Antibody class switching differs among SJL, C57BL/6 and 129 mice

Denise A. Kaminski1,2 and Janet Stavnezer1

1Immunology and Virology Program, Department of Molecular Genetics and Microbiology, University of Massachusetts Medical School, Worcester, MA 01655, USA

2Present address: Trudeau Institute, Saranac Lake, NY 12983, USA

Keywords: B cells, inbred mouse strains, isotype switch

Abstract

Inbred mouse strains used for gene manipulation studies vary in many respects, including immune system function. These differences can interfere with data interpretation unless the mice are well backcrossed. Here, we show that antibody class switching to IgG3 in cultured splenic B cells from Swiss James Lambert (SJL) and 129/Sv mice is 2- to 6-fold less efficient compared with C57BL/6 (B6). Under optimal stimulation conditions, IgA switching is also 2- to 19-fold lower in SJL and 129/Sv B cells. Splenic B cells from SJL mice express higher levels of CD19 and CD21 compared with B6, and their CD21highCD23low B cells have little CD9 expression, suggesting atypical marginal zone (MZ) B cells. However, sort purification of splenic B cell subsets did not equalize in vitro class switching to IgG3 or IgA between SJL and B6. 129/Sv spleens have a 3-fold greater number of MZ B cells compared with B6, with similar CD9 expression. Poor IgG3 switching by 129/Sv B cells is specific to CD23high follicular B cells, whereas similar changes in IgA switching are seen in both CD21high and CD23high B cell subsets from 129/Sv. Therefore, the functions and phenotypes of mature B cells differ among three common strains of mice.

Introduction

B cell activation can result in a switch of antibody heavy-chain isotypes from IgM/IgD expression to IgG, IgE or IgA (1, 2). The heavy-chain class determines the antibody effector function, and therefore its mode of pathogen elimination. Understanding the regulation and mechanisms of class switching is facilitated by gene-targeted and transgenic mouse models. Although strains with mixed genetic backgrounds are thought to be preferential for deriving genetically manipulated animals (3–5), the combination of alleles from the different backgrounds can complicate interpretation of experimental results. In such cases, it is important to know the particular characteristics of each strain.

The Swiss James Lambert (SJL) strain is commonly used for creating transgenic mice (5), and is also used for experimental autoimmune encephalomyelitis, an inducible model of multiple sclerosis, because these mice have a pre-existing T cell repertoire against neuronal antigens (6). Additionally, the SJL genome contains a retroelement-encoded superantigen (sAg), which stimulates Vβ16 CD4+ T cells to secrete cytokines that support growth of B cell lymphomas that arise in these mice (7, 8).

SJL mice have a glycine to valine amino acid substitution in the constant region of the Ig λ1 light-chain gene (9). This substitution impairs intracellular calcium responses to anti-Ig λ stimulation of cultured B cells (10), and disrupts B cell development in Ck−/− mice (10, 11). Correspondingly, SJL mice have 10-fold less serum λ1+ antibody compared with C57BL/6 (B6) (12).

λ light chains are used more frequently by B1 B cells compared with conventional B2 B cells. SJL mice have 10-fold fewer peritoneal B1 cells compared with BALB/c (13). This deficiency may be due to the defective λ1 being less likely to be selected for survival and self-renewal of B1 cells (13).

In contrast to BALB/c, SJL mice have no detectable serum antibody responses to prior protein (14). It is unclear whether this observation is due to the λ1 mutation or to another defect. SJL B cells also have a 2.8-fold lower splenic antibody response to α1 → 3 dextran than B6, although neither strain utilizes λ light chains for this response (15). Therefore, not all differences in B cell function between SJL and B6 are attributable to the λ1 protein. Although SJL mice are used for transgenesis (3, 5), and despite the use of
transgenic mice to study class switch recombination (CSR) (16, 17), class switching in unmanipulated SJL mice has not been reported.

129 mice, which are commonly used for gene targeting (18), also differ in mature B cell function compared with B6 mice. 129 (both Sv and Ola substrain) splenocytes have a modest decrease in proliferative responses to anti-IgM compared with B6 (19). 129 splenocytes also have a defective proliferative response to IL5 in the presence of anti-CD38 stimulation. This defect is associated with lower expression of the IL5 receptor α and common γ chain mRNA in 129 B cells compared with B6 (19). This poor IL5 response may explain a paucity of B1 cells in most 129 mice, although the reduction is not consistently found (19).

