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Maternal B Lymphocytes Specific for Paternal Histocompatibility Antigens Are Partially Deleted During Pregnancy[1]

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Although genetically different from its mother, a mammalian fetus bearing paternal alloantigens is normally not rejected. To investigate one of the many possible mechanisms involved in this important biologic phenomenon, we analyzed the consequences of fetal alloantigen recognition on maternal B lymphocytes. We used transgenic mice expressing a unique B cell receptor with a relatively high affinity for the MHC class I molecule H-2K^k on most B lymphocytes. We provide the first evidence for an alloantigen-specific B cell deletion in the spleens and bone marrow of transgenic mothers bearing H-2K^k-positive fetuses. This highly reproducible deletion affects ≤80% of Id-bearing B cells, starts at midpregnancy, and is only observed until term. Such a specific maternal B cell deletion could contribute to the success of the fetal allograft. *The Journal of Immunology,* **1998, 161: 2677–2683.**

Reproduction by viviparity is an important biologic phenomenon; the detailed mechanisms of this phenomenon are not completely understood by immunologists or developmental biologists. The following question has challenged biologists for several decades: how does the semiallogeneic fetus manage to escape the immune rejection of its mother? Several hypotheses have been put forward, some of which have since been rejected, such as the total lack of immunogenic molecules on the developing fetus or the ignorance of the immune system of the mother toward the conceptus (1). The placenta forms the interface between the mother and its semiallogeneic fetus and has been the focus of numerous studies (2–5). Despite much earlier controversy (6, 7), the current weight of experimental evidence is in favor of the placenta being devoid of highly polymorphic MHC class I and class II Ags during the entire time of pregnancy in humans and mice (4, 5, 8). The placenta also appears to be the site of immune regulation by complex cellular and molecular interactions that are still poorly defined (9).

It is now well-documented in mice that the fetal expression of polymorphic MHC class I Ags starts at around day 9 postcoitum and slowly increases during the second half of gestation, never exceeding one-fiftieth of the adult level in any given tissue (10, 11). This low level of expression does not prevent fetal tissues from being able to trigger a cytotoxic response (12). It is also well-established that the fetus can be recognized as foreign by the immune system of the mother. In addition, there are numerous reports of the passage or effraction of fetal cells into the blood or lymphoid organs of the mother, although with variable frequency (13, 14). A reverse passage has also been reported (15).

We have been interested in the consequences of a semiallogeneic gestation on the specific immune repertoire and function of the mother. Such a question could only be addressed in a transgenic (Tg)[3] animal in which a major proportion of mature lymphocytes bear a unique specificity, since the expression of Tg AgR molecules shuts off endogenous receptor rearrangements. In non-Tg animals, the proportion of specific T or B lymphocytes is too small to be followed.

We have used a previously described Tg B cell mouse model (16, 17) in which fully rearranged Ig heavy and light chain genes have been introduced into a mouse genome. The Tg Igs (IgM and IgD) are expressed on the B cell surface and present a relatively high affinity for H-2K^k molecules, a lower affinity for H-2K^b molecules, and a very low affinity for H-2D^k molecules (18, 19). They bear the 3.83 Id that is specifically bound by the 54.1 mAb. Such mice have already been used successfully to analyze the mechanisms of tolerance to self Ags at the B cell level (16–20). We have crossed age-matched 3.83 (anti-H-2K^k) Tg females (on the B10.D2 background, H-2^d) with B10 MHC-congenic males of H-2^d, H-2^k, or H-2^f haplotypes. Thus, we were able to analyze the fate of 3.83-positive B lymphocytes during syngeneic (H-2^d) or semiallogeneic gestations. In the latter case, two different situations were compared: Semiallogeneic fetuses bearing MHC class I H-2K molecules were either recognized (H-2K^k) or not recognized (H-2K^f as a "third-party" control) by the Tg B cell receptors (BCRs). We show here for the first time that the presence of semiallogeneic fetuses recognized by the Tg BCR significantly alters the fate of specific B lymphocytes (compared with syngeneic or third-party control fetuses); this effect is observed primarily in the spleens of pregnant females starting at midgestation. An average of

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[3] Abbreviations used in this paper: Tg, transgenic; LN, lymph node; BM, bone marrow; sIg, surface Ig; BCR, B cell receptor.

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70% of Tg B cells are deleted from the spleens of H-2K^k-fetus-bearing females starting at around 10 days of gestation. Such effects are fully reversed as early as 4 days postpartum, even in lactating females in which the hormonal down-regulation of B lymphopoiesis is maintained (21, 22).

Materials and Methods

Animals

3.83 anti-H-2K^k-$\mu\delta$ Tg mice have been described previously (16). B10.BR (H-2^k), B10.D2 (H-2^d), and B10.M (H-2^f) congenic mice were obtained from Harlan (Gannat, France). All animal care and handling was performed according to institutional guidelines.

