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IL-4 Promotes Stat6-Dependent Survival of Autoreactive B Cells In Vivo Without Inducing Autoantibody Production

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Persistent cross-linking of hen egg lysozyme (HEL)-specific B cell membrane Ig (mIg) in double transgenic mice that express soluble HEL as a self Ag (HEL-Ig mice) decreases B cell mIgM expression, responsiveness, and life span. Because in vitro treatment with IL-4 inhibits T cell apoptosis through a Stat6-independent mechanism, increases mIg expression, and suppresses activation-induced B cell death, we studied IL-4 effects on B cell mIg expression, survival, and Ab secretion in Stat6-sufficient and deficient HEL-Ig mice. IL-4 treatment nearly normalized B cell number and greatly increased the percentage of mature B cells in HEL-Ig mice, but failed to normalize mIgM expression or spontaneous LPS-induced IgM secretion. IL-4 effects on B cell survival and maturation were CD4+ T cell independent, but Stat6 dependent, and did not involve receptor editing. IL-4 had to be present while B cells were generated to have a detectable effect on autoreactive B cell survival; however, the survival of B cells generated in the presence of IL-4 was substantially increased even after IL-4 was withdrawn. These observations suggest that: 1) activation-induced B cell death and anergy are independent processes; 2) B cells that survive to maturity develop increased resistance to Ag-induced deletion; and 3) IL-4 promotes B and T cell survival through different mechanisms. 


B cell tolerance is induced by exposure to Ag in the absence of additional stimuli protects against the development of humoral autoimmunity (1–3). The characteristics of Ag-induced tolerance depend on the extent of B cell membrane Ig (mIg) cross-linking by Ag. Exposure to polyvalent, particulate self Ag strongly cross-links mIg and deletes newly generated Ag-specific B cells before they leave the bone marrow (3–7), while less intense cross-linking of mIg by a soluble oligovalent Ag allows Ag-specific B cells to mature to the point in which they can migrate from bone marrow to the spleen (1, 8). Most Ag-specific B cells exposed to oligovalent Ag, however, become hyporesponsive (anergic); they secrete less Ig than naive B cells in response to in vitro stimulation with LPS or Th cells, and proliferate poorly in response to anti-Ig Ab stimulation (9). These hyporesponsive B cells lose all or most of their mIgM (8, 10–12) and are deleted within a few days after migrating to the spleen (12–15), before they acquire the mature B cell phenotype. The small percentage of Ag-specific B cells that survives to maturity in the presence of oligovalent Ag predominantly does so by further decreasing its responsiveness to Ag, either by undergoing receptor editing (8) or by down-regulating its expression of mIgD as well as mIgM (12).

Ag-induced B cell anergy and deletion are prevented if Ag-exposed B cells are simultaneously stimulated by T cells (16, 17). The help provided by T cells that prevents B cell anergy and deletion includes humoral factors (cytokines) as well as membrane-associated costimulatory molecules, such as CD40 ligand (18). Because one T cell-produced cytokine, IL-4, enhances B cell mIg expression, inhibits B and T cell apoptosis in vitro, and stimulates humoral autoimmunity if overexpressed in vivo (19–23), it seemed possible that in vivo treatment with IL-4 could prevent Ag-induced B cell tolerance. No prevention of B cell deletion was observed, however, in one study in which B cells in IL-4-overexpressing mice were exposed to a particulate polyvalent self Ag (23). This negative result did not rule out the possibility that IL-4 might inhibit the more subtle form of tolerance that develops when B cells are exposed to an oligovalent, soluble self Ag. To study this issue, we have treated double transgenic (Tgn) mice that express soluble hen egg lysozyme (HEL) and whose B cells express mIgM and mIgD that bind HEL with high affinity (HEL-Ig mice) with a long-acting form of IL-4 (24). Results of these studies demonstrate that IL-4 treatment prevents premature B cell death and allows B cells to acquire a mature phenotype through a Stat6-dependent mechanism, but does not prevent the loss of mIgM or decreased responsiveness to LPS.