Because genetically manipulated mice are important for studying CSR, it is important to establish how well different strains of mice undergo class switching. Here, we compare the phenotypes and in vitro responses of splenic B cells among SJL, B6 and 129/Sv (hereafter, 129) mice. Our observations have important implications for using different inbred mouse strains in studies of B cell function, including isotype switching.

**Methods**

**B cell preparation, culture and flow cytometry**

Mice were housed for at least 2 weeks in our specific pathogen-free facility prior to experiments. Mice were bred and used under guidelines formulated by the University of Massachusetts Animal Care and Use Committee. T-depleted total splenic B cells from 8- to 12-week old B6 (National Cancer Institute (NCI) or Charles River), SJL (NCI) and 129/Sv (Charles River) mice were prepared as described (20). Results using B6 mice from either source did not significantly differ and the data were pooled. For B6 and SJL, results between male and female mice did not differ and were also pooled. Only female 129 mice were used and the results from these mice differed from both female and male B6 mice to the same degree (D. A. Kaminski, unpublished data). Sort-purified splenic B cells were prepared by CD43 depletion by MACS (Miltenyi Biotech, Auburn, CA, USA) followed by staining in 1% FCS in RPMI with anti-B220apc (RA3-6B2, Caltag, Burlingame, CA, USA), anti-CD23 PE (2G8, Southern Biotechnology Associates, Birmingham, AL, USA) and anti-CD21 FITC (7G6, BD PharMingen, San Jose, CA, USA) for cell sorting using a FACSVantage cell sorter (BD PharMingen).

B cells were plated at 10^5 ml^-1 and stimulated with LPS, B lymphocyte stimulator (BLyS), IL4, IL5, transforming growth factor β (TGFβ) and anti-IgD–dextran (αD Dex) as described (21). SJL and B6 mice are IghB, 129 mice are IghA. The αD Dex preparation is a mixture of anti-IgDa (Hαa/1) and anti-IgDb (AF3). Flow cytometry to measure switched isotypes on cultured cells was performed as described (20). For the analysis, 50,000–75,000 events per sample were acquired. The frequency of switched cells was determined by gating on IgG1 IgM^- or IgA1 IgM^- events, as double-positive events are detectable in similar samples from class switching-defective aid^-/- mice (D. A. Kaminski, unpublished data).

Lymph nodes (LN) were homogenized between frosted glass slides and cell suspensions in 1% FCS/PBS were passed over glass wool. Peyer’s patches were repeatedly digested with collagenase (1 mg ml^-1 in serum-free RPMI) at 37°C, passed over glass wool and washed in 1% FCS/PBS. Spleen, LN and Peyer’s patch cells were analyzed by flow cytometry with antibodies against CD19PECy5 (eBioscience, San Diego, CA, USA), CD23PEb (eBioscience, San Diego, CA, USA), CD9 Alexa647 (MZ3) (22), a gift from John Kearney, University of Alabama at Birmingham, IgAPE (Southern Biotechnology Associates), HSAbiotin (Caltag) / streptavidinPerCP (BD PharMingen), CD21FITC and CD23PE.

**Fig. 1.** IgG switching in splenic B cell cultures from SJL, B6 and 129 mouse strains. T-depleted splenocytes, prepared as described in Methods, were cultured for 4 days as indicated, and analyzed by flow cytometry. Lymphoid cells were gated by forward and side scatter. αD Dex was used at 0.3 ng ml^-1 and IL4 at 2400 U ml^-1. (A) Flow cytometry profiles of IgG and IgM expression in B6 and SJL cells. (B) Compiled results showing the frequency of class-switched cells in splenic B cell cultures. Mean ± SD from four to nine mice. *P = 0.047, **P < 0.0045 and ***P < 0.00084.
Carboxy-fluorescein diacetate, succinimidyl ester staining
Freshly isolated splenic B cells were washed with HBSS and incubated in 0.25 μM carboxy-fluorescein diacetate, succinimidyl ester (CFSE, Invitrogen–Molecular Probes, Eugene, OR, USA) in HBSS at 37°C for 15 min, and then washed with and re-suspended in complete media for culture.

