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IL-21 Induces the Apoptosis of Resting and Activated Primary B Cells

Devangi S. Mehta,* Andrea L. Wurster,*, Matthew J. Whitters,† Deborah A. Young,† Mary Collins,† and Michael J. Grusby2*‡

Cytokines play an important role in regulating the development and homeostasis of B cells by controlling their viability. In this study, we show that the recently described T cell-derived cytokine IL-21 induces the apoptosis of resting primary murine B cells. In addition, the activation of primary B cells with IL-4, LPS, or anti-CD40 Ab does not prevent IL-21-mediated apoptosis. The induction of apoptosis by IL-21 correlates with a down-regulation in the expression of Bcl-2 and Bcl-xL, two antiapoptotic members of the Bcl-2 family. Furthermore, the reconstitution of Bcl-xL or Bcl-2 expression protects primary B cells from IL-21-induced apoptosis. In addition, a short-term preactivation of B cells with anti-CD40 Ab confers protection from IL-21-mediated apoptosis through the up-regulation of Bcl-xL. These studies reveal a novel pathway that mediates B cell apoptosis via the IL-21R and suggest that IL-21 may play a role in regulating B cell homeostasis. The Journal of Immunology, 2003, 170: 4111–4118.

Interleukin-21 and its class I cytokine receptor (IL-21R) have been recently described. The cytokine is a product of activated T cells and has significant homology to IL-4, IL-2, and IL-15. The IL-21R, which has homology to the IL-2R and IL-15R β-chains and the IL-4R α-chain, is selectively expressed in lymphoid tissues and has been shown to interact with the common γ chain (γC)1–3. Ligand binding to the IL-21R can transduce signals through Janus kinases 1 and 3, STAT1, STAT3, and STAT51–3. In vitro experiments demonstrated that human IL-21 acts as a costimulus in anti-CD3-mediated T cell proliferation and also as a promoter of the proliferation and maturation of NK cells from bone marrow progenitors. In contrast to the response of human NK cells to IL-21, murine in vitro experiments showed that IL-21 inhibited rather than enhanced the IL-15-dependent expansion of resting and activated NK cells. Murine IL-21 was able to enhance the effector function of previously activated NK cells but did not promote their viability in culture. In addition, we have recently generated IL-21R-deficient (IL-21R−/−) mice and found that these mice have normal lymphoid compartments. In particular, the IL-21R−/− mice had no NK cell deficiency or any defect in NK cell activation and cytolytic activity (4). Taken together, these results suggest that the biological effects of this cytokine may diverge between different species.

The biological effects of IL-21 on B cells, another lymphoid population that expresses the IL-21R, has not been extensively characterized. Initial studies demonstrated that IL-21 costimulates human B cell proliferation induced by anti-CD40 Ab, whereas it inhibits proliferation induced by anti-IgM Ab and IL-4 (2). Recently, it has been shown that human IL-21 induces proliferation and inhibits apoptosis of the IL-6-dependent human myeloma cell lines ANBL-6, IH-1, and OH-2 (5). Because IL-21 seems to elicit contrasting responses from human and murine NK cells, we were interested in characterizing the effects of IL-21 on primary murine B cells to determine whether the biological role of IL-21 also diverges between human and murine B cells.

In this report, we demonstrate that, in contrast to that seen with human IL-21, murine IL-21 does not enhance the proliferation of anti-CD40 Ab-costimulated murine B cells, but does inhibit proliferation induced by anti-IgM Ab and IL-4. We also show that IL-21 induces the apoptosis of primary resting and activated B cells. Moreover, IL-21 can induce the apoptosis of primary B cells even in the presence of prosurvival costimulatory factors such as IL-4, LPS, or anti-CD40 Ab. We show that IL-21-induced apoptosis correlates with a down-regulation in the expression of Bcl-2 and Bcl-xL, two antiapoptotic members of the Bcl-2 family. Furthermore, the reconstitution of Bcl-xL or Bcl-2 expression, either by retroviral overexpression or by preactivation with anti-CD40 Ab, protects primary B cells from IL-21-induced apoptosis. These results suggest that murine IL-21 is an effector rather than inhibitor of murine B cell apoptosis whose potential physiological role may be controlling homeostasis of the B cell compartment.

