Brief Definitive Report

Somatic Hypermutation Shapes the Antibody Repertoire of Memory B Cells in Humans

Eric Meffre,[1] Nadia Catalan,[3] Françoise Seltz,[3] Alain Fischer,[2,3] Michel C. Nussenzweig,[1] and Anne Durandy[3]

[1]*Laboratory of Molecular Immunology, The Rockefeller University, Howard Hughes Medical Institute, New York, NY 10021*

[2]*Unite d'Immunologie-Hematologie and* [3]*Institut National de la Santé et de la Recherche Médicale (INSERM) U429, Hopital Necker-Enfants Malades, Paris 75015, France*

Abstract

High-affinity antibodies produced by memory B cells differ from antibodies produced in naive B cells in two respects. First, many of these antibodies show somatic hypermutation, and second, the repertoire of antibodies expressed in memory responses is highly selected. To determine whether somatic hypermutation is responsible for the shift in the antibody repertoire during affinity maturation, we analyzed the immunoglobulin lambda light chain (Igλ) repertoire expressed by naive and antigen-selected memory B cells in humans. We found that the Igλ repertoire differs between naive and memory B cells and that this shift in the repertoire does not occur in the absence of somatic hypermutation in patients lacking activation-induced cytidine deaminase (AID). Our work suggests that somatic hypermutation makes a significant contribution to shaping the antigen-selected antibody repertoire in humans.

Key words: immunoglobulin repertoire • activation-induced cytidine deaminase • somatic hypermutation • memory B cell • affinity maturation

Introduction

The affinity of antibodies for their cognate antigens increases during immune responses (1). In depth analysis of hybridoma antibodies specific for influenza hemagglutinin or for small chemical haptens such as 2-phenyl-5-oxazolone or 4-hydroxy-3-nitrophenyl acetyl (NP) revealed that somatic hypermutation is one of the mechanisms that produce this increased affinity (2–9). For example, the V_H186–2 + Igλ antibodies dominate the initial antibody response to NP, and mutation from Trp^{H33} to Leu^{H33} brings about a 10-fold increase in affinity of these V_H186–2 + Igλ antibodies (8, 10–13). Increased affinity is also accompanied by a shift in the antibody repertoire, and secondary high-affinity responses to NP are dominated by Igκ antibodies and not Igλ, suggesting that repertoire shifts contribute to affinity maturation (3, 10, 14, 15). Little is known about this shift in the repertoire and how it relates to somatic hypermutation.

Activation-induced cytidine deaminase (AID) is a germinal center B cell–restricted molecule that carries cytidine deaminase activity and is required for switch recombination and somatic hypermutation in mice and humans (16–18). In the absence of AID, B cells are unable to undergo somatic hypermutation or produce secondary antibodies despite germinal center formation (17, 18). Here we report on the Igλ antibody repertoire in humans deficient in AID. We find that AID is essential for the shift in repertoire between naive and antigen-selected memory B cells.

Materials and Methods

Patient Samples and Cell Preparation. AID-deficient patients and AID mutations have been described (18). Patients P1, P13, P14, P17, and P18 were 10, 11, 4, 14, and 2 yr old, respectively at the time of blood donation, and they did not suffer from chronic infections. They were treated with intravenous Ig supplementation. Control donors C1, C2, C3, C4, C5, C6, and C7 were healthy and 32, 11, 35, 2, 28, 41, and 33 yr old, respectively when blood samples were obtained. Blood mononuclear cells were isolated on Ficoll gradients. Control $CD19^+$ B cells were fractionated into naive $CD19^+IgM^+CD27^-$ and memory $CD19^+IgM^+CD27^+$ B cells by cell sorting on FACS Vantage™. Due to absence of secondary isotypes in AID-deficient patients, AID B cells are all IgM^+ and were therefore sorted into naive $CD19^+CD27^-$ and memory $CD19^+CD27^+$ B cells without IgM staining. Antibodies used for staining were FITC–anti-CD19,

M.C. Nussenzweig and A. Durandy contributed equally to this work.

