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Identification of a Precursor to Phosphatidyl Choline-Specific B-1 Cells Suggesting That B-1 Cells Differentiate from Splenic Conventional B Cells In Vivo: Cyclosporin A Blocks Differentiation to B-1[1]

Larry W. Arnold, Suzanne K. McCray, Calin Tatu,[2] and Stephen H. Clarke[3]

The origin of B-1 cells is controversial. The initial paradigm posited that B-1 and B-2 cells derive from separate lineages. More recently it has been argued that B-1 cells derive from conventional B cells as a result of T-independent Ag activation. To understand B-1 cell differentiation, we have generated Ig transgenic (Tg) mice using the H and L chain genes (V_H12 and $V_\kappa4$) of anti-phosphatidyl choline (anti-PtC) B cells. In normal mice anti-PtC B cells segregate to B-1. Segregation is intact in V_H12 (6-1) and $V_H12/V_\kappa4$ (double) Tg mice that develop large numbers of PtC-specific B cells. However, if B-1 cell differentiation is blocked, anti-PtC B cells in these Tg mice are B-2-like in phenotype, suggesting the existence of an Ag-driven differentiative pathway from B-2 to B-1. In this study, we show that double Tg mice have a population of anti-PtC B cells that have the phenotypic characteristics of both B-2 and B-1 cells and that have the potential to differentiate to B-1 (B-1a and B-1b). Cyclosporin A blocks this differentiation and induces a more B-2-like phenotype in these cells. These findings indicate that these cells are intermediate between B-2 and B-1, further evidence of a B-2 to B-1 differentiative pathway. ***The Journal of Immunology*****, 2000, 164: 2924–2930.**

B-1 cells constitute a small but nevertheless significant subset in mice (1). They differ from the more abundant conventional or B-2 cells in a number of respects that presumably reflect their different roles in the immune system. In addition to their differences in cell-surface phenotype and anatomical distribution (1–3), they have characteristics suggesting that they are Ag-selected. For example, V gene analysis suggests that some have undergone Ag-driven clonal expansion (4, 5), and they contribute the majority of circulating IgM (1, 6, 7). Moreover, they are resistant to anti-IgM-induced tolerance (8), and those in the peritoneum express constitutively phosphorylated STAT-3 (9). Both of these characteristics are shared with Ag-activated B cells.

A further argument for Ag selection is that B cells of certain specificities segregate to B-1. These specificities include polyreactive and autoreactive B cells. Among the latter are B cells specific for single-stranded DNA, rheumatoid factor, Thy-1, red blood cells, and the common membrane phospholipid, phosphatidyl choline (PtC)[4] (7, 10–14). Among anti-Thy-1 B cells, Thy-1 itself is responsible for the accumulation of these cells in the B-1 subset, indicating that self-Ags play a role in segregation (13). PtC-specific B cells are particularly notable because they can comprise as much as 10% of the B-1 repertoire in unmanipulated mice (12) due to clonal selection and expansion after birth (15), but they are undetectable among conventional B cells (12, 15).

We have been interested in how B cells of certain specificities segregate to B-1. The mechanism of segregation is intrinsically linked to the relationship between B-1 and B-2 cells. There are two principal hypotheses to explain this relationship, each of which predicts a different mechanism of segregation. One is that B-1 and B-2 cells derive from separate lineage-committed precursors (the lineage hypothesis) (16–18). Other researchers (19, 20) and Haughton et al. (21) have proposed that B-1 cells derive from B-2 cells and are induced to differentiate to B-1 by stimulation with thymus-independent type 2 (TI-2) Ags (the induced differentiation hypothesis). By this hypothesis, B-2 cells are referred to as B-0 cells to reflect their potential to differentiate to B-1 (21). The evidence of Ag selection described above does not discriminate between these hypotheses. Discrimination will require identification of the precursors of B-1 cells.

To understand the origin of B-1 cells, we have examined the differentiation of PtC-specific B cells through the use of anti-PtC transgenic (Tg) mice. Mice carrying the anti-PtC H and L chain genes V_H12 and $V_\kappa4$ were generated (15). Our analysis indicates that segregation to B-1 occurs after Ig gene rearrangement and that segregation appears to be a function of the ability to bind PtC; cells that do not bind PtC or that bind only weakly are B-0, whereas those that bind well are B-1 (22). The role of Ag binding is further suggested by Lam and Rajewsky (23) in their report showing direct correlation between V_H12 receptor surface density and B-1 cell development in mice carrying a V_H12 transgene that has been inserted into an endogenous J_H locus. A similar conclusion about surface receptor density was reached in an analysis of anti-red blood cell B-1 cells (24). The segregation of PtC-binding B cells to B-1 could be explained by either negative selection of anti-PtC B-0 cells (the lineage hypothesis) or differentiation of anti-PtC B-0

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[4] Abbreviations used in this paper: PtC, phosphatidyl choline; Tg, transgenic; TI-2, thymus-independent type 2; *xid*, X-linked immunodeficiency; Dbl, double; CsA, cyclosporin A; int, intermediate.

