Deficiency in CD22, a B Cell–specific Inhibitory Receptor, Is Sufficient to Predispose to Development of High Affinity Autoantibodies

By Theresa L. O’Keefe, Gareth T. Williams, Facundo D. Batista, and Michael S. N euberger

From the Medical Research Council Laboratory of Molecular Biology, Cambridge CB2 2QH, United Kingdom

Summary

CD22 is a B cell–specific transmembrane glycoprotein that acts to dampen signals generated through the B cell antigen receptor (BCR): B cells from CD22-deficient mice give increased Ca\(^{2+}\) fluxes on BCR ligation. Here we show that this B cell hyperresponsiveness correlates with the development of autoantibodies. After the age of eight months, CD22-deficient mice developed high titers of serum IgG directed against double-stranded DNA; these antibodies were of multiclonal origin, somatically mutated, and high affinity. Increased titers of antibodies to cardiolipin and myeloperoxidase were also noted. The results demonstrate that a single gene defect exclusive to B lymphocytes is, without additional contrivance, sufficient to trigger autoantibody development in a large proportion of aging animals. Thus, CD22 might have evolved specifically to regulate B cell triggering thresholds for the avoidance of autoimmunity.

Key words: B lymphocyte • autoimmunity • threshold • CD22 • inhibitory receptor

Several autoimmune diseases are characterized by the presence in serum of high affinity antibodies to self antigens, with the B cells producing them having undergone heavy chain class switching and somatic hypermutation. This suggests that T cell help has been available, at least under these pathological conditions, to facilitate maturation of an anti-self response. The defect is, however, unlikely to lie simply in the inappropriate provision of T cell help: several models reveal that multiple loci can contribute to predisposition to autoimmune disease (1–4), with intrinsic defects in the B cell lineage able to play an important role (5, 6).

Although defects in B cell apoptosis can certainly accelerate autoimmune disease (7), several lines of evidence suggest that an intrinsic hyperresponsiveness of B cells to antigen encounter could also be a contributory cause of autoimmunity. Thus, genetic dissection of the contributing loci in a mouse model of systemic lupus erythematosus reveals that one of the loci (Sle2) leads to B cell hyperactivity (8). Furthermore, the response to self antigen by B cells that express an autoreactive immunoglobulin transgene can be significantly affected by mutations that affect B cell antigen receptor (BCR) \(^{1}\) signaling (9, 10).

We were interested in determining whether mutations affecting B cell signaling would be sufficient to predispose autoantibody development in an otherwise normal mouse. Is a hyperresponsiveness that is restricted to the B cell compartment nevertheless sufficient to so perturb the immune system that the necessary help is recruited to allow development of high affinity anti-self antibodies?

To this end, we made use of CD22-deficient mice, which exhibit a relatively mild B cell hyperresponsiveness (11–14). CD22 is a B cell–specific transmembrane glycoprotein that associates with BCR and possesses an extracellular domain that binds \(\alpha-2,6\)-sialylated glycoconjugates (15–18). It acts as a negative regulator of antigen receptor signaling, with levels of BCR cross-linking that are too low to generate a detectable signal in B cells from control mice, nevertheless giving rise to a calcium flux with CD22-deficient B cells (11–14). Indeed, even halving the abundance of CD22 on the cell surface leads to enhanced BCR signaling (19). In our initial characterization of CD22-deficient mice, we noted a small (approximately twofold) increase in total serum IgM (but not IgG) together with a corresponding increase in total Ig anti-DNA titers in 5-mo-old animals that might be ascribable to an expanded B1 cell population (11). However, here we show that as the CD22-deficient mice age, they have a dramatically increased likelihood of producing somatically mutated, high affinity autoreactive IgG. Thus, it appears that there is tight regulation of BCR signaling and that the perturbations caused by CD22 deficiency can trigger the development of autoimmunity.

