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Immunology

High copy number *I-Ab* transgenes induce production of IgE through an interleukin 4-dependent mechanism

(major histocompatibility complex/B lymphocytes/immunodeficiency)

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ABSTRACT **To better understand the role of class II major histocompatibility complex molecules in both normal and autoimmune responses, we have produced a series of *I-Ab* transgenic mice. One of these transgenic constructs, designated NOD.PD, has the sequence of the NOD β chain ($A\beta^{g7}$) except at positions 56 and 57, where Pro-Asp replaces His-Ser. Several NOD.PD transgenic lines have been produced. One line of these mice carried a very high number of copies (>50) of the NOD.PD transgene. As has been described in other mice carrying high copy numbers of *I-Ab* transgenes, B-cell development was abnormal. The steady state numbers of mature B cells (IgM^+/IgD^{hi}) in the periphery were greatly reduced in transgenic mice compared to nontransgenic littermates. Surprisingly, rather than being accompanied by a generalized hypogammaglobulinemia, this B-cell deficiency was accompanied by elevated concentrations of IgG1 and IgE in the serum. Conversely, the levels of IgG2a were reduced in transgenic mice compared to nontransgenic littermates. Because this isotype pattern was characteristic of interleukin (IL)-4-induced class-switching, we then investigated the role of IL-4 in causing the observed phenotype. We crossed the high copy number transgenic mice with an IL-4-deficient strain of mice. As expected, the elevated levels of IgE in high copy number transgenic mice were eliminated when the IL-4 gene was inactivated. However, the reduction in the number of B cells was not ameliorated. These data indicate that the primary defect caused by the transgene was to reduce the number of B cells in these mice. This reduction was accompanied by a secondary increase in IL-4 production, which drove the remaining B cells toward the production of IgG1 and IgE.**

Class II major histocompatibility complex (MHC) molecules are important regulators of the development and function of the immune system. They participate in shaping the mature $CD4^+$ T-cell repertoire by both positively and negatively selecting T cells in the thymus (1, 2). They also determine the ability of an individual to respond to particular protein antigens by binding short polypeptides and presenting them to T cells in the periphery (3). Recently, an additional role for these molecules in delivering intracellular signals to B cells during T-cell–B-cell cognate interactions has been described (4–6). In addition to these functions, the genes within the class II region of the MHC have been associated with susceptibility to many autoimmune diseases, including insulin-dependent diabetes mellitus, systemic lupus erythematosus, and rheumatoid arthritis (7). However, it is unclear whether these disease associations are due to the above mentioned functions of class II molecules, to as yet undescribed functions of these molecules, or to genes that are in linkage disequilibrium with the associated class II alleles rather than to the class II molecules themselves.

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Our approach to better understanding the role of class II MHC molecules *in vivo* has been to produce transgenic mice by using different alleles of *I-Ab*. In one set of experiments, Gilfillan *et al.* (8, 9) examined a series of $I\text{-}Ab^k$ transgenic mice carrying between 1 and 65 copies of the transgene. Mice carrying >40 copies of the transgene overexpressed $I\text{-}Ab^k$ mRNA in spleen cells, but surface expression of I-A heterodimers did not increase since surface expression was limited by the availability of A_α chains (8). Interestingly, these mice exhibited several abnormalities in their immune systems (9). The most prominent defect was a reduction in the number of mature IgM^+/IgD^{hi} B cells in the spleen, lymph nodes, and bone marrow of these mice. Because class II expression is turned on in B-cell ontogeny at the time when they acquire this mature phenotype, the reduced number of mature B cells was hypothesized to result from toxicity of excess, unpaired A_β chains for the B cells (8). There was also a concomitant increase in the number of eosinophils and granulocytes in the spleens of these transgenic animals. Finally, the mice all died by 5 months of age from unknown causes, although some evidence for the presence of infectious agents was obtained.

While producing additional *I-Ab* transgenic mice in order to study the role of I-A molecules in the pathogenesis of autoimmune diabetes in the nonobese diabetic (NOD) mouse (S.M.S. *et al.*, unpublished data), we generated a line of mice (NOD.PD-SU) carrying >50 copies of the transgene. These mice appeared identical to those described by Gilfillan *et al.* (8, 9). They have reduced numbers of mature IgM^+/IgD^{hi} B cells in the periphery, increased numbers of eosinophils and granulocytes in the spleens, and early mortality. Additional analysis of these mice found elevated levels of IgG1 and IgE in their serum. Because the production of both of these immunoglobulin isotypes is up-regulated by interleukin IL-4 (10), we have investigated the relationship between B-cell numbers, IL-4 production, and serum immunoglobulin levels in high copy *I-Ab* transgenic mice. In this report, we provide evidence suggesting that the decreased B-cell numbers observed are due, at least in part, to an increased rate of differentiation of IgM^+/IgD^{hi} B cells into more terminally differentiated cell types, and that a secondary increase in the production of IL-4, and perhaps IL-5 and IL-6, leads to the other pathologies that are observed.