Results
Class switching in splenic B cells from SJL, B6 and 129 mice
In studies of mice on a mixed B6 and SJL genetic background, we observed considerable variation in the frequency of antibody class switching (D. A. Kaminski, unpublished data). To ask whether this variation is due to genetic differences, we examined class switching in B cells from each parent strain. T-depleted splenocytes were stimulated for 4 days to induce class switching, and then analyzed by flow cytometry. Figure 1 shows that the frequency of B cells that class switched to IgG3 after stimulation with BlyS and LPS ± αDex was at least 4-fold lower in SJL cultures compared with B6. The frequency of IgG2b+ cells was 1.9-fold lower in SJL B cells compared with B6 when stimulated with BlyS/LPS and TGFβ and class switching to IgA was 2.8- to 19-fold lower in SJL B cells depending on the induction conditions (Fig. 2A and B). Additionally, the frequency of IgA+ B cells in the Peyer’s patches was 3.7-fold lower (Fig. 2C and Supplementary Table S1, available at International Immunology Online), suggesting less efficient IgA class switching in vivo. Of the four isotypes tested in culture, only IgG1 switching induced by BlyS/LPS/IL4 was similar between the two strains (Fig. 1B).

We next asked whether class switching differs between B cells derived from 129 and B6 mice. As in SJL mice, 129-derived T-depleted splenocytes cultured for 4 days in BlyS/LPS ± αDex had a lower frequency of IgG3 switching compared with B6, although the effect was less pronounced (Fig. 1B). Also similar to SJL, 129-derived B cells had 1.6-fold lower IgG2b switching, and no change in IgG1 compared with B6.

In contrast, IgA switching was 2.5-fold higher in 129 compared with B6 B cells stimulated with BlyS/LPS/TGFβ alone (Fig. 2B). However, IgA switching was not enhanced in 129 cells by further addition of either IL4/IL5/0.3 ng αDex or with 3 ng αDex. Thus, 129 B cells have 2- to 3-fold lower IgA switching than B6 cells under these ‘optimal’ stimulation conditions. The frequency of IgA+ Peyer’s patch B cells appeared higher in 129 than in B6 mice, but the difference was not significant (Supplementary Table S1, available at International Immunology Online).

Therefore, compared with B6 mice, B cells from SJL and from 129 mice have class switching defects for IgG3 and IgG2b. IgA switching is also reduced in SJL B cells, but in 129 B cells, IgA switching is higher than in B6 when minimal stimulation is used, and is not enhanced by additional activators. Because IgG1 switching is similar among the three strains, we infer that genes encoding the basic CSR

Fig. 2. IgA switching in splenic B cell cultures from three inbred mouse strains. Cultures and analyses were performed as in Fig. 1. αDex was used at 0.3 or 3 ng ml⁻¹ as indicated. IL4 was used at 800 U ml⁻¹. (A) Flow cytometry profiles of IgA and IgM expression. (B) Compiled results showing the frequency of class-switched cells in splenic B cell cultures. Mean ± SD from four to seven mice. *P ≤ 0.03, **P ≤ 0.0058, ***P ≤ 0.00061 and §P = 0.0000025 versus BlyS/LPS/TGFβ/3 ng αDex from B6. (C) IgA and IgM expression on B220+ Peyer’s patch lymphocytes. Representative of nine B6 and three SJL mice.
machinery, e.g. activation-induced cytidine deaminase and DNA repair genes, are similar among these strains, and once induced, the gene products can function equivalently.

Proliferation differences among strains do not account for differences in class switching
Because class switching correlates with cell division (23), we asked whether in vitro B cell proliferation differs among these mouse strains. Figure 3(A) shows that [3H]thymidine ([3H]TdR) incorporation was ~2- to 3-fold lower in SJL B cells compared with B6 when stimulated with BLyS/LPS or BLyS/LPS/TGFβ for 48 h. This lower proliferation correlates with lower IgG3, IgG2b and IgA switching in SJL compared with B6 B cells cultured in these conditions. However, when αDex was added to BLyS/LPS, B6 and SJL B cells proliferated equally well, although IgG3 class switching was 3-fold lower in SJL B cells (compare Fig. 1 with Fig. 3A). Likewise, proliferation in response to BLyS/LPS/TGFβ/IL4/IL5/αDex was similar in SJL and B6 cultures, despite lower IgA switching in SJL cells under these conditions (compare Fig. 2A and B with Fig. 3A). Therefore, although SJL B cells proliferate poorly relative to B6 in some culture conditions, proliferation does not consistently correlate with class switching. [3H]TdR incorporation was comparable between 129 and B6 at 48 h for all conditions tested (Fig. 3B).