\(^{1}\)Abbreviations used in this paper: BCR, B cell antigen receptor; ds, double-stranded; ES, embryonic stem cell.
Materials and Methods

Mice. Mice were generated from chimeras established using a previously described embryonic stem cell (ES) clone (11) containing a targeted integration of a tk-neo cassette into C22. The chimeras (created using C57BL/6 blastocysts) were bred against both C57BL/6 and BALB/c mice, and mice from the F2 generation were maintained for up to 20 mo with tail bleeds taken every 4–6 wk. A cohort of control (129 × C57BL/6) F2 mice (that do not carry any targeted gene alteration) was established analogously. Animals were either bred in our own conventional facility or in a specific pathogen-free (barrier) unit following delivery by Caesarian section and fostering onto C57BL/6 × CBA females in isolators.

Analysis of Autoantibodies. Serum titers of IgG anti-double-stranded (ds) DNA were measured as described elsewhere (20) using alkaline phosphatase-conjugated goat anti–mouse IgG (Sigma Chemical Co., Ltd.). Sera from four MRL/lpr mice were always titered in parallel, with one of these sera assigned a titer of 5 U/ml. The assay was calibrated using a high affinity IgG2a monoclonal anti-dsDNA antibody (S22) from mouse 9612 (see below); 1 U/ml in the ELISA was given by 24 μg/ml of S22. Titers of other IgG autoantibodies were similarly determined using plates that had been coated with either cardiolipin (Sigma Chemical Co.; 100 μg/ml in ethanol) or myeloperoxidase (Calbiochem Corp.; 250 ng/ml in sodium bicarbonate, pH 9.2). Antibody isotypes were determined using reagents from Pharmingen.

Hybridomas were established from unimmunized mice by fusion with NS0 cells and autoantibodies in the supernatants monitored by ELISA developing with biotinylated goat anti–mouse IgG. The binding of monoclonal anti-DNA antibodies at 20°C to a 5'-biotinylated ds 48mer oligonucleotide that had been immobilized on a streptavidin-coated chip (SA-Biacore chip; Pharmacia) was monitored by surface plasmon resonance as previously described (21).

Sequencing of Expressed V_H Segments. Oligo-dT–primed cDNA prepared from RNA extracted from the hybridomas was PCR amplified using a consensus V_H oligonucleotide for forward priming (5'-CGGATCCTGAGGTGCAGCTGGAGGAGTC [22]; upstream) and the C22 linker H2 primer (5'-CGGAATTCCGGGACCAGTGGATAGAC or 5'-CGGATTTCGGGACCAAGGATAGAC for priming back from the C_V1 domain of C-γ1, γ2a, and γb or of C-γ3, respectively). PCR products were sequenced directly as well as ligated into pUC18 with multiple DNA clones sequenced from each hybridoma.

Results

We have previously described (11) an ES line (derived from 129 mice) that carries a targeted integration of a neomycin resistance gene into the CD22 gene; this cell line was used to establish chimeric mice by injection into C57BL/6 blastocysts and germline transmission of the targeted allele (yielding CD22−/− heterozygotes) obtained following breeding with both C57BL/6 and BALB/c females. Cohorts of animals from the F2 generations of both sets of breedings were followed with time for the development of IgG anti-dsDNA antibody. On both backgrounds, high titers of anti-DNA antibodies developed with age (particularly after 8 mo) in many of the CD22−/− animals but not in the CD22+/− litter-matched controls. That the development of these autoantibodies was due to the targeted integration into the CD22 gene is confirmed by the fact that IgG anti-dsDNA was not detected in the sera of control (129 × C57BL/6) F2 mice (Fig. 1).