Materials and Methods

Animals

Female C57BL/6 mice, obtained from the Small Animals Division of the National Cancer Institute, National Institutes of Health (Bethesda, MD), were bred in the Cincinnati Veterans Affairs Medical Center animal facility to male C57BL/6 mice that were hemizygous for both the MD4 anti-HEL Ig H and L transgene and the ML5 soluble HEL transgene (a gift of C. Goodnow, Australian National University, Canberra, Australia) to generate mice that carry only the MD4 transgene (Ig Tgn mice), and mice that carry both the MD4 and ML5 transgenes (HEL-Ig mice). C57BL/6-Stat6-deficient and BALB/c-Stat6-deficient mice were originally obtained from M. Grubbs (Boston, MA). C57BL/6-Stat6-deficient mice were crossed to C57BL/6-HEL-Ig Tgn mice, and their offspring were backcrossed to C57BL/6-Stat6-deficient mice to generate C57BL/6-Stat6-deficient, Ig Tgn mice and Stat6-deficient, HEL-Ig mice. BALB/c Stat6-deficient mice were bred at the Cincinnati Veterans Affairs Medical Center animal facility, and
BALB/c wild-type mice were obtained from the Small Animals Division of the National Cancer Institute, National Institutes of Health. Mice were used at 8–34 wk of age. Mice were age and sex matched in individual experiments.

**Typing of Tgn mice**

Mice that expressed the HEL transgene and/or the anti-HEL transgene were identified by PCR (25). DNA was isolated with QIAamp tissue kits for DNA isolation (Qiagen, Santa Clara, CA). PCR was performed as described (12). Stat6 deficiency was determined by PCR. The following three oligonucleotides were used in Stat6 PCR: Stat6 upper, 5′-GAAGGGGGGACCACCGGG-3′; Stat6 lower, 5′-GTGACGAGGGACACACACGGC-3′; and Neo, 5′-GCACCCCTGAATTTGCTGAAGAGG-3′. PCR amplification of cells from Stat6-deficient mice yields a 225-bp product; PCR amplification of cells from wild-type mice yields a 100-bp product. Oligonucleotide primers were produced by the BIC synthesis center at the Uniformed Services University of the Health Science (Bethesda, MD).

**Experimental conditions**

All mice, except for BALB/c wild-type and Stat6-deficient mice, were maintained on drinking water that contained 25 mM ZnCl2 for at least 3 days before the initiation of other treatments and for the duration of each experiment to maximize serum HEL levels in HEL-Ig mice (15). In experiments in which newly generated B cells were identified by 5-bromo-2′-deoxyuridine (BrDU) incorporation (7, 13), 0.8 mg/ml BrDU (Sigma-Aldrich, St. Louis, MO) was also added to drinking water for a defined period of time. BrDU-containing water also contained 2 ng/ml glucose (except for the experiment in Fig. 7D) and was shielded from light and changed every third day.

**Abs and immunological reagents**

The following hybridomas were obtained and grown as ascites in either allotype-specific mice, were obtained from ascites by (NH4)2SO4 precipitation. Oligonucleotide primers were produced by the BIC synthesis center at the Uniformed Services University of the Health Science (Bethesda, MD).

**Results**

To determine whether treatment with a long-acting formulation of IL-4 would increase the low splenic B cell number in HEL-Ig mice, Ig Tgn and HEL-Ig mice were left untreated or were treated with IL-4C (0.5 μg IL-4 + 3 μg anti-IL-4 mAb) three times per week for 5 or 14 days. IL-4C treatment had little effect on splenic B cell number in Ig Tgn mice, but caused a doubling of splenic B cell number in HEL-Ig mice in 5 days (Fig. 1A) and fully corrected splenic B cell number in HEL-Ig mice after 14 days (Fig. 1B). This effect of IL-4 was CD4+ T cell independent, as it was not blocked

**Preparation of cytokine/anti-cytokine Ab complexes**

IL-4 (200–1000 μg/ml) was mixed at a 2:1 molar ratio (1:6 weight ratio) with neutralizing anti-IL-4 mAb (BVD4-1D11.2) to prepare IL-4/anti-IL-4 mAb complexes (IL-4C), which greatly increase the in vivo t½ and activity of IL-4 (24). After 2 min at room temperature, complexes were diluted with 1% C57BL/6 serum or 1% BALB/c serum to a concentration of 2.5 or 10 μg IL-4/ml, for injection into mice. Complexes were always freshly prepared before use.

