haemopoietic cells via receptors other than the MHC-I-specific receptors, yet reject only those cells that fail to engage their MHC-I-specific receptors. This model accounts for several observations, including hybrid resistance and the striking finding that B6 mice transgenic for the D^d MHC-I gene reject B6 marrow grafts[5]. A second model has MHC-I proteins on the cell surface masking non-MHC-I antigens that serve as targets for marrow graft rejection. This 'masking' model was proposed in a different context[16–18], to account for the finding that loss of MHC-I expression by tumour cell lines augments their sensitivity to natural killer cells[16–20]. The 'missing self' model can also explain these results. Finally, it has been proposed that haemopoietic cells express target antigens encoded by allelically variable recessive genes (*Hh-1* genes) in the MHC that are distinct from conventional MHC genes[21,22].

Our data on rejection of MHC-I-deficient marrow are most consistent with the 'missing-self' or 'masking' models. Either could have evolved to eliminate variant cells that lose MHC-I expression by mutation or as a result of viral infection[23], thereby evading conventional cytotoxic T lymphocyte immunity[5,20,24]. Because it is hard to reconcile all marrow rejection data with any one model, it is possible that rejection of haemopoietic cells by $NK1.1^+$ cells involves several mechanisms.

It is interesting that −/− mice fail to reject their own marrow, as evidenced by their lack of haemopoietic deficiencies, and by the experiment shown in Fig. 2*d*. One interpretation of this is that the definition of 'self' with respect to marrow transplantation is determined by the environment in which the effector cells mature, by a tolerance-inducing and/or positive-selection process. Differentiation of the effector cells mediating rejection may require interaction with MHC-I molecules, as we found for $CD8^+$ mature T cells, which fail to develop in −/− mice[7]. We are currently investigating the role of MHC-I expression in the development of natural killer cells and its influence on the rejection of allogeneic marrow transplants. □

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1. Cudkowicz, G. & Stimpfling, J. H. *Nature* **204,** 450–453 (1964).
2. Cudkowicz, G. & Bennett, M. *J. Exp. Med.* **134,** 1513–1528 (1971).
3. Murphy, W. J., Kumar, V. & Bennett, M. *J. exp. Med.* **166,** 1499–1509 (1987).
4. Yankelevich, B., Knobloch, C., Nowicki, M. & Dennert, G. *J. Immun.* **142,** 3423–3430 (1989).
5. Ohlen, C. *et al. Science* **246,** 666–668 (1989).
6. Zijlstra, M., Li, E., Sajjadi, F., Subramani, S. & Jaenisch, R. *Nature* **342,** 435–438 (1989).
7. Zijlstra, M. *et al. Nature* **344,** 742–746 (1990).
8. Koller, B. H. & Smithies, O. *Proc. natn. Acad. Sci. U.S.A.* **86,** 8932–8935 (1989).
9. Koller, B. H., Marrack, P., Kappler, J. W. & Smithies, O. *Science* **248,** 1227–1230 (1990).
10. Klein, J., Flaherty, L., VandeBerg, J. L. & Schreffler, D. C. *Immunogenetics* **6,** 489–512 (1978).
11. Koo, G. C., Dumont, F. J., Tutt, M., Hackett, J. Jr & Kumar, V. *J. Immun.* **137,** 3742–3747 (1986).
12. Kiessling, R. *et al. Eur. J. Immun.* **7,** 655–663 (1977).
13. Murphy, W., Kumar, V. & Bennett, M. *J. exp. Med.* **165,** 1212–1217 (1987).
14. Herberman, R. B. & Ortaldo, J. R. *Science* **214,** 24–30 (1981).
15. Dennert, G., Knobloch, C., Sugawara, S. & Yankelevich, B. *Immunogenetics* **31,** 161–168 (1990).
16. Storkus, W. J., Howell, D. N., Salter, R. D., Dawson, J. R. & Cresswell, P. *J. Immun.* **138,** 1657–1659 (1987).
17. Shimuzu, Y. & DeMars, R. *Eur. J. Immun.* **19,** 447–451 (1989).
18. Stumhöfel, K. & Hämmerling, G. J. *Eur. J. Immun.* **20,** 171–177 (1990).
19. Harel-Bellan, A. *et al. Proc. natn. Acad. Sci. U.S.A.* **83,** 5688 (1986).
20. Kärre, K., Ljunggren, H. G., Piontek, G. & Kiessling, R. *Nature* **319,** 675–678 (1986).
21. Cudkowicz, G. & Lotzova, E. *Transplant. Proc.* **4,** 1399 (1973).
22. Rembecki, R. M., Bennett, M., Kumar, V. & Potter, T. A. *J. Immun.* **138,** 2734–2738 (1987).
23. Schrier, P. I., Bernards, R., Vaessen, R. T. M. J., Houweling, A. & van der Eb, A. J. *Nature* **305,** 771–775 (1983).
24. Höglund, P. *et al. J. exp. Med.* **168,** 1469–1474 (1988).
25. Liao, N.-S., Maltzman, J. & Raulet, D. H. *J. exp. Med.* **170,** 135–143 (1989).
26. Fowlkes, B. J., Edison, L., Mathieson, B. J. & Chused, T. M. *J. exp. Med.* **162,** 802–822 (1985).
27. Stallcup, K. C., Springer, T. A. & Mescher, M. F. *J. Immun.* **127,** 923–930 (1981).