MATERIALS AND METHODS

Production and Screening of Transgenic Mice. Original breeding pairs of NOD/Lt mice ($H\text{-}2^{g7}$: K^d, A^{g7}, D^b) were kindly provided by E. Leiter. The IL-4-deficient strain IL4T (129 background; ref. 11) was obtained from Klaus Rajewsky via R. Coffman and was embryo derived into our facility to

Abbreviations: MHC, major histocompatibility complex; IL, interleukin; NOD, nonobese diabetic; FITC, fluorescein isothiocyanate.

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ensure the absence of pathogens. The construction of the NOD.PD transgene will be described elsewhere (S.M.S. et al., unpublished). NOD.PD is an *I-Ab^{g7}* genomic sequence in which residues 56 and 57 have been mutated from His and Ser to Pro and Asp, respectively. Tail DNA of offspring was digested with the restriction enzyme *Bgl* II, electrophoresed on agarose gels, transferred to nylon membranes, and hybridized to a ^{32}P-labeled *I-Ab^{k}* cDNA. Transgenic males were backcrossed to NOD females for most experiments. To generate IL-4-deficient mice carrying the transgene, heterozygous NOD.PD transgenic females were first mated with homozygous IL4T males. Male offspring carrying the NOD.PD transgene were then mated to nontransgenic IL4T +/− females in order to produce mice that were homozygous for the IL-4 gene defect and that carried the NOD.PD transgene. The IL4T allele was distinguished from the wild-type NOD IL-4 allele using a *Bgl* II restriction fragment length polymorphism detected with an IL-4 cDNA probe. All transgenic mice analyzed were heterozygous for the NOD.PD transgene.

Antibodies and Conjugates. Monoclonal anti-I-A^{g7} (10-2.16; ref. 12) and anti-mouse IgE (EM95; ref. 13) were purified from ascites fluid and tissue culture supernatant, respectively, by ammonium sulfate precipitation and protein A chromatography. Antibodies were conjugated to *N*-hydroxysuccinimide biotin (Pierce) or fluorescein isothiocyanate (FITC; Becton Dickinson) as described (14). Monoclonal rat anti-mouse IgE (Pharmingen), FITC-conjugated sheep anti-mouse IgD (Nordic), biotin-conjugated goat anti-mouse IgM (The Binding Site, San Diego), biotin-conjugated goat anti-mouse IgE (The Binding Site), biotin-conjugated anti-Thy1.2 (Becton Dickinson), and avidin Texas Red (Vector Laboratories) were obtained commercially.

Histology. Spleens were removed from 12-week-old mice and fixed in phosphate-buffered 10% formalin. Specimens were embedded in paraffin and 6-μm sections were cut, mounted on slides, deparaffinized, and stained with hematoxylin and eosin.

Dendritic Cell Purification. Dendritic cells were purified essentially as described (15). Spleen cells were cultured overnight in plastic dishes to allow attachment of adherent cells. Nonadherent cells were collected by gentle shaking and centrifuged on 14.5% metrizamide gradients in RPMI 1640 medium. Buoyant cells generally consisted of 70–80% dendritic cells as judged by morphology.

Flow Cytometry. Single cell suspensions of spleen were prepared, and erythrocytes were lysed in 140 mM NH_4Cl 17 mM Tris·HCl, pH 7.5. Cells were stained with appropriate concentrations of the antibodies described. Data were collected on a FACS IV (Becton Dickinson) and analyzed using FACS-Desk software (Stanford University Shared FACS Facility). Cells that stained with propidium iodide were excluded from analyses.

Serum Immunoglobulin ELISA. The relative levels of IgM, IgA, IgG1, IgG2a, IgG2b, and IgG3 were measured with a monoclonal antibody isotyping kit (Pierce) as directed by the manufacturer. IgE levels were measured by a capture ELISA; plates were coated with anti-IgE monoclonal antibody (10 μg/ml) and bound IgE was detected with biotinylated anti-mouse IgE followed by avidin alkaline phosphatase (Extravidin; Sigma) and para-nitrophenylphosphate (pNPP-Fast; Sigma) as substrate. A_{405} was measured with a plate reader (Dynatech).

RESULTS

During our studies on the role of class II MHC molecules in autoimmune diabetes in NOD mice, we obtained a line of transgenic mice, designated NOD.PD-SU, carrying >50 copies of the NOD.PD transgene (Fig. 1). Our initial characterization of these mice showed that they were very similar to the mice described by Gilfillan et al. (8, 9). The number of mature IgM$^+$/IgD^{hi} B cells in the spleens, lymph nodes, and bone marrows of these animals was greatly reduced compared to nontransgenic mice (data not shown). These mice also displayed eyelid inflammation and abnormal granulopoeisis and eosinophilia in their spleens as previously described (Fig. 2; data not shown). Because both the transgenes and mouse strains used in the earlier experiments from this laboratory and in the present work were different, these data confirm that the effects seen in the high copy number transgenic mice were not due to the particular *I-Ab* alleles or host strains used.

