Pregnancy-induced alterations of B cell maturation and survival are differentially affected by Fas and Bcl-2, independently of BcR expression

Stéphane M. Caucheteux¹, Marie-Claude Gendron² and Colette Kanellopoulos-Langevin¹

¹Laboratory of Immune Regulations and Development, Department of Developmental Biology and ²Flow Cytometry Unit, Institut J. Monod, UMR 7592 (CNRS and Universities Paris 6 and 7), 2 place Jussieu, 75251 Paris cedex 05, France

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Abstract

In the present work, we have analyzed the roles of two molecules involved in the regulation of cell survival, Bcl2 and Fas, in the pregnancy-induced down-regulation of B lymphopoiesis in mice. Our results show that the overexpression of the anti-apoptotic molecule Bcl2 in Bcl2-transgenic (Tg) B cells is able to protect ‘D’ fraction pre-B cells from pregnancy-induced deletion. In contrast, in Faslpr/lpr mice bearing a mutated cell death receptor Fas, such B cell targets are not protected. Moreover, bone marrow B cell sub-populations at both ends of the differentiation pathway, i.e. pre-pro ‘A’ and mature ‘E–F’ fraction B cells, which are not the major targets of the pregnancy-induced down-regulation, are doubled during pregnancy in Faslpr/lpr mice only. Altogether, these data strongly suggest that B cell down-regulation during pregnancy is due to apoptotic events blocked by Bcl2, but does not depend on a functional Fas receptor. The expression of a transgenic BcR in the 3-83 BcR-Tg mouse model yields similar observations, which indicates that early BcR expression does not alter bone marrow B cell fates during pregnancy.

Introduction

During pregnancy, the maternal immune system must perform the double task of protecting the mother against infections while not rejecting the fetus (1). While numerous mechanisms have been proposed to be working locally at the fetal–maternal interface [for reviews see (2–6)], others appear to act on the maternal immune system as a whole, and in particular at the level of T and B lymphopoiesis. It has been shown that the considerable increase in steroid hormone levels associated with pregnancy has a negative impact on the production of new clones of maternal T and B lymphocytes, through thymic involution (7,8) and down-regulation of B lymphopoiesis in the bone marrow (9), respectively. Work by P. Kincade's laboratory has shown that pregnancy estrogen levels cause a major decrease in the production of B cell progenitors (‘B’ to ‘D’ fraction B cells) (10). The same group has shown that hormonal levels maintained during lactation prolong this effect. Their results also indicate that a single injection of estrogens can selectively reduce B cell progenitors in normal and Ig-transgenic mice (11,12). However, pregnancy is a particular physiological situation accompanied by multiple changes in various cytokines and hormones, including estrogens, progesterone and prolactins. Thus, the mechanisms involved in the fine tuning of bone marrow B cell production and homeostasis, in the special context of pregnancy, remain to be elucidated. We have analyzed the influence of pregnancy on bone marrow B cell subpopulations from mice homozygous for the Faslpr/lpr mutation (13,14) or transgenic for the anti-apoptotic molecule Bcl2 (Bcl2-Tg) specifically expressed in B cells (15), and we have compared them to wild-type B10.D2 mice. Bcl-2 and Fas (16,17) are known to be expressed in bone marrow B cells during their normal maturation (18,19) and to play an important role in the regulation of B cell production and homeostasis (20–22). To examine the role of BcR expression, the same experiments have been performed in 3-83μδ BcR-transgenic mice (23) on the B10.D2 background of the three genotypes (wild type, Bcl2-Tg and Faslpr/lpr).

Our results show that during pregnancy, the homozygous Faslpr mutation does not prevent the decrease of B cell lymphopoiesis, while the overexpression of Tg Bcl2 restricted to B cells protects them from this down-regulation. We also present evidence that the Faslpr mutation causes the selective

Correspondence to: C. Kanellopoulos-Langevin; E-mail: kanellopoulos@ijm.jussieu.fr

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expansion in pregnant mice of pre-pro B cells as well as mature slgM+ B cell subpopulations. The same conclusions hold true in 3-83μ BcR-Tg B cells despite the earlier than normal expression of their BcR. Our results indicate that the pregnancy down-regulation of B lymphopoiesis involves apoptotic mechanisms inhibitable by Bcl2 but does not require functional Fas receptors. Interestingly, although both strains of mice present lupus-like autoimmunity, the Faslpr/lpr mutation only causes a pregnancy-specific expansion of bone marrow B cell subpopulations, not seen in Bcl2-Tg pregnant mice.