To further examine whether the differences in class switching among SJL, B6 and 129 B cells could be due to differences in cell division, we used CFSE labeling to follow class switching as cells divided (23). Figure 4(A) shows that SJL B cell cultures have a lower percentage of IgG3+ cells at each cell division than do B6 when stimulated with BLyS/LPS ± αDex. Similar results were observed for B6 compared with 129 B cells (Fig. 4B). Therefore, poor IgG3 switching in SJL and 129 B cells is unlikely due to a difference in cell division. For B6 cells, but not for SJL or 129, αDex increases the frequency of IgG3+ cells per cell division. Lower IgG2b switching in SJL and 129 cells cultured with BLyS/LPS/TGFβ also appears to result from division-independent mechanisms (Fig. 4C and D).

In optimal stimulation conditions, the relatively poor IgA switching in SJL, B6 and 129 B cells could be due to differences in cell division, we used CFSE labeling to follow class switching as cells divided (23). Figure 4(A) shows that SJL B cell cultures have a lower percentage of IgG3+ cells at each cell division than do B6 when stimulated with BLyS/LPS ± αDex. Similar results were observed for B6 compared with 129 B cells (Fig. 4B). Therefore, poor IgG3 switching in SJL and 129 B cells is unlikely due to a difference in cell division. For B6 cells, but not for SJL or 129, αDex increases the frequency of IgG3+ cells per cell division. Lower IgG2b switching in SJL and 129 cells cultured with BLyS/LPS/TGFβ also appears to result from division-independent mechanisms (Fig. 4C and D).

In optimal stimulation conditions, the relatively poor IgA switching in SJL (Fig. 5C and E) and 129 (Fig. 5D and F) B cells compared with B6 is also independent of cell division. In contrast, IgA switching induced by the minimal conditions of BLyS/LPS/TGFβ is similar at each cell division between SJL and B6 B cell cultures (Fig. 5A). Therefore, poor proliferation of SJL B cells likely contributes to the lower IgA switching under minimal conditions, but does not account for lower IgA switching under optimal stimulation. Higher BLyS/LPS/TGFβ-induced IgA switching in 129 compared with B6 B cells appeared to be independent of cell division (Fig. 5B). Collectively, these results suggest that IgA switching under minimal conditions is regulated not only by cell division, but also by other mechanisms.

Freshly isolated splenic B cells differ among mouse strains
To determine whether class switching among mouse strains correlates with differences in B cell phenotype, we used flow cytometry to characterize freshly isolated splenic B cells based on cell-surface antigen expression. The frequency of splenocytes expressing the B cell markers B220 or CD19 is somewhat lower in SJL spleens compared with B6 (Fig. 6A). This difference is much greater in inguinal LN (Supplementary Table S2, available at International Immunology Online).

One speculation is that the SJL B cells might have a trafficking defect. Another difference between SJL and B6 is that the fluorescence intensity of CD19 is higher on splenic B cells from SJL [mean fluorescence intensity (MFI) = 1149 ± 372] than from B6 (654 ± 237, P = 0.031) and also on LN B
cells (Fig. 6A and unpublished data). Expression of CD21, a complement receptor that forms a signaling complex with CD19 (24, 25), is also higher on splenic B cells from SJL (MFI = 97.7 ± 20.3) than B6 (40.0 ± 8.6, P = 0.027) (Fig. 6B). It is unclear whether differential expression of these molecules between SJL and B6 B cells is related to the differences in LPS responsiveness observed. The expression of HSA (or CD24) is similar between the two strains, and CD23 (FcεRII) expression is modestly lower on SJL B cells (Fig. 6B).