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Expression of anti-DNA immunoglobulin transgenes in non-autoimmune mice

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SELF-REACTIVE B cells can be regulated by either deletion or inactivation[1]. These manifestations of self-tolerance have been dramatically shown in transgenic mice in which the number of self-reactive cells has been artificially expanded[2,3]. We have now extended these models to ask if B-cell tolerance as described for non-disease-associated antigens also operates for the targets of autoimmunity. The target we have chosen is DNA. Anti-DNA antibodies are diagnostic of certain autoimmune syndromes in humans and are a characteristic of the murine model of systemic autoimmunity, the MRl/lpr mouse[4]. Antibodies to both single-stranded and double-stranded DNA have been implicated in disease[5,6]. By generating anti-DNA transgenic mice, we have addressed the question of whether DNA-specific B cells are regulated in normal (non-autoimmune) mice. We indeed found that most transgenic B cells bind DNA, yet we failed to detect secreted anti-DNA. We suggest that as a consequence of their self-reactivity these B cells are developmentally arrested.

Transgenic mice were constructed using the heavy (H) chain variable (VH) gene of a monoclonal anti-DNA antibody, 3H9, which arose spontaneously in a diseased MRL/lpr mouse[7]. The VH gene coding for 3H9 is repeatedly used in anti-DNA antibodies from this autoimmune strain where it is associated with a variety of light (L) chains including representatives of the Vκ4, Vκ8, Vκ9, and Vκ23 groups (ref. 8 and J. E., manuscript in preparation). The variety of L chains which can combine with the VH3*H*9 to yield an anti-DNA antibody has been extended by transfection to include members of Vκ3 and Vκ21 groups and Vλ1 (ref. 30). Most of these VH3H9/VL combinations result in antibodies that bind both single-stranded (ss) and double-stranded (ds) DNA[8,30], but one combination, VH3H9/Vκ8, binds only ssDNA. The dominant role of the 3H9 H chain in determining DNA binding allows us to study the regulation of different kinds of anti-DNA antibodies. For example, by mating the VH3H9 transgenic to a pre-existing Vκ8 transgenic (C.C., S.A.C., J. J. Mackle, W. U. Gerhard and M.W., manuscript submitted) we would expect to generate a monospecific anti-ssDNA transgenic mouse[30]. In a VH3H9-only transgenic, in which the VH3H9 is combined with many endogenous L chains, we would expect both anti-ssDNA antibodies as well as antibodies that bind both ss and dsDNA. This report focuses on two transgenic lineages: the VH3H9 H-chain-only transgenic and the progeny (VH3H9/Vκ8) of mating this VH3H9 transgenic to the Vκ8 transgenic.

The H-chain transgene dominates the preimmune repertoire in both the VH3H9 and VH3H9/Vκ8 transgenics, as shown in three separate analyses. First, VH3H9 messenger RNA comprises the majority of splenic immunoglobulin mRNA (Fig. 1*b*). Second, lymphocytes from these mice lack surface IgD (Fig. 2*a*). As the VH transgene construct does not include the IgD constant region gene (Fig. 1*a*), had IgD expression been observed it would have originated from endogenous genes. Finally, few, if any, complete endogenous immunoglobulin gene rearrangements are found among hybridomas from either the VH3H9 or VH3H9/Vκ8 transgenics (Table 1). Hybridomas derived from VH3H9/Vκ8 also showed little endogenous κ chain rearrangement (Table 1).