Cell suspensions

Nucleated cell suspensions were prepared from the blood, spleens, paraaortic lymph nodes (LNs), and bone marrow (BM) of 3.83 Tg pregnant or nonpregnant females. Heparinized blood was obtained from anesthetized animals by intracardiac puncture. BM samples were flushed from the long leg bones (femurs and tibias) with HEPES-buffered 199 tissue-culture medium (Life Technologies, Cergy Pontoise, France). E were eliminated from the blood and spleen cell suspensions by osmotic shock in lysing buffer (0.17 M Tris and 8.3 g/L ammonium chloride, mixed 1 volume/9 volumes, pH 7.3).

Immunofluorescence staining and flow cytometry analyses

The incubation of 4×10^5 nucleated cells per sample was performed with the following mAbs: 54.1 rat mAb specific for the 3.83 BCR Id was revealed by an FITC-labeled mouse anti-rat κ light chain Ab (PharMingen, San Diego, CA), biotin-coupled anti-B220 Abs (PharMingen) (in the presence of a 10-fold excess of normal mouse serum Ig for single labelings) were followed by phycoerythrin-labeled streptavidin (Southern Biotechnology Associates, Birmingham, AL), and goat Abs to mouse μ-chain were coupled to FITC (PharMingen). Flow cytometry profiles were obtained on an Epics Elite-ESP flow cytometer that was equipped with a 488-nm argon laser (Coultronics, Margency, France). The cell populations analyzed were