Studies in rats and BALB/c mice showed that CD21 expression in the spleen is highest in the marginal zone (MZ) B cell population (26, 27). These cells, in contrast to B cells in the follicles, express little or no CD23 (27). Although SJL B cells express high levels of CD21, the distribution of CD21highCD23low and CD21lowCD23high populations were similar between SJL and B6 when the analysis gates were adjusted to the individual contours (Fig. 6C), suggesting that the frequency of MZ and follicular (FO) B cells are similar between the two strains. We thus asked whether the CD21highCD23low population in SJL mice has characteristics of MZ B cells as previously described for B6 and BALB/c mice. In both B6 and SJL spleens, CD21highCD23low B cells have a higher forward scatter than CD21lowCD23high B cells, indicating larger cell size (Fig. 6D). Most B6 CD21high, CD23low B cells express the tetraspanin CD9, whereas the CD21lowCD23high B cells express little to none of this marker (Fig. 6D), similar to published results (22). In contrast, SJL CD21highCD23low B cells expressed very little CD9; however, its levels on non-B cells were similar between B6 and SJL (Fig. 6E). Although there is no known function in B cells for CD9 (28), our result suggests that this SJL population does not share all characteristics as previously described for MZ B cells.

A similar analysis of 129 mice is shown in Fig. 7. B6 and 129 mice have similar proportions of B cells in the spleen (Fig. 7A, Supplementary Table S2, available at International Immunology Online). Splenic B cells in adult B6 mice are divided into an HSAlow population consisting of relatively mature B cells and an HSAhigh population consisting of immature (newly formed) and MZ B cells, as previously described for BALB/c mice [Fig. 7B and (29)]. 129 B cells showed instead a broad distribution of HSA expression whose MFI, 26.4 ± 0.14, was intermediate to the two populations in the B6 samples (8.16 ± 0.31 and 80.5 ± 0.85). This difference, shown previously (19), suggests that 129 and B6 splenic B cells have dissimilar differentiation programs. This hypothesis is supported by a larger CD23− B cell population in 129 relative to B6 spleens (Fig. 7B). CD21highCD23low (MZ) B cells in the 129 mice showed similar forward scatter and CD9 expression compared with the same cells from B6 mice (Fig. 7D); however, these cells were 2.7-fold more frequent and their absolute number was 3.1-fold greater in 129 compared with B6 (Fig. 7C and E). Newly formed (CD21lowCD23low) B cells were also increased (1.7-fold) in 129 compared with B6 (Fig. 7C and E).

The observations from Figs 6 and 7 demonstrate that splenic B cells in SJL, B6 and 129 mice differ in surface phenotype and distribution of B cell sub-populations. Together
with our class switching experiments, these results suggest that splenic B cells among these three mouse strains have distinct differentiation patterns and functional capabilities.

**Class switching in sort-purified B cell subsets differs among mouse strains**

We next asked whether unequal class switching observed among these inbred mouse strains could be due to differences in specific B cell sub-populations. Splenic B cells were sort purified into B220$^{+}$CD21$^{\text{high}}$CD23$^{\text{low}}$ (CD21$^{\text{high}}$ for brevity) and B220$^{+}$CD21$^{\text{low}}$CD23$^{\text{high}}$ (CD23$^{\text{high}}$) fractions, as indicated in Fig. 8, to test the ability of these purified populations to isotype switch. IgG3 switching stimulated by BLyS/LPS was at least 6-fold lower in each SJL B cell population than in the corresponding population from B6 mice (Fig. 9A and data not shown), similar to total B cell cultures (Fig. 1). Although IgG3 switching in the 129 CD23$^{\text{high}}$ population was 2.2- to 3.5-fold less than in the B6 CD23$^{\text{high}}$ B cells, IgG3 switching was similar in CD21$^{\text{high}}$ B cells from B6 and 129 mice (Fig. 9A). Therefore, the defect in IgG3 switching in SJL mice is observed in both CD21$^{\text{high}}$ and CD23$^{\text{high}}$ populations, but in 129 mice, the defect is specific to the CD23$^{\text{high}}$ FO B cells. IgG2b switching was 2-fold lower in both CD21$^{\text{high}}$ and CD23$^{\text{high}}$ B cells from SJL mice compared with B6, and was also lower in these populations from 129 (Fig. 9B).

IgA switching was 5.8-fold more efficient in CD21$^{\text{high}}$ than in CD23$^{\text{high}}$ cells from B6 mice when each population was stimulated with BLyS/LPS/TGF$\beta$ (solid gray bars in Fig. 9C; (30)). However, in SJL, there was no difference in IgA switching between CD21$^{\text{high}}$ and CD23$^{\text{high}}$ cells, and the

---

**Fig. 5.** IgA switching during cell division. As in Fig. 4. (A, C and E) B6 versus SJL and (B, D and F) B6 versus 129 in the culture conditions indicated.
level of switching was very low in both subsets (Fig. 9C). Therefore, B6 MZ B cells are more poised to switch to IgA upon minimal stimulation compared with FO B cells, and the cause of this readiness is lacking in the corresponding B cell population from SJL mice.

