Fig. 9A). Importantly, addition of IL-21R-Fc to cultures of T cells and B cells did not inhibit T cell activation as determined by cell size, and up-regulation of CD25, CD69, and CD154. Notably, addition of anti-CD154 to cultures of T cells and B cells blocked CD40/CD154 interaction and thus CD154 down-regulation, but had no effect on expression of other T cell-activation molecules. These results clearly demonstrate that neutralization of IL-21 does not affect the ability of the T cells to become activated by anti-CD3.

Importantly, however, in the same cultures, IL-21 neutralization dramatically inhibited B cell activation (Fig. 9B). At the initiation of culture, B cells were small and expressed low levels of CD25 and CD69. Following 48 h, the B cells cultured alone did not enlarge nor up-regulate either CD25 or CD69 expression. Upon addition of anti-CD3-activated CD4⁺ T cells, the B cells dramatically increased in cell size as well as up-regulated CD25 and CD69 expression. IL-21 blockade inhibited T cell-induced B cell activation as manifested by a reduction in cell size as well as CD25 expression and to a lesser extent CD69 expression (Fig. 9B). BAFFR-Fc had minimal effects on B cell activation, while blockade of CD154/CD40 interaction inhibited T cell-induced B cell

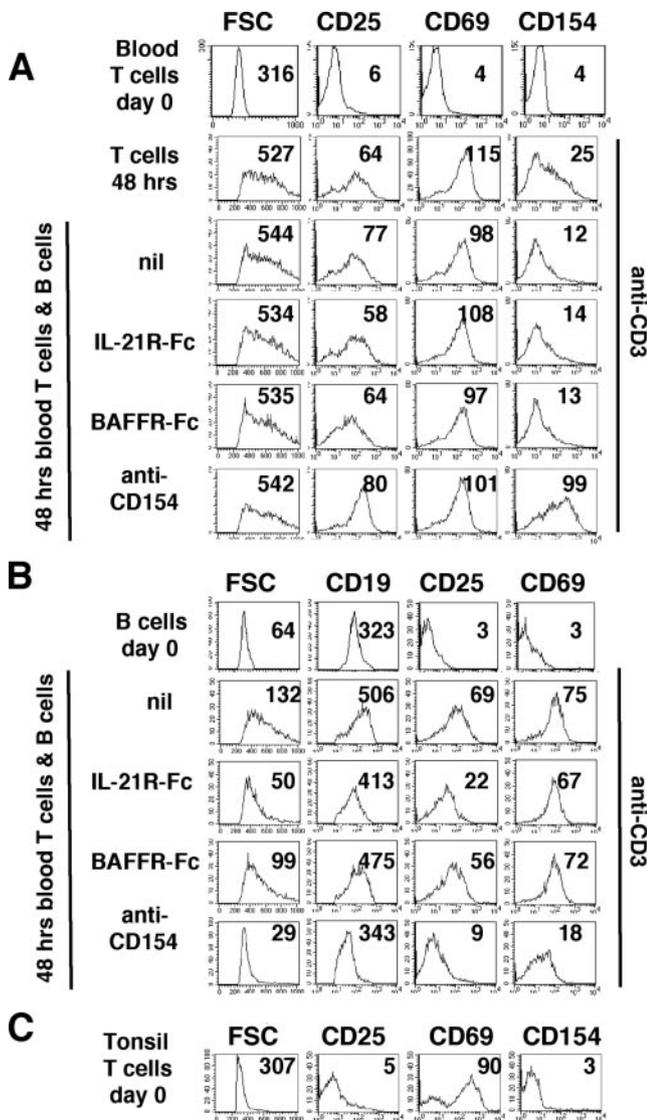


FIGURE 9. IL-21R-Fc inhibits B cell, but not anti-CD3-induced T cell activation. B cells positively selected with CD19 beads and anti-CD3-activated mitomycin C-treated autologous CD4⁺ T cells were cultured as described in Fig. 1. *A*, CD4⁺ T cells and B cells were cultured for 48 h as indicated, and stained with mAb specific for CD4, CD19, and either CD25, CD69, or CD154. Histograms shown are gated on CD4⁺CD19⁻ T cells. *B*, Histograms shown are gated on CD4⁺CD19⁺ B cells. Data from peripheral blood T and B cells are from one of four experiments with similar results. *C*, Freshly isolated tonsillar T cells were stained for markers indicated. Histograms shown are gated on CD4⁺ T cells. Data represent one of two experiments for CD25 and CD69 expression and one of four experiments for CD154 expression. Numbers in each panel represent the median fluorescence intensity of staining with the respective mAb.

activation as expected. These data demonstrate that neutralization of IL-21 directly inhibited B cell activation and PC differentiation without affecting T cell stimulation.