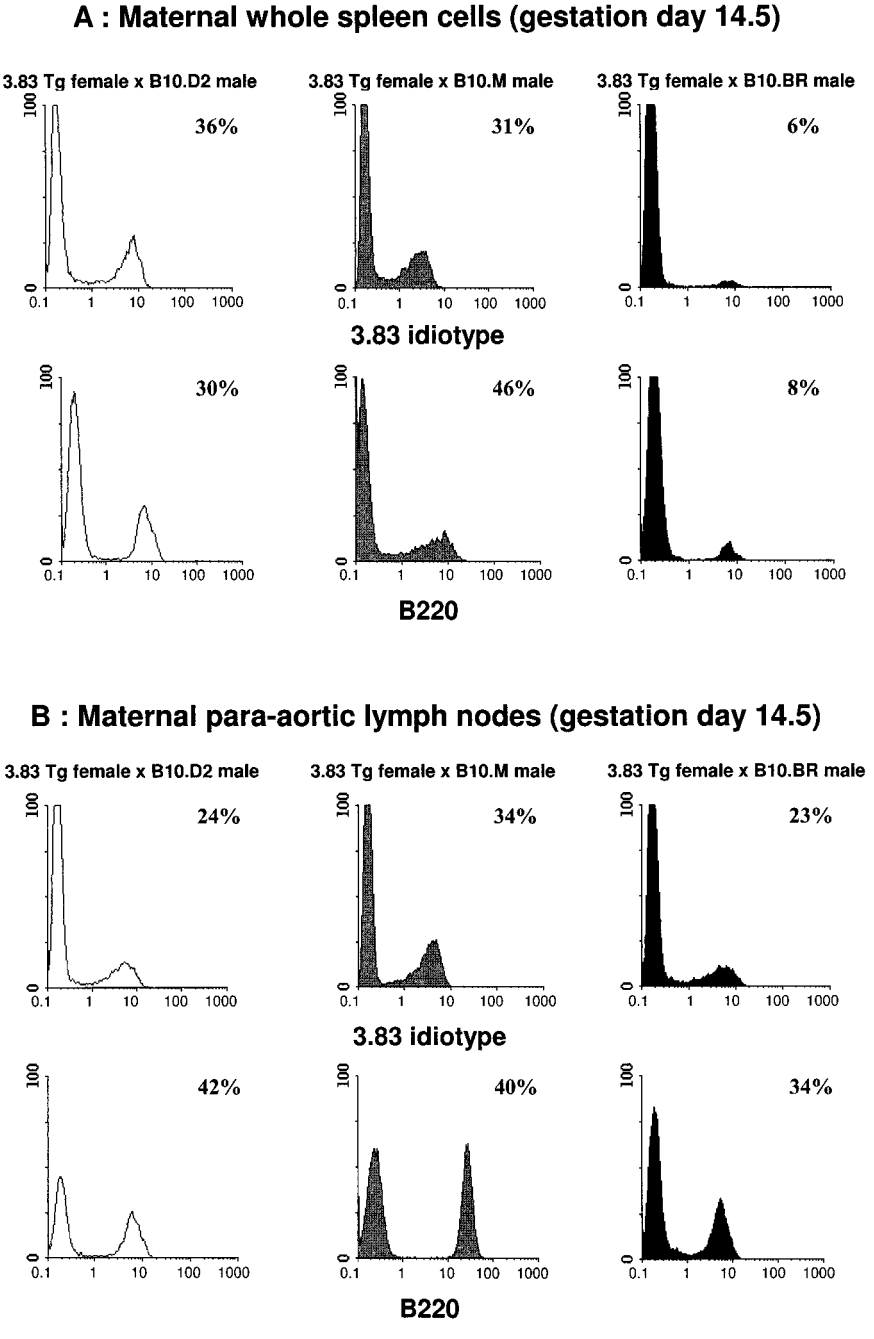

FIGURE 1. Flow cytometry profiles of anti-H-2K^k 3.83 Tg pregnant mouse spleen cells. Three 3.83 Tg pregnant females were analyzed individually at 14.5 days postmating with a syngeneic B10.D2 male (*left column*, white profiles), an allogeneic (third-party) B10.M male (*middle column*, gray profiles, from a separate experiment), or an allogeneic B10.BR male bearing H-2K^k Ags recognized by the 3.83 BCR (*right column*, black profiles). Flow cytometry profiles and the percentages of positive cells in maternal whole spleen cells (*A*) or paraaortic LN cells (*B*) are shown. Cell numbers (on the *y*-axis) are plotted vs the relative fluorescence intensity in logarithmic scale on the *x*-axis ($\geq 10^4$ cells were analyzed in each case): *upper rows* represent staining with 54.1 rat mAbs specific for the 3.83 BCR Id; *lower rows* indicate staining with anti-B220 Abs. Background staining was <1% in each case. The total cell numbers in the spleen or LNs were expanded 1.5 to 2.5 times in all pregnant mice compared with nonpregnant controls.

gated on the basis of forward and side (90° angle) scatter criteria to avoid potential contamination by dead cells or debris.

ELISA

Microtiter plates (Luxlon, CML, Nemours, France) were coated with 54.1 purified rat mAb (0.25 μg per well). Duplicate serum dilutions were added to each well, and bound Abs were revealed by peroxidase-coupled rat anti-mouse κ light chain mAbs (Serotec, Oxford, U.K.). The enzymatic activity was measured after the addition of the substrate *O*-phenylenediamine in 0.1 M citrate buffer containing 0.03% H_2O_2 at 490 nm using a microplate reader (MR 5000, Dynatech, Saint-Cloud, France). One single pool of CosLin D1 supernatant in serial twofold dilutions was used as a positive control to obtain a standard reference curve in every assay. This supernatant originated from transfected cells secreting the 3.83 IgM only (16, 17).

Statistical analyses

Mean values were compared using the Student's *t* test.

Results

We crossed anti-H-2K^k 3.83 Tg virgin females with B10 congenic males that were homozygous for H-2^d (B10.D2), H-2^k (B10.BR), or H-2^f (B10.M) MHC haplotypes. The beginning of gestation was ascertained by the observation of a vaginal plug (counted as day 0.5 of gestation). Tg pregnant and nonpregnant mice (used as controls) were analyzed at various times of gestation. Their blood and lymphoid organs were removed for analysis by flow cytometry after incubation with various Abs that were specific for B lymphocyte differentiation Ag. Figure 1*A* shows spleen B cell populations in pregnant (day 14.5 of gestation) mice. A significant decrease in the percentage of 3.83-positive cells (80% in this experiment) could be seen reproducibly in the spleens of Tg females that had been mated with B10.BR males. The same observations could be made when we labeled the cells with anti-IgM Abs. Pregnant females that had been mated with B10.D2 or B10.M males did not show this effect, and their B cell percentages were comparable with those of nonpregnant mice. To see whether this lack of 3.83-positive and IgM$^+$ cells was due to a modulation of their receptors or to the disappearance of the cells, we labeled the cells with the B220 cell marker. It was determined that the percentage of cells bearing the pan B cell marker B220 was also diminished in a comparable proportion (Fig. 1*A*). These results indicated that a significant proportion of anti-H-2K^k Tg B cells had disappeared from the spleen in B10.BR-mated pregnant females only. In contrast, when the paraaortic LNs removed from the same animals were subjected to the same analyses, no significant difference could be observed between control and experimental Tg animals (Fig. 1*B*). The same results were obtained on other peripheral LNs from the same animals.

The decrease in specific Tg B cells was seen reproducibly in the spleens of B10.BR pregnant females from day 12.5 until day 18.5 of gestation. The amplitude of this decrease was not correlated with the size of the litters. As can be seen from the compiled data of 12 separate experiments presented in Figure 2, only B10.BR-mated females (seven animals) undergo a significant loss of Tg B cells in their spleens (70%, $p \leq 0.001$) (Fig. 2*B*) and BM (66%, $p \leq 0.001$) (Fig. 2*C*); no loss was observed in the LNs of these mice (Fig. 2*A*). This B cell deficit remains at the same amplitude until day 18.5, as can be inferred from the small SD values. In contrast, the average percentages of CD4$^+$ or CD8$^+$ T cells are not significantly affected in the same spleens (Fig. 2*E*). Moreover, the subpopulation of non-Tg B lymphocytes bearing an endogenously rearranged, Id-negative BCR is not different in the three groups (Fig. 2*D*). The ratio of IgM to B220 labeling remained close to 1 at all time points, indicating that ~70% of the Tg receptor-bearing B cells had disappeared from the spleen and BM between days 12.5 and 18.5 of gestation. This Ag-specific deletion was also observed in blood leukocytes from the same animals (data not