CD23high B cells from both SJL and B6 mice responded to IL4/IL5/0.3 ng αDex in BLyS/LPS/TGFβ cultures, increasing IgA switching by ~12-fold, although the resulting frequency of IgA was still lower in the SJL cultures (Fig. 9C). Addition of 3 ng αDex to BLyS/LPS/TGFβ cultures resulted in 34-fold more IgA switching in B6 CD23high B cells compared with SJL. Therefore, IL4/IL5/αDex, but not high-dose αDex, can enhance IgA switching in CD23high B cells from SJL mice, similar to total B cell cultures.

IgA switching by 129 CD23high cells stimulated with BLyS/LPS/TGFβ was higher than in B6 CD23high cells (Fig. 9C). Therefore, the higher IgA switching observed in unsorted 129 B cells under these conditions (Fig. 2B) is unlikely due to the over-representation of MZ B cells. CD21high B cells from 129 mice switched more frequently to IgA than the same subset from B6, although the difference was not significant (Fig. 9C). Stimulation with the optimal conditions does not increase IgA switching by 129 CD23high cells. Therefore, CD23high cells from 129 mice switch well under minimal conditions, but unlike the same subset from B6, they did not respond to further stimulation with either IL4/IL5/αDex or with αDex at a higher dose.

Discussion

Class switching differs among three inbred mouse strains

We have shown that splenic B cells from both SJL and 129 mice undergo poor IgG3 and IgG2b, but similar IgG1 class switching compared with B cells from B6 mice when stimulated under identical culture conditions. Differences in IgA switching were also observed, and varied from modest to extreme, depending upon the stimuli used. Whereas IgA switching is lower in both SJL and 129 compared with B6 under optimal stimulation conditions (BLyS/LPS/TGFβ with IL4/IL5/0.3 ng αDex or with 3 ng αDex), IgA switching induced by the minimal stimuli of BLyS/LPS/TGFβ is highest in 129 B cells, intermediate for B6 and poor for SJL.

Sort purification of splenic B cells showed that IgG3, IgG2b and IgA switching are inefficient in both CD21high (MZ) and CD23high (FO) B cells from SJL mice, similar to results for total splenic B cells. In 129, low IgG3 switching appears specific to the FO B cells, and low IgG2b switching is specific to MZ B cells. IgA switching is higher in 129 compared with B6 and SJL in both B cell subsets cultured with...
minimal stimuli. However, IgA switching in 129 B cells is not further increased with IL4/IL5/αβDex (unlike B6 or SJL) or with high-dose αβDex (unlike B6).

We have previously shown that MZ and B1 B cells from B6 mice switch to IgA with greater efficiency than FO B cells when each is given minimal stimulation (30). Therefore, MZ B cells in B6 mice either have an IgA-promoting factor that is lacking in FO B cells or MZ B cells lack an IgA-suppressive factor that is present in FO B cells. Both CD21^hi MZ-like and CD23^hi FO B cells from SJL mice switch poorly to IgA under both minimal and optimal stimulation conditions, suggesting a mechanism that does not differ between these two populations of B cells. However, CD23^hi FO B cells from 129 mice switch to IgA almost as efficiently as their MZ B cells under minimal conditions. This observation suggests that the FO B cells of 129 mice either have the IgA-promoting factor of MZ B cells or lack the IgA-suppressive factor possessed by FO B cells from B6 mice. B cells deficient in the histone methyltransferase Suv39H1 (on the B6 background) have less efficient IgA class switching than Suv39H1-sufficient cells (31); however, this difference is observed in both MZ and in FO B cells (D.A.K., unpublished data). An interesting candidate suppressor is Late-SV40 Factor (LSF/CP2), which suppresses IgA class switching in the I.29μ B cell lymphoma (32). A systematic screen of genes and signaling events induced in B6 versus 129 FO B cells is likely to reveal regulators of IgA class switching. We have not examined B1 B cells in the SJL and 129 mice because each has a paucity of such cells.