Methods

Mice
C57Bl/10.D2/nOlaHsd (B10.D2) congenic mice were purchased from Harlan (UK). 3-83μ (23), Faslpr/lpr and Bcl-2 (Eμ-bcl-2-22 line) (15) transgenic (Tg) females kindly provided by D. Nemazee were maintained on the B10.D2 background. These mice were used as single Tg 3-83μ, Bcl-2 and Faslpr/lpr, and as double Tg (DTg) (Bcl-2, 3-83μ) and (Faslpr/lpr, 3-83μ). All mice were housed in the animal facilities at the J. Monod Institute and cared for in accordance with the institutional guidelines for animal welfare. Virgin females were initially used between 6 to 10 weeks of age and mated with B10.D2 males. Vaginal plug observed the following morning was considered as day 0.5 of pregnancy. Non-pregnant control mice were pseudo-pregnant age-matched females mated on the same day as experimental mice. Control and pregnant mice were euthanized and analyzed between days 16.5 and 18.5 of gestation. Mice examined during the postpartum were allowed to nurse for 1 week.

Mice were screened by polymerase chain reaction (PCR) on tail DNA samples. PCR assays were performed in a final volume of 25 μl containing 100 ng of purified genomic DNA in PCR buffer, 250 nM of dNTPs, 500 nM of each oligonucleotide primer, and 1 U of Taq DNA polymerase (Applied Biosystem, Courtaboeuf, France). Oligonucleotide primers used to detect the 3-83μ transgene are 5′-CAGCTTCCTGCATATCGTGCC and 3′-TGTCCTCCCTCCGAACTGTG, 5′-CTTCCAGAGACAGCCAGGAGAAATC and 3′-TCATGTGTGTGGAGAGCGTCACC to amplify the human Bcl-2 transgene. PCR products of each transgene were generated using the following conditions: 94°C for 1 min, 60°C for 1.5 min, 72°C for 1 min for a total of 30 cycles.

Cell suspensions
Bone marrow (BM) cell suspensions were prepared by flushing the long bones (femurs and tibias) from both hind legs with HBSS, 4% heat-inactivated FCS, 0.1% NaN3. Erythrocytes were eliminated by osmotic shock in lysis buffer (10% 0.17 M Tris in 8.3 g/l NH4Cl). Aliquots of 10⁶ nucleated cells were incubated for each staining.

Flow cytometry analyses
BM cells were stained with the following antibodies: polyclonal goat anti-mouse IgM, monoclonal anti-HSA (30F1), monoclonal anti-493 (24), all labeled with FITC, Spectral Red labeled monoclonal anti-B220 (RA3-6B2) (Southern Biotechnology Associates, France), biotin-coupled anti-B220 (BD-Biosciences, France) and biotin-coupled anti-CD43 (S7) (25). S7 and 493 antibodies were purified and coupled in our laboratory. The cell surface marker 493 has been described by Rolink et al. (24) as the complement component C1qlike receptor. It is present on pro-, pre- and ‘immature’ B cells (slgMhigh, slgDneg or low, short-lived B cells which are about to leave the bone marrow or have just entered the spleen).

Biotin-coupled antibodies were revealed by phycoerythrin-labeled streptavidin (BD). For cytoplasmic μ staining, after surface staining with anti-B220 antibody, intracellular staining was performed by using the intracellular fixation and permeabilization (IC Fix/IC Perm) buffer kit from Biosource (Biosource, Cliniscience, France). BM cells were fixed with paraformaldehyde for 10 min, and subsequently permeabilized with saponin in FCS-buffered saline solution, as recommended by Biosource. Cells were analyzed after FITC-coupled anti-IgM antibody staining.

Cell suspensions were analyzed on an Epics Elite-ESP flow cytometer (Beckman-Coulter, France). Non-lymphoid cells, dead cells, debris and aggregates were excluded by gating on forward and side scatter parameters. Because of the differences in B cell numbers between non-pregnant and pregnant animals, we routinely acquired gated events until we had analyzed at least 10 000 B220+ cells from every bone marrow sample.