**Immunofluorescence staining**

Spleen cells were depleted of erythrocytes, filtered through nylon gauge, and suspended at 20 × 10^6 cells/ml in HBSS with 10% newborn bovine serum and 0.2% NaN3 (HNA). A total of 100 μl cell suspension was stained for 30 min on ice with 1 μg each of appropriately labeled Abs. Cells were washed twice with HNA, then, if appropriate, exposed to streptavidin-R-PE (S-PE, purchased from BD Immunocytochemistry Systems) for 30 min on ice. All staining was performed in the presence of 10 μg/ml unlabeled anti-FcγRIII mAb (24G2). After washing once with HNA, all samples were prepared for cell staining by 5-bromo-2′-deoxyuridine (BrdU) incorporation, were washed once with HBSS/0.2% sodium azide, then fixed in PBS/2% paraformaldehyde. Staining for BrdU was performed as previously described (12). All samples were analyzed with either a FACSscan or a FACSCalibur Analyzer equipped with a red diode laser (BD Biosciences, Mountain View, CA). Data were analyzed with Lysis II or CellQuest software. Light scatter gates were set to exclude most nonlymphoid cells and cells that had died before fixation. Cells that had been stained with a single fluorochrome-labeled Ab were used to determine compensation for overlap between emission spectra. Percentages of specifically stained cells and the mean and/or median fluorescence intensities of specifically stained cells were determined.

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Defects in these B cells that result from chronic cross-linking of B cell maturity in HEL-Ig mice suggested that it might also correct other defects observed in most experiments (see, for example, Figs. 5 and 7).

The ability of IL-4 to increase splenic B cell number and level of B cell mIgM (but not mIgD) that results primarily from a block in the terminal glycosylation and membrane insertion of \( \mu \)-chain (11). This was not the case: although IL-4C enhanced both mIgM and mIgD expression by Ig Tgn B cells and enhanced mIgD expression by HEL-Ig B cells, it failed to increase the very low expression level of HEL-Ig B cell mIgM (Fig. 3).

**IL-4 does not affect serum HEL levels in HEL-Ig mice**

IL-4 enhancement of B cell survival and mIgD expression in HEL-Ig mice might reflect either increased B cell resistance to mlg cross-linking-induced cell death or suppression of HEL synthesis, which would decrease B cell mlg cross-linking. To differentiate between these possibilities, we measured serum HEL levels in untreated and IL-4C-treated HEL-Ig mice (Fig. 4). IL-4C treatment had no detectable effect on serum HEL levels ( \( p = 0.52 \) ), indicating that decreased Ag-induced mlg cross-linking is not responsible for the effects of IL-4 on B cell survival.

**IL-4 selectively increases the number of mature B cells in HEL-Ig mice through a Stat6-dependent mechanism**

Our observation that IL-4 increases B cell survival in HEL-Ig mice suggested that this cytokine selectively increases mature splenic B cell number in these mice. Furthermore, observations that IL-4 cell mlg, including the selective decrease in expression of B cell mlgM (but not mlgD) that results primarily from a block in the terminal glycosylation and membrane insertion of \( \mu \)-chain (11). This was not the case: although IL-4C enhanced both mIgM and mIgD expression by Ig Tgn B cells and enhanced mIgD expression by HEL-Ig B cells, it failed to increase the very low expression level of HEL-Ig B cell mIgM (Fig. 3).

**IL-4 fails to increase B cell mIgM expression in HEL-Ig mice**

The ability of IL-4 to increase splenic B cell number and level of maturity in HEL-Ig mice suggested that it might also correct other defects in these B cells that result from chronic cross-linking of B cell mIg, including the selective decrease in expression of B cell mIgM (but not mIgD) that results primarily from a block in the terminal glycosylation and membrane insertion of \( \mu \)-chain (11). This was not the case: although IL-4C enhanced both mIgM and mIgD expression by Ig Tgn B cells and enhanced mIgD expression by HEL-Ig B cells, it failed to increase the very low expression level of HEL-Ig B cell mIgM (Fig. 3).
increases splenic T cell survival through a Stat6-independent mechanism (21) and enhances anti-Ig Ab-induced B cell proliferation in the absence of Stat6 (31) suggested that the effects of IL-4 on splenic B cell survival and maturation would also be Stat6 independent. To test these hypotheses, we bred HEL-Ig mice and Ig Tgn mice with Stat6-deficient mice (all on a C57BL/6 background) and compared the responses of Stat6-deficient and Stat6-sufficient HEL-Ig and Ig Tgn mice to IL-4. Results of these studies confirmed the first hypothesis, but refuted the second. Although splenic B cells that have an immature (B220<sup>dull</sup>HSA<sup>bright</sup>) phenotype are normal in number in Stat6-sufficient HEL-Ig Tgn mice and do not appreciably increase in number following IL-4C treatment (Fig. 5, top panel), IL-4C causes a large increase in the low number of mature splenic B cells in these mice (Fig. 5, middle panel). Contrary to our expectations, both the IL-4-induced increase in the number of mature HEL-Ig splenic B cells and the IL-4-induced decrease in their mean splenic B cell HSA expression were completely Stat6 dependent (Fig. 5, middle and bottom panels).