Even though tonsil T cells constitutively expressed IL-21 mRNA (Fig. 2*B*), they were small cells that expressed the activation marker CD69, but not CD25 or CD154 (Fig. 9*C*). When cultured with B cells in the absence of anti-CD3 stimulation, only a modest differentiation of PC was induced (data not shown). The inability of freshly isolated tonsillar T cells to induce efficient PC differentiation was presumably because of minimal CD154 expression by these T cells. Notably, however, the modest capacity of these cells to induce PC differentiation was substantially reduced by

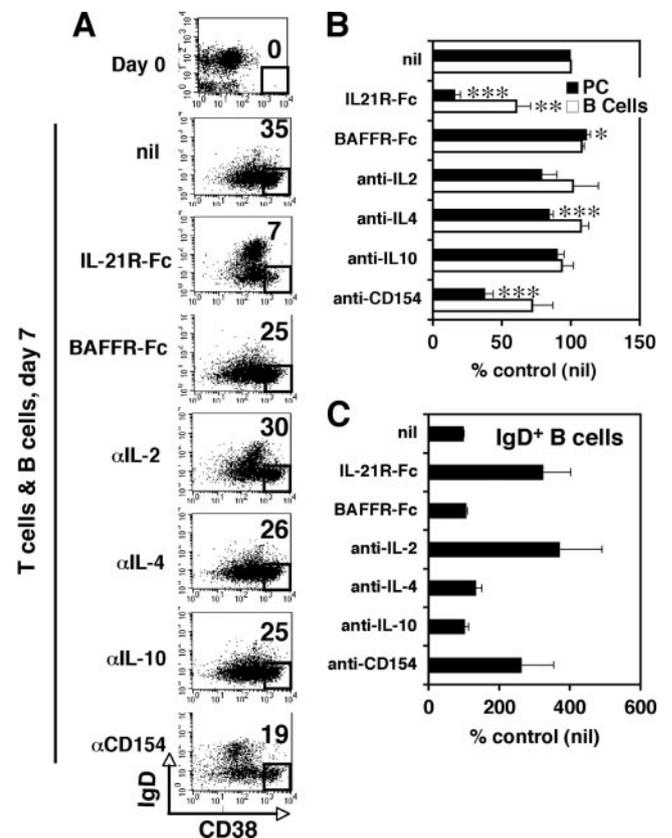


FIGURE 10. IL-21 is the major cytokine directing PC differentiation. *A*, Positively selected B cells and mitomycin C-treated autologous T cells and were cultured as described in Fig. 1 and specific cytokine or interaction inhibitors were added as indicated. Nil indicates control cultures without inhibitors. *A*, Following 7 days of culture, cells were harvested and assessed for IgD and CD38 expression gated on CD4⁺CD19⁺ B cells. The phenotype of the B cells before culture (day 0) is shown for comparison. PC are IgD⁻CD38^{high} in the boxed region, and numbers represent percent of PC. *B* and *C*, Following 7 days of culture, B cell, PC, and IgD⁺ B cell numbers were determined by the total numbers of CD19⁺ cells, CD19⁺CD4⁻IgD⁻CD38^{high} PC, or CD19⁺CD4⁻IgD⁺ B cells, respectively. Mean ± SEM of the percent of response (compared with cultures with no inhibitors, nil) was determined from five independent experiments ($n = 4$ for cultures with anti-CD154). *, Significant difference between cultures with nil and inhibitors; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.005$.

IL-21R-Fc (data not shown), demonstrating that these cells not only express mRNA, but also produce IL-21 protein.