Materials and Methods

Mice

C57BL/6 mice were obtained from Taconic Farms (Germantown, NY). IL-21R−/− mice on the C57BL/6 background were generated and maintained as previously described (4).

B lymphocyte preparation and culture

Splenic B lymphocytes were purified to 95% purity by depletion of CD43+ cells using MACS magnetic beads specific for CD43 (Miltenyi Biotec, Auburn, CA) per the manufacturer’s instructions. B lymphocytes were cultured at 1–2 × 10^6 cells/ml in RPMI 1640 supplemented as described previously (6). Recombinant IL-21 (R&D Systems, Minneapolis, MN) was added to indicated cultures at a concentration of 200 ng/ml. Anti-CD40 Ab (BD PharMingen, San Diego, CA) was added to indicated cultures at a...
concentration of 5 μg/ml. Anti-IgM Ab (Fab’)_2 fragment; Southern Biotechnology Associates, Birmingham, AL) was added to indicated cultures at a concentration of 5 μg/ml. Recombinant IL-4 (PeproTech, Rocky Hill, NJ) was added to indicated cultures at a concentration of 10 ng/ml. LPS (Sigma-Aldrich, St. Louis, MO) was added to indicated cultures at a concentration of 10 μg/ml. Boc-D(OMe)-FMK; Calbiochem, La Jolla, CA) was added to indicated cultures at a concentration of 20 μM.

B cell proliferation assay

For stimulations, CD43-depleted splenic B cells were cultured in either 5 μg/ml anti-CD40 Ab or 5 μg/ml anti-IgM Ab and 10 ng/ml IL-4 in the presence of varying amounts of IL-21 (0–200 ng/ml). Proliferation was assessed by the incorporation of [3H]thymidine (50 μCi/well) during the last 18 h of a 90 h culture. Stimulations were conducted in triplicate.

Propidium iodide analysis

Lymphocytes were cultured as described in the figures, pelleted by centrifugation and fixed in 40% EtOH. The cells were treated with RNase A (50 μg/ml) for 45 min at 37°C and subsequently stained with 700 mM propidium iodide in 3.8 × 10^-5 M sodium citrate. Analysis was performed on a FACSscan flow cytometer (BD Biosciences, Mountain View, CA).

Annexin V staining

Lymphocytes were cultured as described in the figures, pelleted by centrifugation, and resuspended in 100 μl of 1× binding buffer (10 mM HEPES (pH 7.4), 140 mM NaCl, and 2.5 mM CaCl_2) and stained with 5 μl of annexin V-PE (BD PharMingen) for 15 min at room temperature in the dark. Cells were diluted in 1× binding buffer and analyzed on a FACSscan flow cytometer (BD Biosciences).

Immunoblot analysis

Immunoblots were performed as previously described using Bcl-xL-, Bcl-2-, or Hsp90-specific Ab (Santa Cruz Biotechnology, Santa Cruz, CA) diluted 1/1000 in blocking buffer (6). Changes in protein levels relative to Hsp90 were determined by densitometry using NIH Image 1.62 software.

RNase protection assay

Total RNA was isolated using TRIzol RNA isolation reagent (Invitrogen, San Diego, CA). RNase protection assays were performed according to the manufacturer’s instructions using a RiboQuant RNase protection kit, 5 μg of total RNA per sample, and the mouse Apo-2 probe set (BD PharMingen). The resulting gel was dried and developed by autoradiography. Changes in mRNA levels relative to L32 and GAPDH were determined by densitometry using RiboQuant RNase protection kit. The resulting gel was dried and developed by autoradiography.