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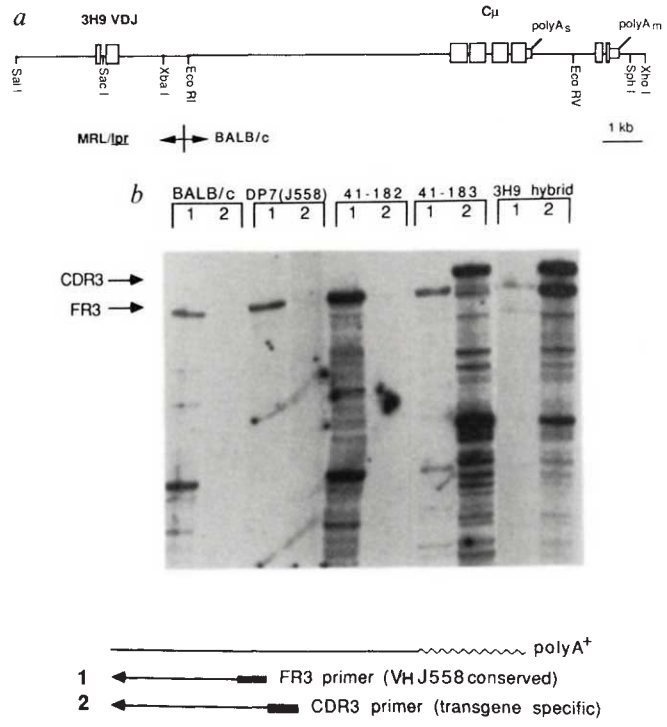

FIG. 1 The VH3H9 transgene construct and its transciption. *a*, The restriction map of the VH3H9 construct. The productive H-chain gene from the 3H9 MRL/lpr anti-DNA hybridoma was joined to the BALB/c IgM constant region[22]. This construct includes the secreted and membrane exons and 3′ untranslated sequences of IgM but does not include IgD. Unique restriction enzyme sites and enzymes cutting only once in the VDJ region are indicated. *b*, Expression of the transgene was evaluated by primer extension analysis of spleen cell poly $(A)^+$ RNA. Identical amounts of poly $(A)^+$ RNA were annealed to the framework region (FR3) primer, homologous to a conserved region of VH J558 genes (lanes 1), or the complementarity-determining region (CDR3) primer, homologous to the junctional diversity region of the VH3H9 transgene (lanes 2). In a normal mouse the J558 family comprises the majority of splenic immunoglobulin mRNA (~50%)[23], yet the VH3H9-specific RNA is undetectable: no signal in CDR3 lanes is seen with BALB/c RNA, RNA from a non-transgenic littermate (41–182) or from a hybridoma that uses a different J558 gene (DP7). Extension with both primers is seen only when RNA from a transgene-positive mouse (41–183) or RNA from the 3H9 hybridoma is used. The majority (62%) of J558 mRNA in transgenic spleen 41–183 is transcribed from the transgene. Therefore, at least 31% of total mRNA is 3H9 derived. This is certainly an underestimate. We think it is unlikely that the VH3H9 transgene exerts its influence just on the J558 family. Furthermore, plasma cells contribute disproportionately to splenic immunoglobulin mRNA levels[24] and may overrepresent endogenous VH genes.

METHODS. *a*, The productively rearranged 3H9 VDJ region was isolated from a size-enriched λ phage library as a 4.4-kilobase *Eco*RI fragment. Subsequently, the 5′ *Eco*RI site was removed using exonuclease III and mung bean nuclease, and a *Sal*I restriction site linker was inserted. The 3H9 VDJ region was joined to the 11.6-kb *Eco*RI–*Xho*I Cμ fragment from BALB/c[22]. The *Sal*I–*Xho*I fragment containing the entire VH3H9 construct was purified from plasmid sequences and microinjected into pronuclei of fertilized (C57BL6 X SJL) embryos[25]. Thirteen founder animals were obtained; the progeny of one of these (no. 41) is described in this report. *b*, Poly $(A)^+$ RNA was prepared from spleens or hybridoma cells. RNA (3 μg) was annealed to 5 pg ^{32}P-labelled oligonucleotides using either the FR3 (5′-TCTTGCACAGTAATAGACTGCAGAGTC-3′) or the CDR3 primer and converted to cDNA. Reaction products were analysed by PAGE and autoradiography. Full-length cDNA was quantitated for each RNA sample by digitized image scanning of the FR3 and CDR3 lanes. The proportion of VH3H9-derived message among the J558 transcripts in transgenic mice was calculated from the FR3/CDR3 cDNA signal ratio of 3H9 hybridoma transcripts (*A*), and the corresponding ratio of signals obtained using 41–183 splenic transcripts (*B*) according to the formula: $X = 1 - (B - A)/B$.