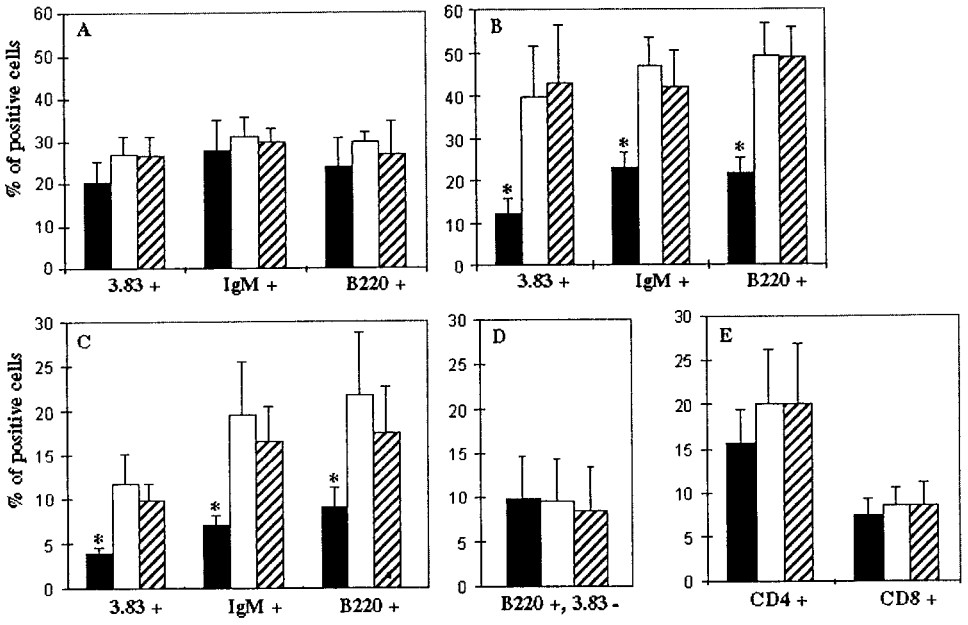

FIGURE 2. Flow cytometry analyses of 3.83 Tg lymphoid cells after midpregnancy. The histogram bars (*A–C*) represent the mean percentages (± SEM) of 3.83-positive, sIgM$^+$, or B220$^+$ B cells. Flow cytometry analyses were performed on cells that had been prepared from different lymphoid organs from individual pregnant mice between days 12.5 and 18.5 of gestation. *A*, Paraaortic LNs; *B*, Spleen; *C*, BM. While the overall deletion of B220$^+$ cells was ~50% in B10.BR-mated females compared with control pregnant mice, the deletion in B220^{low} cells reached 72% on average (also see Fig. 5 below). *D* and *E*, Spleen. The histogram bars indicate the mean percentages (± SEM) of B220$^+$ and 3.83 Id-negative cells (*D*) or of CD4$^+$ or CD8$^+$ T cells (*E*) in the same spleens as in *B*. The black bars correspond to allogeneic B10.BR-mated females, the hatched bars correspond to allogeneic third-party B10.M-mated mice, and the white bars correspond to syngeneic B10.D2-mated females. Each column represents the average of at least seven individual animals. * = $p < 0.001$; all other differences are not statistically significant (Student's *t* test).

shown). The phenomenon is reversible, since splenic B cell populations are not different from controls at 4 days postpartum, even in lactating females (data not shown).

The analysis of double-labeled (B220 vs surface IgM (sIgM)) spleen cells by flow cytometry revealed that the $sIgM^{high}$ subpopulation was primarily affected in B10.BR matings compared with other pregnancies (Fig. 3, showing a typical result on day 18.5 of gestation). Again, no such differences could be detected in the LNs of the same animals.

We subsequently studied the appearance of this B cell deletion during embryonic development. The deletion was clearly detectable in both the BM and spleen at midgestation in ~30% of B10.BR pregnant females (day 10.5). Interestingly, the percentages of 3.83, IgM, and $B220^+$ cells were diminished in the BM only in another 30% of animals (Fig. 4, *C* vs *D*); percentages in the spleen did not differ significantly between control and experimental crosses (Fig. 4, *A* and *B*). In these B10.BR-mated females, the fluorescence labeling intensity of the 3.83-positive or IgM^+ spleen cells was shifted toward lower levels compared with the control pregnant animals analyzed in the same experiment, suggesting that the cells had begun to encounter Ag (Fig. 4, *A* vs *B*). The remaining one-third of animals did not differ from controls at this stage.

The serum of each mouse was tested by ELISA to determine their levels of 3.83-positive IgM. All pregnant females presented a 3.83-positive IgM level that was at least 50% lower than the level observed for nonpregnant age-matched females. No significant difference relating to the alloantigens borne by fetuses was observed among the various pregnant female groups (Table I).