Statistical analyses
Comparisons of mean values between pregnant and non-pregnant animals within each one of the three groups was performed using the Student’s t-test. All analyses used Statview 5.0.1 software for Macintosh.

Results

Only B220+, slgM− B cells are diminished in pregnant Bcl2-Tg, or Faslpr/lpr mice

We have followed the fate of bone marrow B cells at various stages of their differentiation pathway (18,19) in pregnant wild-type B10.D2 female mice. As previously described by Medina et al. in Balb/c mice (9), we observed a down-regulation of B lymphopoiesis during pregnancy, and saw that immature bone marrow B cell subpopulations are selectively sensitive to the influence of gestation. As shown in Fig. 1(A) (left), in a representative experiment comparing pregnant (day 18.5 of gestation) versus non-pregnant B10.D2 mice, B220+, slgM− as well as B220low, slgM− immature B cells were diminished to 4.6% from 18.9% (P < 0.001) and down to 2.7% from 7% (P < 0.01), respectively, while more mature B220high, slgM+ cells were not significantly affected. We then compared how pregnancy affects B lymphopoiesis in two different mouse strains on B10.D2 background: one which overexpresses the anti-apoptotic molecule Bcl2 under the control of the Eμ enhancer in Tg B cells (15), and the other bearing the homozygous Faslpr/lpr mutation (14). It has been well established that both mechanisms play an important role in the physiological regulation of B cell production and survival, outside of the particular context of gestation (26,27). In both pregnant Bcl2-Tg and Faslpr/lpr mice (Fig. 1A, center and
right), B220+, slgM− B cells were still decreased, to 6.4% from 12.9% (P < 0.01) and to 6.6% from 24.3% (P < 0.01), respectively. However, in Bcl2-Tg mice, B220low,slgM+ B cells were not affected anymore, just like B220high,slgM+ mature cells. The comparison of percentages of the various B cell subpopulations from six to nine pregnant mice within each of the three different groups is presented in Fig. 5(A). The comparison of absolute bone marrow B cell numbers supports the same conclusions (data not shown).

To pursue our analysis further, we distinguished B220+ B cells on the basis of their content of intra-cytoplasmic μ chains (Cμ). We found that Fas<sup>+/−</sup> Cμ+ cells are affected by pregnancy as much as in B10.D2 control mice, while Bcl2-Tg Cμ+ cells were not significantly affected. In line with the results from Fig. 1(A), B220+, Cμ− B cells were significantly down-regulated by pregnancy in all three groups (Fig. 2).

During pregnancy, 493+ B cells are protected by the overexpression of Bcl2 while 493− cells are expanded in Fas<sup>+/−</sup> mice

We studied B cell populations on the basis of the cell surface marker 493, which has been described by Rolink et al. (24) as the complement component C1qlike receptor. It is present on pro-, pre- and ‘immature’ B cells (slgMhigh, slgDneg or low, short-lived B cells which are about to leave the bone marrow or have just entered the spleen). Mature B cells do not express the 493 marker (28,29). Figure 1(B) shows that bone marrow B220+493+ cells are markedly decreased during gestation in wild-type B10.D2 mice (9.1% vs 31.6%, P < 0.001). In Bcl2-Tg females, this cell population gets some degree of protection (16% vs 27%, P < 0.02) which is not observed in Fas<sup>+/−</sup> females (12.7% vs 36.2%, P < 0.01). More mature B220+493− cells are unaffected by pregnancy in B10.D2 or Bcl2-Tg mice but it is interesting to note that this population is expanded in Bcl2-Tg females compared to B10.D2 mice. In contrast, this 493− B cell frequency is lower in non-pregnant Fas<sup>+/−</sup> mice than in B10.D2 mice. Pregnancy causes a significant expansion (8.3% vs 4%, P < 0.001) which brings it back to the same frequency as in wild-type pregnant controls. Compiled data (from 7 to 17 animals per group) are presented in Fig. 5(A). The comparison of absolute B cell numbers yields similar results (data not shown).