Address correspondence to Michel C. Nussenzweig, Department of Molecular Immunology, The Rockefeller University, RRB Rm. 470, Box 220, 1230 York Ave., New York, NY 10021. Phone: 212-327-8106; Fax: 212-327-8370; E-mail: nussen@mail.rockefeller.edu

PE–anti-CD27 (Immunotech/Beckman Coulter), and biotin-conjugated anti-IgM mAb (PharMingen), which was visualized with Streptavidin Red 670 (GIBCO BRL).

Reverse Transcription PCR, Cloning, and Sequencing. Total RNA was extracted from 10^4–10^5 purified cells using TRIzol Reagent (GIBCO BRL) and reverse transcribed in a 10-μl reaction with Superscript II (GIBCO BRL). For reverse transcription (RT)-PCR reactions, 1 μl of cDNA was amplified for 30–35 cycles of 30 s at 94°C, 30 s at 58°C (V_H1-Cμ) or at 55°C (V_λ-C_λ) and 30 s at 72°C with a final 10-min extension at 72°C using HotStarTaq™ DNA polymerase (QIAGEN) and the following primers: $V_\lambda1-8$ family consensus sense, 5′-GGG(G/A)TC(T/C)CTGA(C/T/G)CG(A/C/G)TTCTCTGG(C/G)TCC-3′; $V_\lambda9$ sense, 5′-ATCCCTGATCGCTTCTCAGTCTTG-3′; $V_\lambda10$ sense, 5′-GATCTCAGAGAGATTATCTGCATCC-3′; and C_λ antisense, 5′-CACAC(T/C)AGTGTGGCCTTGTTG-GCTTG-3′. Sense FR1 V_H1 and antisense Cμ primers were as described previously (19, 20). RT-PCR products were run on 2% agarose gels, and PCR products were gel purified (Qiaquick™; QIAGEN) and cloned into TA vectors (Invitrogen). Double-stranded DNA sequences were obtained using antisense Cμ or Cλ primers and Dye Terminator Cycle Sequencing (PE Applied Biosystems). Sequences were analyzed using Ig BLAST®. When two or more identical sequences were found, they were counted as a single clone. Sequences were considered mutated when they displayed two or more nucleotide differences from their germline counterparts. Differences in gene distribution between naive and memory B cells were analyzed with chi-square tests (Cochran-Mantel-Haenszel test) adjusted by the Bonferroni method for multiple testing and they were considered significant when *P* values were equal to or less than 0.05.