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cells to B-1 cells (the induced differentiation hypothesis). To discriminate between these possibilities, we combined the anti-PtC Tgs with the X-linked immunodeficiency (*xid*) mutation (25), a null mutation of Bruton's tyrosine kinase (26–29). This kinase is a component of the signaling pathway from the IgM receptor complex (30). The loss of Bruton's tyrosine kinase results in impaired TI-2 responses and poor B-1 cell development (1, 31). Our analysis indicated that the majority of splenic anti-PtC B cells have the conventional B cell phenotype of *xid* mice (25), suggesting that B-0 cells are precursors to B-1 cells and that differentiation is dependent on signaling from surface IgM. The lineage hypothesis does not predict that anti-PtC B cells with the *xid* mutation would have a B-0 phenotype.

To identify precursors of B-1 cells in non-*xid* mice in vivo, we focus in this report on a population of PtC-specific B cells in double (Dbl) Tg mice that have an ambiguous phenotype (25). We demonstrate by adoptive transfer and treatment with cyclosporin A (CsA) that these cells are intermediate in differentiation between B-0 and B-1, indicating a line of differentiation between these two subsets consistent with a single B cell lineage.

Materials and Methods

Mice

The Dbl Tg mice used in this study were bred and maintained in our animal colony as described (15). C.B17 recipients were purchased from Taconic (Germantown, NY).

Flow cytometry

The Abs used for immunofluorescence were against IgM^a (DS-1), IgM^b (AF6-78), B220 (RA3-6B2), CD5 (53-7.3), CD43, and CD23, were obtained from PharMingen (San Diego CA), and were fluoresceinated, biotinylated, or conjugated to PE. In three-color experiments, directly fluoresceinated, PE-conjugated, and biotinylated Abs were combined. The biotinylated Ab binding was revealed by addition of streptavidin-RED670 (Life Technologies, Gaithersburg, MD). To detect PtC-binding B cells, liposomes encapsulating carboxyfluorescein were used as previously described (15). Contours are 5% probability.

Cell sorting and adoptive transfer

Splenic B cells from Dbl Tg or BALB/c mice were stained with anti-B220-FITC and anti-CD23-PE and the $B220^+$, $CD23^{int}$, and $B220^+$ $CD23^-$ cells were sorted separately on a MoFlo (Cytomation, Ft. Collins, CO) high-speed sorter. A total of $2–4 \times 10^6$ sorted Dbl Tg $CD23^{int}$ cells or BALB/c $CD23^+$ cells were injected i.v into C.B17 mice that had been irradiated with 500 rad 2 days before cell transfer. The sorted Dbl Tg $CD23^{int}$ cells were contaminated with 3–5% $CD23^-$ B-1 cells, and therefore, B-1 cell control transfers were with 2×10^5 $CD23^-$ B-1 cells into C.B17 mice that had been irradiated as described above. Mice were analyzed by flow cytometry 3–5 days posttransfer. All experiments were performed three or more times.

CsA treatment

C.B17 mice received i.p. injections of 50 mg/kg CsA (Novartis Pharmaceuticals, Basle, Switzerland) in 30 μl beginning the day before transfer and then daily for 4 days. After 4 days, spleen and peritoneal cells were taken for flow cytometry analysis. Dbl Tg mice were treated for 2 wk with daily doses of CsA as above and were analyzed by flow cytometry. For the transfers of CsA-treated cells from Dbl Tg mice, mice were treated with daily injections of CsA for 2 wk, and the spleen cells were stained for B220 and CD23. The $B220^+$ $CD23^+$ cells were sorted and $\sim 4 \times 10^6$ cells were injected i.v. into irradiated C.B17 mice as described. After 6 days, spleen cells were taken and stained for analysis by flow cytometry.