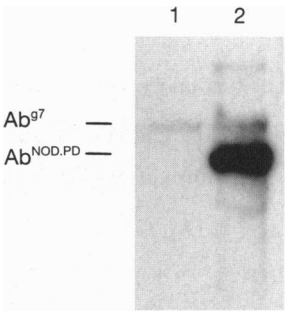

FIG. 1. Southern analysis of transgene integration. Southern blotting of nontransgenic (lane 1) and NOD.PD-SU transgenic (lane 2) DNA was performed as described. The transgene migrates at a lower molecular size than the endogenous gene because *Bgl* II digests the construct only once and the transgene has integrated into the germline in a head-to-tail configuration, thus producing a fragment the size of the injected construct.

There are two basic ways in which the number of mature IgM$^+$/IgD^{hi} B cells could be reduced: either the rate at which pro-B, pre-B and/or immature B cells differentiate into mature B cells could be reduced or the rate at which mature B cells die or differentiate into plasma cells could be increased. Gilfillan et al. (9) suggested that impaired B-cell development and increased B-cell death were the most likely causes for the reduced B-cell numbers, since high levels of *Ab* mRNA expression were observed in the transgenic B cells and since similar overexpression of transgenes was known to be toxic in other systems. To investigate this point, we initially examined the effect of the NOD.PD-SU transgene on other class II-expressing cells. If overexpression of A_β chains was toxic for B cells, one might expect to see similar toxicity for macrophages and dendritic cells as well, since they also express I-A molecules. This effect might be especially pronounced in dendritic cells since they normally express much more (5- to 10-fold) I-A than B cells. However, the numbers of macrophages and dendritic cells recovered from the spleens of transgenic and nontransgenic mice were similar (Fig. 3; data not shown). Also, the level of expression of class II on these cells was similar in transgenic animals compared to controls (Fig. 3; data not shown). Thus, either B cells are more susceptible to the toxic effects of excess A_β-chain synthesis or toxicity was not the underlying cause of the observed immunodeficiency.

To further examine the extent of B-cell differentiation in NOD.PD-SU mice, we determined the levels of serum immunoglobulin in transgenic and nontransgenic animals. If the number of plasma cells being produced was decreased in parallel with the reduction in IgM$^+$/IgD^{hi} B cells, then one might expect the amount of serum immunoglobulin to be reduced. To our surprise, we discovered that immunoglobulin levels were either increased or decreased in transgenic mice, depending on the isotype examined. While IgM and IgA levels appeared relatively unchanged between transgenic and nontransgenic mice (Fig. 4 *A* and *F*), the levels of IgG1 and IgE were much higher in transgenic mice than in controls (Fig. 4 *B* and *G*). In contrast, the levels of IgG2a, IgG2b, and IgG3 were reduced in the transgenic mice (Fig. 4 *C–E*). However,