Bone marrow B cell sub-populations are differentially affected by pregnancy in the Bcl2-Tg or Fas<sup>+/−</sup> contexts

In order to determine precisely at what stage(s) differentiating B cells are affected by the pregnancy-induced down-regulation in the presence of the Fas<sup>+/−</sup> mutation or the Bcl2 transgene, we analyzed the various bone marrow B cell sub-populations, corresponding to ‘A’ to ‘F’ fractions as described by Hardy et al. (18). As presented in Fig. 3(A), B220+, CD43+ or CD43− cells populations have been gated and then subdivided into HSAlow or HSAhigh cells. Thus, one can differentiate CD43+ cells into A (HSAlow) and B–C (HSAhigh), and CD43− cells into D (HSAhigh) and E–F (HSAlow) B cell sub-populations. The pregnancy-induced variation in their respective absolute numbers can be compared between pregnant (day 16.5 to 18.5) and non-pregnant animals in the three genetic contexts (cf. Fig. 3 and Table 1). Figure 3(A) shows flow cytometry results from individual mice in a representative experiment, and Table 1 presents a compilation of the data obtained from triple labeling of whole bone marrow cells within the three groups. Moreover, Fig. 3(B) presents the data from these same experiments plotted as the ratio of cell numbers in pregnant versus non-pregnant animals, for each B cell subpopulation from each one of the three groups of mice under study.

As shown in Fig. 3(A), in pregnant B10.D2 mice, primarily B–C and D sub-populations are markedly diminished (1.1 × 10<sup>5</sup> vs 4.9 × 10<sup>5</sup>, P < 0.001 for B–C; and 0.7 × 10<sup>6</sup> vs 3.1 × 10<sup>6</sup>, P < 0.01 for D). In contrast, in Bcl2-Tg mice, D fraction cells are reproducibly protected (1.2 × 10<sup>5</sup> vs 1.5 × 10<sup>5</sup> NS) and B–C fraction cells remain affected, although to a lesser degree (1.2 × 10<sup>5</sup> vs 2.6 × 10<sup>5</sup>, P < 0.001). In Fas<sup>+/−</sup> pregnant mice, a decrease in both B–C (2.2 × 10<sup>5</sup> vs 6.9 × 10<sup>5</sup>, P < 0.01) and D cells was still observed (1.1 × 10<sup>6</sup> vs 5.7 × 10<sup>5</sup>, P < 0.01).
Bcl2 overexpression blocks the pregnancy-induced down-regulation of B220+, Cμ+ bone marrow cells. The same experiment as in Fig. 1 is presented except bone marrow cells have been stained for the B220 surface antigen and intra-cytoplasmic μ chains. Numbers in each quadrant correspond to cell percentages contained within each quadrant. Statistically significant differences are noted by an asterisk. Control groups comprise 2–6 animals and experimental groups, 7–12 mice. Mean percentages (pregnant vs non-pregnant) for Cμ+ B cells: B10.D2, 10.2 ± 0.6% vs 19.7 ± 2.9%, P < 0.001; Bcl2-Tg, 38 ± 3.7% vs 42.4 ± 3.2%, NS; Faslpr/lpr, 7.9 ± 0.6% vs 20.5 ± 0.1%, P < 0.001.

Our observations are summarized in Fig. 3(B), where we plotted the ratio of pregnant to non-pregnant cell numbers for each B cell subpopulation within each of the three animal groups. Compared to the B10.D2 situation, it is clear that fraction B–C and fraction D are partially protected in Bcl2-Tg pregnant mice; the remaining difference for fraction D is not statistically significant. In contrast, these B–C and D populations are greatly reduced in Faslpr/lpr pregnant mice as well as in B10.D2 controls. Finally, a marked and significant expansion of fractions A and E–F was clearly seen in pregnant Faslpr/lpr mice only.

Role of sIgM in the phenomenon: analysis in 3-83μα BcR-Tg mice

From previously published results (12,33,34) and the data reported above (Figs 2 and 3), it appears that bone marrow B cells affected by estrogens (12) or targeted by pregnancy-induced down-regulation (33,34) have started to rearrange their BcR and contain intra-cytoplasmic μ chains. Thus, it was important to check how pregnancy would affect bone marrow B cells expressing a transgenic BcR earlier than their endogenous receptor in the two experimental genetic contexts. It was important to examine whether these BcR-Tg cells would have the same regulatory mechanisms, including regulation of survival, at work as their non-Tg counterparts.