The titer of anti-DNA antibody in the CD22-deficient mice is, in many cases, of a comparable order to that found in 12-mo-old MRL/lpr mice. By 18 mo of age, over 70% of the CD22-deficient mice have at some time shown evidence of IgG anti-dsDNA antibody in their sera at concentrations >1.5 U/ml; none of the 42 control mice revealed titers of this magnitude (Fig. 2). We have also followed a limited number of CD22+/− heterozygotes and found that

![Figure 1. IgG anti-dsDNA antibodies in the sera of CD22-deficient and control mice. Serum IgG anti-dsDNA was measured by ELISA and compared in CD22−/− (center panels; ■) and CD22+/− litters (left panels; ○). These mice derive from the F2 generation of conventionally housed, CD22-targeted ES chimeric mice bred against BALB/c (top panels) or C57BL/6 (bottom panels). These cohorts included 16 CD22-deficient and 17 CD22+/− mice from the BALB/c breedings and 21 CD22-deficient animals (25 CD22+/−) from the C57BL/6 breedings. Elevated titers of IgG anti-dsDNA were not detected in sera of aged C57BL/6 or 129 control mice. In addition, the titer of IgG anti-dsDNA was monitored in the sera of 18 control (129 × C57BL/6) F2 mice that did not carry a targeted gene modification (bottom right panel) to exclude the possibility that a C22-linked polymorphism might result in autoantibody development in the context of a (129 × C57BL/6) F2 background. The titers of IgG anti-DNA in sera of four MRL/lpr mice are indicated (○). Definition of units and calibration of the assay is described in Materials and Methods. Autoantibody titers did not differ significantly between males and females.](image-url)
3/11 had developed IgG anti-dsDNA by 12 mo of age (not shown).

Life expectancy among CD22-deficient mice was decreased (10/43 weaned CD22-deficient mice having died by 15 mo of age compared with 1/43 CD22+/+ controls), with at least 4 of the deaths due to infection. However, all but one of these deaths occurred after 7 mo of age. Furthermore, we did not detect proteinuria or antibody deposition in glomeruli in the mice harboring autoantibodies. This lack of pathology may well correlate with the fact that anti-DNA titers do not simply rise with age but, in individual animals, often rise, regress, and rise again.

dsDNA is not the sole target of autoantibody development. Mice were also monitored for the development of antibodies to cardiolipin and myeloperoxidase; a clear distinction between the CD22-deficient and control siblings was found here as well (Table I and Fig. 3 A). The largest cohort of animals was followed under conditions of conventional housing, but we also compared autoantibody development in CD22-deficient and control mice housed in a barrier unit. The results (Table I) reveal that autoantibodies also develop under these cleaner conditions.

Subclass typing of serum autoantibodies revealed that, in both the C57BL/6 and BALB/c breedings, IgG2a anti-DNA was found in ~80% of the autoimmune animals. However, >50% of the autoimmune mice contained anti-DNA antibodies of multiple IgG subclasses. To obtain more detail about the nature of these antibodies, hybridomas were established from two immunized, 18-mo-old, CD22-deficient females (mouse 9612 from the BALB/c breedings and mouse 9714 from the SPF facility). These hybrids all expressed IgG antibodies (subclass and V<sub>H</sub> sequences are given in Fig. 5), but S14 and S28 were Card-specific IgMs with unassigned V<sub>H</sub>s.

Table I. Autoantibodies in the Sera of CD22-deficient and Control Mice

| Antigen      | Conventionally housed | Barrier housed |
|--------------|-----------------------|---------------|
|              | 12-mo-old             | 18-mo-old     | 14-mo-old     | 12-mo-old             | 18-mo-old     | 14-mo-old     |
|              | CD22+/+               | CD22−/−      | CD22+/+      | CD22−/−              | CD22+/+      | CD22−/−      |
| Cardiolipin  | 0/19*                 | 1/16         | 0/17         | 3/14                 | 0/5          | 2/7          |
| Myeloperoxidase | 0/19                 | 3/16         | 0/17         | 3/14                 | 0/5          | 6/7          |
| dsDNA        | 0/25                  | 10/19        | 0/25         | 10/15                | 0/5          | 4/7          |

Serum IgG autoantibodies were monitored at the ages indicated. All mice are from the C57BL/6 breedings. Two of the conventionally housed 18-mo-old as well as two of the barrier-housed 14-mo-old CD22-deficient mice simultaneously harbored antibodies against cardiolipin, myeloperoxidase, and DNA.