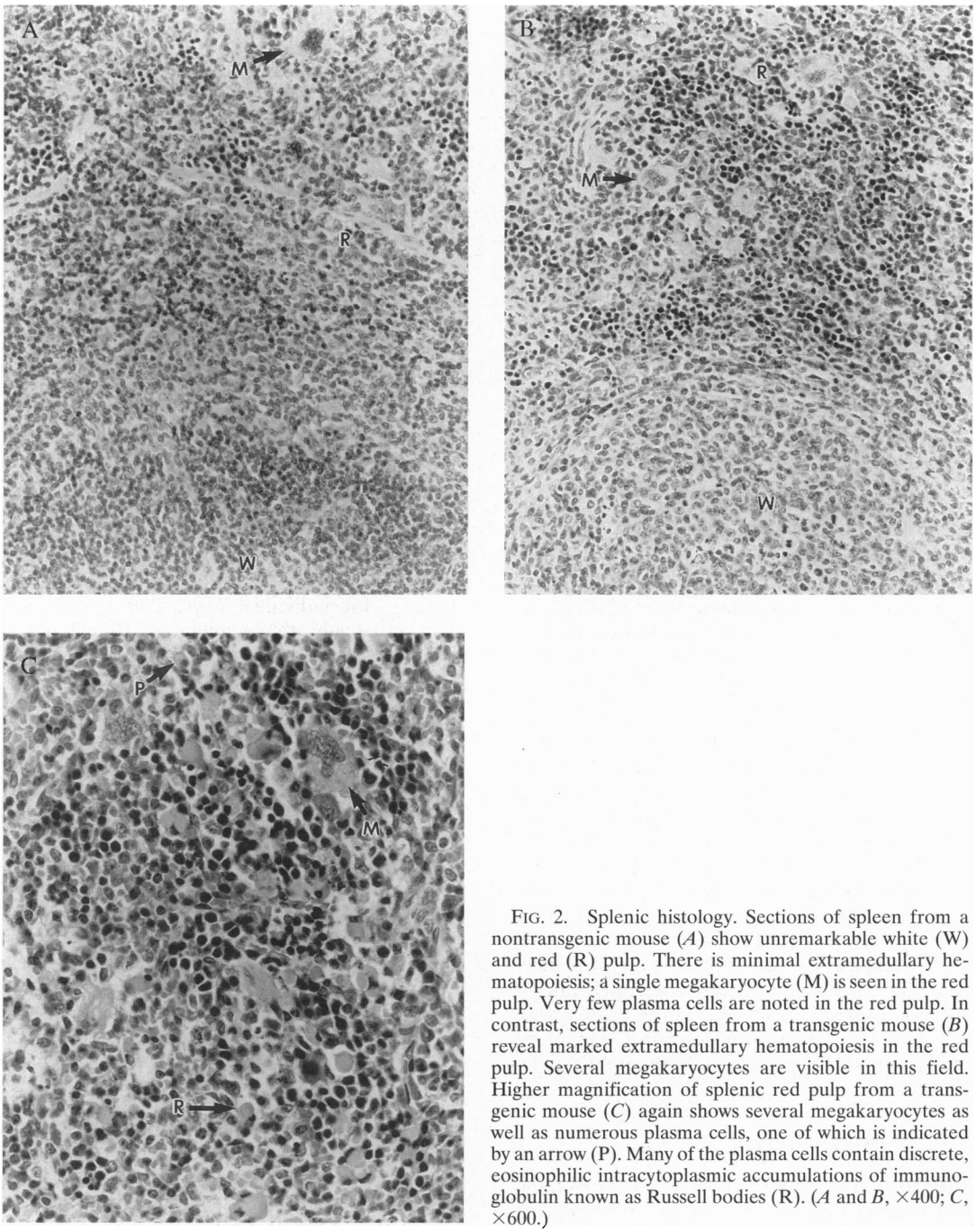

FIG. 2. Splenic histology. Sections of spleen from a nontransgenic mouse (*A*) show unremarkable white (W) and red (R) pulp. There is minimal extramedullary hematopoiesis; a single megakaryocyte (M) is seen in the red pulp. Very few plasma cells are noted in the red pulp. In contrast, sections of spleen from a transgenic mouse (*B*) reveal marked extramedullary hematopoiesis in the red pulp. Several megakaryocytes are visible in this field. Higher magnification of splenic red pulp from a transgenic mouse (*C*) again shows several megakaryocytes as well as numerous plasma cells, one of which is indicated by an arrow (P). Many of the plasma cells contain discrete, eosinophilic intracytoplasmic accumulations of immunoglobulin known as Russell bodies (R). (*A* and *B*, ×400; *C*, ×600.)

the total amount of IgG present in the serum was similar in transgenic and nontransgenic mice, indirect evidence that the number of plasma cells was not changed (data not shown). Histologic examination of spleens from transgenic and nontransgenic mice clearly showed a greater number of plasma cells in the red pulp of transgenic spleen than in the nontransgenic spleen (Fig. 2). Since the number of plasma cells in the transgenic spleen was moderately increased, but the steady-state number of plasma cell precursors was reduced, this suggests that the rate of differentiation from mature B cells to plasma cells is increased in these mice. Thus, toxicity of the transgene product for B cells cannot fully explain their absence in high copy number transgenic mice.

The isotype pattern observed was striking in that the isotypes known to be induced by IL-4 were increased, while those induced by interferon-γ were decreased (10). This suggested that an IL-4-dependent mechanism might be responsible, at least in part, for the phenotype observed. Alternately, B cells that switch to particular isotypes might be more susceptible to the toxicity of excess A_β-chain synthesis. To determine whether IL-4 was indeed responsible for the altered pattern of immunoglobulin isotypes being produced, we introduced the high copy number NOD.PD transgene onto an IL-4-deficient background (11). NOD.PD-SU mice homozygous for the defective IL-4 allele lost the spontaneous production of elevated levels of IgE compared to transgenic mice carrying one copy of a functional IL-4 gene (Fig. 4*H*). IgG1 levels were also reduced in both transgenic and nontransgenic mice lacking IL-4 (data not shown). Effects on other isotypes were more difficult to discern, perhaps due to the impact of other genes segregating in these F_2 progeny. Thus, despite our inability to detect IL-4 in the sera of NOD.PD-SU mice (data not shown), the clear reduction of IgE levels indicated that the increase in IgE seen earlier was due to an IL-4-dependent mechanism.