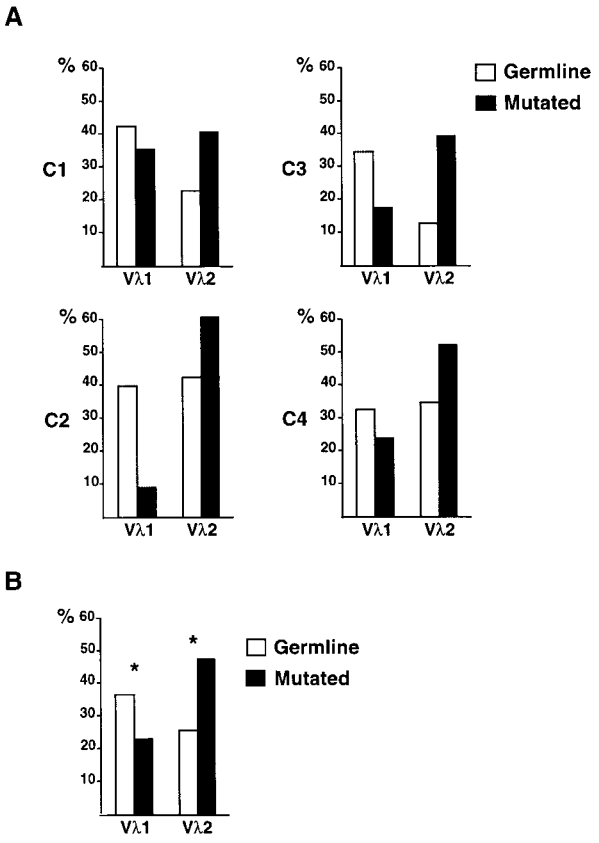

Figure 1. Igλ repertoire expressed in peripheral B cells from control donors. (A) Vλ1 and Vλ2 gene usage in germline-encoded (open bars) and mutated (solid bars) sequences from $CD19^+$ peripheral B cells in four unrelated controls. 25, 15, 46, and 34 germline $V_\lambda J_\lambda$ sequences from donors C1, C2, C3, and C4 were compared with 33, 23, 33, and 29 mutated $V_\lambda J_\lambda$ sequences from the same individuals. The percent V_λ utilization is indicated on the y axis. (B) Combined total of Vλ1 and Vλ2 gene usage in germline and mutated $V_\lambda J_\lambda$ sequences from control donors. 121 germline-encoded and 118 mutated sequences were obtained from the four control donors. Asterisk (*) indicates statistically significant difference (Vλ1, $P = 0.022$; Vλ2, $P = 0.0004$).

Results and Discussion

To determine whether there is a shift in repertoire between naive and antigen-selected B cell compartments in humans, we compared the unmutated germline Igλ sequences to mutated Igλ sequences obtained from $CD19^+$ peripheral B cells from four control donors. In humans, Igλ light chains are found in 30–40% of all antibodies, and among the 10 Vλ gene families three (Vλ1, Vλ2 and Vλ3) represent >80–90% of all Vλ genes (21, 22). We found that the distribution of Vλ1 and Vλ2, two of the most frequently used human Vλ families, differs between germline-encoded and mutated antibodies (Fig. 1 A; total of 239 individual sequences): Vλ1 is decreased and Vλ2 increased

