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Immunology

Induction of tolerance to an IgG autoantibody

(double-stranded DNA antibody/B-cell tolerance/anergy/allelic exclusion)

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ABSTRACT **Nonautoimmune mice transgenic for the heavy chain of an IgG2b anti-double-stranded-DNA antibody express the transgene in lymphoid organs and display partial allelic exclusion of this γ2b transgene. The spleens of these mice are characterized by marked B-cell depletion. Although there are B cells in these mice that express the transgene and recognize double-stranded DNA, they are anergic *in vivo*. Recovery from the state of anergy occurs *in vitro* after lipopolysaccharide stimulation. Thus this transgenic model demonstrates the induction of self tolerance to an IgG autoantibody.**

Anti-DNA antibodies are characteristic of the disease systemic lupus erythematosus (SLE) and of the lupus-like syndromes in autoimmune strains of mice. The pathogenic autoantibodies of SLE are usually high-affinity IgG antibodies that bind to double-stranded DNA (dsDNA) and are encoded by somatically mutated immunoglobulin variable region genes.

It is believed the presence of anti-dsDNA antibodies in the serum of patients with lupus is due to a defect in regulation resulting in the breakdown of tolerance. Tolerance has been attributed to three basic mechanisms: deletion, anergy, and suppression. Deletion represents the elimination of autoreactive clones and is a major mechanism for negative selection of autoreactive T cells in the thymus (1, 2). It has been observed for B cells as well (3, 4). Anergy, defined as long-term functional silencing or unresponsiveness, has also been observed among autoreactive T (5, 6) and B cells (7, 8). Although studies on suppression have been more controversial, there appears to be a subpopulation of T cells that can acutely down-regulate T- and B-cell autoresponsiveness (9, 10).

It has been difficult to study the regulation of B cells secreting autoantibodies *in vivo*, since they comprise a small percentage of the total lymphocyte population. Several groups have used the transgenic mouse model to look at the regulation of IgM autoantibodies and have observed anergy or deletion depending, in part, upon whether the autoantigen is soluble or membrane bound (3, 4, 7, 8). Tolerance induction is dependent on antigen-receptor-mediated crosslinking and subsequent signal transduction (11, 12). IgM antibodies can be expressed on the membrane only in association with an α/β heterodimer. The α subunit is encoded by the *mb-1* gene. IgG2b, however, does not need the *mb-1* gene product for transport to the membrane and, hence, does not require heterodimer association for surface expression (13). In addition, the glycosylation of the *mb-1*-encoded protein that associates with IgM may differ from that of the *mb-1*-encoded protein that associates with IgG. These differences between membrane IgM and membrane IgG could affect transmembrane signaling and result in the use of different pathways of regulation.

We have used the transgenic model to study the regulation of a potentially pathogenic IgG anti-dsDNA antibody. This laboratory has characterized (14) anti-dsDNA antibodies from hybridomas derived from BALB/c mice after immunization with phosphorylcholine or with an anti-I-J antibody. These antibodies were initially selected for their expression of a member of the S107 heavy chain variable region (V_H) gene family, as the S107 family has been shown to be involved in anti-DNA responses in (MRL × lpr) and (NZB × NZW)F_1 mice (15–18). They are distinguished by the fact that they bind dsDNA strongly, are IgG but do not display any somatic mutation, and are derived from a nonautoimmune strain. We have produced nonautoimmune transgenic mice containing the R4A V_H11 gene utilized by one of these anti-DNA antibodies ligated to a γ2b constant region gene. We now discuss the regulation of anti-DNA antibodies formed by the random association of this transgenic IgG heavy chain with endogenous light chains and present evidence for the induction of self tolerance to autoreactive anti-DNA IgG antibodies in these mice. This study demonstrates that B cells producing an IgG autoantibody can be rendered anergic.

MATERIALS AND METHODS

Transgene Construction and Generation of Transgenic Mice. A genomic clone containing a 3.5-kilobase (kb) *Eco*RI fragment consisting of the rearranged VDJ (variable–diversity–joining) region and the heavy chain enhancer region was isolated from the R4A hybridoma, which secretes an anti-dsDNA antibody, ligated to a 6.8-kb fragment containing both the membrane and secreted forms of γ2b, and cloned into Bluescript. This entire 10.3-kb insert was removed from the vector by digestion with *Not* I and *Sal* I restriction enzymes, purified by electroelution, and microinjected into the male pronucleus of C57BL/6 × CBA fertilized eggs, as described (19).