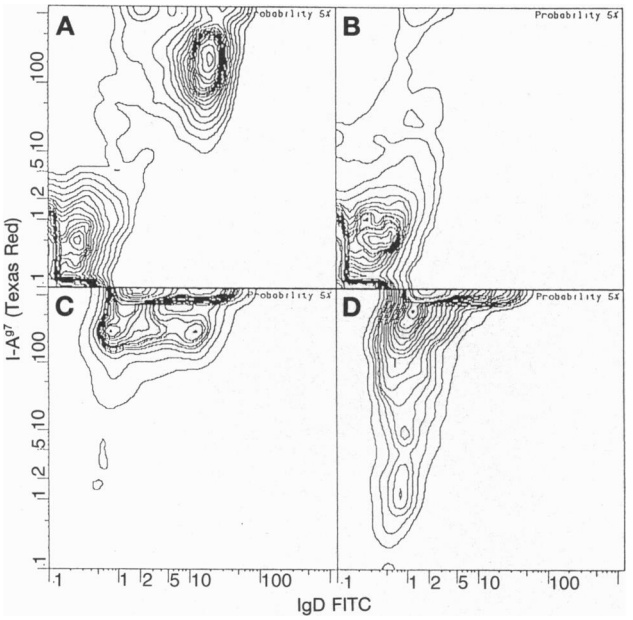

FIG. 3. Dendritic cell populations in spleens of NOD.PD-SU mice. Total splenocytes (*A* and *B*) or purified dendritic cells (*C* and *D*) from nontransgenic (*A* and *C*) or NOD.PD-SU transgenic (*B* and *D*) mice were stained with FITC-conjugated anti-IgD and biotin-conjugated anti-I-A^{g7} followed by avidin Texas Red. Contour plots represent 5% probability intervals.

In addition to its role in influencing the pattern of isotypes produced during immunoglobulin class switching, IL-4 is also able to accelerate the rate of differentiation of B cells to plasma cells (16). We therefore wanted to determine whether such an acceleration in B-cell differentiation was responsible for the decrease in B-cell numbers observed in transgenic mice. However, the reduction in the number of IgM$^+$/IgD^{hi} B cells observed in IL4 (−/−) NOD.PD transgenic mice was identical to that seen in IL4 (+/−) NOD.PD transgenic mice when compared with nontransgenic mice with or without a functional IL-4 gene (Fig. 5). Thus, while an accelerated rate of B-cell differentiation cannot be excluded as the cause of the observed B-cell deficiency, such an acceleration cannot be attributed to the increased IL-4 production alone in NOD.PD-SU mice.

DISCUSSION

What is the mechanism responsible for reduced B-cell numbers in high copy number *I-Ab* transgenic mice? We believe it unlikely that the reduced numbers of B cells in the transgenic mice are completely due to a block in B-cell development resulting from the toxicity for B cells of excess synthesis of I-A$_\beta$ chains. No toxicity was seen for class II-expressing cells other than B cells, despite the fact that the level of I-A expression on dendritic cells is 5–10 times higher than that on B cells. Thus, either B cells are especially sensitive to the toxic effects of I-A$_\beta$ chain overexpression, or toxicity is not the full answer to the B-cell deficiency in NOD.PD-SU mice. Furthermore, the mice were not hypogammaglobulinemic, as was seen in other mice lacking mature B cells. Such B-cell-deficient mice have been generated by treatment with anti-IgM antibodies (17), by inactivation of the RAG-1 (18), RAG-2 (19), or λ5 (20) genes, by the *scid* mutation (21), or by a human λ light-chain transgene (22). The normal levels of serum immunoglobulin in our system imply that plasma cells are being produced in normal numbers, an idea inconsistent with a complete block in B-cell development.

Instead of blocking B-cell development, an alternative explanation for the reduced B-cell numbers is that the IgM$^+$/IgD^{hi} B cells in transgenic mice are differentiating more rapidly than normal and are thus disappearing from the steady state populations in the spleen. These cells are normally relatively long lived (23), and modest increases in their rate of differentiation might therefore account for the significant reduction in B-cell numbers seen. Increases in the rate of differentiation could be due to an intrinsic property of the transgene in these mice, or it could be secondary to an indirect effect on T cells of transgene expression on antigen presenting cells. Indeed, a direct role for class II MHC molecules in B-cell development has been described. Cross-linking of class II MHC molecules delivers activation signals to B cells via a protein kinase C-dependent pathway (4), and this signaling, in conjunction with cross-linking of surface immunoglobulin has been shown to induce B-cell differentiation *in vitro* (24). Cross-linking of class II MHC molecules can also inhibit B-cell responses, depending on the activation state of the B cell at the time when signaling occurs (25, 26). Thus, it is possible that overexpression of I-A$_\beta$ during B-cell development could lead to aberrant signaling in B cells, resulting in both toxicity for some B cells and the accelerated differentiation of the remaining B cells, depending on the presence or absence of additional signals—e.g., IL-4 or immunoglobulin cross-linking—for individual cells. This would then lead to the reduced steady state numbers of B cells observed. Interestingly, transgenic mice carrying high copy numbers of a mutated *I-Ab^b* transgene which does not encode the cytoplasmic tail required for intracellular signaling do not display any of the phenotypes noted in this report (27).