Other cytokines known to be important in B cell responsiveness play a minimal role in T cell-induced PC differentiation

To determine whether blockade of other T cell-derived cytokines known to be important in B cell responsiveness modulated T cell-B cell collaboration, inhibitory Abs to IL-2, IL-4, and IL-10 were compared with IL-21R-Fc. Whereas IL-21R-Fc significantly inhibited T cell-induced PC differentiation, blockade of neither IL-2, IL-4, nor IL-10 overtly suppressed PC differentiation (Fig. 10*A*). As expected, blocking CD40/CD154 interactions reduced T cell-dependent B cell expansion and PC differentiation (Fig. 10*A*). As above and in Fig. 10*B*, IL-21R-Fc consistently and significantly inhibited B cell expansion and PC differentiation. None of the other cytokine inhibitors tested consistently had the same effect, although blockade of IL-4 did result in a modest, but significant inhibition of PC differentiation (Fig. 10*B*). In three of four experiments, Abs to anti-CD154 reduced B cell expansion and in four of four experiments inhibited PC differentiation, although not to the

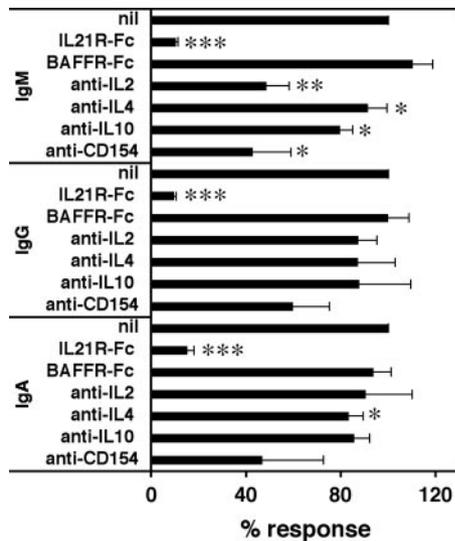


FIGURE 11. IL-21 is the major cytokine inducing IgG and IgA production, whereas IgM secretion is influenced by many cytokines. T cells and B cells were cultured as described in Fig. 1 and specific inhibitors were added as indicated. Nil indicates control cultures without inhibitors. Following 10 days of culture, cell supernatants were harvested and IgM, IgG, and IgA were assessed. Data are expressed as mean \pm SEM of the percentage of Ig found in cultures with inhibitors compared with cultures with no inhibitor (nil). Data represent results from four independent experiments with all conditions other than cultures with anti-CD154, where $n = 3$. *, Significant difference between cultures with nil and inhibitors; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.005$. Range value for Ig in T cell/B cell “nil” cultures was IgM: 1.8–11.8 $\mu\text{g/ml}$; IgG: 2.5–20.5 $\mu\text{g/ml}$; IgA: 2.3–13.7 $\mu\text{g/ml}$.

same extent as IL-21 blockade (Fig. 10B). To compare IL-21 to the other cytokines with regard to B cell expansion and PC differentiation, a separate analysis was performed directly comparing the effect of IL-21R-Fc to each of the other conditions. Importantly, IL-21R-Fc was found to be significantly more effective at decreasing B cell and PC numbers compared with anti-IL-2, anti-IL-4, and anti-IL-10. Of interest, addition of BAFFR-Fc to these cultures caused a small, but significant, increase in PC generation (Fig. 10B). This significant increase of PC following BAFFR-Fc addition was only noted when nil cultures were normalized to 100% (thus removing interexperiment variation), suggesting that the effect of BAFF inhibition was modest (compare Figs. 5B with 10B).

Previously, we have reported that IL-21 induces IgD downmodulation (12). As shown in Fig. 10C, blockade of de novo IL-21 production by activated T cells resulted in an increase in the numbers of IgD⁺ B cells compared with cultures with no inhibitors. Blockade of IL-2 or CD154 also inhibited IgD downmodulation, but not neutralization of IL-4 or IL-10 (Fig. 10C). Notably, IL-21 neutralization significantly inhibited T cell-dependent-IgM, IgG, and IgA production, whereas neutralization of IL-2, IL-4, or IL-10 inhibited IgM (but significantly less than IL-21 neutralization), but not IgG production (Fig. 11). Anti-IL-4, but not anti-IL-2 or IL-10, had a modest, but significant, effect on IgA secretion.

IL-21 is involved in the generation of PC from both naive and memory B cells

Previously, we reported that IL-21 has the capability to induce PC differentiation from both naive and memory human peripheral blood B cells (12). Therefore, we sought to examine the role of IL-21 in T cell-dependent generation of PC from naive and memory B cells. As shown in Fig. 12, the ability of activated T cells to