Results

The existence of a B-1 intermediate cell in V_H12 Tg mice

B-0 and B-1 cells are distinguishable using an array of cell-surface markers. B-0 cells are IgM^{low}, $B220^{high}$, $CD23^+$, $CD43^-$, and $CD5^-$, whereas B-1 cells are IgM^{high}, $B220^{low}$, $CD23^-$, and often $CD43^+$ and $CD5^+$ (25). In addition, B-1 cells are larger and more granular than B-0 cells. Although the majority of anti-PtC B cells in Dbl Tg mice have the typical B-1 phenotype (15), 5–20% have an unusual phenotype. These cells express CD23 typical of B-0 cells, albeit at lower levels, and express the B-1 cell markers CD5 and CD43 at levels equivalent to those of B-1 cells (Ref. 25 and Fig. 1). They are also intermediate to B-0 and B-1 in B220 expression ($B220^{int}$) and cell size, and they express less IgM than B-1 cells do. Thus, their phenotype suggests that they are cells in transition from B-0 to B-1 ($B\text{-}1^{int}$).

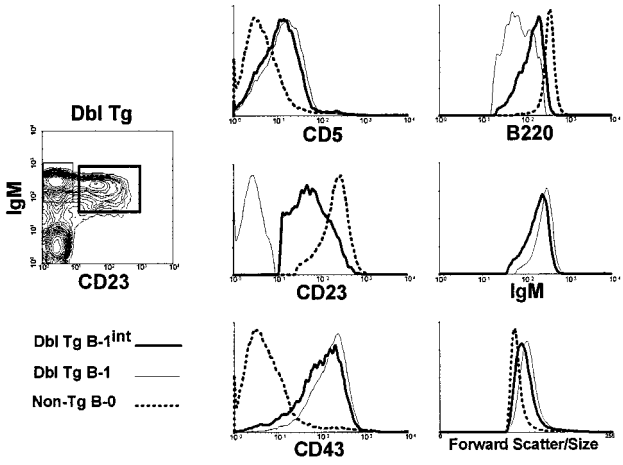

FIGURE 1. Dbl Tg mice have a population of PtC-specific B cells with a phenotype intermediate to B-0 and B-1 ($B\text{-}1^{int}$). The phenotype of IgM^{a+} $CD23^-$ B-1 cells (Dbl Tg B-1) and IgM^{a+} $CD23^+$ $B\text{-}1^{int}$ cells (Dbl Tg $B\text{-}1^{int}$) shown in the boxes on the two-parameter histogram (*left*) are compared with each other and with those of B-0 cells from a non-Tg littermate (Non-Tg B-0). Expression levels of CD5, CD23, CD43, B220, IgM^a, and cell size are shown using one-parameter histograms. The IgM level on non-Tg B-0 cells is not shown because they are IgM^b.

To test the possibility that $B\text{-}1^{int}$ cells are precursors to B-1, the $B220^+$, $CD23^{int}$ cells from Dbl Tg spleens were sorted (Fig. 2*A*) and adoptively transferred to sublethally irradiated C.B17 mice. Three to five days later, spleen cells from recipient mice were analyzed by flow cytometry. The recovered donor cells were PtC-specific at all time points. At 3 days posttransfer, most transferred IgM^a cells were similar in phenotype to the starting population, i.e., $CD23^{int}$, $CD5^+$, and $CD43^+$, although some had decreased expression of CD23 (Fig. 2*B*). However, by day 5 most transferred cells had lost CD23 expression. In addition, they had increased IgM expression and cell size, and some had decreased B220 (Fig. 2*B*). Interestingly, a substantial fraction of the recovered B cells had lost expression of CD5 but retained expression of CD43. Thus, it appears that $B\text{-}1^{int}$ cells can give rise to both B-1a ($CD5^+$) and B-1b ($CD5^-$) cells. B-1 cells that contaminate the sorted $B\text{-}1^{int}$ population are not responsible for these results because transfer of B-1 cells at a number equal to that determined by flow cytometry analysis to contaminate (3–5%) the sorted $B\text{-}1^{int}$ cell populations yielded few or no recoverable cells 5 days posttransfer (data not shown). Thus, between 3 and 5 days posttransfer, $B\text{-}1^{int}$ cells had differentiated to B-1, supporting the claim that they are precursors to B-1. Sorted BALB/c splenic B-0 cells transferred to C.B17 mice did not change within this time period, indicating that the manipulations (staining with Abs, cell sorting, etc.) have not induced differentiation to B-1 (Fig. 2*B*).