Our results collectively indicate that isotype-specific switching differs in a B cell-autonomous manner among three commonly used inbred mouse strains. Differences in B cell proliferation among SJL, B6 and 129 B cells do not account for the differences in isotype switching. Collectively, our results may have important implications for studies using mice on mixed genetic backgrounds.

SJL mice develop T cell-dependent B cell lymphomas (7, 8, 10, 11). It is unclear whether B cells of this strain have a higher propensity to be transformed or if spontaneous transformation is similar to other strains and instead the tumor propagates due to the presence of sAg-activated SJL T cells. Since we find a poor proliferative response of SJL B cells to BLyS/LPS, the latter explanation would seem more likely.

In mice, including SJL, the Igλ light chain is expressed on only ~5% of splenic B cells and accounts for ~5% of serum Ig (11). This biased usage, together with the fact that in our
experiments we activate B cells principally through Toll-Like Receptors and cytokine receptors, indicates that the defect previously described for $\lambda_1$ protein in SJL mice is unlikely to account for the observed differences in class switching.

SJL B cells responded well to $\alpha_\omega$Dex by increased proliferation in the presence of BLyS/LPS; however, this reagent had little effect on IgG3 or IgA switching in SJL, unlike B6 B cells. These results suggest that $\alpha_\omega$Dex enhances isotype switching in B6 by a mechanism other than its ability to promote B cell proliferation, a concept supported by increased IgG3 per cell division in B6 B cells treated with BLyS/LPS/ $\alpha_\omega$Dex compared with BLyS/LPS alone. B cells from 129 mice have lower levels of mRNA for IL5 receptor subunits associated with a lack of IL5 response during anti-CD38-induced spleen cell proliferation (19). We found that IgA switching induced by BLyS/LPS/TGFβ is not enhanced in 129 cells by IL4/IL5/$\alpha_\omega$Dex, differing from both B6 and SJL. IgA switching is also not enhanced in 129 B cells in response to high-dose $\alpha_\omega$Dex, unlike B6, but similar to SJL. Therefore, 129 B cells appear unresponsive to IL4/IL5/ $\alpha_\omega$Dex, which may reflect the previously described paucity of the IL5 receptor on these cells (19). Additionally, the 129 B cells have an impaired response to high-dose $\alpha_\omega$Dex, which suggest a defect in B cell receptor signaling.

Serum IgG3 levels in 129 mice were reported to be comparable to B6 (19). However, we find poor IgG3 switching in total splenic B cell cultures from this strain, which is largely attributable to FO B cells that constitute as much as 80% of splenic B cell preparations. Thus, in 129 mice, IgG3 secretion from the larger population of MZ B cells, which are known to rapidly differentiate into plasma cells (33), may compensate for poor IgG3 switching by the FO B cells, to generate a total serum level similar to that of B6. Although serum IgG1 is slightly increased in 129 mice (19), we find that IgG1 switching in culture is similar between 129 and B6. Furthermore, serum IgG levels are also known to be regulated by their uptake from serum by the FcRn receptor (34). These results clearly indicate that Ig levels in non-immune serum and secretions are not adequate measures of isotype switching.

Fig. 8. Sort-purified splenic B cells from (A) B6 and SJL or (B) B6 and 129 mice. CD43-depleted splenocytes were gated on B220+ lymphocytes to sort purify the indicated populations. Right-hand profiles show color overlays of sorted and unsorted populations. Red events are the unsorted cells in the starting population, blue events are the sorted CD21$^{\text{high}}$ B cells and green events are the sorted CD23$^{\text{high}}$ B cells.

IgG1 class switching was equivalent in all three mouse strains examined. This result indicates that the basic recombination machinery is expressed and functional to a similar degree in all the mice. It also suggests that the basic signaling pathways downstream of TLR4 and the IL4 receptor function
with similar efficiency. The differences that we observe for other isotypes among mouse strains emphasize the uniqueness of how each is regulated. Our observations suggest that gene products from allelic variants (differing either in expression levels or gene product quality) in these strains regulate isotype specificity during CSR. It will be interesting to determine whether these influences are at the level of germ line transcription, switch region chromatin accessibility or perhaps of other levels. Ultimately, it will be important to determine (by linkage analysis or expression arrays) what these alleles are, and whether they encode protein or regulatory RNAs, which will provide valuable information for how isotype-specific class switching is regulated.