Southern and Northern Blot Analysis. DNA was prepared from tails of 4-week-old mice (19), digested with *Kpn* I, transferred to nitrocellulose after electrophoresis, (20) and probed with a ^{32}P-labeled 312-base-pair *Sst* I fragment containing most of the γ2b CH3 heavy chain constant region domain (21). Total RNA was isolated from the spleen, kidney, heart, brain, thymus, and liver of a transgenic mouse and from the spleen of a nontransgenic littermate, electrophoresed on a denaturing formaldehyde gel, transferred to nitrocellulose (22), and hybridized to a γ2b CH3 probe (described above) or to a probe specific for the S107 V_H gene family.

Flow Cytometry Analysis. Spleen cells from 6- to 10-week-old transgenic mice and their nontransgenic littermates were depleted of erythrocytes and resuspended at 1×10^7 cells per ml. Cells were incubated for 30 min on ice with the following antibodies: fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse IgG $F(ab')_2$ (Cappel Laboratories), biotiny-

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Abbreviations: FITC, fluorescein isothiocyanate; LPS, lipopolysaccharide; SLE, systemic lupus erythematosus; dsDNA, double-stranded DNA; V_H, heavy chain variable region.

lated goat anti-mouse IgM $F(ab')_2$ (Cappel Laboratories), or phycoerythrin-conjugated anti-B220 (PharMingen, San Diego). Biotinylated antibodies were counterstained with phycoerythrin-conjugated streptavidin (Fisher). Cells that were to be double stained were incubated for another 30 min on ice with a second antibody or with phycoerythrin-conjugated streptavidin. Cells were then fixed overnight in phosphate-buffered saline (PBS)/1% paraformaldehyde. Fluorescence was analyzed using a FACScan flow cytometer (Becton Dickinson) and LYSYS software programs.

Cytoplasmic Immunofluorescent Staining. Spleen-cell suspensions were prepared as described above and resuspended in PBS/5% (vol/vol) fetal calf serum at 0.5×10^6 cells per ml. Slides were prepared using a Cytospin 2 (Shandon, Southern Products Ltd., Cheshire, England). Cells were fixed in 95% ethanol/5% acetic acid (vol/vol) for 20 min on ice and immunostained for 30 min with goat anti-mouse IgG $F(ab')_2$-FITC or with goat anti-mouse IgM $F(ab')_2$-rhodamine (Cappel Laboratories) or double stained with both antibodies diluted 1:100 in PBS/1% bovine serum albumin.

Lipopolysaccharide (LPS) Stimulation. Spleen-cell suspensions from transgenic and nontransgenic littermates were prepared as described. Cells were resuspended in RPMI 1640 medium supplemented with 10% fetal calf serum, 2 mM glutamine, 1% penicillin/streptomycin, 1% nonessential amino acids (GIBCO), 1% pyruvate, and 50 μM 2-mercaptoethanol. Approximately 1.0×10^6 cells per ml were plated per well in 24-well flat-bottom tissue culture plates. Wells were cultured in duplicate in the absence of and presence of LPS (Sigma) at 0.1–25 μg/ml. Supernatants were collected at day 3 and day 6 of culture and assayed by ELISA for dsDNA binding (14).

Quantitative IgG γ2b ELISA. A 96-well polystyrene flat-bottom ELISA plate (Costar) was coated with a 1:1000 dilution of either goat anti-mouse IgG (γ2b) (Fisher) or goat anti-mouse IgM (Fisher) for 2 h at 37°C. Plates were blocked for 2 h with PBS/1% bovine serum albumin, washed with PBS/0.05% Tween, and incubated for 2 h at 37°C with 1:10 dilutions of sera or with 1:2 dilutions of a commercially purified γ2b standard. Wells were incubated for 2 h with a 1:1000 dilution of goat anti-mouse IgG γ2b conjugated to alkaline phosphatase, washed, and developed with alkaline phosphatase substrate tablets. Absorbance of triplicate wells was measured at 405 nm.

RESULTS

R4A γ2b Transgenic Mice. The R4A γ2b construct encoding both membrane and secreted γ2b was injected into (C57BL/B6 × CBA)F_1 fertilized eggs (Fig. 1*A*). Southern blot analysis on tail DNA digested with *Kpn* I and probed with a γ2b CH3 heavy chain constant region fragment demonstrated the presence of the transgene integrated into the genome at a single site (Fig. 1*B*). Densitometry revealed six to seven copies of the transgene arrayed in a head-to-tail fashion.