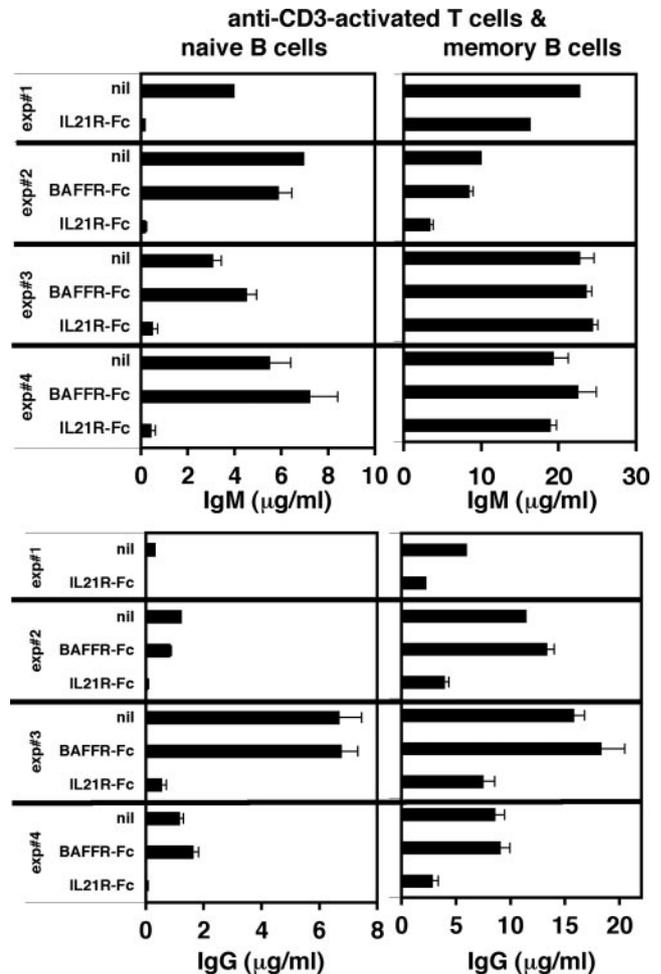


FIGURE 12. T cell-induced IgG production from both naive and memory B cells requires IL-21. Sorted naive and memory B cells were cultured with mitomycin C-treated T cells and cultured for 11–12 days in anti-CD3 pre-coated microtiter wells. Ig content was determined and data are expressed as mean \pm SEM of Ig concentration of triplicate cultures from the same experiment with no inhibitor (nil), BAFFR-Fc, or IL-21R-Fc.

induce naive B cells to undergo class switch recombination, differentiate into PC, and produce both IgM and IgG is strictly dependent on the presence of IL-21. Moreover, the ability of memory B cells to differentiate into PC and produce IgG was also partially dependent on IL-21. However, the ability of CD27⁺ memory B cells to produce IgM was only marginally affected by neutralization of IL-21 (Fig. 12). These data strongly suggest that IL-21 differentially regulates responses of naive and memory B cells, with naive B cells being almost completely dependent on IL-21, whereas Ig production from memory B cells being somewhat less dependent on IL-21.

Discussion

These studies used an in vitro model of human T cell-dependent B cell responses to explore the comparative role of IL-21 in the differentiation of PC. Although a great deal is known regarding T cell-B cell collaboration, including the importance of cell-cell contact involving CD40/CD154 and CD11a/CD18-CD54 interactions (17, 26, 27), the critical soluble factors that mediate this process have not been completely elucidated.

IL-21 has clearly been shown to be a major contributor to activation, differentiation, and death of purified human and mouse B

cells in vitro (30). Moreover, the role of IL-21 in humoral immunity has also been delineated both in vitro and in vivo in mice (45, 46). Specifically, IgG, but not IgM is greatly reduced in IL-21R^{-/-} mice, whereas IgE is increased. In IL-4R/IL-21R double-deficient mice, a reduction of all Ig isotypes is observed (45). Thus, in mice both IL-21 and IL-4 appear to play important roles in PC differentiation. The relevance of IL-21 in direct human T cell-B cell interaction and function has not been determined. To examine this, we used a previously described model of human T cell-B cell collaboration in which anti-CD3-activated T cells drive B cell responses in the absence of BCR engagement (22). Importantly, in this model system, the B cell is not required for T cell activation or cytokine production but rather acts as a recipient of contact-dependent and soluble T cell signals and undergoes activation, proliferation, and PC differentiation, each of which can be measured. Notably, activation of nearly all B cells occurred in this system as well as multiple rounds of B cell proliferation and differentiation of CD38^{high}CD27^{high} PC, a portion of which expressed CD138.