**IL-4 inhibits in vivo deletion of mature B cells by anti-IgD mAb**

To determine whether the B cell-sparing effect of IL-4 could be observed with normal, as well as Ig Tgn B cells that were activated by mlg cross-linking, we studied a system in which treatment of BALB/c mice with an anti-IgD mAb causes deletion of mature B cells (32). Our initial experiment (Fig. 6, left panels), which used our standard dose of IL-4C, showed that anti-IgD mAb had its expected effect on mature splenic B cell number in wild-type BALB/c mice. However, IL-4C treatment did not increase mature splenic B cell number significantly more in anti-IgD Ab-treated mice than in mice that did not receive anti-IgD Ab (p = 0.091). Because anti-IgD mAb cross-links mIg on normal B cells to a much greater extent than HEL cross-links mlg on HEL-Ig B cells (18), we reasoned that more IL-4 signaling might be required to rescue anti-IgD mAb-ligated wild-type B cells than to rescue HEL-ligated B cells in HEL-Ig mice. For this reason, we increased the quantity of IL-4C used to treat anti-IgD mAb-injected mice 4-fold. Although this treatment still did not fully negate anti-IgD mAb depletion of mature splenic B cells (Fig. 6, right panels), depletion was significantly inhibited (p = 0.029). Furthermore, this higher dose of IL-4C increased the number of mature splenic B cells in anti-IgD mAb-treated mice more than in otherwise untreated mice (p = 0.001). Thus, there appears to be a relationship between the intensity of mlg cross-linking and the concentration of IL-4C that is required to inhibit mlg cross-linking-induced B cell deletion. Studies with anti-IgD mAb also provided confirmation that IL-4 prevents mlg cross-linking-induced B cell deletion through a Stat6-dependent mechanism (Fig. 6).

**IL-4 selectively prolongs the survival of Ag-activated B cells generated in its presence**

Six experiments were performed to determine the kinetics of the relationship between IL-4C treatment and increased survival of

**FIGURE 4.** IL-4 treatment has little effect on serum HEL levels in HEL-Ig mice. Serum HEL levels were determined by ELISA for Ig Tgn and HEL-Ig mice (3–5/group) that were left untreated or were injected i.p. three times per week with IL-4C that contained 0.5 μg mouse IL-4 for 16 days before bleeding. Differences in serum HEL levels in untreated and IL-4C-treated HEL-Ig Tgn mice were not significant (p = 0.52).

**FIGURE 5.** Stat6 is required for IL-4-induced normalization of mature splenic B cell number in HEL-Ig Tgn mice. Wild-type and Stat6-deficient Ig Tgn and HEL-Ig mice (10–12/group) were left untreated or were injected i.p. three times per week for 14 days with IL-4C that contained 0.5 μg mouse IL-4. Mice were sacrificed 14 days after initiation of IL-4C treatment. Spleen cells from individual mice were counted and stained with anti-B220 (either Cy5 or PerCP labeled), FITC-anti-HSA, and PE-anti-CD19 mAbs and analyzed by flow cytometry to determine the percentage of B220<sup>−</sup>CD19<sup>+</sup> cells that were B220<sup>dull</sup>HSA<sup>dull</sup> (mature) or B220<sup>dull</sup>HSA<sup>bright</sup> (immature). Numbers of mature and immature splenic B cells were determined as in Fig. 1. HSA expression (MFI) was determined for B220<sup>−</sup>CD19<sup>+</sup> splenic B cells.