In addition to an intrinsic abnormality in B-cell differentiation, other cells may contribute to increased B-cell development as a consequence of transgene expression on macrophages, dendritic cells, or residual B cells. The most likely factors that could induce B-cell differentiation are the Th2-derived cytokines IL-4, IL-5, and IL-6 (16). Indeed, transgenic mice that grossly overexpress IL-4 exhibit a phenotype almost indistinguishable from that seen in I-A$_\beta$ overexpressing mice (28). However, our data clearly show that in our system the reduction in B-cell numbers is proximal to any effects of IL-4. The eosinophilia present in NOD.PD-SU mice suggests that IL-5 may also be overproduced as a consequence of transgene expression. Cytokines other than or in conjunction with IL-4, therefore, could induce accelerated differentiation of B cells and may be responsible for the decreased number of B cells observed.

The production of IL-4 and IL-5 would imply that Th2 cells are being induced in the NOD.PD-SU mice. This idea is also supported by the high levels of serum IgE and the eosinophilia present in these mice, clear evidence for IL-4 and IL-5 production. One possible mechanism for increased Th2 responses as a consequence of transgene expression is that there is some toxicity for developing B cells as discussed above and that the reduced number of B cells in a mouse induces the up-regulation of IL-4, IL-5, and IL-6 as a compensatory mechanism for maintaining B-cell numbers. B-cell-deficient mice exist in several varieties. Mice carrying either the *xid* (29) or *scid* (21) mutations have reduced numbers of mature B cells as do mice treated from birth with antibodies to IgM (17). Mice in which genes important in B-cell differentiation have been inactivated by gene targeting, including RAG-1 (18), RAG-2 (19), Ig μ (30), Ig δ (31), the IgH J region (32, 33), and λ5 (20), also lack or have reduced numbers of mature B cells. Finally, in addition to high copies of class II transgenes, a human λ light-chain transgene (22) and the IL-4 transgene described above (28) also caused reduced B-cell numbers. However, only in the *I-Ab* transgenic mice and the IL-4 transgenic mice was the reduction in B-cell numbers accompanied by elevated levels of IgG1 or IgE (28). While increased levels of IgG1 and