Cells at various stages of differentiation to B-1 appear to be evident in V_H12-only Tg (6-1) mice. In 6–1 mice, the transgene-encoded H chain can associate with multiple L chains and generate both PtC-binding and -nonbinding B cells (15). Cells that stain

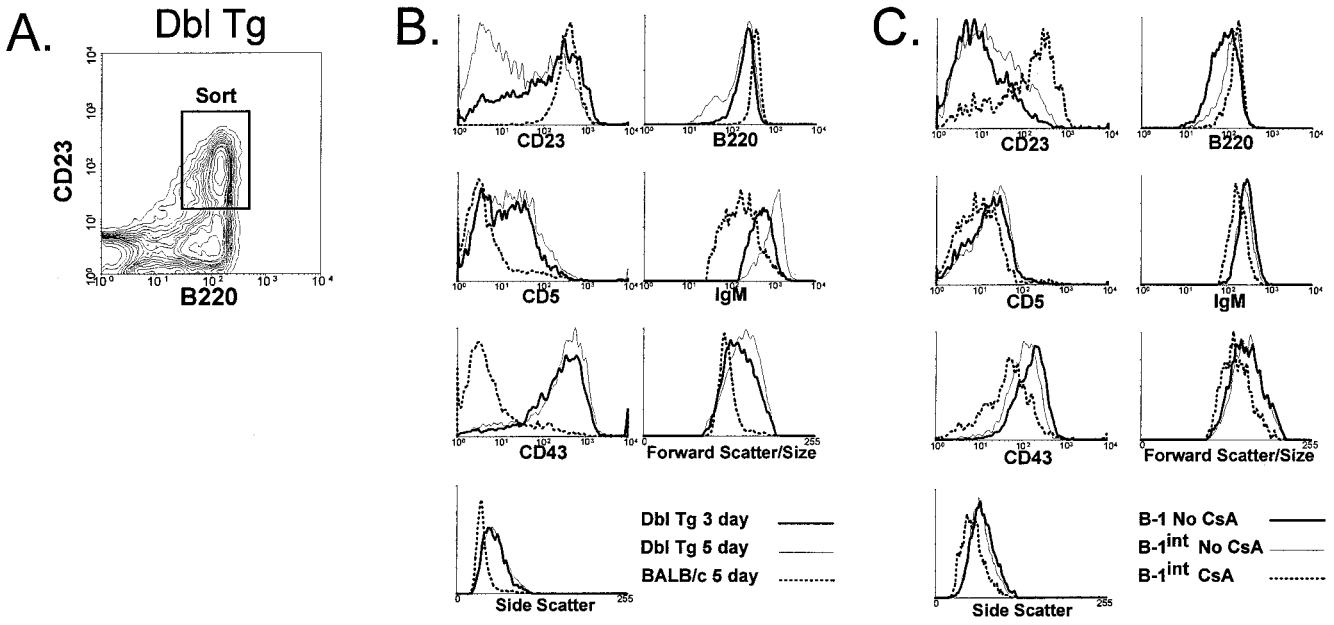

FIGURE 2. Adoptively transferred Dbl Tg B-1^{int} cells differentiate to B-1 cells. *A*, The CD23^{+} B-1^{int} cell population used in the cell transfer experiments is indicated. *B*, Sorted B-1^{int} cells were injected i.v. into sublethally irradiated C.B17 mice. After 3 or 5 days the transferred cells in the spleens of recipient mice were analyzed by flow cytometry. Shown is the phenotypic comparison of cells 3 and 5 days after transfer of Dbl Tg B-1^{int} cells with BALB/c CD23^{+} splenic B-0 cells taken 5 days posttransfer. *C*, B-1^{int} cells from Dbl Tg mice were transferred to sublethally irradiated mice that were (B-1^{int} CsA) or were not (B-1^{int} No CsA) treated daily with CsA beginning the day before transfer. The phenotypes of the cells of these populations are compared with that of B-1 cells from Dbl Tg mice (B-1 No CsA).

brightly with liposomes (PtC^{bright}) are B-1, whereas those cells that stain less brightly (PtC^{int}) or not at all (PtC^{-}) are B-0 (15, 22). The difference in liposome binding ability is due to L chain use. The PtC^{bright} cells predominantly use the V_{κ}4/5H gene (15). This is the gene used by anti-PtC B-1 cell lymphomas and hybridomas, and it is the V_{κ} transgene in Dbl Tg mice (9, 15). Based on CD23 expression, there are three subpopulations of PtC^{bright} cells: CD23^{high}, CD23^{int}, and CD23^{-} (Fig. 3). The CD23^{-} cells are the majority cell type in these mice and are B-1 (i.e., CD5^{+}, CD43^{+}, B220^{low}, and IgM^{high}), whereas the CD23^{int} cells have a phenotype identical with the B-1^{int} cells of Dbl Tg mice (i.e., CD5^{+}, CD43^{+}, and B220^{int}). The CD23^{high} cells express CD23 at levels nearly equivalent to those of B-0 cells, but many are CD43^{+}, CD5^{+}, and B220^{int} (Fig. 3). However, a smaller proportion of CD23^{high} cells has this phenotype relative to the B-1^{int} population, suggesting that PtC^{bright} CD23^{high} cells are at an earlier stage of B-1 cell differentiation than are B-1^{int} cells.