TABLE 1 Summary of transgenic hybridomas

	VH3H9/Vκ8	VH3H9
(*a*) Immunoglobulin$^+$ total (%)	80	88
Transgene$^+$/immunoglobulin$^+$(%)		
VH3H9	83	95
Vκ8	93	NA*
H^0/H^0, H^0/H^r, H^r/H^r, $H^r/-$†	16, 17, 12, 9‡	17, 33, 17, 18‡
K^0/K^0, $K^0/-$§ (%)	83	NA*
(*b*) Specificity		
ssDNA (%)	84	52
dsDNA (%)	0	0
Cardiolipin (%)	84	30

Hybridomas were derived from LPS-stimulated spleen cells from both the VH3H9/Vκ8 and the VH3H9 transgenics. One-hundred-and-sixteen hybridomas were analysed from the VH3H9 transgenic and seventy-five from the VH3H9/Vκ8 transgenic. Immunoglobulin-secreting hybridomas were assayed for the presence of the transgene(s) and for the extent of endogenous immunoglobulin gene rearrangement. *a*, VH3H9 transgene positive animals were first identified by Southern blot analysis and subsequently using polymerase chain reaction (PCR)[16] with the following primers: 5-CTGTCAGGAACTGCAGGTAAGG-3′ (VH3H9 leader intron), and 5′CATAACATAGGAATATTTACTCCTCGC-3′ (VH3H9 CDR3). Vκ8 transgene animals were identified using PCR with Vκ8-specific primers: 5′-GGTACCTGTGGGACATTGTG-3′ (Vκ8 leader), 5′-AGCACCGAACGTGAGAGG-3′ (Vκ8-JK5). Hybridomas were generated from VH3H9/Vκ8 and VH3H9 transgenic spleen cells 3 days after an injection of 50 μg LPS as described[17]. The presence of the transgene(s) and the extent of endogenous immunoglobulin gene rearrangement was determined using Southern blot analysis as described[18]. Messenger RNA sequence analysis was performed on three of the VH3H9/Vκ8 hybridomas and four of the VH3H9 hybridomas, establishing that the transgenes are expressed and that the expressed transgene(s) are unaltered[8]. Messenger RNA primer extension analysis from 32 additional VH3H9 hybridomas confirmed the use of the VH3H9 transgene in 31 of the 32 hybridomas (data not shown). *b*, The hybridoma monoclonal antibodies were assayed for their ability to bind ssDNA, dsDNA, and cardiolipin. Only hybridomas which had retained the transgene(s) DNA are included. The secretion and specificity of the monoclonal antibodies was determined in the enzyme-linked immunoabsorbent assay (ELISA) essentially as described for dsDNA[19], ssDNA[20], and cardiolipin[21].

* NA, not applicable.

† Number of hybridomas with the following genotype: H^0/H^0, both endogenous heavy chain alleles are in germ-line configuration; H^0/H^r, one allele is in germ-line configuration and the other is rearranged; H^r/H^r, both alleles are rearranged; $H^r/-$, one allele is absent, presumably owing to chromosome loss in the hybridoma, and the other is rearranged.

‡ 33% of the endogenous heavy chain rearrangements identified in the VH3H9 hybridomas and 43% in the VH3H9/Vκ8 hybridomas have restriction fragments similar in size to those identified as incomplete, D to JH rearrangements[15].

§ K^0/K^0, both endogenous kappa alleles are in germ-line configuration; $K^0/-$, one endogenous κ allele is in germ-line configuration, the other is absent, presumably as a result of chromosomal loss.

The overwhelming degree of endogenous gene exclusion predicts that the VH3H9/Vκ8 transgenic mice should essentially produce monospecific anti-DNA immunoglobulins. This prediction was tested by examining the specificity of spleen cell surface immunoglobulins as well as antibodies produced by hybrids derived from the transgenics. In these mice the majority (60–69%) of the IgM^{++} cells bound ssDNA (Fig. 2*b*). Similarly, most hybridomas derived from lipopolysaccharide (LPS)-activated spleen cells express anti-DNA antibodies (Table 1). Moreover, these antibodies have the features of the antibodies expressed by the VH3H9/Vκ8 transfectoma[30], in that they bind ssDNA and cardiolipin but not dsDNA. The frequencies of anti-DNA expression are substantially higher than those found in non-transgenic littermates (Fig. 2*b*) or among LPS hybrids from normal mice[9,10].