We also investigated the status of the BM cells from which mature B lymphocytes originate. Medina et al. have shown that B lymphopoiesis is greatly reduced by estrogens produced at much higher levels than normal during pregnancy (21, 22). Despite the low B cell numbers left in the BM due to hormonal down-regulation, a comparison of the B220/sIgM double-labeling plots from females bearing $H\text{-}2K^k$-positive fetuses with the plots from control pregnant females showed a massive reduction in relatively immature $sIgM^+$, $B220^{low}$ B cells (Fig. 5, *lower right plot*). In contrast, $B220^{high}$ cells appeared to be only marginally affected. Moreover, an important decrease in 3.83 Id-positive B cells was observed in B10.BR-mated pregnant females only (80% on average, $p \leq 0.001$) (cf Fig. 4*D*).

Discussion

Despite numerous studies since the hypotheses proposed by Medawar (1), the detailed cellular and molecular mechanisms that allow the semiallogeneic fetus to be tolerated by the immune system of the mother remain unclear. In particular, the precise immune status of the maternal lymphocytes that were specific for the paternal alloantigens borne by the fetus has not been addressed.

A report using Tg mice for a TCR anti-$H\text{-}2K^b$ has shown that maternal T lymphocytes do recognize paternal alloantigens during pregnancy (23). This "awareness" leads to a TCR down-modulation on $H\text{-}2K^b$-reactive T cells around midgestation (days 9–11 postcoitum) in the spleens of mice with $H\text{-}2K^b$-bearing fetuses. Moreover, such mice do not reject $H\text{-}2K^b$-bearing P815 tumor cell

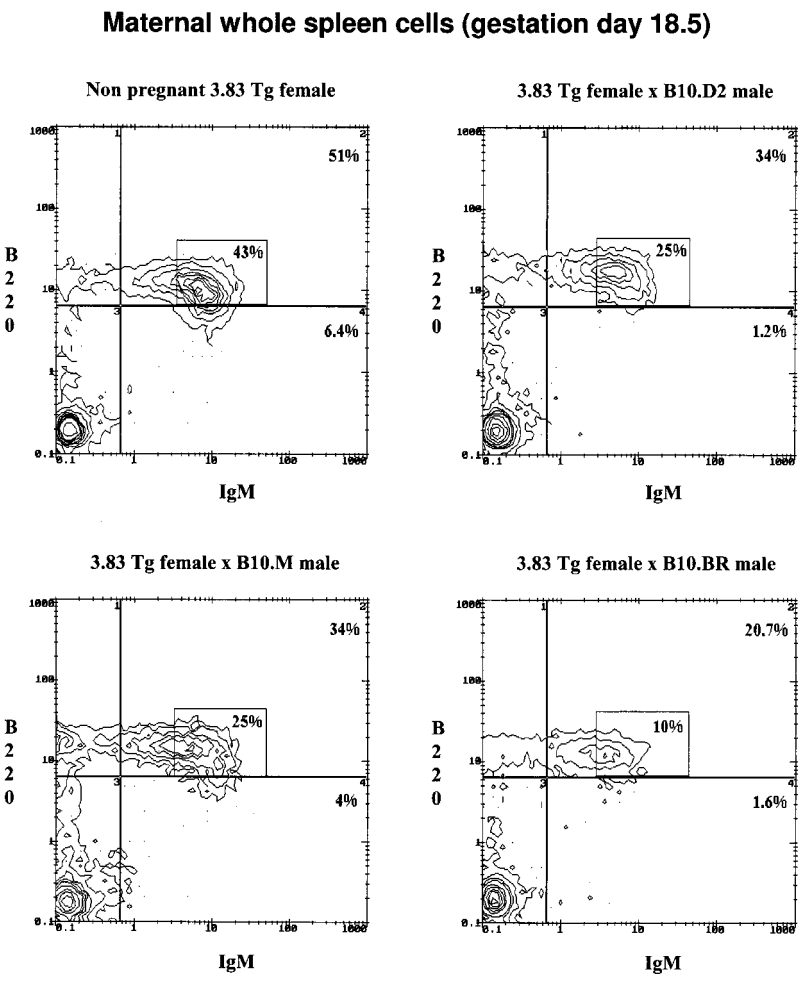

FIGURE 3. Double-labeling of 3.83 Tg pregnant mouse spleen cells. A typical experiment in which spleen cells from nonpregnant or pregnant females (day 18.5 of gestation, same crosses as in previous figures) were double-labeled for the B220 Ag (*y*-axis) as well as for sIgM (*x*-axis) is shown. The fluorescence intensities have been plotted on a logarithmic scale in arbitrary units. The percentages of cells present in each quadrant are indicated in the *upper right corner* of each plot. Boxes highlight cells with higher levels of sIgM and show their percentages in each case. The same results were obtained seven times between days 12.5 and 18.5 of gestation.