Retroviral transduction of B cells

The GFP-RV bicistronic vector was obtained from K. Murphy (St. Louis, MO) (7) and the Phoenix-Eco packaging cell line was obtained from G. Nolan (Stanford, CA) (8). The murine Bcl-xL-cDNA was cloned into GFP-RV (6). The human Bcl-2 cDNA cloned into MSCV-I-GFP was kindly provided by S. Korsmeyer (Boston, MA) (9). Transfection of the packaging cell line was performed using Effectene (Qiagen, Valencia, CA), and viral supernatants were harvested 48–72 h later. Anti-CD40 Ab (5 μg/ml) and LPS-activated (10 μg/ml) purified splenic B cells were retrovirally transduced by incubation of 1×10^7 B cells at 1×10^9/ml with an equal volume of viral supernatant and 2 μg/ml polybrene. The cultures were centrifuged at 500 × g for 1 h at room temperature. The culture medium was changed after 24 h. Green fluorescent protein (GFP)-positive cells were sorted by FACScan (BD Biosciences) 36 h after transduction and cultured in the presence or absence of IL-21 (200 ng/ml) for 24 h. Cells were subsequently analyzed for apoptosis by propidium iodide analysis.

Results

**IL-21 does not enhance anti-CD40 Ab-mediated proliferation of primary murine B cells, but does inhibit the proliferation induced by anti-IgM Ab and IL-4**

To identify any differences between murine and human B cell responses to IL-21, we examined the ability of IL-21 to co-stimulate B cell proliferation. Splenic B cells from wild-type and IL-21R-/- mice were purified and cultured with graded doses of IL-21 (0–200 ng/ml) in the presence of anti-CD40 Ab or anti-IgM Ab and IL-4. In contrast to that seen with human IL-21, murine IL-21 did not enhance the proliferation of anti-CD40 Ab-costimulated wild-type B cells (Fig. 1A). However, similar to human IL-21, murine IL-21 did inhibit anti-IgM Ab- and IL-4-induced proliferation of wild-type B cells in a dose-dependent manner (Fig. 1B). The IL-21R-/- B cells were not susceptible to the antiproliferative effects of IL-21.

**IL-21 induces the apoptosis of resting and activated primary murine B cells**

Considering that murine IL-21 does not costimulate the proliferation of B cells, it is possible that IL-21 could instead be inducing death. To determine what effect IL-21 has on the viability of primary murine B cells, splenic B cells from wild-type and IL-21R-/- mice were purified and cultured in the presence or absence of IL-21 for 24 h. Subsequently, the cultures were analyzed for the presence of apoptotic cells by propidium iodide analysis. As expected, a significant proportion of the population of untreated cells was found to be subdiploid. However, the addition of IL-21 to wild-type B cell cultures yielded a 2-fold increase in the number of apoptotic cells (Fig. 2A). In contrast, there was no difference in the percentage of apoptotic cells between untreated and IL-21-treated cultures of IL-21R-/- B cells indicating the specific requirement of this receptor in order for IL-21 to transduce an apoptotic signal. In addition to inducing apoptosis in resting B cells, IL-21 treatment also induced the apoptosis of anti-CD40 Ab-activated B cells (Fig. 2B). Wild-type B cells cultured in the presence of IL-21 for 24 h after activation had a 5-fold increase in the number of subdiploid cells compared with anti-CD40 Ab alone. Interestingly, IL-21 treatment of anti-CD40 Ab-activated wild-type B cells also induced a 3-fold increase in the number of cells transitioning from the G_1/G_0 phase to S phase of the cell cycle (IL-21: 6.2% ±
The dual effects of IL-21 on anti-CD40 Ab-activated B cells may explain why IL-21 does not inhibit anti-CD40 Ab-mediated proliferation even though it induces apoptosis in these cells.

These results were confirmed by annexin V staining, an early marker of apoptosis that detects the translocation of phospholipid phosphatidylserine from the inner to the outer surface of the plasma membrane (Fig. 3, A and B). IL-21 treatment of resting B cells resulted in a 50% increase of apoptotic cells compared with untreated B cells within 6 h of IL-21 treatment. In addition, the induction of apoptosis by IL-21 is dependent on the activation of caspases, known effectors of apoptosis (Fig. 3C). When resting B cells are treated with IL-21, the percentage of annexin V-positive cells increases significantly. This increase is blocked by a broad-spectrum caspase inhibitor, indicating that caspase activation is essential for IL-21-induced apoptosis. These findings suggest that IL-21 plays a dual role in B cell responses, promoting both proliferation and apoptosis, depending on the activation status of the cells.