In this study, we found that T cell-derived IL-21 was a crucial component of CD4⁺ T cell-directed B cell activation and differentiation. Neutralization of IL-21 with an IL-21R-Fc fusion protein significantly inhibited the ability of activated T cells to induce B cell activation, proliferation, PC differentiation, and Ig production. IL-21 played a role in responses of both naive and memory B cells, although class switch recombination and PC differentiation by naive cells exhibited a much greater dependence on IL-21 than that of memory B cells. No further inhibition of PC differentiation was noted when anti-IL-4 or anti-IL-10 was added to the cultures blocked with IL-21R-Fc (our unpublished observations). However, in the majority of experiments, the combination of anti-IL-2 and IL-21R-Fc resulted in greater inhibition of PC differentiation than IL-21R-Fc alone. Consistent with these data, we have reported that the combination of IL-21 and IL-2, but not IL-4 and IL-21 or IL-10 and IL-21, was more efficient than IL-21 alone at inducing PC differentiation in the presence of anti-CD40 (Ref. 12, and data not shown). Importantly, IL-21 blockade inhibited IgM, IgG, and IgA production. These data are consistent with our previous findings demonstrating that IL-21 was capable of costimulating PC differentiation and IgM and IgG production from human peripheral blood B cells (12). Although we had previously found that IL-21 and anti-CD40 induced IgA production from human peripheral blood B cells (12), others have reported that IL-21 and anti-CD40 did not induce IgA production from human splenic B cells (15). The current results confirm a role of IL-21 in IgA secretion during human T cell-B cell collaboration.

Many T cell-derived cytokines have been implicated in human B cell activation and differentiation. These include IL-2, IL-4, IL-6, and IL-10 (25, 41, 47–49). Recently, we directly compared the ability of peripheral blood B cells to be induced to differentiate into PC following activation with anti-CD40 and the combination of a number of cytokines. Our results showed that while IL-2, IL-4, or IL-6 with anti-CD40 did not induce effective PC differentiation, the combination of IL-2 and IL-10 was able to induce some differentiation and Ig production of B cells activated via CD40 engagement (12). In striking contrast, IL-21 drove substantial proliferation, class switch recombination, PC differentiation, and Ig production from anti-CD40-stimulated blood B cells (12). Consistent with these results, we found that T cell-directed activation, proliferation and differentiation of CD19⁺ peripheral blood B cells was dependent on IL-21 and CD40/CD154 interactions, whereas, neutralization of IL-2, IL-4, and IL-10 had little effect, consistent with other findings (29). These data reiterate the importance of

IL-21 as a major component of the helper function of T cells that regulate B cell responsiveness.

IL-21 not only was a critical component in T cell-dependent PC differentiation, but also was important in regard to IgD down-modulation. Moreover, IL-21 played an important role in B cell, but not T cell, activation. Inhibition of T cell-derived IL-21 with IL-21R-Fc significantly inhibited B cell activation as manifested by reduced cell size as well as lower density of CD19 and CD25 expression. CD69 expression, however, was directly induced by CD40 engagement, and was unaffected by IL-21 blockade. Neutralization of de novo-produced IL-2 did not consistently affect PC differentiation. However, blockade of IL-2 activity resulted in reduced IgD down-modulation. These findings suggest that IL-2 may stimulate activated naive B cell expansion without inducing the initial steps in maturation as manifested by decreased expression of IgD. IL-21, of all the cytokines examined, most efficiently stimulated IgD down-modulation and PC differentiation. Although neutralization of IL-21, and to a much lesser extent IL-4, affected PC numbers, blockade of all cytokines tested significantly reduced IgM production. In contrast, IgG secretion was inhibited only by neutralization of IL-21, but not the other cytokines. These data suggest that IgM production induced by activated T cells and supported by IL-2, IL-4, and/or IL-10 is largely derived from lymphoblasts or pre-PCs, whose ongoing activity can be driven by a variety of cytokines. Although these cytokines support IgM production from preswitched B cells, the data suggest that they play a much lesser role in PC differentiation and IgG production. Production of IL-21, in contrast, is clearly a major inducer of class switch recombination, PC differentiation from naive B cells as well as IgM, IgG, and IgA secretion, as our previous data using a T cell-free system would predict (12). Responses of memory B cells and especially IgM secretion from these cells was much less dependent on IL-21, suggesting that other cytokines, and particularly the combination of IL-2 and IL-10 might play a relatively larger role in responses of memory B cells in humans as we have shown previously (12).