CsA blocks differentiation to B-1

TI-2 Ags are proposed to be responsible for driving differentiation to B-1 (19–21). Because B cell responses to CsA in vivo and in vitro (32, 33) can block TI-2 stimulation, we examined the effect of CsA treatment on B-1 cell differentiation. Dbl Tg B-1^{int} cells were transferred to sublethally irradiated C.B17 mice that received daily CsA treatment beginning the day before transfer. As shown in Fig. 2*C*, the transferred cells did not differentiate to B-1 after 4 days, even though B-1^{int} cells transferred into untreated mice differentiated to B-1. Relative to B-1^{int} cells transferred into untreated mice, CsA-treated cells showed essentially no change in CD23 levels, a decrease in CD5, CD43, and IgM expression, a decrease in cell size and granularity, and an increase in B220 expression. Thus, CsA blocks differentiation to B-1 and appears to cause B-1^{int} to acquire a more B-0-like phenotype, suggesting that continual Ag stimulation is required for differentiation to B-1.

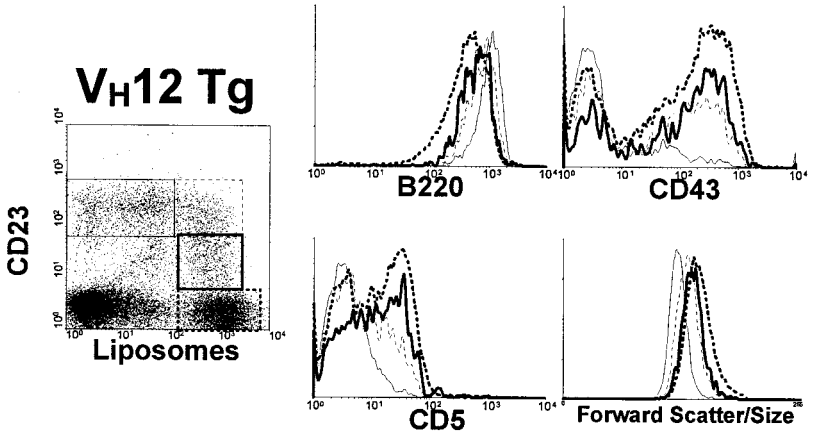

FIGURE 3. 6-1 mice show a progression of differentiation of PtC^{bright} cells from B-0 to B-1. 6-1 mice have multiple populations of B cells when stained with liposomes and CD23 as indicated by the boxes in the two-parameter histogram (*left*). These populations differ in phenotype as indicated by the one-parameter histograms to the *right*. The populations are coded as shown with the boxes in the two-parameter histogram.

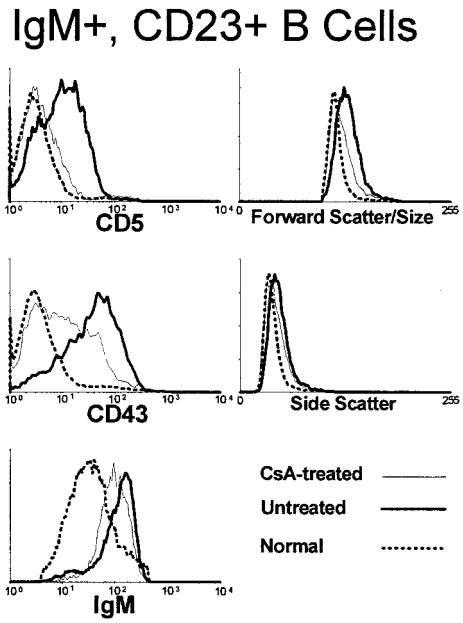

FIGURE 4. Long-term treatment of Dbl Tg mice with CsA leads to an accumulation of PtC-specific cells that have a B-0 phenotype. Dbl Tg mice treated daily with CsA or left untreated were compared by flow cytometry for phenotypic differences. Shown is the phenotype of the IgM^+, $CD23^+$ population. For comparison, the $CD23^+$ cells of a non-Tg littermate are shown (Normal).