* Mice harboring autoantibodies/total mice.
9449 from the C57BL/6 breedings), as well as two CD22<sup>+/+</sup> litter-matched controls. No anti-dsDNA IgG was detected in the supernatants from 198 wells from the control fusions; strong titers, however, were detected in 20/302 wells from the CD22-deficient mice (18 of these from mouse 9612; 2 from mouse 9449; Fig. 3). Similarly, whereas no cardioplip-specific hybrids were detected in the control fusions, 19 positives were obtained from the CD22-deficient mice (10 from mouse 9612; 9 from mouse 9449). The majority of these cardiolipin-specific antibodies were IgMs, although mouse 9612 gave two IgA and mouse 9449 gave two IgG3 antcardiolipin antibodies.

The hybridomas were then expanded for further characterization. Analysis of the anti-DNA antibodies by surface plasmon resonance using a biotinylated oligonucleotide as antigen revealed that several bound DNA very tightly, with dissociation half-lives in the range of 8–500 min (Fig. 4). To ascertain whether the B cells producing these antibodies were clonally related and whether they had undergone somatic hypermutation, the V<sub>H</sub> sequences were determined from several of the anti-DNA antibodies from mouse 9612. The results demonstrate that the anti-DNA antibodies within a single CD22-deficient mouse derive from multiple, clonally expanded B cell progenitors that have undergone class switching and somatic hypermutation. Thus, for example, hybridoma S48 appears to be derived from S30, as they carry the same V<sub>H</sub>36–60/J<sub>H</sub>2 rearrangement but with S48 harboring multiple additional somatic mutations (several to arginine), which could account for its increased affinity (Fig. 5 A). Similarly, S31 (IgG1) and S35, S66, S11, and S15 (all IgG2a) all express the same (V<sub>H</sub>158 family member)/J<sub>H</sub>2 rearrangement with an arginine-rich CDR 3 (characteristic of many anti-DNA antibodies [23–25]): the individual antibodies differ, however, in the extent of accumulated somatic mutations (Fig. 5 B). In contrast, S20 and S22 carry distinct rearrangements of the same V<sub>H</sub>7183 family member (Fig. 5 C). Thus, paralleling observations previously made with other autoimmune mice (25), multiple independent B cells appear to have seeded an ongoing anti-DNA response. Similarly, in respect of the two cardioplip-specific IgG3s, analysis of their V<sub>H</sub> sequences revealed them to be clonally related (Fig. 5 D).

**Discussion**

The development of autoantibodies in CD22-deficient mice reveals that a single gene defect exclusive to B cells is sufficient to trigger autoimmunity in a large proportion of mice. This presumably means that the restriction of the provision of T cell help to foreign antigens is intrinsically imperfect: T cell help for an autoantibody response can be elicited by a hyperreactive B cell compartment.

The CD22-deficient animals do not, however, go on to develop autoimmune disease. This is consistent with genetic analyses of predisposition to systemic autoimmune disease in lupus-prone mouse strains, which reveal a role for multiple genetic loci (1–4, 26). Indeed, one of the loci contributing to autoimmunity in NZM mice (SlX3) has been mapped to a region of chromosome 7 in the vicinity of Cd22 and, when bred into C57BL/6 mice, causes production of IgG anti-dsDNA antibodies as well as lupus nephritis (27). It will be interesting to ascertain whether this, at least in part, reflects a functionally relevant C d22 polymorphism. By analogy with studies in the MRL mouse (28), it will also be interesting to ascertain whether mutations in Fas or its ligand exacerbate autoimmunity in CD22-deficient mice.