Despite the high frequency of anti-DNA B cells in the VH3H9/Vκ8 transgenics, anti-DNA serum titres are not higher than those found in normal mice (Fig. 3*a*). In this regard our mice are different from other immunoglobulin transgenics, such

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as the anti-lysozyme and anti-H-2^k transgenics, which have high serum levels of the transgene-encoded antibody[2,3]. That the VH3H9/Vκ8 mice show no increase in anti-DNA titres over the background levels observed in normal mice, and also display reduced serum IgM levels (Fig. 3*b*), suggests that the anti-DNA B cells are blocked in their ability to differentiate into antibody-secreting cells. In this respect the VH3H9/Vκ8 B cells are similar to the anti-lysozyme B cells in mice that also carry a transgene coding for the neo-self-antigen, hen-egg lysozyme[11]. By analogy with the lysozyme/anti-lysozyme tolerance model, we propose that the anti-DNA B cells have encountered antigen, for example, DNA or DNA-protein complexes, and this encounter has left them functionally silent. It is possible that the inability to detect anti-DNA activity, along with the abnormally low level of IgM, is due to immune complexes and/or tissue deposits being formed between the transgenic antibody and self-antigen, thereby masking serum anti-DNA activity. We think this is unlikely because anti-DNAs are readily detectable in autoimmunity[5]. Future studies on the expression of the VH3H9/Vκ8 antibodies on the genetic background of the autoimmune mouse should resolve this issue.

Comparison with the lysozyme/anti-lysozyme transgenics shows a difference in phenotype of self-reactive B cells. Whereas B-cell anergy in the lysozyme/anti-lysozyme transgenics correlates with a low density of membrane IgM[11], this is not the case for the VH3H9/Vκ8 transgenic B cells. Here the B cells which bind ssDNA have a high density of surface IgM (Fig. 2*b*). This indicates that B-cell anergy is not always correlated with down-regulation of membrane IgM.

The VH3H9-only transgenic mice provide a model for surveying the potential range of specificities that result from pairing VH3H9 with the endogenous L-chain pool. As discussed, natural and transfectoma antibodies in which this H chain is paired with a variety of L chains bind both ds and ssDNA, and when paired with other L chains, bind only ssDNA[30]. Specificity analysis of VH3H9-only transgenic spleen cells shows that 63% (range 38–86%) of the IgM^{++} B cells bound ssDNA, and that the remainder did not bind DNA at all (Fig. 2*b*). This latter category shows that association with certain L chains can abolish DNA binding. Similarly, hybridomas from the VH3H9 mice express both DNA-binding and non-DNA-binding antibodies (Table 1). Although there are a variety of L chains which in combination with the VH3H9 can bind both ss and dsDNA[30], we found no dsDNA-binding monoclonal antibodies. One possible explanation is that B cells with this specificity (as opposed to anti-ssDNA only) are deleted. Alternatively, anti-dsDNA lymphocytes may exist but escape our notice because they are expressed in a population of cells refractory to LPS activation[12] and/or fusion. If this population is deleted or nonfunctional then we would expect the remaining Vκ repertoire to be reduced. In fact it is: mRNA sequence analysis of L-chain genes from 32 hybrids reveals use of a limited variety of L chains in both the non-DNA-binding and the ssDNA-binding hybrids (M.Z.R., J.E. and M.W., manuscript in preparation).

Fifty-two per cent of the VH3H9 hybrids secrete anti-ssDNA antibodies, a frequency similar to the average number of DNA-binding spleen cells (Fig. 2*b*), yet, like the VH3H9/Vκ8 transgenics, the VH3H9 mice show no increase in anti-DNA serum titres above those seen in normal mice (Fig. 3*a*). Therefore, anergy applies not just to the VH3H9/Vκ8 anti-DNAs, but also