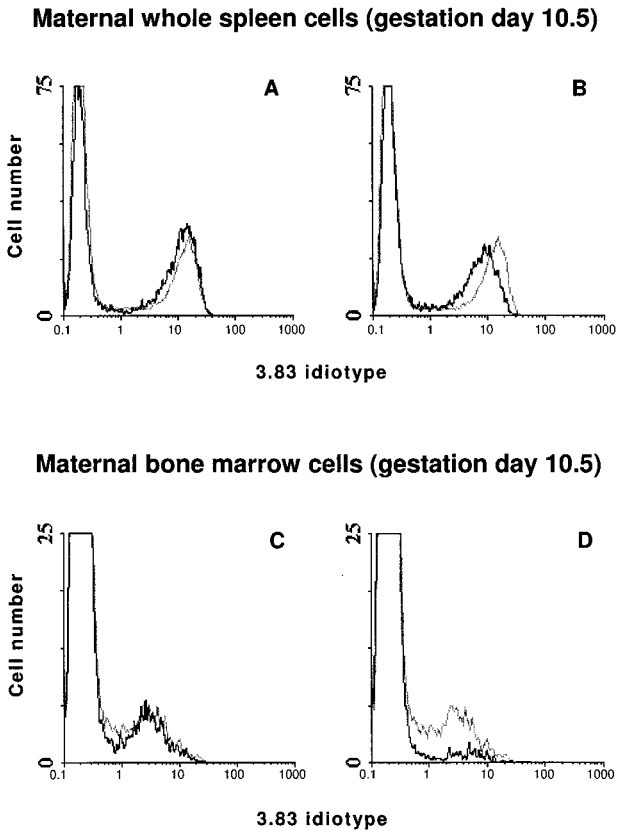

FIGURE 4. Flow cytometry profiles of anti-H-2K^k 3.83 Tg pregnant mouse cells (day 10.5 of gestation). The experiment was performed as described in Figure 1. The flow cytometry profiles of the cell surface staining for the 3.83 Id in the spleen (*A* and *B*) or BM (*C* and *D*) have been superimposed. *A* and *C*, Comparison between B10.D2 (black line) and B10.M (gray line) crosses. *B* and *D*, Comparison between B10.M (gray line) and B10.BR (black line) crosses. The percentages of 3.83-positive spleen (*A* and *B*) cells were as follows: 45% (B10.D2), 33% (B10.M), and 38% (B10.BR), with fluorescence peaks at positions 150, 160, and 92, respectively. The percentages of 3.83-positive BM (*C* and *D*) cells were as follows: 11% (B10.D2), 13% (B10.M), and 3% (B10.BR), with fluorescence peaks at positions 28, 43, and 23, respectively.

grafts. These observations suggest that a transient state of maternal tolerance to paternal alloantigens is established during the second half of gestation.

We have studied Tg mice bearing a major population of B lymphocytes with IgM and IgD receptors of a relatively high affinity for H-2K^k molecules (19). Our results are in agreement with the data obtained on T cells (23). We also observed a specific effect on maternal splenic lymphocytes that started around midgestation and was reversible after delivery. However, the T cell phenomenon was reportedly at its peak between days 9 and 11 of gestation and was limited to an AgR down-modulation. The important but incomplete B cell deletion we have observed is seen reproducibly from day 10.5 of gestation in the BM and from day 12.5 in the spleen and blood until day 18.5. It is dependent upon the presence of alloantigen-bearing fetuses in utero, since it is not seen after delivery. To our knowledge, this is the first report on such a phenomenon. In addition, our results suggest that this phenomenon starts at midgestation with a down-modulation of Id-bearing IgRs on the B cell surface. This possibility was observed in the B10.BR-mated females, in which no deletion of spleen Tg B cells had occurred at that time. When total cell numbers instead of frequencies were compared among the different groups, the same basic

Table I. *Comparison of 3.83-positive Ig serum levels*[a]

Mating	*n*	3.83 Id (μg/ml)
Tg × B10.BR	9	9 (± 4)
Tg × B10.D2	7	8 (± 3)
Tg × B10.M	5	8 (± 2)
Tg nonpregnant	5	17 (± 5)

[a] *n* = number of animals tested in each group.
SEMs are shown in parentheses. Tg = transgenic female.

conclusions could be reached. However, cell frequency data are more accurate for two reasons: 1) they are obtained from more homogeneous cell samples that are gated on lymphoid cells by scatter criteria on the flow cytometer, and 2) although total spleen and LN cell numbers are increased routinely in the pregnant females of all three groups compared with nonpregnant females, the absolute cell numbers present rather substantial variations from one animal to the next, even within the same group. These variations are probably due to the increase in erythropoietic activity in the spleens of pregnant mice. Fowler and Nash have reported that spleen weight and cellularity are increased in parallel to erythropoiesis during gestation in mice, with a peak on day 12 and a positive correlation with litter size (24).

It is interesting to note the similarity in the timing of the onset of B cell deletion and of the first appearance of MHC class I mRNA and surface molecules in mouse embryos; both of these events occur around day 9.5 of gestation (10, 11, 25, 26). The receptor modulation is observed first and is replaced shortly thereafter by the deletion of ≤70% of BM and splenic B cells until term. This partial effect is different from previously described complete (and thus irreversible) Tg B cell eliminations in an H-2^k or an H-2^b mouse (16, 17). It is probably due to the low antigenic load coming from the semiallogeneic fetuses vs the large numbers of specific B lymphocytes present in such Tg mice. It is noteworthy that a partial deletion (although restricted to peripheral lymphoid organs) was also observed in the same Tg mouse model in the case of a peripheral expression of low levels of H-2K^b Ag in the liver (18).