Spleen Histology. The weight of the spleens of all the transgenic animals analyzed was 14–39% the weight of the spleens of their nontransgenic littermates (Fig. 2). Fifty to 70% fewer total cells were present in the transgenic spleens than in the nontransgenic spleens. Furthermore, histopathologic studies revealed a paucity of B cells in follicles in the spleen of transgenic mice and a skewing of the B cells toward an immature stage of development.

RNA Expression. Expression of the R4A γ2b transgene was demonstrated by Northern blot analysis of splenic RNA probed with a CH3 fragment and a fragment specific for the S107 V_H gene family (Fig. 3*A*). On an overexposed blot, we observed RNA expression of both the membrane and secreted forms of γ2b in the transgenic mouse spleen, corresponding to molecular sizes of 3.9 kb and 1.9 kb, respectively (data not shown). RNA from liver, kidney, brain, heart, thymus, and spleen of a transgenic mouse was also probed with the γ2b fragment (Fig. 3*B*). RNA expression was highest in the spleen but some γ2b RNA was present in the thymus as well. Expression of a heavy-chain transgene in the thymus has been observed by others and is probably due to the presence of similar transactivating factors in both T and B cells (23, 24). Low levels of γ2b expression were observed in other organs but this was presumably due to infiltration by B cells and plasma cells.

Flow Cytometry Analysis. Flow cytometry analysis was performed on splenic cells of 6- to 10-week-old mice to quantitate the percentage of transgenic B cells that were positive for membrane IgG. In one assay representative of several others, 26% of transgenic splenic cells that were positive for the B220 B-cell marker were surface-positive for IgG and 10% of the B220-positive splenic cells from their nontransgenic littermates were IgG surface-positive.

Cytoplasmic Immunofluorescence Staining. Cytoplasmic staining of splenic B cells with FITC-labeled goat anti-mouse IgG $F(ab')_2$ fragments demonstrated that 10–15% of total transgenic spleen cells stained positively for IgG compared to <1% of total cells from the spleens of nontransgenic littermates (Fig. 4 *A* and *B*). Cells were double-stained with anti-mouse IgG $F(ab')_2$-FITC and anti-mouse IgM $F(ab')_2$

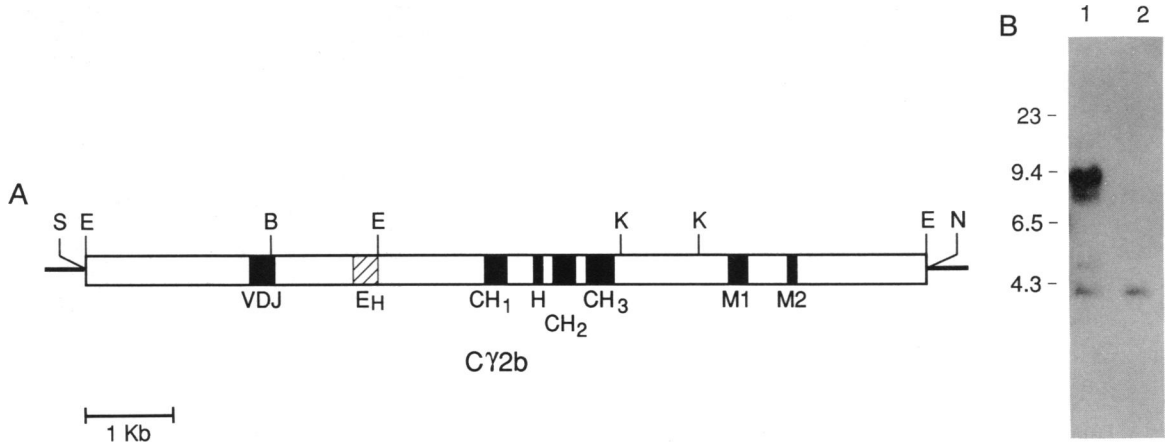

FIG. 1. (*A*) Map of the R4A γ2b heavy chain transgene. A 3.5-kb *Eco*RI fragment containing the rearranged VDJ (variable–diversity–joining) and heavy chain enhancer (E_H) regions was ligated to a 6.8-kb *Eco*RI fragment containing the γ2b constant (CH) region including the membrane exons (M1 and M2). Solid boxes depict exons. Open boxes depict introns. B, *Bam*HI; E, *Eco*RI; K, *Kpn* I; S, *Sal* I; N, *Not* I. (*B*) Southern blot of tail DNA digested with *Kpn* I and hybridized to a γ2b CH3 fragment. Lanes: 1, DNA from a transgenic mouse (the transgene is indicated by a band at 9.3 kb); 2, DNA from a nontransgenic littermate. Molecular sizes in kb are shown.