**FIGURE 3.** The apoptosis of primary B cells is induced within 6 h of treatment with IL-21 and can be inhibited by a broad-spectrum caspase inhibitor. Primary B cells purified from wild-type mice were cultured in the presence or absence of IL-21 (200 ng/ml) over a time course of 3, 6, and 12 h (A) and then stained with annexin V to detect the percentage of apoptotic cells (gated population). The percentage of annexin V-positive cells over hours of treatment with IL-21 is represented graphically in B. Primary B cells purified from wild-type mice were cultured in the presence of absence of a Boc-D(OMe)-FMK (20 μM), a caspase inhibitor, for 30 min and then treated with IL-21 for 24 h (C). Propidium iodide staining was used to assess the percentage of apoptotic cells. The results are representative of two independent experiments.
cells were pretreated with a broad-spectrum caspase inhibitor (Boc-D(OMe)-FMK) before culturing with IL-21, there was a complete inhibition of apoptosis when compared with cells that were not treated with the caspase inhibitor.

IL-21 down-regulates the mRNA and protein expression of Bcl-2 and Bcl-xL

In an effort to determine the mechanism underlying the apoptotic effects of IL-21 signaling, we examined whether IL-21 affects the expression of various members of the Bcl-2 family. The Bcl-2 family is a group of mitochondrial membrane proteins composed of both pro- and antiapoptotic members that can suppress or promote mitochondrial dysfunction. It is the relative intracellular levels of these pro- and antiapoptotic proteins that can determine whether or not apoptosis is activated or suppressed (10, 11). RNase protection assays showed that, within 3 h of IL-21 treatment, several of the antiapoptotic Bcl-2 members are down-regulated in both resting B cells and in B cells preactivated with anti-CD40 Ab for 24 h (Fig. 4A). In both resting and activated B cells, Bcl-2 mRNA levels were most dramatically decreased upon IL-21 stimulation, representing a 7-fold reduction to essentially nondetectable levels of mRNA, whereas Bcl-xL mRNA levels were downregulated ~2-fold. These results were confirmed by quantitative real-time PCR (data not shown). In addition, the mRNA levels of Bfl-1 and Bcl-w, also antiapoptotic factors, were reduced ~2-fold with IL-21 treatment in resting B cells. Within 6 h of IL-21 stimulation of primary B cells, both Bcl-2 and Bcl-xL protein levels were significantly decreased (Fig. 4B), which correlates with the onset of apoptosis as determined by annexin V staining (Fig. 3A). These experiments show that, in primary B cells, IL-21 signaling instructs the rapid repression of multiple antiapoptotic factors allowing for the activation of cell death.
Overexpression of Bcl-xL and Bcl-2 rescues B cells from IL-21-induced apoptosis

The studies in the previous section suggest that IL-21 signaling results in the repression of Bcl-xL and Bcl-2 expression and subsequently cell death. To determine whether overexpression of Bcl-xL or Bcl-2 could prevent IL-21-induced apoptosis, we used a retroviral gene expression system to overexpress these prosurvival factors in primary B cells. Bcl-xL or Bcl-2 cDNA were cloned into a bicistronic retroviral expression construct that allows for the co-expression of the antiapoptotic factor and GFP within the same cell. Purified wild-type B cells were first activated with LPS and anti-CD40 Ab for 24 h, and then transduced with retrovirus containing either the empty GFP vector or the vector including the antiapoptotic factor and GFP. GFP-positive cells were sorted by flow cytometry to 95% purity and then cultured in the presence or absence of IL-21 for 24 h. The presence of apoptotic cells was detected by propidium iodide analysis. B cells transduced with GFP alone resulted in a 2- to 3-fold increase in apoptotic cells after stimulation with IL-21 (Fig. 5). However, when Bcl-xL or Bcl-2 was coexpressed with GFP, there was no difference in the percentage of apoptotic cells between cultures with or without IL-21. These data indicate that either Bcl-xL or Bcl-2 overexpression is sufficient to rescue primary B cells from IL-21-induced apoptosis, providing additional evidence that the apoptotic effect of IL-21 is mediated through the down-regulation of these prosurvival proteins.