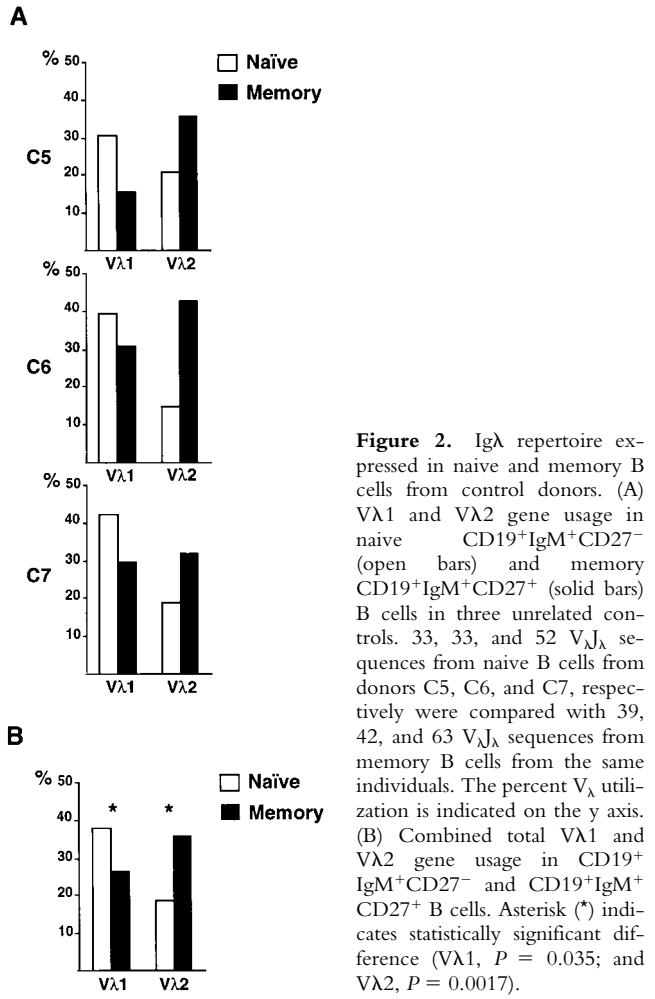

Figure 2. Igλ repertoire expressed in naive and memory B cells from control donors. (A) Vλ1 and Vλ2 gene usage in naive $CD19^+IgM^+CD27^-$ (open bars) and memory $CD19^+IgM^+CD27^+$ (solid bars) B cells in three unrelated controls. 33, 33, and 52 $V_\lambda J_\lambda$ sequences from naive B cells from donors C5, C6, and C7, respectively were compared with 39, 42, and 63 $V_\lambda J_\lambda$ sequences from memory B cells from the same individuals. The percent V_λ utilization is indicated on the y axis. (B) Combined total Vλ1 and Vλ2 gene usage in $CD19^+IgM^+CD27^-$ and $CD19^+IgM^+CD27^+$ B cells. Asterisk (*) indicates statistically significant difference (Vλ1, $P = 0.035$; and Vλ2, $P = 0.0017$).

among mutated Igλs, and this difference is independent of the age of the donors (Fig. 1, A and B).

To further analyze the shift in Igλ repertoire between naive and memory B cells, we fractionated peripheral B cells using CD27 memory marker and isolated naive ($CD19^+IgM^+CD27^-$) and memory ($CD19^+IgM^+CD27^+$) B cells from control donors (23, 24). The difference in Vλ distribution was also found when comparing naive and memory B cell compartments (Fig. 2; total of 262 sequences). Antibodies cloned from memory B cells were predominantly mutated and showed decreased Vλ1 and increased Vλ2 gene usage (Fig. 2, A and B). We conclude that there is a shift in the Igλ repertoire between the naive and antigen-selected memory B cell compartments in humans.

To determine whether the shift in Igλ repertoire between the naive and memory compartments is related to somatic hypermutation, we analyzed the Igλ genes expressed in naive and memory B cells from patients lacking activation-induced deaminase (AID) (17, 18). AID has been shown to be essential for both hypermutation and switch recombination but does not appear to be necessary for normal B cell development in mice and humans (17, 18). Patients with AID deficiency showed no secondary antibodies and no somatic mutation; nevertheless, these individuals displayed enlarged tonsils with germinal centers and showed normal numbers of $CD19^+CD27^+$ B cells (18). The $CD27^+IgM^+$ B cells found in AID-deficient patients resembled authentic $CD27^+IgM^+$ memory B cells in that they showed normal selection against V_H1–69, a V_H gene that is frequently found in B lymphoid chronic lymphocytic leukemias producing autoreactive antibodies (Fig. 3) (19, 25, 26). However, the antibodies expressed in antigen-selected memory B cells in five AID-deficient patients differed from the three controls in that they showed no mutations, and there was no shift in the Vλ repertoire between naive B cells and antigen-selected memory B cells (compare Figs. 2 and 4; total of 330 sequences). In particular, there was no increase in Vλ2 gene expression and no relative decrease in Vλ1 (Fig. 4). In addition, V_H5–51 gene usage was favored in the memory $CD27^+$ B cells from AID-deficient patients but not in normal controls (Fig. 3).

Somatic hypermutation is known to increase antibody affinity during immune responses. However, the contribution of mutation to shaping the antibody repertoire has not been determined. We have found a global shift in the Igλ