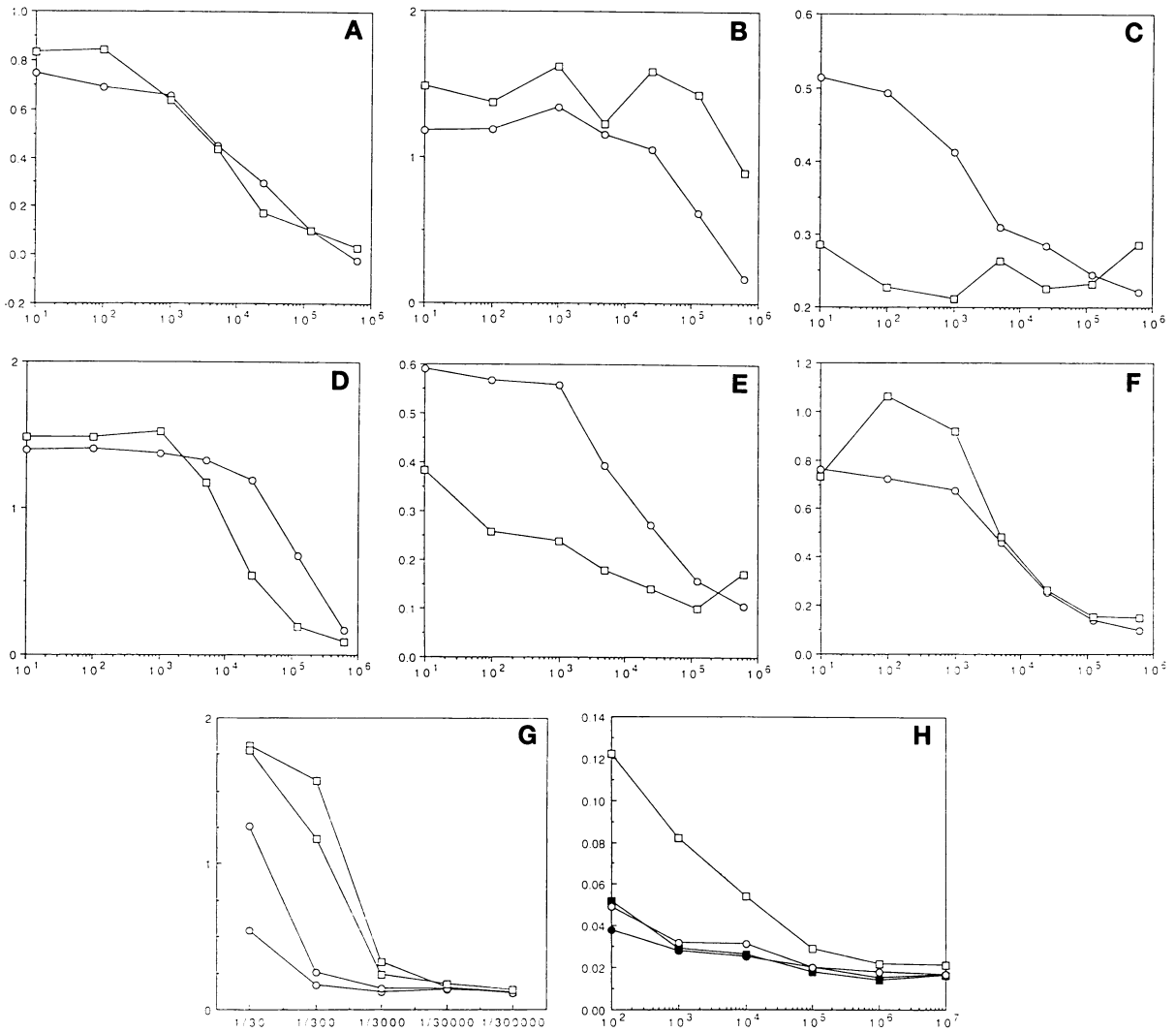

FIG. 4. Serum immunoglobulin levels in NOD.PD-SU mice. Sera of 12-week-old female nontransgenic (○) or NOD.PD-SU transgenic (□) mice were serially diluted and assayed for IgM (*A*), IgG1 (*B*), IgG2a (*C*), IgG2b (*D*), IgG3 (*E*), IgA (*F*), and IgE (*G* and *H*) as described. Solid symbols in *H* represent IL-4 −/− mice; open symbols are IL-4 +/− mice. Ordinates represent reciprocal dilutions of serum and abscissas represent the A_{405} values.

IgE in IL-4 transgenic mice are not very surprising, the remaining data argue that the class II transgene is acting to up-regulate IL-4 production through a mechanism unrelated to a reduction in the number of B cells.

Rather than compensating for a low number of mature B cells, we can propose two other explanations for the increased production of IL-4 in high copy number *I-Ab* transgenic mice. First, the expression of transgenic I-A molecules on dendritic cells, macrophages, or remaining B cells in the class II transgenic mice might favor induction of Th2 cells. While there is no obvious mechanism to explain why this might occur, changes in the structure of class II molecules, in the density of class II molecules on the surface of antigen presenting cells, or in the types of antigen presenting cells presenting antigen to T cells have all been proposed to affect the regulation of Th1 and Th2 development (34–37). It is unlikely that this is due to the structure of the transgenic I-A molecule since this phenotype has been observed in transgenic mice carrying high copy numbers of $I\text{-}Ab^{k}$, $I\text{-}Ab^{d}$, and $I\text{-}Ab^{g7.pd}$ transgenes. Alterations in class II density and the antigen presenting cells may still account for the different Th development observed.

The second mechanism for Th2 induction in I-A transgenic mice is that overexpression of $I\text{-}A_{\beta}$ on B cells could again lead to aberrant signaling and activation of those B cells that remain in NOD.PD-SU mice. These activated B cells would then be able to induce Th2 cells. While the full range of effects of class II-mediated signaling on B cells is still unknown, expression of the T-cell costimulatory molecule B7 is known to be up-regulated following cross-linking of class II molecules (5). Furthermore, T-cell costimulation by B7 is important in regulating cytokine production (38). It is interesting to compare the results from NOD.PD-SU transgenic mice to those of mice treated with antibodies against IgD. Anti-IgD treatment produces a state of polyclonal B-cell activation leading to large increases of serum IgE levels in an IL-4-dependent manner (39). In that case, it was suggested that B-cell activation due to cross-linking of membrane IgD leads to the up-regulation of IL-4. This is consistent with a model in which polyclonal B-cell activation in NOD.PD-SU mice, due to aberrant signaling by overexpressed $I\text{-}A_{\beta}$ molecules, leads to increased IL-4 production. Again it is significant that transgenic mice carrying truncated *I-Ab* transgenes do not display this phenotype (27).