**FIGURE 6.** Prevention of in vivo deletion of B cells in anti-IgD mAb-treated mice requires an increased amount of IL-4 and is Stat6 dependent. Wild-type and Stat6-deficient BALB/c mice (4/group) were treated with anti-CD4 mAb (1 mg i.v./wk) and anti-IL-7 mAb (3 mg i.p. three times per week), starting 1 wk before other treatments. All mice received anti-FcRII mAb (0.5 mg i.p.) on the same day that additional treatments were begun. Mice received no additional treatment (untreated), 200 μg HB67 (anti-δ mAb) i.v., IL-4C that contained 0.5 or 2 μg IL-4 i.p. every other day, or both HB67 and IL-4C. All mice were sacrificed 5 days after the initiation of either HB67 or IL-4C treatment. Spleen cells from individual mice were counted and stained with PerCP-anti-B220, FITC-anti-HSA, and PE-anti-CD19 mAbs and analyzed by flow cytometry to determine the percentage of B220<sup>−</sup>CD19<sup>+</sup> cells that were B220<sup>dull</sup>HSA<sup>dull</sup> (mature) or B220<sup>dull</sup>HSA<sup>bright</sup> (immature). Numbers of mature and immature splenic B cells were determined as in Fig. 1. Treatment with IL-4C that contained 2.0 μg IL-4 increased mature B cell number in anti-δ-treated wild-type mice significantly more than in mice that did not receive anti-δ (p = 0.001); no significant difference was seen with IL-4C that contained 0.5 μg IL-4 (p = 0.091). IL-4C that contained 2 μg IL-4 significantly blocked anti-δ depletion of mature splenic B cells (p = 0.029).
Ag-activated B cells. These studies used BrdU labeling to identify cells that had divided while BrdU was being administered (predominantly newly generated B cells) (33) and anti-IL-7 mAb to inhibit B lymphopoiesis in the bone marrow (28). An initial study, in which Ig Tgn and HEL-Ig mice were treated with BrdU ± IL-4C ± anti-IL-7 mAb for the 14 days before sacrifice (Fig. 7A), demonstrated that IL-4C treatment selectively increased the number of mature HEL-Ig splenic B cells that were generated while IL-4C was being administered (BrdU⁺ B cells). Treatment with anti-IL-7 mAb blocked this effect completely, presumably by blocking B lymphopoiesis. In contrast, IL-4C treatment, initiated 1–2 wk after HEL-Ig B cells were generated, had no significant effect (p = 0.314) on splenic B cell survival (Fig. 7B; note log scale on abscissa). When IL-4C was administered to HEL-Ig mice for 16 days, mature splenic B cells generated during the first 7 days or the last 9 days of this period were both considerably increased in number (Fig. 7C). The same was true for mature B cells generated during the first 3 days of a 14-day period of IL-4C treatment (Fig. 7D; note log scale on abscissa). Increased survival of HEL-Ig Tgn B cells generated in the presence of IL-4C was more directly demonstrated by an additional experiment (Fig. 7E) that measured the percentages of splenic B cells that were BrdU⁺ and 11 days after a 3-day BrdU pulse in mice that did or did not receive IL-4C for the entire experiment. Although the percentage of splenic B cells that were BrdU⁺ was higher in untreated than in IL-4C-treated mice 4 days after the pulse (34 vs 25%, respectively), the percentage of BrdU-labeled splenic B cells declined 11 days after the pulse to 3% in untreated mice vs 6.5% in IL-4C-treated mice. Thus, survival of BrdU-labeled B cells over this time period increased ~3-fold as a result of IL-4C treatment. Similar results were obtained when calculations were based on absolute numbers of splenic BrdU⁺ B cells, rather than percentages of splenic B cells that were BrdU⁺ (data not shown).

To determine whether the continuing survival of Ag-stimulated B cells generated during a period of IL-4C treatment depends upon continuing IL-4C stimulation, Ig Tgn and HEL-Ig mice were treated with BrdU and IL-4C for 5 days and sacrificed 11 days after termination of BrdU treatment (Fig. 7F, note log scale on abscissa). Even though this short period of IL-4C treatment had no effect on total splenic B cell number in either mouse strain, it significantly increased the number of mature, BrdU⁺ splenic B cells in HEL-Ig mice (p = 0.039); this increase (2.1-fold) was not as large (3.6-fold) as that observed when IL-4C treatment was continued until the time of sacrifice (Fig. 7, compare D and F); however, the magnitudes of these increases were not significantly different (p = 0.21). Thus, if newly generated, Ag-stimulated B cells are induced by IL-4 to survive and mature, they develop increased ability to survive continuing Ag activation. This ability to survive may be increased further, however, if Ag-activated B cells continue to be stimulated by IL-4 after they have matured.