Prosurvival factors IL-4, LPS, and anti-CD40 Ab do not protect primary B cells from IL-21-induced apoptosis

To further explore the capacity of IL-21 to induce the apoptosis of B cells, we asked whether or not other known B cell costimulatory factors such as IL-4, LPS, and anti-CD40 Ab, or B cell receptor (BCR) activation could inhibit IL-21-induced apoptosis. Primary resting B cells were cultured in the presence of IL-21 and either IL-4, LPS, anti-CD40 Ab, or anti-IgM Ab for 24 h. B cells were also pretreated with IL-4, LPS, anti-CD40 Ab, or anti-IgM Ab for 6 h followed by the addition of IL-21 for 24 h. Subsequently, the cultures were analyzed for the presence of apoptotic cells by propidium iodide analysis.

Although BCR activation of resting B cells results in the induction of apoptosis, the addition of IL-21 to these cultures did not result in an increase in the percentage of apoptotic cells (Fig. 6A). The lack of an additive effect in the presence of IL-21 suggests either that the apoptotic programs induced by IL-21 and anti-IgM Ab stimulation overlap or that the maximum susceptibility of B cells to apoptosis has already been reached by each individual stimulus.

IL-4 protects B cells from apoptosis, but the addition of IL-21 can overcome the prosurvival effects of IL-4 even when cells are pretreated with IL-4 (Fig. 6B). LPS also promotes B cell survival; however, the addition of IL-21 to LPS-treated cultures still results in the apoptosis of B cells (Fig. 6C). In addition, anti-CD40 Ab treatment, which also protects B cells from apoptosis, does not protect against IL-21-induced apoptosis when the two factors are added to the culture simultaneously (Fig. 6D). However, when B cells are first pretreated for 6 h with anti-CD40 Ab and then cultured in IL-21 for 24 h, there is complete protection from apoptosis. Interestingly, this is in contrast to B cells that are preactivated with anti-CD40 Ab for 24 h, where IL-21 is still capable of inducing apoptosis (Fig. 2B). These data suggest that early pretreatment of B cells with anti-CD40 Ab for 6 h allows enough time for the accumulation of an anti-CD40 Ab-induced prosurvival factor.

**FIGURE 6.** Prosurvival costimulatory factors do not protect primary B cells from IL-21-induced apoptosis. Primary B cells purified from wild-type mice were cultured in the presence or absence of IL-21 with or without anti-IgM Ab (A), IL-4 (B), LPS (C), and anti-CD40 Ab (D) for 24 h. Cells were also pretreated with each costimulatory molecule for 6 h followed by the addition of IL-21 to cultures for 24 h. Propidium iodide staining was used to assess the percentage of apoptotic cells. The results are representative of three independent experiments. The error bars indicate SDs.
that can overcome the apoptotic program that is initiated by IL-21 within 6 h of cytokine stimulation.

**Pretreatment of primary B cells with anti-CD40 Ab up-regulates Bcl-x<sub>L</sub> expression resulting in protection from IL-21-induced apoptosis**

We have shown that the pretreatment of primary resting B cells with anti-CD40 Ab for 6 h is sufficient to protect these cells from IL-21-induced apoptosis (Fig. 6D). However, the simultaneous culturing of B cells with anti-CD40 Ab and IL-21 does not provide protection from apoptosis. Additionally, we have also shown that a longer activation of B cells with anti-CD40 Ab (24–36 h) does not alter the susceptibility of these cells to IL-21-induced apoptosis (Fig. 2B). To determine the minimal time of anti-CD40 Ab pretreatment required for maximal protection from IL-21-induced apoptosis, we conducted a time course of pretreatment of splenic B cells with anti-CD40 Ab for 0–6 h before the introduction of IL-21 for an additional 24 h and then analyzed for apoptosis by propidium iodide staining. We found that the percentage of apoptotic B cells induced by IL-21 linearly decreases with the increasing length of time of anti-CD40 Ab pretreatment, and that 6 h of anti-CD40 Ab pretreatment was required for maximal protection (Fig. 7A).