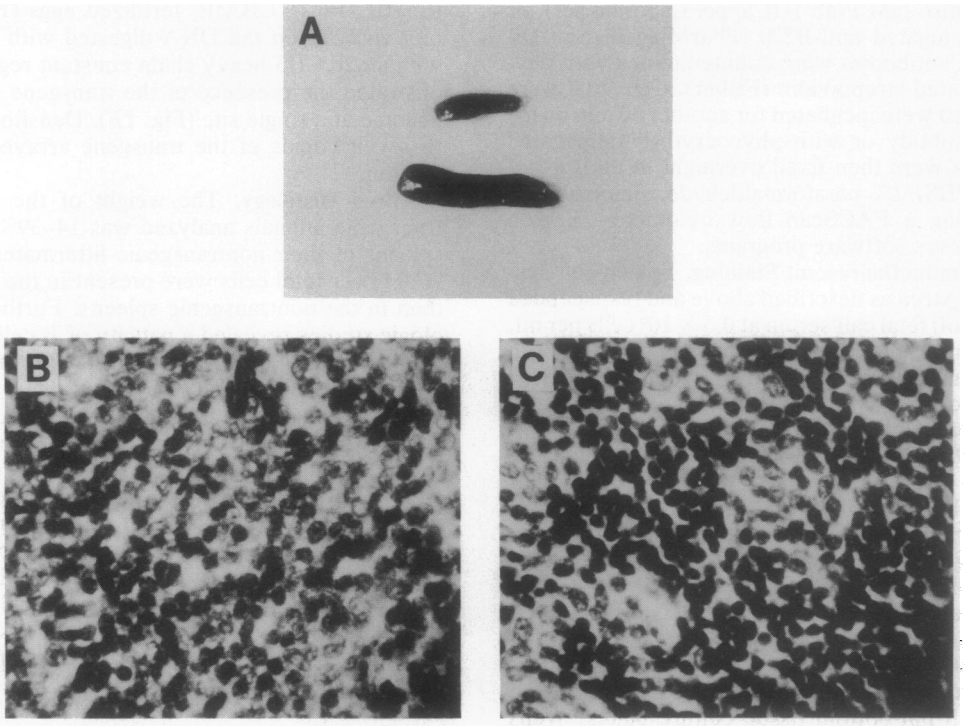

FIG. 2. (*A*) Spleen of a transgenic mouse (upper spleen) is smaller than that of its nontransgenic littermate (lower spleen). Hematoxylin and eosin-stained spleen section from transgenic mouse (*B*) and a normal mouse (*C*). There are fewer mature cells and more cells with abundant cytoplasm in the transgenic spleen than in the normal spleen. (×400.)

conjugated to rhodamine to determine whether the cells expressing IgG were allelically excluded. Many of the IgG-positive cells of the transgenic mice appear to be allelically excluded, as they did not immunofluoresce with anti-IgM (Fig. 4 *C* and *D*). Although there is variability from mouse to mouse, in most mice, 25–50% of the cells expressing the transgene display allelic exclusion.

Assays of Serum Immunoglobulin. Since the R4A transgene encodes a heavy chain that associates with an unmutated $V_\kappa 1$ light chain variable region to form an anti-dsDNA antibody in the R4A hybridoma (14), we presumed that R4A γ2b–κ light chain associations would occur in the transgenic mouse that