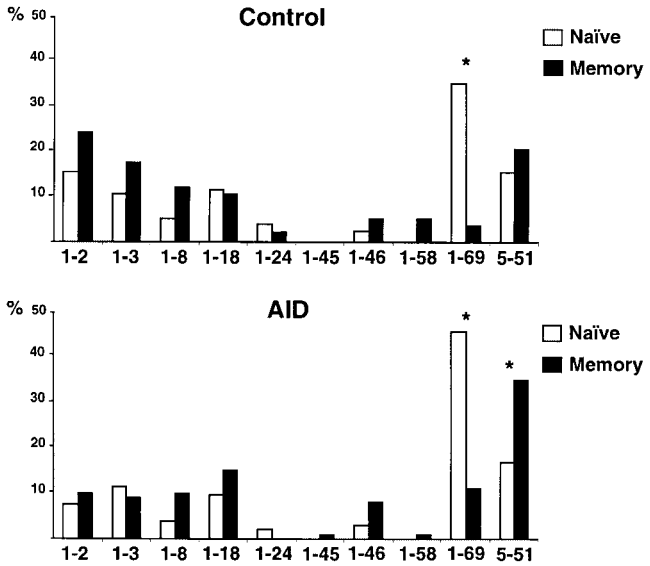

Figure 3. V_H1 and V_H5 repertoire analysis of naive and memory B cells from control donors and AID-deficient patients. 77 control C5, C6, and C7 (top) and 105 AID (bottom) V_H1 and V_H5 sequences from naive B cells (open bars) are compared with 58 control and 112 AID sequences from memory B cells (solid bars). The percent V_H1 and V_H5 utilization is indicated on the y axis. Asterisk (*) indicates statistically significant difference (V_H1–69, $P < 0.0001$ for both controls and AID-deficient patients; V_H5–51, $P = 0.03$ for AID-deficient patients).

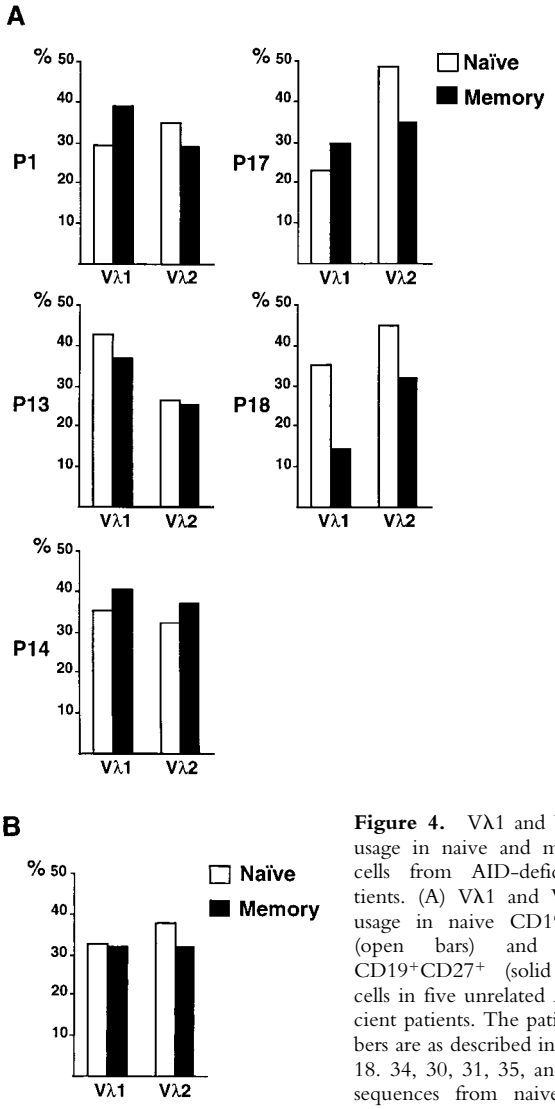

Figure 4. Vλ1 and Vλ2 gene usage in naive and memory B cells from AID-deficient patients. (A) Vλ1 and Vλ2 gene usage in naive $CD19^+CD27^-$ (open bars) and memory $CD19^+CD27^+$ (solid bars) B cells in five unrelated AID-deficient patients. The patient numbers are as described in reference 18. 34, 30, 31, 35, and 31 $V_\lambda J_\lambda$ sequences from naive B cells from AID-deficient patients P1, P13, P14, P17, and P18, respectively were compared with 31, 35, 32, 37, and 34 $V_\lambda J_\lambda$ sequences from memory B cells from the same individuals. The percent V_λ utilization is indicated on the y axis. (B) Combined total Vλ1 and Vλ2 gene usage in $CD19^+CD27^-$ and $CD19^+CD27^+$ B cells for AID-deficient patients.

antibody repertoire between naive and memory B cells from normal donors. This shift in repertoire is associated with somatic hypermutation and is AID dependent. We conclude that AID and hypermutation make a significant contribution to shaping the antigen-selected memory B cell repertoire in humans.