The precise mechanism by which CD22 deficiency predisposes to autoimmunity remains to be definitively identified, but we believe the hyperresponsiveness of CD22-deficient B cells to BCR ligation is likely to be of central importance. Phosphorylation of CD22 on its cytoplasmic tyrosines following BCR ligation is mediated by the Lyn kinase and leads to the recruitment of the phosphatase SHP1 (29–34). It is therefore notable that deficiencies in either Lyn or SHP1 both lead to autoimmunity (35–38). However, this autoimmunity is more severe than that in CD22-deficient animals and is most unlikely to simply reflect defects in CD22-mediated regulation of BCR. Indeed, the increased severity probably correlates with both Lyn and SHP-1 being implicated in signal transduction through multiple cell-surface receptors, with their functions not being limited to the B cell lineage.

Thus, the significance of the autoantibody development in CD22-deficient mice lies in the fact that these autoantibodies arise as a consequence of a relatively mild perturbation that is exclusive to B lymphocytes and that affects the BCR signaling threshold. Experiments performed using transgenic mice that have been engineered to express high affinity autoreactive specificities on a substantial proportion of their B cells have revealed that the fate of such B cells is sensitive to modifications in CD22, Lyn, and SHP-1 as well as other genes that affect BCR signaling (9, 10). Our findings are entirely consistent with these earlier studies but reveal that CD22 deficiency alone, without additional con-
trivance, is sufficient to predispose autoimmunity in normal animals.

It is attractive to speculate from our results that the major physiological function served by CD22 in normal mice is to mediate the avoidance of autoimmunity. In light of the diminished level of CD22 expression in immature B cells (39), we previously suggested (11) that CD22 plays a role in raising the threshold of sensitivity to antigen that accompanies differentiation of an immature B cell (sensitive to tolerization/deletion by low affinity antigen) into a mature B cell that awaits triggering by exogenous antigen (40). Such a proposal could well explain the autoimmunity in CD22-deficient mice. However, a role for CD22 should also take into account the specificity of its extracellular domain for \( \alpha_{2,6}\)-sialyloconjugates (18). Intriguingly, the sialylated moieties present on eukaryotic membranes enhance the interaction between complement components C3b and factor H, thereby leading to inhibition of the alternative complement pathway; this serves to bias activation of the innate immune system toward microbial infection and away from autoreactivity (41, 42). Maybe CD22 recognition of the sialoglycoconjugates expressed on mammalian cells serves an analogous role in the adaptive immune system, damping the BCR signaling that might otherwise be triggered by low affinity autoantigens. It will be interesting to ascertain whether making mutations in the CD22 extracellular domain that abolish recognition of sialoglycoconjugates will be sufficient to predispose autoimmunity.

We thank Michael Ehrenstein for provision of (129 × C57BL/6)F2 control mice and Angela Middleton and Theresa Langford for animal husbandry. T.L. O’Keefe was supported by an Oliver Bird Fund fellowship and an International Research Scholar’s award from the Howard Hughes Medical Institute (to M.S. Neuberger). F.D. Batista was supported by fellowships from the European Molecular Biology Organization and the Arthritis Research Campaign.

Address correspondence to Michael Neuberger, MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, United Kingdom. Phone: 44-1223-402245; Fax: 44-1223-412178; E-mail: msn@mrc-lmb.cam.ac.uk

Theresa L. O’Keefe’s present address is LeukoSite Inc., 215 First Street, Cambridge, MA 02144.

Received for publication 21 December 1998 and in revised form 4 February 1999.
References

1. Kono, D.H., R.W. Burlingame, D.G. Owens, A. Kuramoto, R.S. Balderas, D. Balomenos, and A.N. Theofilopoulos. 1994. Lupus susceptibility loci in New Zealand mice. Proc. Natl. Acad. Sci. USA. 91:10168–10172.

2. Moore, L., U.H. Rudofsky, J.A. Longmate, J. Schifflerbauer, and E.K. Wakeland. 1994. Polygenic control of susceptibility to murine systemic lupus erythematosus. Immunity. 1:219–229.