In BcR-transgenic 3-83μα mice, the rearranged BcR transgenes are expressed very early during B cell development, at stages normally deprived of endogenous surface immunoglobulin expression. Nevertheless, such cells are equally sensitive to pregnancy-associated down-regulation (34). We have analyzed how the fate of these cells is modified in the presence of the Bcl2-Tg or the Faslpr/lpr mutation. As shown in Fig. 4(A) (left), IgM+B220lo B cells: in B10.D2, 19.1% vs 9.3%, P < 0.05, as well as in (Faslpr/lpr, 3-83μα) mice (2.2% vs 10.5%, P < 0.01) (Fig. 4A, right). In contrast, B220hi/lo, IgM+ B cells are fully protected in (Bcl2, 3-83μα) mice (21.3% vs 19.7%, NS) (Fig. 4A, center). Moreover, a remarkable expansion (×2, 10.1% vs 5.2%, P < 0.02) of more mature B220hi, IgM+ cells was observed in the bone marrow of pregnant (Faslpr/lpr, 3-83μα) mice. In keeping with these results (Fig. 4B, left and right panels), a significant decrease was observed in 493+, B220+ bone marrow B cells in (B10.D2, 3-83μα) and in (Faslpr/lpr, 3-83μα) Tg mice (4% vs 7.9%, P < 0.05; 4.8% vs 14.4%, P < 0.02, respectively). Again (Fig. 4B, center), this population is fully protected in (Bcl2, 3-83μα) Tg mice (5.7% vs 6.2%, NS). In (Faslpr/lpr, 3-83μα) mice, the expansion of more mature B220hi, IgM+ B cells is confirmed as 493+ cells are increased in pregnant females (12.2% vs 7.5%, P < 0.05). A compilation of
the percentages of each B cell population in the three groups of 3-83 μg Tg mice has been plotted in Fig. 5(B). The comparison of absolute numbers from three to seven pregnant mice within each of the three different groups leads to the same conclusions (data not shown).

Thus, our observations in 3-83 μg-Tg animals are in line with our data from non-BcR-Tg mice (33,34). The Bcl2-Tg overexpression protects bone marrow BcR-Tg B cells from the pregnancy-induced down-regulation. In contrast, the Faslpr/lpr mutation, which inactivates 95% of the Fas death receptor activity, does not prevent the down-regulation of 'B–D' fraction B cells, but it is responsible for an expansion of the 'A' fraction cells, as well as more mature ('E–F' fraction) cells, in pregnant animals. Moreover, these results confirm that an earlier than normal transgenic BcR expression does not alter the fate of slgM+ B cells during pregnancy. Thus, these data indicate that early BcR expression and signaling do not interfere with the regulation of B cell differentiation.

Discussion

New B cell clones are produced in the bone marrow throughout adult life. This multi-step process of proliferation and differentiation from continuously produced B cell precursors is highly regulated. This tight control is needed to maintain the homeostasis of the system and prevent the selection of potentially harmful auto-reactive B lymphocytes. Both cell autonomous events such as anergy or apoptotic death, and external stimuli like stromal cell contact, cytokines and hormones participate in the fine tuning of B cell production. These regulations operate both at the level of transition from the stem cell to the early B cell lineage as well as further down the B cell differentiation pathway (30,31). Steroid hormones are known to play important roles in these regulations at the various stages of differentiation, including recently characterized early B cell precursor stages (30–32). Apoptosis has also been shown to play a role at various steps...
of B cell maturation (26,27), eliminating cells with unproductive Ig gene rearrangements via death by neglect and clearing auto-reactive B cells via activation-induced cell death. The cell death receptor Fas is expressed during the whole B cell differentiation (22), while the anti-apoptotic molecule Bcl2 is expressed endogenously in Pro-B and mature B cells (21,35). Thus, both molecules are important participants in the regulation of B cell survival. Pregnancy is a unique physiological situation with multiple changes in the expression of cytokines and hormones, which lead to a thymic involution (8,36) and a striking decrease of bone marrow B cell production, targeting mainly pre-B and immature (fractions B–D) bone marrow B cells of pregnant mice (9). However, it remained unclear whether apoptosis was involved in this pregnancy-induced down-regulation, what molecules were involved and at what particular differentiation stage(s) they were acting. In order to address these important questions, we have studied B lymphopoiesis in pregnant mice either bearing a mutated cell death receptor, Faslpr/lpr, or overexpressing the anti-apoptotic molecule Bcl2 via a transgene targeted to B lymphocytes only. We then compared their various B cell sub-populations with age and sex-matched non-pregnant counterparts.