Increased survival of IL-4-stimulated, Ag-activated B cells is not a result of increased receptor editing

Receptor editing (replacement of an Ig L chain that allows for autoreactivity with one that does not) has been demonstrated in mice that have autoreactive, Tgn mlg (34, 35). Receptor-edited B cells can escape deletion by self Ag, because their mlg no longer reacts (or reacts less avidly) with self Ag. To determine whether IL-4 enhances B cell survival by increasing receptor editing, we evaluated the ratio of HEL-binding capacity to mlg expression in mature splenic B cells from HEL-Ig mice. Previous studies have established that non-receptor-edited B cells in these mice demonstrate a linear relationship between HEL binding and mlg expression, while receptor-edited B cells express a lower ratio of HEL binding to mlg expression (12). Using this technique, we found that IL-4C treatment selectively increases the number of mature, non-receptor-edited splenic B cells as well as the percentage of mature splenic B cells that is not receptor edited in HEL-Ig mice, and that the IL-4-induced increases in the non-receptor-edited B cell population is selectively suppressed by anti-IL-7 mAb treatment (Fig. 7). Thus, IL-4C treatment does not increase HEL-Ig B cell survival by increasing receptor editing.

IL-4 does not increase spontaneous IgM secretion or the IgM secretory response of HEL-Ig splenic B cells to LPS

The ability of IL-4 treatment to enhance maturation and survival of autoreactive B cells suggested that this cytokine might also prevent autoantigen-induced B cell unresponsiveness. Three similar experiments addressed this issue by determining whether IL-4C treatment would increase spontaneous IgM secretion or the B cell secretory response to LPS by cultured spleen cells from HEL-Ig mice. IL-4C treatment failed to increase the negligible levels of IgM anti-HEL Ab in HEL-Ig serum or in 1- or 3-day culture supernatants of unstimulated HEL-Ig spleen cells (data not shown) and failed to increase LPS-induced IgM secretion by cultured HEL-Ig splenic B cells in two of three experiments (Fig. 9). In contrast, Ig Tgn B cells consistently responded to LPS, although IL-4 treatment decreased IgM secretion by LPS-stimulated Ig Tgn B cells through a Stat6-dependent mechanism (Fig. 9).

Discussion

Our studies support five conclusions: 1) IL-4 inhibits the early death and promotes the maturation of Ag-specific B cells that are chronically exposed to a soluble, oligovalent Ag; 2) these effects are Stat6 dependent; 3) enhanced survival is achieved primarily by allowing immature B cells to mature to a developmental stage that has increased resistance to Ag-induced cell death; however, IL-4 also enhances the survival of B cell receptor-activated mature B cells; 4) higher concentrations of IL-4 are required to prevent in vivo deletion of B cells by stronger mlg cross-linking; and 5) IL-4 enhancement of B cell survival and maturation is not accompanied by normalization of mlg expression, spontaneous Ig secretion, or responsiveness to LPS. These observations will be discussed in turn.