3. Drake, C.G., S.K. Babcock, E. Palmer, and B.L. Kotzin. 1994. Genetic analysis of the NZB contribution to lupus-like autoimmune disease. Proc. Natl. Acad. Sci. USA. 91:4062–4066.

4. Hogarth, M.B., J.H. Slingsby, P.J. Allen, E.M. Thompson, P. Chandler, K.A. Davies, E. Simpson, B.J. Morley, and M.J. Waipart. 1998. Multiple lupus susceptibility loci map to chromosome 1 in BXSB mice. J. Immunol. 161:2753–2761.

5. Perkins, D.L., R.M. Glaser, C.A. Mahon, J. Michaelson, and R.A. Eisenberg. 1991. An intrinsic B cell defect is responsible for the production of autoantibodies in the development of murine systemic autoimmune disease. J. Exp. Med. 173:1441–1449.

6. Sobel, E.S., T. Katagiri, K. Katagiri, S.C. Morris, P.L. Cohen, and R.A. Eisenberg. 1997. Genetic dissection of systemic lupus erythematosus pathogenesis: Sle2 on murine chromosome 4 leads to B cell hyperactivity. J. Immunol. 159:454–465.

7. Cornall, R.J., J.G. Cyster, M.L. Hibbs, A.R. Dunn, K.L. Otipoby, E.A. Clark, and C.C. Goodnow. 1998. Polygenic autoimmune traits: Lyn, CD22 and SHP-1 are limiting elements of a biochemical pathway regulating BCR signaling and selection. Immunity. 8:497–508.

8. Inaoki, M., S. Sato, B.C. Eintraub, C.C. Goodnow, and T.F. Tedder. 1997. CD19 regulates signaling thresholds control peripheral tolerance and autoantibody production in B lymphocytes. J. Exp. Med. 186:1923–1931.

9. O'Keeke, T.L., S. Bandypadhyay, S.K. Datta, and T. Imanishi-Kari. 1997. Antigen receptor-evoked calcium influx is enhanced in CD22-deficient B cell lines. J. Exp. Med. 175:1470–1480.

10. Mohan, C., Y. Shi, J.D. Laman, and S.K. Datta. 1995. Interaction between CD40 and its ligand gp39 in the development of murine lupus nephritis. J. Immunol. 154:1470–1480.

11. Batista, F.D., and M.S. N euberger. 1998. Affinity dependence of the B cell response to antigen: a threshold, a ceiling, and the importance of off-rate. Immunology. 8:751–759.

12. Dattamajumdar, A.K., D.P. Jacobson, L.E. Hoo d, and G.E. Osman. 1996. Rapid cloning of any rearranged mouse immunoglobulin variable genes. Immunogenetics. 43:141–151.

13. Ellat, D., D.M. W ebelster, and A.R. Rees. 1998. V region sequences of anti-DNA and anti-β2-microglobulin antibodies from BN1/NZW F1 mice. J. Immunol. 141:1745–1753.

14. O'Keeke, T.L., S. Bandypadhyay, S.K. Datta, and T. Imanishi-Kari. 1990. V region sequences of an idiotypically connected family of pathogenic anti-DNA autoantibodies. J. Immunol. 144:4275–4283.

15. Shlomchik, M., M. Macselli, H. Shan, M.Z. Radi c, D. Pesiksky, A. Marashk-Rothstein, and M. Weigert. 1990. Anti-DNA antibodies from autoimmune mice arise by clonal expansion and somatic mutation. J. Exp. Med. 171:265–292.

16. Vyse, T.J., and B.L. K otzin. 1998. Genetic susceptibility to systemic lupus erythematosus. Annu. Rev. Immunol. 16:261–292.

17. Morel, L., C. Mohan, Y. Yu, B.P. Croker, N. Tian, A. Deng, and E.K. Wakeland. 1997. Functional dissection of systemic lupus erythematosus using congenic mouse strains. J. Immunol. 158:6019–6028.