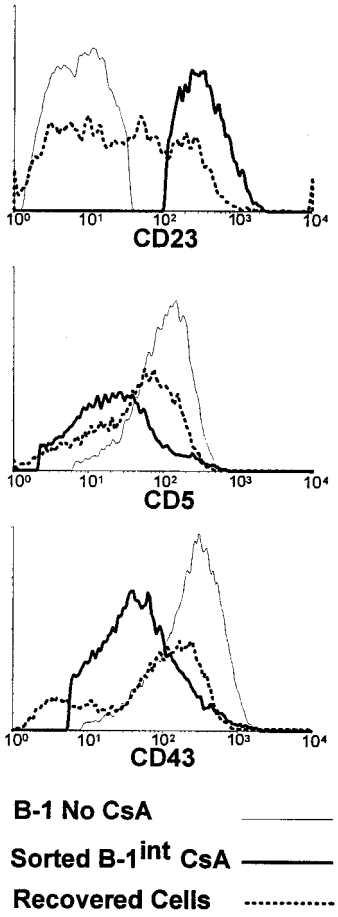

FIGURE 5. PtC-specific B-0 cells from CsA-treated Dbl Tg mice differentiate to B-1 after adoptive transfer to sublethally irradiated mice. Shown are the phenotypic comparison of the $CD23^+$ cells from CsA-treated Dbl Tg mice (Sorted $B\text{-}1^{int}$ CsA) and the cells recovered from recipient mice 6 days later (Recovered Cells). For reference, the phenotype of B-1 cells (IgM^+ $CD23^-$) from a Dbl Tg mouse that have not been treated with CsA (B-1 No CsA) is included.

Daily treatment of Dbl Tg mice with CsA for 2 wk results in the accumulation of PtC-specific cells with a more B-0-like phenotype (Fig. 4). The $B\text{-}1^{int}$ cells from CsA-treated mice are $CD5^-$ and IgM^{low}, and all have reduced their CD43 levels with many showing little or no CD43 expression. Despite a more B-0-like phenotype, CsA-treated cells nonetheless retain the ability to differentiate to B-1 (Fig. 5). Transferred $CD23^+$ cells from CsA-treated Dbl Tg mice decreased CD23 expression and increased CD5 and CD43 expression (Fig. 5).

Discussion

In this paper, we describe anti-PtC B cells that have the phenotypic characteristics of both B-0 and B-1 cells. Our analysis of these cells suggests that they are intermediate ($B\text{-}1^{int}$) in a differentiative pathway from B-0 to B-1; they differentiate to B-1 upon adoptive transfer, and they acquire a more B-0-like phenotype upon exposure to CsA. However, CsA does not induce a complete loss of CD5 and CD43 expression in the transfer experiments (Fig. 2*C*), and CD43 expression is not completely lost by long-term CsA treatment (Fig. 4). We attribute this to the influence of constant Ag stimulation driving differentiation toward B-1, and to the short time frame of the transfer experiments. However, a more complete block in differentiation, mediated by the *xid* mutation, results in PtC-specific B cells that have the phenotype of conventional (B-0) cells of *xid* mice and that do not express either CD5 or CD43 (25). Taken together then, we conclude that anti-PtC B cells differentiate from B-0 to B-1 as a result of signaling through IgM. We further conclude, based on the effect of CsA on $B\text{-}1^{int}$ cells, that $B\text{-}1^{int}$ differentiation is reversible. Whether differentiation of cells that have reached the B-1 cell stage is reversible is presently unknown. This pathway, which is illustrated in Fig. 6, was initially proposed by Wortis and coworkers (19, 20) as a result of their observation that splenic B-0 cells can be induced to differentiate to B-1 in vitro by treatment with anti-IgM and IL-6.

Our findings have bearing on the relationship between B-1a and B-1b cells. Transferred $B\text{-}1^{int}$ cells give rise to both B-1a and B-1b cells. Thus, cells of these B-1 subsets must diverge late in differentiation from B-0. Moreover, because $B\text{-}1^{int}$ cells are $CD5^+$, these data indicate that B-1b cells initially express CD5 and then lose CD5 expression during differentiation. The significance of CD5 loss to the function of these cells is unknown. One possibility is that CD5 may regulate the differentiation to Ab-secreting cells because CD5 is a negative regulator of IgM receptor signaling (34). Regardless of its role, because the $B\text{-}1^{int}$ cells in this study are all PtC-specific, the divergence to the B-1a and B-1b subsets must not be on the basis of specificity.