Our data demonstrate that, in contrast to normal BcR-Tg or non-Tg animals, Bcl2-Tg mice do not have their B cell progenitors selectively reduced by pregnancy. Although one previous study reported the protective effect of Bcl-2 on B cells (12), this protection was observed following estrogen

### Table 1. Pregnancy-induced variations in total cell numbers from bone-marrow B cell sub-populations in Bcl-2-Tg and Faslpr/lpr mice

<table>
<thead>
<tr>
<th>B220+</th>
<th>CD43+</th>
<th>CD43–</th>
<th>Total bone marrow B220+ B cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B10.D2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>non-pregnant (3)</td>
<td>0.6 ± 0.1</td>
<td>4.5 ± 0.6</td>
<td>2.5 ± 1.1</td>
</tr>
<tr>
<td>pregnant (7)</td>
<td>0.6 ± 0.1</td>
<td>1.3 ± 0.3</td>
<td>0.6 ± 0.2</td>
</tr>
<tr>
<td>Bcl-2 Tg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>non-pregnant (7)</td>
<td>10.6 ± 3</td>
<td>2.4 ± 0.2</td>
<td>2.6 ± 1.8</td>
</tr>
<tr>
<td>pregnant (8)</td>
<td>9.4 ± 2</td>
<td>1.2 ± 0.4</td>
<td>1.6 ± 1</td>
</tr>
<tr>
<td>Faslpr/lpr</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>non-pregnant (4)</td>
<td>0.6 ± 0.1</td>
<td>5.5 ± 0.2</td>
<td>4.3 ± 1.3</td>
</tr>
<tr>
<td>pregnant (7)</td>
<td>1.0 ± 0.2</td>
<td>2.4 ± 0.8</td>
<td>1.3 ± 0.4</td>
</tr>
</tbody>
</table>

aNumber of mice tested.
bCell numbers (×10⁶ ± SEM) calculated as shown in Fig. 3(A). Significantly different values (compared to non-pregnant controls within each group) are underlined.
cP < 0.001.
dP < 0.01.
eP < 0.05.

Fig. 4. Role of sIgM in pregnancy-induced B cell down-regulation: analysis in 3-83μ6 BcR-Tg mice. The same experiment as in Fig. 1 has been performed in (B10.D2, 3-83μ6) Tg mice, in the presence or absence of the Bcl2 transgene or the Faslpr/lpr mutation. Numbers on each plot correspond to cell percentages contained within each box or quadrant. Because of the dispersion of B220 expression levels on (Bcl2,3-83μ6)Tg cells, no attempt has been made to distinguish sIgM+cells on the basis of their B220 level. Each plot comes from a single mouse, representative of two or three animals (non-pregnant control groups) and three to seven animals (pregnant groups, on days 16.5–18.5 of gestation).
injections, and in a different Bcl2-Tg mouse line, where the Bcl2 molecule is overexpressed in both T and B cells. Thus, our results are the first to yield information on the effect of Bcl2 on B cells during pregnancy. Moreover, we show that in lpr/lpr mice, despite the modifications of lymphoid subsets due to a mutated Fas receptor, pregnancy downmodulation of B cell precursors is still clearly at work. To the best of our knowledge, this is also the first report of such observations.