Differentiation of PC has been shown to require antecedent B cell proliferation (25). Moreover, we have reported that IL-21 and anti-CD40-induced PCs arise from dividing precursors (12). Because IL-21R-Fc blocked B cell proliferation, the inhibitory effect on PC differentiation could have been secondary to diminished B cell expansion. However, late addition of IL-21R-Fc after extensive B cell proliferation had occurred, caused substantial inhibition of PC differentiation, suggesting a requirement for IL-21 in PC differentiation independent of its role in B cell expansion. This finding is consistent with previous observations that IL-21 is a potent inducer of BLIMP-1, a required regulator of the PC genetic program (12, 46).

Various phenotypic markers have been used to assess the stage of PC differentiation. Expression of CD138 has been shown to be expressed by bone marrow PC and, therefore, has been used to identify terminally differentiated, nondividing, long-lived PC (37). It is, therefore, notable that anti-CD3-activated T cells induced the differentiation of CD138-expressing PC in an IL-21-dependent manner. This is consistent with our previous observation that IL-21 can induce the differentiation of nondividing, long-lived PC from anti-CD40-activated B cells (12).

BAFF/BLYS belongs to the TNF family and binds three receptors, BAFFR, transmembrane activator and calcium modulator cyclophilin ligand interactor (TACI), and B cell maturation Ag (50). In humans, dendritic cells are the major producers of BAFF/BLYS (50), although, many other cell types including T cells and a small subset of B cells also produce BAFF/BLYS (51, 52). Our data suggest that in purified cultures of T cells and B cells, BAFF/BLYS

had a minimal contribution to T cell-dependent B cell differentiation. We noted that in some experiments, addition of BAFFR-Fc resulted in a modest, but significant, increase in PC differentiation. BAFF/BLyS is a known survival factor for human B cells (50). Thus, we assumed that if small amounts of BAFF/BLyS were produced in these cultures, addition of BAFFR-Fc would decrease, not increase, the response. However, BAFF/BLyS can also stimulate T cell proliferation and cytokine production (52, 53). Moreover, BAFFR, but not TACI, is expressed by subpopulations of human T cells (51). These data suggest that BAFF/BLyS in the T/B culture system may negatively impact the ability of T cells to induce PC differentiation. We are currently exploring this possibility.

The majority of B cell expansion, class switch recombination, and PC differentiation occurs in the germinal center and is largely dependent on CD4⁺ Th cell function (54). Peripheral blood T cells have been shown to be capable of initiating B cell activation and PC differentiation following anti-CD3 activation in vitro (22, 25). Moreover, specialized CXCR5⁺ follicular Th cells constitutively produce IL-21 and are found within B cell areas of lymphoid tissue, specifically the mantle zone, the outer zone and light zone of the germinal centers (32, 54). These T cells have been shown to be capable of stimulating B cells to express activation-induced cytidine deaminase expression, class switch recombination, and Ig production (54). In this regard, in vitro-activated tonsillar CD57⁺ germinal center Th cells also have the capacity to induce activation-induced cytidine deaminase expression and IgM, IgG, IgA, and IgE production from germinal center tonsillar B cells in an IL-4- and IL-10-independent manner (29). Furthermore, CXCR5⁺CD57⁺ T follicular helper cells are capable of inducing spontaneous Ig production from tonsillar B cells (55). Our data suggest that the ability of helper follicular T cells to induce PC differentiation is dependent on their capacity to produce IL-21.

Notably, we found that freshly isolated tonsillar T cells contained as much IL-21 mRNA as do anti-CD3-activated peripheral blood T cells. Furthermore, these tonsillar T cells did not express IL-2 mRNA (nor CD25) ruling out a general up-regulation of cytokine production, although they were partially activated as assessed by CD69 expression. The IL-21 from freshly isolated tonsillar T cells was presumably produced by CXCR5⁺ T follicular helper cells that have been shown to express this cytokine mRNA without in vitro activation (32), and to have spontaneous B cell helper activity (55). However, tonsil T cells were relatively ineffective inducers of PC differentiation when tested immediately ex vivo in the absence of anti-CD3 activation. This is likely to reflect the finding that freshly isolated tonsil T cells are largely devoid of CD154 expression. Notably, however, the small degree of PC differentiation they induced was inhibited by IL-21R-Fc, consistent with the conclusion that they constitutively secrete IL-21.

Taken together, our data show that IL-21 produced by human activated T cells is a critical component of T cell-directed B cell activation, proliferation, class switch recombination, and PC differentiation both in vitro, and presumably in vivo, during germinal center reactions.

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Disclosures

The authors have no financial conflict of interest.

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