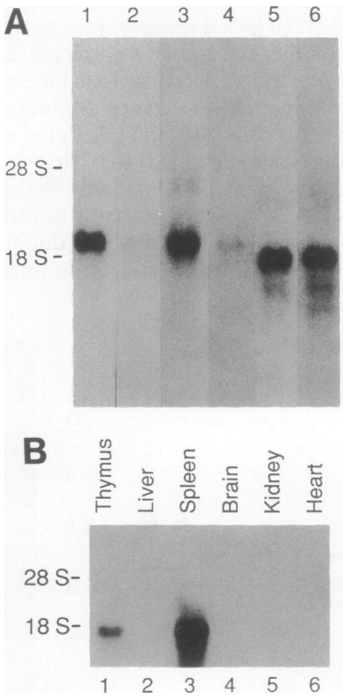

FIG. 3. (*A*) Expression of the R4A γ2b transgene as demonstrated by Northern blot analysis. RNA was hybridized with probes specific for the S107 V_H gene family (lanes 1 and 2), γ2b (lanes 3 and 4), and actin (lanes 5 and 6). RNA from a transgenic spleen is in lanes 1, 3, and 5, and RNA from the spleen of a nontransgenic littermate is in lanes 2, 4, and 6. (*B*) Tissue-specific expression of the R4A γ2b transgene. Tissues are indicated.

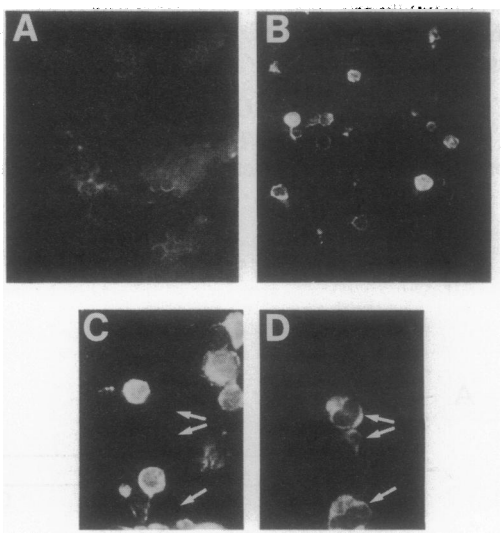

FIG. 4. Cytoplasmic immunofluorescent staining of spleen cells. Splenic cells from transgenic (*A*) and nontransgenic (*B*) mice were immunostained with goat anti-mouse IgG $F(ab')_2$-FITC for detection of the γ2b transgene. Little immunostaining for IgG was observed in the nontransgenic mouse (*A*), but 10–15% of the cells stained positively for IgG in the transgenic mouse (*B*). (×25.) Allelic exclusion of cells expressing the γ2b transgene. Photographs in *C* and *D* represent the same field shot with different filters to observe IgM-positive cells (*C*) and IgG-positive cells (*D*). Arrows in *D* indicate allelically excluded B cells expressing IgG only. (×60.)

would lead to DNA binding. We, therefore, used an ELISA to assay sera from transgenic mice and normal controls for their titers of circulating anti-DNA γ2b antibodies (Table 1). The concentrations of total γ2b isotype in the sera of these mice were determined. Table 1 presents results of one such assay. It includes two mice with slightly elevated titers of γ2b anti-dsDNA antibody. They also showed elevated titers of total γ2b. Only one transgenic mouse had slightly elevated γ2b titers without any increase in γ2b anti-DNA activity. No other sera tested showed dsDNA binding. No transgenic mouse had a titer of γ2b anti-DNA activity similar to that present in serum of (NZB × NZW)F_1 mice, despite having equivalent or higher titers of serum γ2b. Transgenic and nontransgenic mice showed similar concentrations of IgM in their sera (≈0.22 mg/ml).

LPS Stimulation. Splenic lymphocytes from transgenic mice and from their nontransgenic littermates were cultured with increasing concentrations of LPS to see whether B cells could be activated to secrete anti-dsDNA antibodies (Fig. 5). After 3 and 6 days in culture with LPS (0.1–25 μg/ml) only the transgenic B cells could be stimulated to secrete γ2b anti-dsDNA antibodies. This indicates that at least some of the transgenic B cells are present in a state of anergy from which they can recover by mitogen stimulation.

Renal Pathology. Neither transgenic mice nor their nontransgenic littermates displayed proteinuria or renal pathology.

DISCUSSION

While it is clear there is a genetic predisposition to the development of SLE, there is little evidence to date that immunoglobulin gene polymorphisms play a role in the genetic predisposition. Furthermore, it is clear that the immunoglobulin genes of a nonautoimmune animal can encode autoantibodies but do so *in vivo* only in an animal with a genetic background that predisposes to autoimmunity (25). These observations and others provide strong support for a regulatory defect in SLE. It has, however, been difficult to study autoantibody regulation because even in an animal with autoimmune disease, only a very small percent of the B cells are producing autoantibodies (26). Transgenic mice have provided an important approach to studying regulation of autoreactive B cells and mechanisms of B-cell tolerance.