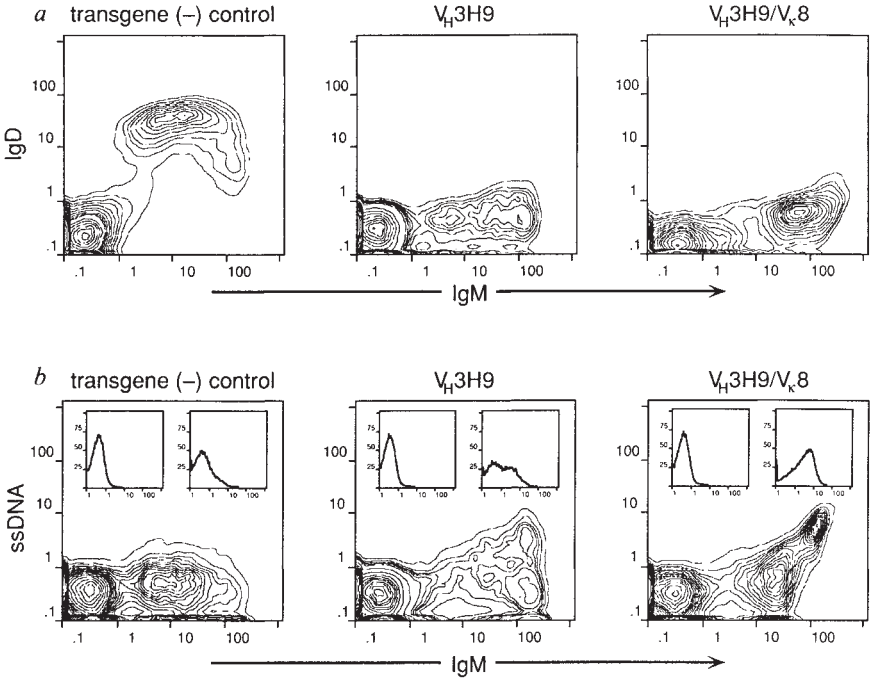

FIG. 2 Fluorescence-activated cell sorter (FACS) analysis of spleen cells. *a*, Cell-surface analysis shows that splenic cells from a non-transgenic animal express both IgM and IgD isotypes[26] whereas cells from the VH3H9/Vκ8 and VH3H9 transgenic mice express IgM alone. On the basis of the distribution of B220, Ly-1, Thy-1, CD3, CD4 and CD8, the B- and T-cell composition of these transgenics is equivalent to normal mice (data not shown). Non-transgenic animals express a range of IgM and IgD cell-surface densities. The majority of the IgM^+ cells in the VH3H9/Vκ8 mice have a high density of surface IgM. Although the VH3H9 mice also have many cells with a high IgM surface density, there are also cells with lower levels of IgM. *b*, Spleen lymphocytes were analysed for cell-surface binding to ssDNA and IgM. The inset panels display DNA binding on the *x*-axis and relative cell number on the *y*-axis. Threshold for detection in the FACS DNA assay is dependent on surface immunoglobulin density. Therefore, only IgM^{++} cells (defined as >50 fluorescence units on the IgM scale) were quantitated. The left inset shows DNA binding in the IgM^- cell population and the right inset is for the IgM^{++} cell population. Non-transgenic animals showed very little binding (8–17%) to ssDNA in either cell population. In the example shown here, 67% of IgM^{++} cells in the VH3H9/Vκ8 transgenic bound ssDNA (range 60–69%). The VH3H9 transgenics have two populations of IgM^{++} cells: 40% that bind ssDNA and 60% that do not. There was considerable variation in the precentage of DNA^+ cells among VH3H9 transgene animals, range 38–86%. All the DNA-binding cells in both transgenics were IgM^+, ruling out the possibility that the (non-immunoglobulin) DNA receptor that has been reported on splenic T cells was being detected[27].

METHODS. *a*, Offspring from matings between F_1 (VH3H9$^+$C57BL6/SJL × MRL+/+) and $Vκ8^+$ BALB/c mice were genotyped as described in Table 1. Spleen cells from animals either negative for both transgenes, positive for both (VH3H9/Vκ8), or positive for only the VH3H9 transgene were stained simultaneously with fluorescein-conjugated anti-IgD^a allotype (AMS 15.1), biotin-anti IgD^b allotype (AF6-122) (revealed by Texas-Red avidin) and allophycocyanin (APC)-anti-IgM (331.1.2), and then analysed using a dual-laser FACSTAR$^+$ (refs 28, 29). A total of eight negative, nine VH3H9, and five VH3H9/Vκ8 animals were analysed. The examples shown are stained with anti-IgD^a; the anti-IgD^b showed no staining for this set of mice. In all FACS analyses, only cells falling within normal lymphocyte values for both forward and large-angle scatter were included (eliminating granular and dead cells). Propidium iodide was also included to exclude dead cells. Staining intensities are expressed in arbitrary fluorescence units on four-decade logarithmic scales. *b*, Spleen cells were stained simultaneously with APC-anti-IgM and 10 μg biotin-ssDNA (revealed by Texas Red–avidin) and analysed by FACS. Biotinylated DNA was prepared as previously described[19]. Independent experiments using the following sources of DNA gave similar results: S1 or mung-bean nuclease treated sonicated salmon sperm DNA; S1 or mung-bean nuclease-treated *Xba*I-digested λ phage DNA; S1-treated *Hae*III-digested salmon sperm DNA. DNA was prepared by heating to 95°C for 5 min and then rapidly chilled before use.