B220 was chosen as it is one early B cell marker whose expression level augments with B cell maturation. Our results show that a majority of Bcl2-overexpressing bone marrow B cells are protected from the negative influence of pregnancy. In particular, D fraction cells are no longer significantly affected (Fig. 3B). We made the same observations in non-Tg or 3-83ld-Tg mice, which indicates that the early expression of a fully rearranged and functional BcR does not modify the fate of B cells under these conditions. Only Cγ-negative B cells are not protected, most probably because the overexpression level of Bcl2 in such cells is null or insufficient, as the Eμ enhancer is just starting to be active in the B fraction (37). This protection strongly suggests that apoptotic mechanisms normally responsible for the elimination of cells during pregnancy are inhibited by the overexpressed Bcl2 molecule. The Bcl2 molecule is normally expressed in B cells undergoing maturation. Interestingly, it is upregulated in anti-DNA peripheral B cells activated by estrogens (38). In Bcl2-Tg mice, it is expressed at high levels in all stages of B cell differentiation. It has also been shown to regulate B lymphopoiesis by prolonging the quiescent state before entry in cell cycle and delaying transition steps at various maturation stages. Thus, during pregnancy, the overexpression of Bcl2 in B cells leads to a blockade of apoptotic cell death, and the decrease in BrdU incorporation in those mice (our unpublished results) suggests that the turn-over of B cells is slower. Both mechanisms result in the protection of Pro- and Pre-B cells in which the Eμ enhancer drives a sufficient level of Tg Bcl2 expression (39). Finally, it is noteworthy that the degree of protection we have observed, although highly significant and reproducible, does not reach 100%, which suggests that other apoptotic mechanisms not regulated by Bcl2 also operate to control B cell production during pregnancy (40).

In contrast, in Faslpr/lpr pregnant mice, cells from both fractions B–C and D are decreased to the same extent as in wild-type control mice. In the light of our present data in Bcl2-Tg mice, this observation suggests that apoptotic mechanisms which do not involve the Fas receptor play a role in this down-regulation and are inhibitable by Bcl2. Interestingly, A and E–F fractions, which are not the major targets of pregnancy down-regulation in normal mice, are significantly and selectively augmented by pregnancy in Faslpr/lpr mice, which again is a previously unreported finding. Although the most immature subpopulation we have studied in the present report (‘pre-pro’ or ‘A’ cells) is increased, major populations (B–C and D) are down-regulated, so the total number of bone marrow B cells is diminished during pregnancy. As mentioned above, this A fraction also includes precursors of T and NK cell lineages (30–32), so it remains possible that none or only part of the cellular increase affects early B cell precursors. In
addition, we show that ‘E–F’ cells (Fig. 3A), also corresponding to mature 493-low or negative (Figs 1 and 5) B220hi cells, are doubled during pregnancy in fas−/−/ld and non-BcR-Tg mice. Interestingly, how some B cell subpopulations derived from decreased precursor compartments remain unchanged or are even increased during pregnancy is currently unknown. This maintainance or accumulation of specific cell subpopulations could be due to a lack of negative selection or resistance to apoptosis induced by pregnancy. The transitory nature of the effect of pregnancy might also play a role, as some developmental compartments with a lower turn-over rate might be less affected. Alternatively, one could speculate that under the particular influence of pregnancy, some cells receive activating signals (from hormones, their BcR or death receptors) which could lead to an upregulation of FLICE-inhibitory protein (FLIP) (41). In normal mice, FLIP has been shown not only to block apoptosis triggered by Fas, TRAIL or the TNF-receptor, but also to promote cell proliferation and differentiation (42). It is important to note that both experimental mouse strains, Tg Bc12 and fas−/−/ld, are auto-immune prone and present clinical signs of autoimmunity increasing in severity with age. Thus, in these auto-immune prone animals, the pregnancy-induced increase in potentially auto-reactive mature B cells could have important consequences on the state of tolerance to self-antigens within the B cell pool.

Another aspect of our work has addressed the role played by the BcR in the regulation of B cell maturation and survival during pregnancy. It is now well established that the expression of a functional BcR on the cell surface is a requirement for B cell survival and further differentiation (43). Previous work from our laboratory has shown that 3-83μ BcR-Tg immature B cells are sensitive to the negative effect of pregnancy (34). It has also been reported that injections of estrogens can globally mimic this effect (12). Our results obtained both from (fas−/−/ld, 3-83μ) and (Bc12, 3-83μ) Tg pregnant mice closely parallel our observations in non-BcR-Tg mice, and indicates that the early expression of the Tg BcR does not affect B cell fate during pregnancy.

In conclusion, the present work confirms and extends previous knowledge on the influence of pregnancy on the mouse B cell compartment. Our results show that mice bearing two different genetic alterations known to trigger age-dependent auto-immune syndromes (21,22) in mice are differentially affected during pregnancy.

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