Autoreactivity can be expressed not only by IgM antibodies but also by IgG. Indeed, some antibodies will not acquire autospecificity until they encounter the T-cell factors that lead to somatic mutation and heavy chain class switching

Table 1. Serum levels of anti-DNA antibodies in transgenic and nontransgenic mice

Mouse	IgG2b, mg/ml	Anti-DNA (IgG2b), A_{405}
Tg+		
1	2.50	0.375
2	1.90	0.346
3	0.42	0.130
4	0.40	0.093
5	0.65	0.113
6	0.47	0.077
Tg−		
7	0.42	0.062
8	0.26	0.088
9	0.35	0.095
(NZB × NZW)F_1	0.35	1.197

Serum levels of anti-DNA γ2b antibodies in transgenic (Tg+) and nontransgenic (Tg−) mice as measured by ELISA and quantification of IgG γ2b concentrations in these sera. Serum from NZB × NZW mouse was a positive control for anti-DNA γ2b binding.

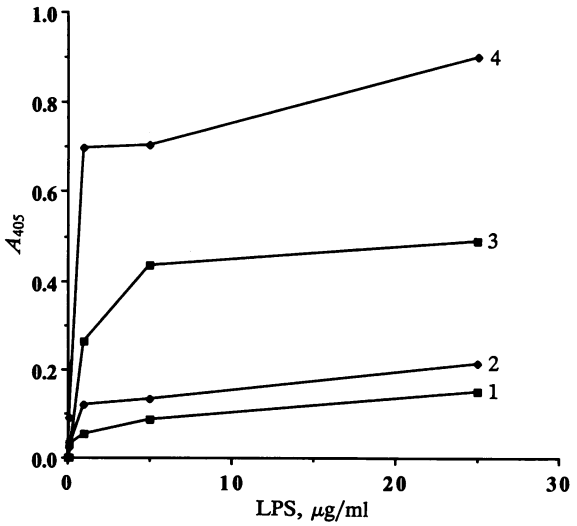

FIG. 5. Measurement of γ2b anti-DNA antibody secreted into culture supernatants after LPS stimulation. Curves: 1, nontransgenic (day 3); 2, nontransgenic (day 6); 3, transgenic (day 3); 4, transgenic (day 6).

(27–30). We have established a transgenic mouse expressing a γ2b heavy chain of an anti-dsDNA antibody to study the regulation of IgG autoantibodies. Because IgG antibodies are produced by activated B cells or at least by B cells that are more mature than IgM-producing B cells and because membrane IgG associates with different cytoplasmic proteins than membrane IgM, it is possible that IgG-producing B cells are regulated differently from IgM-producing cells. Just as mechanisms of central tolerance and mechanisms of peripheral tolerance exist for T cells, central and peripheral tolerance may exist for B cells also. The R4A transgenic mouse provides a model for studying the breakdown in peripheral B-cell tolerance that regulates IgG autoantibodies, just as the IgM transgenic animals previously reported have provided insights into central B-cell tolerance. The defect in SLE appears to be manifest in peripheral B-cell tolerance as the pathogenic antibodies are IgG and appear to be made by B cells activated by antigen and helper T cells.

In the R4A γ2b transgenic mouse, the transgene is expressed in B cells and T cells in the thymus. B cells expressing the transgene display partial allelic exclusion. Allelic exclusion has been seen in μ transgenic mice when the membrane form of the μ chain is present (31). There are few data relevant to allelic exclusion in γ heavy chain transgenic mice. A lack of allelic exclusion has been reported in mice transgenic for a γ1 transgene (32) and generally in mice transgenic for a γ2b transgene (33). Roth *et al.* (34) have found a single founder that did display allelic exclusion of a γ2b transgene. The degree of allelic exclusion we see is variable from animal to animal but in general 30–50% of the IgG-producing B cells do not produce an IgM heavy chain. Partial allelic exclusion has also been demonstrated by assaying hybridomas from a R4A γ2b transgenic mouse. Thirty percent of clones secrete an IgG γ2b heavy chain that has been demonstrated by sequence analysis to be the transgene. Of these γ2b-expressing hybridomas, 70% were shown to secrete only one heavy chain.