We thank Dr. Mila Jankovic, Dr. Bernardo Reina San Martin, Dr. Eva Besmer, and members of the Nussenzweig lab for comments and discussions and Mrs. M. Forveille for excellent technical assistance.

This work was supported by grants from the National Institutes of Health to M.C. Nussenzweig and from Institut National de la Santé et de la Recherche Médicale. M.C. Nussenzweig is an investigator in the Howard Hughes Medical Institute.

Submitted: 1 May 2001
Revised: 13 June 2001
Accepted: 19 June 2001

References

1. Siskind, G.W., and H.N. Eisen. 1965. Effect of variation in antibody-hapten association constant upon the biologic activity of the antibody. *J. Immunol.* 95:436–441.
2. Weigert, M.G., I.M. Cesari, S.J. Yonkovich, and M. Cohn. 1970. Variability in the lambda light chain sequences of mouse antibody. *Nature.* 228:1045–1047.
3. Griffiths, G.M., C. Berek, M. Kaartinen, and C. Milstein. 1984. Somatic mutation and the maturation of immune response to 2-phenyl oxazolone. *Nature.* 312:271–275.
4. Kaartinen, M., G.M. Griffiths, P.H. Hamlyn, A.F. Markham, K. Karjalainen, J.L. Pelkonen, O. Makela, and C. Milstein. 1983. Anti-oxazolone hybridomas and the structure of the oxazolone idiotype. *J. Immunol.* 130:937–945.
5. Kaartinen, M., G.M. Griffiths, A.F. Markham, and C. Milstein. 1983. mRNA sequences define an unusually restricted IgG response to 2-phenyloxazolone and its early diversification. *Nature.* 304:320–324.
6. McKean, D., K. Huppi, M. Bell, L. Staudt, W. Gerhard, and M. Weigert. 1984. Generation of antibody diversity in the immune response of BALB/c mice to influenza virus hemagglutinin. *Proc. Natl. Acad. Sci. USA.* 81:3180–3184.
7. Sablitzky, F., G. Wildner, and K. Rajewsky. 1985. Somatic mutation and clonal expansion of B cells in an antigen-driven immune response. *EMBO J.* 4:345–350.
8. Allen, D., T. Simon, F. Sablitzky, K. Rajewsky, and A. Cumano. 1988. Antibody engineering for the analysis of affinity maturation of an anti-hapten response. *EMBO J.* 7:1995–2001 (published erratum at 8:2444).
9. Milstein, C. 1987. Diversity and the genesis of high affinity antibodies. *Biochem. Soc. Trans.* 15:779–787.
10. Reth, M., G.J. Hammerling, and K. Rajewsky. 1978. Analysis of the repertoire of anti-NP antibodies in C57BL/6 mice by cell fusion. I. Characterization of antibody families in the primary and hyperimmune response. *Eur. J. Immunol.* 8:393–400.
11. Cumano, A., and K. Rajewsky. 1986. Clonal recruitment and somatic mutation in the generation of immunological memory to the hapten NP. *EMBO J.* 5:2459–2468.
12. French, D.L., R. Laskov, and M.D. Scharff. 1989. The role of somatic hypermutation in the generation of antibody diversity. *Science.* 244:1152–1157.
13. Furukawa, K., A. Akasako-Furukawa, H. Shirai, H. Nakamura, and T. Azuma. 1999. Junctional amino acids determine the maturation pathway of an antibody. *Immunity.* 11: 329–338.
14. Boersch-Supan, M.E., S. Agarwal, M.E. White-Scharf, and T. Imanishi-Kari. 1985. Heavy chain variable region. Multiple gene segments encode anti-4-(hydroxy-3-nitro-phenyl)acetyl idiotypic antibodies. *J. Exp. Med.* 161:1272–1292.