IL-4 promotion of autoreactive B cell survival and maturation

Previous studies that demonstrated humoral autoimmunity in Tgn mice that overproduce IL-4 (22, 23) and a decrease in autoantibody production in IL-4-deficient MRL/Mp-lpr/lpr mice (36) hypothesized that IL-4 might contribute to autoimmunity by inhibiting Ag-induced deletion of autoreactive B cells. The only published study that examined IL-4 effects on the deletion of autoreactive B cells (23), however, concluded that even a high level of autologously produced IL-4 did not prevent such deletion. One important difference between that study and ours is the nature of the autoantigen, which was particulate and multivalent in the previous study, but soluble and oligovalent in ours. Comparison of the results in these two studies suggests that the ability of IL-4 to prevent Ag-induced B cell deletion decreases as the intensity of Ag-induced mlg cross-linking increases. Thus, IL-4 might be expected to inhibit the deletion of B cells that are specific for soluble, oligovalent self Ags that are present at relatively low concentration, but not to prevent the deletion of B cells specific for polyvalent cell membrane Ags. This expectation is consistent with the relatively mild autoimmunity that develops in IL-4 Tgn mice (22, 23) and the relatively modest effect that deleting the IL-4 gene has on lpr-associated autoimmunity (36).
FIGURE 7. IL-4 increases the survival of HEL-Ig B cells in HEL-Ig Tgn mice that are generated in its presence. Six separate experiments were performed in which Ig Tgn and HEL-Ig mice (3–5/group) were left untreated, were injected three times per week i.p. with IL-4C that contained 0.5 μg mouse IL-4, or received a combination of both IL-4C and anti-IL-7 mAb (3 mg, three times per week i.p.). Mice were administered drinking water that contained BrdU for the indicated periods. Mice were sacrificed in each experiment at the end of the treatment period or 12 days after the last IL-4C injection (F). Spleen cell suspensions were prepared, counted, stained with biotin-anti-HSA and Cy5-anti-B220 mAbs, followed by S-PE, then fixed, permeabilized, and stained with FITC-anti-BrdU mAb. Percentages of BrdU^+ and BrdU^- HSA^{bright} (immature) and HSA^{dull} (mature) B220^+ cells were determined by flow cytometry and multiplied by total spleen cell numbers to calculate numbers of cells of each phenotype. Information on immature and mature splenic B cell numbers is shown, except for B and E, which depict total splenic B cells.
Stat6 dependence of IL-4 effects on autoreactive B cell survival and maturation

Although in vitro effects of IL-4 on T cell survival have been Stat6 independent (21), our studies demonstrate that IL-4 rescue of Ag-activated B cells is Stat6 dependent. Thus, although IL-4 promotes the survival of both Ag-stimulated B and T cells, our observations suggest that this is accomplished through different molecular mechanisms. Consistent with this, we have observed that splenic T cell numbers are increased to a much greater extent than splenic B cell numbers in IL-4 Tgn Stat6-deficient mice (S. C. Morris and F. D. Finkelman, unpublished observations).

Relationship between B cell survival and maturation

Several studies have suggested that exposure to Ag deletes newly generated B cells more readily than mature B cells (37–40); however, previous studies with Ig Tgn and HEL-Ig mice and with mice injected with anti-IgD mAb have demonstrated that mlg cross-linking in the absence of T cell help also deletes mature B cells (12, 32, 41). Our studies with IL-4 suggest that the difference between the behavior of immature and mature B cells is quantitative rather than qualitative. Treatment of HEL-Ig mice with IL-4 for a few days doubled the number, and presumably, increased the survival, of B cells generated while IL-4 was being administered.
measured by IgM secretion. This latter observation is more difficult to interpret than the former, because IL-4 has an inhibitory effect on LPS-induced IgM secretion by B cells from Ig Tgn mice and because a modest stimulatory effect of IL-4 on LPS induction of IgM secretion by HEL-Ig B cells was observed in one of three experiments. It is impossible to rule out the possibility that IL-4 might enhance differentiation to IgG1 secretion by wild-type B cells that have been exposed to self Ag, because, while IL-4 stimulates normal, LPS-activated B cells to switch to IgG1 secretion (42), the structure of the Ig transgene prevents such switching. Similarly, it is possible that IL-4 may increase the ability of self Ag-exposed B cells to respond to other stimuli that promote Ig secretion, such as type 2 T-independent Ags or T cell-associated CD40 ligand. Regardless of these issues, the greater ability of IL-4 to prevent the deletion of self Ag-exposed B cells than to promote their differentiation to Ab-secreting cells suggests that deletion and anergy are different processes and may account for the limited nature of the autoimmunity that develops in IL-4 Tgn mice.

Although our results indicate that IL-4 can prevent the deletion of autoreactive B cells, we cannot be certain of the physiological importance of this effect because we do not know how levels of IL-4 in IL-4C-treated mice or IL-4 Tgn mice compare with those generated in the immediate vicinity of a B cell that may be exposed to self Ag. Although the decreased autoreactivity that is observed in IL-4-deficient MRL/Mp-jpr/jpr mice (36) suggests that IL-4 has a physiological role in autoimmunity, further studies in autoimmune disease models are required to test the possibility that the deletion-inhibiting effect of IL-4 is of general importance in autoimmune disease and, if so, to determine the relative importance in autoimmunity of the Stat6-dependent effects of IL-4 on B cells and the Stat6-independent effects of IL-4 on T cells.

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References


RESOLVE OF Ag-ACTIVATED B CELLS BY IL-4