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to the variety of anti-DNA antibodies formed by VH3H9 plus endogenous L chains. In contrast to the VH3H9/Vκ8 mice, the VH3H9 mice have near normal levels of serum IgM (fig. 3*b*). We presume that the population of non-DNA-binding cells, identified in Fig. 2*b* and Table 1, is responsible for the normal levels of serum IgM.

These results show that anti-DNA antibodies are regulated in normal (non-autoimmune) animals. Most B cells in VH3H9/Vκ8 and roughly half in the VH3H9 transgenics are specific for ssDNA, but they apparently do not differentiate into immunoglobulin-secreting cells. As these B cells retain a high density of surface IgM, the immunoglobulin on these cells may be uncoupled from the immunoglobulin signalling pathway[13,14] rendering them functionally silent. Hybridomas from the VH3H9 transgenics have suggested an additional mechanism of regulating anti-DNA B cells. Here we find anti-ssDNA antibodies but fail to find anti-dsDNA antibodies.

Most importantly, the results demonstrate that B-cell tolerance to DNA is manifested in ways similar to those described in experimental models of tolerance[1-3]. This observation, that normal mice regulate disease-associated antibodies, implies that autoimmune disease results from a breakdown of regulation of autoantibody expression. □

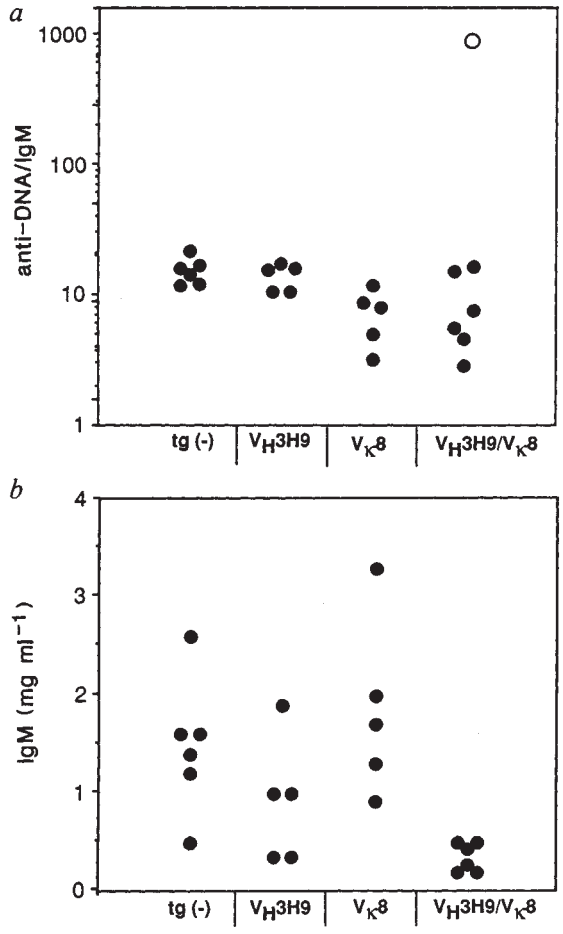

FIG. 3 Relative anti-DNA titres and IgM concentrations in serum from transgene-negative (tg(−)) littermates and VH3H9, Vκ8 and VH3H9/Vκ8 transgenics showing: *a*, at a given IgM concentration there is no increase in anti-DNA titres in the transgenics above the background observed in non-transgenic mice. ○, Value obtained from one of the LPS-induced VH3H9/Vκ8 hybrids at the equivalent IgM concentration. Each symbol represents values obtained for a single mouse. *b*, IgM levels in the non-transgenic, VH3H9 and Vκ8 mice are similar whereas those in the VH3H9/Vκ8 mice are depressed. METHODS. Offspring from matings between F_1 (VH3H9$^+$ C57BL6/SJL × MRL+/+) and Vκ8$^+$ BALB/c mice were assayed as described in Table 1 for the presence of transgenes and bled between 8 and 16 weeks of age. Sera were diluted in 1% BSA in PBS and assayed by ELISA for binding to polyspecific anti-immunoglobulin goat antibodies or heat-denatured calf thymus DNA (50 μg ml^{-1}) absorbed on protamine sulphate (200 μg ml^{-1}) coated plates[20]. Anti-DNA titres represent dilutions required to achieve absorbance of 0.1. Standard IgM concentrations were prepared by dilution of purified antibodies from the murine myeloma 3741. Antibody levels were measured using an ELISA plate reader at 405 nm following incubations with goat anti-mouse IgM-conjugated alkaline phosphatase and substrate.