It is not yet clear why some mice expressing an IgG transgene display allelic exclusion and others do not. It is critical, however, that at least a percentage of the B cells expressing the transgene display allelic exclusion for studies in B-cell regulation to proceed. B cells that do not display allelic exclusion and secrete both protective and potentially pathogenic antibodies may receive contradictory signals regarding immunoglobulin secretion and tolerance induction and not represent a physiologic B-cell subset. Avoidance of

the simultaneous production of protective and pathogenic antibodies may underlie the need for allelic exclusion.

Most of the mice do not display serum titers of anti-dsDNA antibody. Those that do display low titers show no renal pathology, suggesting either that the titers are too low to mediate tissue injury or that the secreted anti-dsDNA antibodies are nonpathogenic. Studies of the serum anti-dsDNA antibodies in these mice should help elucidate characteristics of autoantibodies that escape tolerance such as the anti-mouse erythrocyte antibodies secreted in transgenic mice generated by Okamoto *et al.* (35). It is possible that the expressed γ2b anti-dsDNA antibodies may have somatic mutations that permit them to escape normal regulatory mechanisms. Alternatively, the secreted anti-dsDNA antibodies may be produced by B cells that are not allelically excluded and, because they express two distinct membrane immunoglobulin receptors, may befuddle normal mechanisms of tolerance induction.

The mice we have generated have small spleens that are substantially depleted of B cells. *In vitro* transfection experiments show that the R4A γ2b heavy chain in association with several light chains forms an anti-nuclear antibody (unpublished results). We believe that the B cells expressing these autoreactive heavy and light chain combinations are anergic or deleted in the transgenic animal, thus accounting for the diminished size of the spleen. That anti-dsDNA activity could be induced by LPS stimulation of B cells demonstrates that at least some of the B cells with "forbidden" heavy and light chains are anergic *in vivo* and not deleted. It remains possible, however, that the anergic B cells represent only a fraction of the R4A γ2b–light chain combinations that encode autoreactivity and that B cells with other combinations are indeed deleted. The answer to this question awaits a detailed examination of the light chains present in B cells secreting the R4A γ2b heavy chain *in vivo* and in those B cells that are anergic *in vivo*. If there are light chains that can associate with the R4A γ2b heavy chain *in vitro* but are not seen in either the expressed or anergic antibody repertoire, it is possible that the B cells expressing such light chains are deleted.

Radic *et al.* (36) also demonstrated that the heavy chain of an anti-DNA antibody may bind DNA when associated *in vitro* with a spectrum of light chains. The heavy chain they studied bound single-stranded DNA and dsDNA in association with a number of light chains. In studies of transgenic mice expressing that heavy chain, it appears that any B cell expressing a light chain that leads to DNA binding in combination with the transgenic heavy chain is rendered tolerant through anergy or deletion (8). Cells binding single-stranded DNA could be rescued by fusion, but no dsDNA binding cells were detectable. Perhaps the difference between those mice and the mice we describe is that a larger number of germline-encoded light chains can associate with the R4A heavy chain to encode dsDNA binding. Thus, dsDNA binding B cells are more frequent and more easily detectable. Alternatively, there may be differences in antigenic cross reactivities or fine specificities or in idiotypic regulation that lead to anergy of dsDNA binding B cells in R4A γ2b transgenic animals and not in those described by Erikson *et al.* (8).

The mice reported here confirm the utility of transgenic models for the study of peripheral tolerance. They demonstrate the regulation of an IgG autoantibody heavy chain and show that mechanisms of tolerance exist for IgG autoantibodies and for IgM.