A B-0 to B-1 differentiation pathway provides a B cell activation framework for the interpretation of the numerous B cell receptor signaling and coreceptor knockout models that differentially affect B-1 and B-0 cells. For example, CD19 knockout and CD21 knockout mice lack B-1 cells (35–38), as do mice lacking the cytoplasmic kinases Vav and protein kinase C-βI/II (39–41). Conversely, mice that overexpress CD19 and mice deficient in Src homology domain 2-containing phosphotyrosine phosphatase-1 have excessive numbers of B-1 cells (36, 37, 42). In light of our evidence of a B-0 to B-1 differentiative pathway, the effects of these signaling

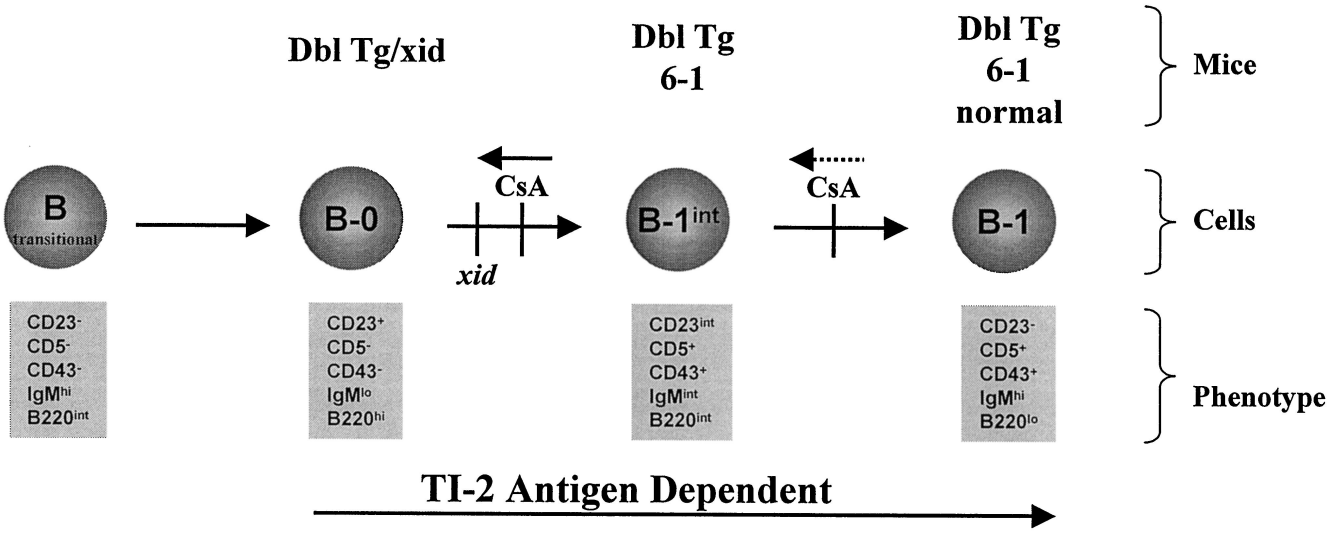

FIGURE 6. Schematic of B-1 cell differentiation based on the analysis of PtC-specific B cell differentiation. In this schematic, it is presumed that PtC-specific B cells pass through a transitional B cell stage before becoming B-0 cells that express CD23, although the existence of PtC-specific transitional cells has not been demonstrated. We propose that B-0 cells will differentiate to B-1 if stimulated by an appropriate Ag, presumably a TI-2 Ag. The mice in whom PtC-specific cells of each type have been observed are given across the top. Normal mice have a significant population of PtC-specific B-1 cells (12), and it is presumed that they have PtC-specific B-1^{int} cells but that their number is too low to detect. *xid* blocks differentiation from B-0 to B-1^{int} (25). Other mutations affect this pathway as discussed in the text. CsA blocks differentiation of B-1^{int} to B-1 and probably blocks differentiation of B-0 to B-1^{int}. It may also cause B-1^{int} cells to revert to B-0. The effect of CsA on cells that have already differentiated to B-1 is not known, as indicated by the dotted reverse arrow.

molecules on B-1 cells should be viewed in the context of Ag-driven B-0 to B-1 cell differentiation rather than as having differential effects on B-0 and B-1 lineages independent of Ag stimulation. This interpretation is consistent with the known roles these molecules play in B cell Ag activation.