The mechanism(s) leading to such a cell disappearance from the spleen are presently unknown. Trivial side effects have been ruled out by the B10.M crosses, which allowed us to study 3.83 Tg pregnant mice bearing semiallogeneic fetuses that were not recognized by the Tg BCR. We have also confirmed that the B10.BR cross by itself did not have any particular influence on B cell populations in pregnant mice in two ways: 1) non-Tg B10.D2 pregnant mice that were crossed with B10.M or B10.BR males did not have significantly different B cell populations in their spleens (data not shown), and 2) as shown in Figure 2, *E* and *D*, the T cells as well as non-Tg B lymphocytes present in the same spleens as the anti-H-2K^k-deleting B cells were not affected. Thus, it appears that this deleting effect is restricted to Id-positive B cells and is most likely Ag-specific. We have verified that these cells are not drawn to or sequestered in other lymphoid organs such as the peripheral LNs (even the paraaortic LNs draining the uterus), blood, or BM. The potential homing of these cells to the placenta appears unlikely: such a possibility has not been reported in the literature, including reports on TCR anti-H-2K^b Tg pregnant mice (23). In humans, it has been shown that the leukocytes that are found in decidual cell suspensions are mostly NK cells, with some macrophages and T cells but virtually no B cells (27). In mice and rats, macrophages represent the vast majority of BM-derived cells in the decidua (28).

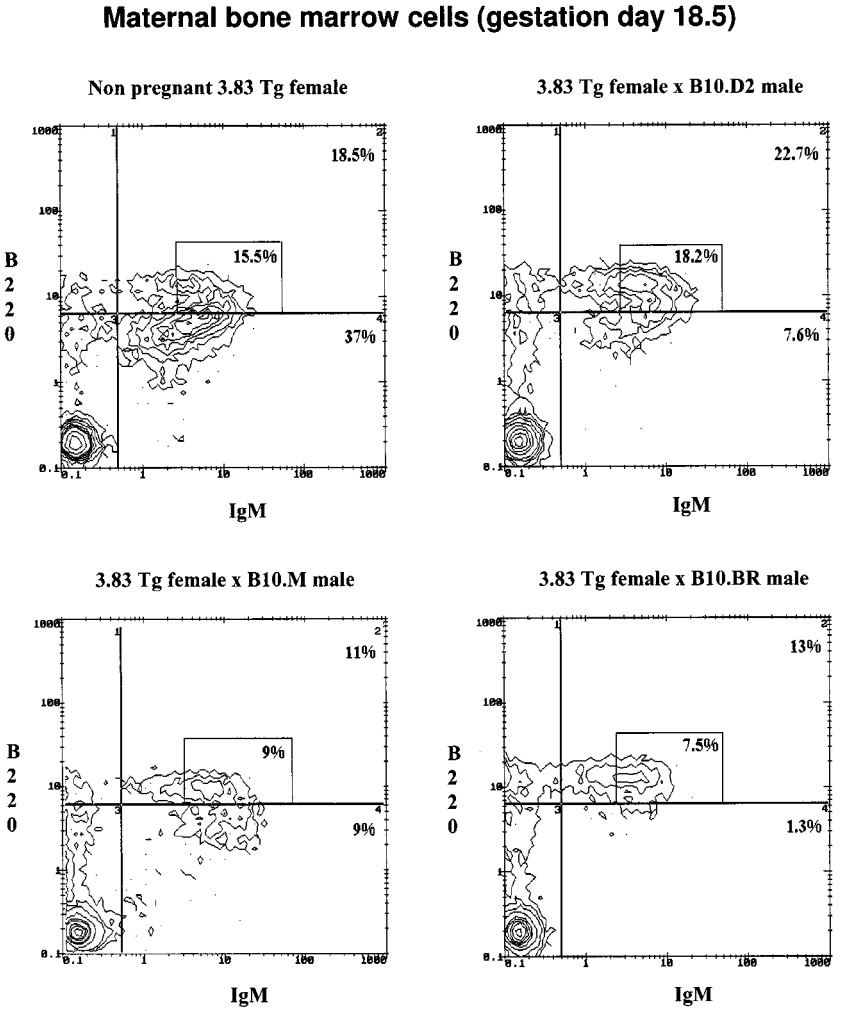

FIGURE 5. Double-labeling of 3.83 Tg pregnant mouse BM cells. In the same experiment as that shown in Figure 3, we have double-labeled BM cells from nonpregnant or pregnant females (same animals and overall procedures and same representation as described in Fig. 3). The total cell numbers were reduced by 50% on average between nonpregnant and pregnant females. The B10.BR-specific reduction in $sIgM^+$, $B220^{low}$ cells (in the fourth quadrant of the *lower right plot*) was 80% in this case. Similar results were obtained in six different experiments.