SJL B cells have higher CD19–CD21 co-receptor levels relative to B6 and 129

In addition to class switching differences, we have observed that SJL B cells express higher levels of the B cell co-receptors CD19 and CD21. Studies with cd19<sup>−/−</sup> mice, CD19 transgenic mice and various doses of the CD19 transgene on a cd19<sup>−/−</sup> or cd19<sup>+/+</sup> background showed that CD19 expression levels positively correlate with LPS responsiveness of B cells in culture (35). Thus, although we do not intentionally stimulate CD19–CD21 during our in vitro experiments, it appears paradoxical that in SJL mice, these molecules are expressed at high levels on B cells that have a poor proliferative response. This high expression may be a compensation mechanism to allow maturation of SJL B cells despite their poor responses to other stimuli. Alternatively, the high levels of CD21–CD19 might alter B cell development, and thereby indirectly cause poor B cell responses. The latter possibility is supported by studies of mice with a human CD19 transgene expressed at normal B cell developmental stages (36). In B6 bone marrow (BM), CD19 levels are modestly lower on early B<sub>220<sup>low</sup></sub> B lineage cells compared with B<sub>220<sup>high</sup></sub> mature B cells [(35) and data not shown]. Expression of transgenic hCD19 results in modest but significant reductions in BM and splenic B cells and more pronounced (up to 5-fold) reductions in peripheral blood B cells (35). We find that CD19 expression is similar on B<sub>220<sup>low</sup></sub> and B<sub>220<sup>high</sup></sub> BM cells of SJL mice, and in each case, this level exceeds that of the B<sub>220<sup>high</sup></sub> population in B6 BM (D. A. Kaminski, unpublished data). This observation suggests that the lower frequency of B cells, and perhaps as a cause or a consequence, poor proliferation, in SJL mice may result from the high CD19 levels.

**Fig. 9.** Class switching in purified CD21<sup>high</sup> and CD23<sup>high</sup> B cell subsets. Cells purified as in Fig. 8 were plated with the indicated stimuli and switched isotypes were assayed by flow cytometry on day 4. Mean ± SD from two to five mice. (A) IgG3 expression. (B) IgG2b expression. (C) IgA expression.
during B cell development. Nevertheless, hCD19 transgenic mice have increased peritoneal cavity and splenic B1 populations (37), in contrast to SJL mice, in which these cells are greatly reduced ([13] and data not shown). Further investigation of B lineage cells in SJL mice compared with CD19-deficient and CD19 transgenic models may clarify whether and how expression of this molecule contributes to the development, homeostasis and function of mouse B cells.

A human CD21 transgene driven by the mouse Vα2 promoter and λ2-4 enhancer is expressed on BM B220<sup>low</sup> cells, which normally do not express endogenous CD21 (38). This premature CD21 expression is associated with reduced B cell frequencies in the peripheral blood, BM and spleen, as well as reduced serum IgG. However, we have observed that CD21 is only expressed on B220<sup>high</sup> BM cells in each strain (D. A. Kaminski, unpublished data), suggesting that the mature B cell defects in SJL mice do not result from aberrant CD21 expression.

Collectively, our results demonstrate significant, B cell-intrinsic differences in antibody class switching among three commonly used strains of inbred mice. These findings have important implications for the use of these strains for studies of mature B cell biology.

**Supplementary data**

Supplementary tables S1 and S2 are available at *International Immunology* Online.

**Acknowledgements**

We thank Erin Linehan for technical assistance, John Kearney, Rachel Gerstein, Robert Woodland and Madelyn Schmidt for reagents and helpful discussions and the UMass FACS Facility for cell sorting. Supported by National Institutes of Health grants F32 AI056806 to D.A.K. and R21 AI062738 to J.S. The authors declare no conflict of interest.

**Abbreviations**

- **BM**: bone marrow
- **BLyS**: B lymphocyte stimulator
- **B6**: C57BL/6
- **CFSE**: carboxy-fluorescein diacetate, succinimidyl ester
- **CSR**: class switch recombination
- **αDex**: anti-IgD-dextran
- **FO**: follicular
- **[3H]TdT**: 3H-thymidine
- **LN**: lymph node
- **MFI**: mean fluorescence intensity
- **MZ**: marginal zone
- **NCI**: National Cancer Institute
- **sAg**: superantigen
- **SJL**: Swiss James Lambert
- **TGFβ**: transforming growth factor β

**References**