18. Theofilopoulos, A.N., and F.J. Dixon. 1985. Murine models of systemic lupus erythematosus. Adv. Immunol. 37:269–390.

19. Doody, G.M., L.B. Justement, C.C. Delibrias, J.R. Mathews, and E.K. Wakeland. 1997. Identification of the tyrosine phosphatase PTP1C as a B cell antigen receptor-associated protein involved in the regulation of B cell signaling. J. Exp. Med. 181:2077–2084.

20. Chan, V.W.F., C.A. Lowell, and A.L. DeFranco. 1998. Defective regulation of antigen receptor signaling in Lyn-deficient B lymphocytes. Curr. Biol. 8:545–553.

21. Smith, K.G.C., D.M. Tarlinton, G.M. Doody, M.L. Hibbs, and D.T. Fearon. 1998. Inhibition of the B cell by CD22: a role in B cell activation for CD22 and the protein tyrosine phosphatase SHP. Science. 269:242–244.

22. Campbell, M.A., and N.R. Klinman. 1995. Phosphorytosine-dependent association between CD22 and protein tyrosine phosphate 1C. Eur. J. Immunol. 25:1573–1579.

23. Pani, G., M. Kozlowski, J.C. Cambier, G.B. Mills, and K.A. Siminovitch. 1995. Identification of the tyrosine phosphatase PTP1C as a B cell antigen receptor-associated protein involved in the regulation of B cell signaling. J. Exp. Med. 181:2077–2084.

24. Nishizumi, H., K. Horikawa, I. Minari-Rascan, and T.A.
Yamamoto. 1998. A double-edged kinase Lyn: a positive and negative regulator for antigen receptor-mediated signals. J. Exp. Med. 187:1343–1348.

35. Shultz, L.D., and M.C. Green. 1976. Motheaten, an immunodeficient mutant of the mouse. II. Depressed immune competence and elevated serum immunoglobulins. J. Immunol. 116:936–943.

36. Hibbs, M.L., D.M. Tarlinton, J. Armes, D. Grail, G. Hodgson, R. Maglito, S.A. Stacker, and A.R. Dunn. 1995. Multiple defects in the immune system of Lyn-deficient mice culminating in autoimmune disease. Cell. 83:301–311.

37. Nishizumi, H., I. Taniuchi, Y. Yamanashi, D. Kitamura, D. Illic, S. Mori, T. Watanabe, and T. Yamamoto. 1995. Impaired proliferation of peripheral B cells and indication of autoimmune disease in lyn-deficient mice. Immunity. 3:549–560.

38. Chan, V.W.F., F. Meng, P. Soriano, A.L. DeFranco, and C.A. Lowell. 1997. Characterization of the B lymphocyte populations in Lyn-deficient mice and the role of Lyn in signal initiation and down-regulation. Immunity. 7:69–81.

39. Stoddart, A., R.J. Ray, and C.J. Paige. 1997. Analysis of murine CD 22 during B cell development: CD 22 is expressed on B cell progenitors prior to IgM. Int. Immunol. 9:1571–1579.

40. Slater, R.A., P.C. Sandel, and J.G. Monroe. 1998. B cell receptor-induced apoptosis in primary transitional B cells signaling requirements and modulation by T cell help. Int. Immunol. 10:1673–1682.

41. Fearon, D.T. 1978. Regulation by membrane sialic acid of beta 1H-dependent decay-dissociation of amplification C3 convertase of the alternative pathway. Proc. Natl. Acad. Sci. USA. 75:1971–1975.

42. Kazatchkine, M.D., D.T. Fearon, and K.F. Austen. 1979. Human alternative complement pathway: membrane-associated sialic acid regulates the competition between B and beta 1H for cell-bound C3b. J. Immunol. 122:75–81.

43. Chukwuocha, R.U., A.B. Hartman, and A.J. Feeney. 1994. Sequences of four new members of the VH 7183 gene family in BALB/c mice. Immunogenetics. 40:76–78.