B-1^{int} cells appear to be present in non-Tg mice. B cells expressing both CD23 and CD5 are present in the spleens of 7- to 10-day-old non-Tg mice (20). These cells may be equivalent to the B-1^{int} cells described here, which would mean that they are in transition to B-1 as suggested (20). They may be visible in very young non-Tg mice because the B-1 population is developing rapidly; B-1 cells reach their highest frequency (40–45%) at day 9 (43). B-1^{int} cells may be visible in adult Dbl Tg mice because, like in young non-Tg mice, a large proportion of the B cells are differentiating to B-1.

Ag appears to be required for anti-PtC B-1 cell differentiation, as suggested by the effects of the *xid* mutation (25) and CsA. In addition, we have shown a correlation between the ability to bind PtC and segregation to B-1 (22), and Lam and Rajewsky (23) have shown that Ag receptor density is important in anti-PtC B-1 differentiation, indicating that signaling through surface IgM is critical. The Ag responsible for anti-PtC differentiation to B-1 is unknown. PtC is an abundant phospholipid, so the Ag could be self. If so, the irradiation of recipient mice for B-1^{int} cell transfers may have increased the levels of this Ag and contributed to driving B-1 differentiation. Transfer to unirradiated mice yielded too few recovered donor B cells to test this possibility. However, involvement of a non-self-Ag cannot be ruled out. In fact, anti-PtC Abs appear to have a protective effect against certain bacterial infections (44), suggesting this possibility.

Whatever the Ag driving PtC-specific B-1 differentiation, it and the other Ags that drive B-1 differentiation are most likely TI-2 Ags; TI-2 stimulation in vitro can induce a B-1 phenotype (19, 20), and B-1 cells are present in nude mice (1). Moreover, *xid* mice, which respond poorly to TI-2 Ags (45), have few B-1 cells (1). The specificities known to be associated with B-1 are typically Ags that cannot elicit T cell responses (carbohydrates, lipids, and DNA (7, 10–14)), further supporting the idea that TI-2 Ags drive B-1 cell differentiation. Both self- and non-self-Ags can be involved in B-1 cell development; Hayakawa et al. (13) have shown that development of B-1 cells specific for a carbohydrate epitope on the self-Ag Thy-1 is dependent on the presence of Thy-1 itself, and Whitmore et al. (46) have shown that immunization with the non-self-Ag polyvinyl pyrrolidone (PVP) produces a population of anti-PVP B-1 cells.

A variety of factors that affect B cell specificity have been suggested to be key to the burst in B-1 cell differentiation in the neonate (20). These include the absence in neonatal lymphocytes of terminal deoxynucleotidyl transferase (47–51), which results in little or no N region addition among neonatal B cells (in contrast to adult bone marrow-derived cells) and a bias in the neonate relative to the adult in V_H gene rearrangements (52–54). But other factors including the availability of Ags and other nonantigenic factors (e.g., cytokines or other cells) that drive B-1 cell differentiation or differences in the specificities produced as mice age could be involved. A recent report suggests that B cell differentiation in the neonate is less dependent on the efficient formation of a pre-B cell receptor than it is in the adult (55). Specifically, B cells expressing the V_H11 gene were favored in the neonate over the adult. Because V_H11 is also used to encode anti-PtC Abs (4, 5), this mechanism may favor the expression of cells that have B-1 specificities. Nevertheless, the neonatal repertoire appears to be biased by a variety of mechanisms that may be evolutionarily selected to rapidly fill the B-1 repertoire, leading to a rapid enlargement of the B-1 compartment relative to the B-0 compartment in neonates. In this regard, we have demonstrated that anti-PtC B cells are selected at multiple differentiative checkpoints to ensure that a high frequency of V_H12-expressing B cells have an anti-PtC V_HCDR3 (56) and express $V_\kappa4/5H$ (22), and Booker and Haughton (57) have shown that the V_H11 and V_H12 genes are evolutionarily conserved. This

is evidence of strong evolutionary selection for the development of an anti-PtC response, presumably to promote the survival of every individual.

B-1 cells may be receptive to T cell help (58). If this is a pathway generally open to these cells and they can be drawn into germinal centers, they may undergo affinity maturation through somatic hypermutation and selection as well as heavy chain class switch. Because there is a bias toward self-specificities among B-1 cells, they could be an important reservoir of anti-self B cells in autoimmune diseases. It is possible that B-1 cells are activated with T cell help and are driven to produce pathogenic Abs through somatic mutation and selection in a germinal center. In light of the evidence that B cells can move between subsets, an assessment of the potential of these B cells to be activated by T-dependent Ags in autoimmunity is warranted.

Acknowledgments

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