It has been shown that soluble Ag such as H-2 (16) or hen egg-white lysozyme (29, 30) are unable to cause cell deletions, except on Ag-activated, high affinity, germinal center B cells (31, 32). As the deletion can be seen in primiparous naive females as soon as the developing fetuses begin expressing $H\text{-}2K^k$ Ags, it is assumed, although not demonstrated, that alloantigen-bearing fetal cells are blood-borne to the lymphoid organs, where they encounter specific B cells. The paraaortic LN B cells draining the uterus do not share the same fate, possibly because they are in a different state of differentiation, since they contain very few cells of the IgM^{high}, $B220^{low}$ phenotype. An alternative explanation might be that these LN cells do not encounter the fetal alloantigen-bearing cells. These as yet unidentified fetal cells are most likely blood-borne from the placenta and reach the spleen and BM first. BM B cells are known to be highly sensitive to tolerization, which is probably why they are affected first and are already deleting on day 10.5 of gestation. Surprisingly, fetal cells do not appear to be prevented from reaching those sites by the anti-$H\text{-}2K^k$ IgM Abs present in plasma. Presumably, these circulating Abs are not of a sufficiently high avidity for the low Ag-expressing cells. They remain as IgMs, since the Tg Ig constructs do not allow a class switch (18, 20). As IgMs do not cross the placental barrier, they remain harmless to the fetuses.

It is well-known that multiparous females frequently display paternal MHC-specific Abs in their serum, apparently without harm to their progeny. Along the same line, earlier work by Mitchison (33) has shown that one can hyperimmunize female mice against paternal Ags without affecting the size or health of subsequent litters. This does not contradict the results that we obtained using naive primiparous females. Moreover, the deletion we observe does not affect all B cells, at least in our present model, and does not occur postpartum. Thus, immunization is most likely to occur at the time of delivery, when fetal cells might be able to enter the maternal circulation in greater numbers.

Interestingly, a recent study by Bonney and Matzinger (14) has reported the presence of fetal cells in the mother (as detected by quantitative PCR) as a relatively rare event. However, fetal cells are responsible for the priming of an anti-HY immune reaction in about one-third of all previously pregnant mice. In our experiments, we observed a specific B cell deletion in all mice studied. The apparent discrepancy between these observations could be due to different mouse strain combinations or to different housing conditions. It could be also the result of a higher level of sensitivity of the immune system of the mother compared with the PCR assay with regard to the detection of fetal cells. We would like to propose an alternate hypothesis that mouse fetal RBCs bearing MHC class I molecules (34, 35) could be responsible, at least in part, for the deletion we have observed. The embryo E are anucleated from day 12.5 of development and would not be detected in the PCR assay.

The results of the ELISAs showed no significant differences between 3.83-positive IgM levels in the various pregnant female groups. This finding could be the result of the absence of a transplacental passage of IgM. The large enough pool of remaining B cells in the peripheral LNs could also be a source of secreted IgM.

A further understanding of this observation will require an investigation of the deletional mechanism(s) as well as of the state of activation or responsiveness of the remaining B cells.

What happens in the BM is particularly interesting, since it is the site of B cell development; it has been shown that B lymphopoiesis is down-regulated by gestational estrogenic hormones. As reported by Medina et al. (21, 22), the high estrogen levels that are attained during gestation lead to a remarkable decrease of B lymphopoiesis in the BM, particularly at the small pre-B cell stage. In contrast, the overall hormonal effect on splenic B cell populations is reportedly marginal. Despite the very low B cell numbers left in the BM, we were able to observe a nearly complete deletion of $B220^{low}$, $sIgM^{+}$-specific Tg B cells; however, this deletion was found only in mothers bearing $H\text{-}2K^{k}$ fetuses. Thus, the phenomenon we report in the present work appears to be distinct from the one described by Medina et al. for the following reasons: 1) it is restricted to pregnant females bearing $H\text{-}2K^{d/k}$ fetuses, 2) it affects spleen and BM cells to the same extent, and 3) the two phenomena have different kinetics; the specific cell deletion starts around day 10 and stops at parturition. However, both phenomena are superimposed during the second half of gestation.

Our data are consistent with the interpretation that the B cells that are eliminated in the maternal tolerance process are recent BM emigrants; such B cells have an IgM^{high} phenotype, are present in the spleen but not in the LNs, and are known to turn over rapidly (36, 37). In this study, the elimination of these cells may be related to their enhanced tolerance sensitivity or to their greater accessibility to fetal Ag. Preliminary evidence from $RAG^{-/-}$, 3.83 Tg mice suggests that other lymphocytes are not required for the specific maternal B cell deletion (our unpublished observations). Both B cell deletion and TCR down-modulation (23) are potentially important mechanisms involved, among many others, in the success of the fetal allograft. Given the importance of this biologic phenomenon for species survival, it is extremely likely that multiple, potentially redundant mechanisms are at work to ensure the success of viviparity. A refinement of the experimental model would require both the presence of fewer Tg B cells in the lymphoid organs and also their capacity to class-switch. These adjustments would bring the model even closer to the physiologic situation.

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