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1. Nossal, G. J. V. *Science* **245,** 147–153 (1989).
2. Goodnow, C. C. *et al. Nature* **334,** 676–682 (1988).
3. Nemazee, D. A. & Bürki, K. *Nature* **337,** 562–566 (1989).
4. Theofilopoulos, A. N. & Dixon, F. J. *Adv. Immun.* **37,** 269–389 (1985).
5. Tan, E. M. *Adv. Immun.* **44,** 93–151 (1989).
6. Pankewycz, O. G., Migliorini, P., & Madaio, M. P. *J. Immun.* **139,** 3287–3294 (1987).
7. Shlomchik, M., Aucoin, A. H., Pisetsky, D. S. & Weigert, M. G. *Proc. natn. Acad. Sci. U.S.A.* **84,** 9150–9154 (1987).
8. Shlomchik, M. *et al. J. exp. Med.* **171,** 265–297 (1990).
9. Souroujon, M., White-Scharf, M. E., Andre-Schwartz, J., Gefter, M. L. & Schwartz, R. S. *J. Immun.* **140,** 4173–4179 (1988).
10. Holmberg, D. *et al. Immun. Rev.* **93,** 147–169 (1986).
11. Basten, A., Brink, R. A., Mason, D. Y., Crosbie, J. & Goodnow, C. C. In *Progress in Immunology VII* (eds Melchers, F. *et al.*) 377–383 (Springer-Verlag, Berlin-Heidelberg, 1989).
12. Chace, J. H., Scott, D. W. *J. Immun.* **141,** 3258–3262 (1988).
13. Campbell, K. S. & Cambier, J. C. *EMBO J.* **9,** 441–448 (1990).
14. Wienands, J., Hombach, J., Radbruch, A., Riesterer, C. & Reth, M. *EMBO J.* **9,** 449–455 (1990).
15. Kurosawa, Y. & Tonegawa, S. *J. exp. Med.* **155,** 201–218 (1982).
16. Saiki, R. K., gelfand, D T., Mullis, K. B. & Erlich, H. A. *Science* **239,** 441–432 (1988).
17. Van Snick, J. L. & Coulie, P. *J. exp. Med.* **155,** 219–230 (1982).
18. Coleclough, C., Perry, R. P., Karjalainen, K. & Weigert, M. *Nature* **290,** 372–378 (1981).
19. Radic, M. Z. *et al. Cold Spring Harb. Symp. quant. Biol.* **54,** 933–946 (1989).
20. Eaton, R. B., Schneider, G. & Schur, P. H. *Arthritis Rheum.* **26,** 52–68 (1983).
21. Loizou, S. *et al. Clin. exp. Immun.* **62,** 738–745 (1985).
22. Marcu, K. B., Banerji, J., Penncavage, N. A., Lang, R. & Arnheim, N. *Cell* **22,** 187–196 (1980).
23. Yancopoulos, G. D., Malynn, B. A. & Alt, F. W. *J. exp. Med.* **168,** 417–435 (1988).
24. Perry, R. P. & Kelley, D. E. *Cell* **18,** 1333–1339 (1979).
25. Camper, S. A. *Biotechiques* **5,** 638–650 (1987).
26. Hardy, R. R., Hayakawa, K., Haaijman, J. & Herzenberg. L. A. *Nature* **297,** 589–591 (1982).
27. Bennett, R. M., Kotzin, B. L. & Merritt, M. J. *J. exp. Med.* **166,** 850–863 (1987).
28. Hayakawa, K. & Hardy, R. R. *J. exp. Med.* **168,** 1825–1838 (1988).
29. Stall, A. M. & Loken, M. R. *J. Immun.* **132,** 787–795 (1984).
30. Radic, M. Z., Mascelli, M. A., Erikson, J., Shan, H. & Weigert, M. *J. Immun.* (in the press).

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Expression of recombinant dystrophin and its localization to the cell membrane

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DUCHENNE's muscular dystrophy (DMD) is an X-linked progressive myopathy caused by a defect in the DMD gene locus[1,2]. The gene corresponding to the DMD locus produces a 14-kilobase (kb) messenger RNA that codes for a large cytoskeletal membrane protein, dystrophin[3,4]. DMD and Becker's muscular dystrophy are the consequences of dystrophin mutations[4,5]. The exact biological function of dystrophin remains unknown but it has been demonstrated that it is localized to the cytoplasmic face of the cell membrane and has direct interaction with several other membrane proteins[6,7]. We report here the synthesis of a 14-kb full-length complementary DNA for the mouse muscle dystrophin mRNA and the expression of this cDNA in COS cells. The recombinant

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