It is well documented that anti-CD40 Ab treatment of B cells up-regulates the expression of the prosurvival factor Bcl-x<sub>L</sub>, protecting B cells from BCR-mediated death (12). In contrast, IL-21 treatment results in the down-regulation of Bcl-x<sub>L</sub> transcription within 3 h and protein expression after 6 h (Fig. 4), which tightly correlates with the onset of apoptosis after 6 h of IL-21 treatment as seen by annexin V staining (Fig. 3, A and B). Therefore, we hypothesized that the short pretreatment of B cells with anti-CD40 Ab may induce the expression of Bcl-x<sub>L</sub> to levels that can compensate for the IL-21-mediated down-regulation of Bcl-x<sub>L</sub>, resulting in protection of B cells from apoptosis. We next examined the protein expression levels of Bcl-x<sub>L</sub> in B cells pretreated with anti-CD40 Ab for 6 h and subsequently cultured with IL-21 for 12 h, where cells are protected from apoptosis, relative to B cells that are treated with anti-CD40 Ab and IL-21 simultaneously for 12 h, where cells are susceptible to IL-21-induced apoptosis. We found that, while IL-21 induces the down-regulation of Bcl-x<sub>L</sub> protein in cells simultaneously treated with anti-CD40 Ab and IL-21, Bcl-x<sub>L</sub> protein levels were maintained in B cells that were treated with anti-CD40 Ab for 6 h before IL-21 stimulation (Fig. 7, B and C). However, IL-21 was still able to induce the down-regulation of Bcl-2 mRNA levels after 6 h of anti-CD40 Ab pretreatment (data not shown), suggesting that Bcl-x<sub>L</sub> is the more critical prosurvival factor that is regulated by IL-21.

**Discussion**

In this study, we examined the effect of IL-21 on primary murine B cell viability. We demonstrated that IL-21 induces apoptosis in both resting and activated primary B cells. We also show that IL-21 stimulation of B cells leads to a rapid decrease of Bcl-2 and Bcl-x<sub>L</sub> mRNA and protein levels, suggesting that the down-regulation of these antiapoptotic proteins is the probable mechanism that promotes IL-21-mediated cell death. Furthermore, we found that the overexpression of Bcl-2 or Bcl-x<sub>L</sub> was sufficient to protect primary B cells from IL-21-induced apoptosis.

We also examined the effects of various costimuli on IL-21-mediated apoptosis of B cells and found that, whereas anti-IgM Ab treatment of B cells induces apoptosis, the addition of IL-21 to anti-IgM Ab-stimulated cultures did not result in an increase in the percentage of apoptotic cells. Moreover, prosurvival costimulatory factors such as IL-4, LPS, and anti-CD40 Ab could not prevent IL-21-mediated apoptosis. Only when B cells were pretreated with anti-CD40 Ab for 6 h before IL-21 stimulation, could CD40 activation prevent apoptosis. Interestingly, B cells subjected to longer pretreatments (24 h) with anti-CD40 Ab were no longer protected from IL-21-induced apoptosis. Perhaps the disparity in protection offered by anti-CD40 Ab stimulation is due to the induction of a prosurvival factor that is transiently increased only in the initial hours of anti-CD40 Ab activation. Alternatively, this prosurvival factor could be repressed or lack stability in late anti-CD40 Ab-activated B cells.

Apoptosis plays a critical role in B cell development and homeostasis and is tightly regulated by many cytokines. IL-4 is a potent antiapoptotic cytokine for B cells that can prevent death induced by Ig cross-linking, glucocorticoids, Fas ligation, and the withdrawal of growth factors from culture (13–16). It has been shown that the antiapoptotic activity of IL-4 in B cells is in part mediated by the induced expression of Bcl-x<sub>L</sub>, a member of the Bcl-2 family (6, 17).
Although IL-4 is a cytokine that promotes B cell viability, there are also cytokines, such as TNF-α, TGFβ, and IFN-γ, that promote B cell death. TNF-α induces cell death via the activation of caspase-8 (18, 19). The overexpression of Bcl-2, Bcl-xL, and Bfl-1 has been shown to inhibit TNF-α-mediated apoptosis (20–24). TGFβ is another cytokine that has been shown to promote apoptosis in resting human B cells, in the murine lymphoma WEHI 231, and in the human B cell lymphoma Ramos (25–27). TGFβ-mediated apoptosis of Ramos cells has been associated with the down-regulation of Bcl-xL expression (28). In addition, studies have shown that IFN-γ also induces the apoptosis of the immature B cell line WEHI 279 and normal murine pre-B cells via the down-regulation of Bcl-2 and Bcl-xL (29). Although the receptors and ensuing signaling cascades for these cytokines are very different, the modulation of Bcl-2 family members appears to be an important common mechanism in determining cell survival.