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1. Kappler, J. W., Roehm, N. & Marrack, P. (1987) *Cell* **49**, 273–280.
2. Kisielow, P., Bluthmann, H., Staerz, U. D., Steinmetz, M. & Boehmer, H. V. (1988) *Nature (London)* **333**, 742–746.
3. Nemazee, D. A. & Burki, K. (1989) *Nature (London)* **337**, 562–566.
4. Hartley, S. B., Crosbie, J., Brink, R., Kantor, A. B., Basten, A. & Goodnow, C. C. (1991) *Nature (London)* **353**, 765–769.
5. Jenkins, M. K. & Schwartz, R. H. (1987) *J. Exp. Med.* **165**, 302–319.
6. Lamb, J. R., Skidmore, B. J., Green, N., Chiller, J. M. & Feldmann, M. (1983) *J. Exp. Med.* **157**, 1434–1447.
7. Goodnow, C. C., Crosbie, J., Adelstein, S., Lavoie, T. B., Smith-Gill, S. J., Brink, R. A., Pritchard-Briscoe, H., Wotherspoon, J. S., Loblay, R. H., Raphael, K., Trent, R. J. & Basten, A. (1988) *Nature (London)* **334**, 676–682.
8. Erikson, J., Radic, M. Z., Camper, S. A., Hardy, R. R., Carmack, C. & Weigert, M. (1991) *Nature (London)* **349**, 331–334.
9. Gershon, R. K. & Kondo, K. (1971) *Immunology* **21**, 181–192.
10. Basten, A., Loblay, R. H., Trent, R. J. & Gatenby, P. A. (1980) *Recent Adv. Clin. Immunol.* **2**, 33–64.
11. DeFranco, A. L., Page, D. M., Blum, J. H. & Gold, M. R. (1989) *Cold Spring Harbor Symp. Quant. Biol.* **54**, 733–740.
12. Scott, D. W., Ales-Martinez, J.-E., Clace, J. H., Lo Cascio, N. J., Silver, L. & Warner, G. L. (1989) *Cold Spring Harbor Symp. Quant. Biol.* **54**, 899–905.
13. Venkitaraman, A. R., Williams, G. T., Dariavach, P. & Neuberger, M. S. (1991) *Nature (London)* **352**, 777–782.
14. Shefner, R., Kleiner, G., Turken, A., Papazian, L. & Diamond, B. (1991) *J. Exp. Med.* **173**, 287–296.
15. Shlomchik, M. J., Aucoin, A. H., Pisetsky, D. S. & Weigert, M. G. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 9150–9154.
16. Behar, S. M. & Scharff, M. D. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 3970–3974.
17. Eilat, D., Webster, D. M. & Rees, A. R. (1988) *J. Immunol.* **141**, 1745–1753.
18. Treppichio, W., Jr., & Barrett, K. J. (1987) *J. Immunol.* **138**, 2323–2331.
19. Hogan, B., Constantini, F. & Lacy, E. (1986) *Manipulating the Mouse Embryo: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
20. Southern, E. M. (1975) *J. Mol. Biol.* **98**, 503–517.
21. Tucker, P. W., Marcu, K. B., Newell, N., Richards, J. & Blattner, F. R. (1979) *Science* **206**, 1303–1306.
22. Thomas, P. S. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 5201–5205.
23. Grosschedl, R., Weaver, D., Baltimore, D. & Constantini, F. (1984) *Cell* **38**, 647–658.
24. Tsang, H., Pinkert, C., Hagman, J., Lostrum, M., Brinster, R. L. & Storb, U. (1988) *J. Immunol.* **141**, 308–314.
25. Gavalchin, J., Nicklas, J. A., Eastcott, J. W., Madaio, M. P., Stollar, B. D., Schwartz, R. S. & Datta, S. K. (1985) *J. Immunol.* **134**, 885–893.
26. Ando, D. G., Ebling, F. M. & Hahn, B. H. (1986) *Arthritis Rheum.* **29**, 1139–1146.
27. Diamond, B. & Scharff, M. D. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 5841–5844.
28. Siekevitz, M., Kock, C., Rajewsky, K. & Dildrop, R. (1987) *Cell* **48**, 757–770.
29. Shimuzu, A. & Honjo, T. (1984) *Cell* **36**, 801–803.
30. Shlomchik, M., Mascelli, M., Shan, H., Radic, M. Z., Pisetsky, D., Marshak-Rotherstein, A. & Weigert, M. (1990) *J. Exp. Med.* **171**, 265–297.
31. Storb, U. (1987) *Annu. Rev. Immunol.* **5**, 151–175.
32. Yamamura, J., Judo, A., Ebihara, T., Kamino, K., Araki, K., Kumahara, Y. & Watanabe, T. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 2152–2156.
33. Storb, U., Engler, P., Manz, J., Gollahon, K., Denis, K., Lo, D. & Brinster, R. (1988) *Ann. N.Y. Acad. Sci.* **546**, 51–56.
34. Roth, P., Lo, D., Hackett, J., Jr., Loglio, L. & Storb, U. (1990) *J. Cell. Biochem. Suppl.* **114D**, 228 (abstr.).
35. Okamoto, M., Murakami, M., Shimizu, A., Ozaki, S., Tsubata, T., Kumagai, S. & Honjo, T. (1992) *J. Exp. Med.* **175**, 71–79.
36. Radic, M. Z., Mascelli, M. A., Erikson, J., Shan, H. & Weigert, M. (1991) *J. Immunol.* **146**, 176–182.