15. Bothwell, A.L., M. Paskind, M. Reth, T. Imanishi-Kari, K. Rajewsky, and D. Baltimore. 1981. Heavy chain variable region contribution to the NPb family of antibodies: somatic mutation evident in a gamma 2a variable region. *Cell.* 24: 625–637.
16. Muramatsu, M., V.S. Sankaranand, S. Anant, M. Sugai, K. Kinoshita, N.O. Davidson, and T. Honjo. 1999. Specific expression of activation-induced cytidine deaminase (AID), a novel member of the RNA-editing deaminase family in germinal center B cells. *J. Biol. Chem.* 274:18470–18476.
17. Muramatsu, M., K. Kinoshita, S. Fagarasan, S. Yamada, Y. Shinkai, and T. Honjo. 2000. Class switch recombination and hypermutation require activation-induced cytidine deaminase (AID), a potential RNA editing enzyme. *Cell.* 102: 553–563.
18. Revy, P., T. Muto, Y. Levy, F. Geissmann, A. Plebani, O. Sanal, N. Catalan, M. Forveille, R. Dufourcq-Labelouse, A. Gennery, et al. 2000. Activation-induced cytidine deaminase (AID) deficiency causes the autosomal recessive form of the Hyper-IgM syndrome (HIGM2). *Cell.* 102:565–575.
19. Fais, F., F. Ghiotto, S. Hashimoto, B. Sellars, A. Valetto, S.L. Allen, P. Schulman, V.P. Vinciguerra, K. Rai, L.Z. Rassenti, et al. 1998. Chronic lymphocytic leukemia B cells express restricted sets of mutated and unmutated antigen receptors. *J. Clin. Invest.* 102:1515–1525.
20. Meffre, E., F. Papavasiliou, P. Cohen, O. de Bouteiller, D. Bell, H. Karasuyama, C. Schiff, J. Banchereau, Y.J. Liu, and M.C. Nussenzweig. 1998. Antigen receptor engagement turns off the V(D)J recombination machinery in human tonsil B cells. *J. Exp. Med.* 188:765–772.
21. Ignatovich, O., I.M. Tomlinson, P.T. Jones, and G. Winter. 1997. The creation of diversity in the human immunoglobulin V(lambda) repertoire. *J. Mol. Biol.* 268:69–77.
22. Farner, N.L., T. Dorner, and P.E. Lipsky. 1999. Molecular mechanisms and selection influence the generation of the human V lambda J lambda repertoire. *J. Immunol.* 162:2137–2145.
23. Klein, U., K. Rajewsky, and R. Kuppers. 1998. Human immunoglobulin (Ig)M^+IgD^+ peripheral blood B cells expressing the CD27 cell surface antigen carry somatically mutated variable region genes: CD27 as a general marker for somatically mutated (memory) B cells. *J. Exp. Med.* 188:1679–1689.
24. Tangye, S.G., Y.J. Liu, G. Aversa, J.H. Phillips, and J.E. de Vries. 1998. Identification of functional human splenic memory B cells by expression of CD148 and CD27. *J. Exp. Med.* 188:1691–1703.
25. Sthoeger, Z.M., M. Wakai, D.B. Tse, V.P. Vinciguerra, S.L. Allen, D.R. Budman, S.M. Lichtman, P. Schulman, L.R. Weiselberg, and N. Chiorazzi. 1989. Production of autoantibodies by CD5-expressing B lymphocytes from patients with chronic lymphocytic leukemia. *J. Exp. Med.* 169:255–268.
26. Borche, L., A. Lim, J.L. Binet, and G. Dighiero. 1990. Evidence that chronic lymphocytic leukemia B lymphocytes are frequently committed to production of natural autoantibodies. *Blood.* 76:562–569.