Like these cytokines, we have demonstrated that IL-21 also promotes the apoptosis of B cells by down-regulating the antiapoptotic molecules Bcl-2 and Bcl-xL. Most interestingly, IL-21 is structurally homologous to IL-4, IL-2, and IL-15, which all belong to the class I receptor cytokine family and utilize the γc to transduce signals. The ability of IL-21 to directly induce apoptosis is a highly unusual and novel property of a cytokine that signals through the γc, considering that this class of cytokines is usually known to induce proliferation and mediate prosurvival effects.

During an immune response, there is a balance between signaling pathways that support cell survival and proliferation and those that culminate in apoptosis to maintain homeostasis of the immune system and prevent the development of autoimmunity. IFN-γ is an important mediator of the Th1 response, which negatively regulates B cell differentiation and proliferation, whereas IL-4, a Th2 cytokine, has been shown to promote B cell proliferation and confer resistance to apoptosis. Interestingly, we have recently demonstrated that IL-21 is preferentially produced by Th2 cells (30). The IL-21-mediated apoptosis of B cells may represent a novel mechanism of cell death regulated by the Th2 response. It is possible that IL-21 is necessary to control any excessive B cell proliferation and Ab production promoted during a Th2 immune response to maintain homeostasis. In support of this notion, the initial characterization of IL-21R−/− mice revealed 3-fold higher levels of serum IgE as compared with those of wild-type mice (4). These data suggest that IL-21 does play a role in regulating B cell responses in vivo, possibly by regulating the survival of Ab-producing B cells.

Our studies show that IL-21 can induce death in normal primary B cells. However, a recent study demonstrated that human IL-21 induced the proliferation and inhibited apoptosis of three of the six human myeloma cell lines tested (5). One possibility is that murine IL-21 has divergent biological effects from the human cytokine. Another explanation for contrasting results is the difference in differentiation status between the myeloma cells, a plasma cell stage, and the primary mature B cells studied here. A last possibility is that the pathophysiological nature of these myeloma cells renders them insensitive to the apoptotic signaling induced by IL-21, possibly through aberrant receptor signaling or the constitutive up-regulation of antiapoptotic factors. TNF-α, another apoptosis-inducing cytokine, also stimulates the proliferation of the same human myeloma cell lines used in this study (5). Interestingly, prior experiments have demonstrated that IL-21 costimulates human B cell proliferation under specific conditions (2). However, our studies show that IL-21 does not costimulate primary murine B cell proliferation (Fig. 1). Taken together, it is possible that IL-21 stimulation could potentially transduce both a proliferative and apoptotic signal, and depending on the status of the cell, there may be variable results in cell viability. It will be interesting to determine whether murine IL-21 induces or inhibits the apoptosis of murine myeloma cell lines.

In conclusion, we have demonstrated that IL-21 induces apoptosis in both resting and activated primary murine B cells, which is correlated with the reduced expression of Bcl-xL and Bcl-2. The IL-21-mediated apoptosis of B cells can be prevented by the reconstitution of Bcl-xL or Bcl-2 expression by either overexpressing these proteins or inducing their expression and accumulation by preactivating the B cells appropriately before IL-21 stimulation. Taken together, these data demonstrate that the apoptotic effect induced by IL-21 is mediated by the down-regulation of the antiapoptotic proteins Bcl-xL and Bcl-2, revealing a novel apoptotic pathway, transduced by the IL-21R, for B lymphocytes. Dysregulation of this pathway could lead to a lack of B cell homeostasis and have implications in diseases such as multiple myeloma.

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