In conclusion, high copy number *I-Ab* transgenic mice have reduced numbers of B cells in their spleens. These mice also exhibit symptoms of an allergic-like disease caused by overproduction of IL-4. These symptoms include eyelid inflammation, eosinophila, and elevated serum levels of IgE. Further studies on high copy number *I-Ab* transgenic mice may prove useful in understanding the regulation of IL-4 and IgE production *in vivo*. They also may provide a useful model in which

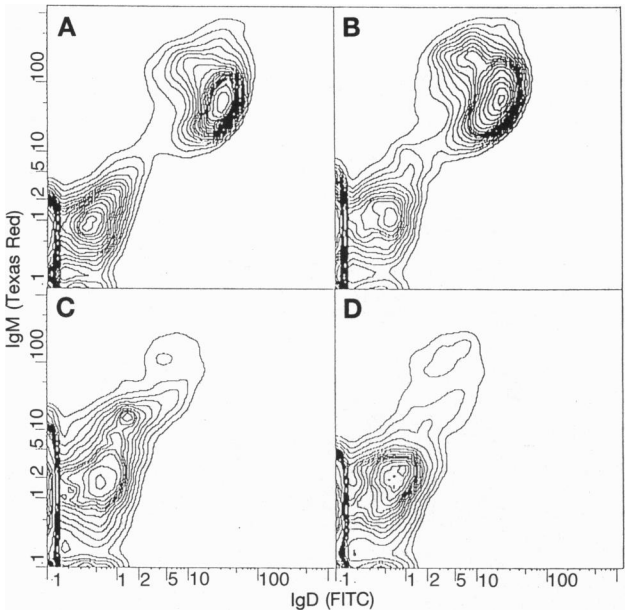

FIG. 5. B-cell populations in spleens of IL-4-deficient NOD.PD-SU mice. Spleen cells from nontransgenic IL4 +/− (*A*), nontransgenic IL4 −/− (*B*), transgenic IL4 +/− (*C*), and transgenic IL4 −/− (*D*) mice were stained with FITC-conjugated anti-IgD and biotin-conjugated anti-IgM followed by avidin Texas Red. Contour plots represent 5% probability intervals. Plots represent lymphocytes only as determined by analysis of forward and obtuse scatter parameters.

to evaluate potential therapeutics for allergic disease. Finally, these mice may provide evidence for an *in vivo* role of I-A_β signaling in B cells.

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1. von Boehmer, H. & Kisielow, P. (1990) *Science* **248,** 1369–1373.
2. Benoist, C. & Mathis, D. (1990) *Ann. Rev. Immunol.* **8,** 681–715.
3. Buus, S., Sette, A., Colon, S. M., Miles, C. & Grey, H. M. (1987) *Science* **235,** 1353–1358.
4. Cambier, J. C., Newell, M. K., Justement, L. B., McGuire, J. C., Leach, K. L. & Chen, Z. Z. (1987) *Nature (London)* **327,** 629–632.
5. Nabavi, N., Freeman, G. J., Gault, A., Godfrey, D., Nadler, L. M. & Glimcher, L. H. (1992) *Nature (London)* **360,** 266–268.
6. St. Pierre, Y., Nabavi, N., Ghogawala, Z., Glimcher, L. H. & Watts, T. H. (1989) *J. Immunol.* **143,** 808–812.
7. Sinha, A. A., Lopez, M. T. & McDevitt, H. O. (1990) *Science* **248,** 1380–1388.
8. Gilfillan, S., Aiso, S., Michie, S. A. & McDevitt, H. O. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 7314–7318.
9. Gilfillan, S., Aiso, S., Michie, S. A. & McDevitt, H. O. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 7319–7323.
10. Coffman, R. L., Seymour, B. W. P., Lebman, D. A., Hiraki, D. D., Christiansen, J. A., Shrader, B., Cherwinski, H. M., Savelkoul, H. F. J., Finkelman, F. D., Bond, M. W. & Mosmann, T. R. (1988) *Immunol. Rev.* **102,** 5–28.
11. Kuhn, R., Rajewsky, K. & Muller, W. (1991) *Science* **254,** 707–710.
12. Oi, V. T., Jones, P. P., Goding, J. W., Herzenberg, L. A. & Herzenberg, L. A. (1978) *Curr. Top. Microbiol. Immunol.* **81,** 115–129.
13. Baniyash, M. & Eshar, Z. (1984) *Eur. J. Immunol.* **14,** 799–807.
14. Harlow, E. & Lane, D. (1988) *Antibodies: A Laboratory Manual* (Cold Spring Harbor Lab. Press, Plainview, NY).
15. Clare-Salzer, M. J., Brooks, J., Chai, A., Van Herle, K. & Anderson, C. (1992) *J. Clin. Invest.* **90,** 741–748.
16. Paul, W. E. (1989) *Cell* **57,** 521–524.
17. Cerny, A., Heusser, C., Sutter, S., Huegin, A. W., Bazin, H., Hengartner, H. & Zinkernagel, R. M. (1986) *Scand. J. Immunol.* **24,** 437–445.
18. Mombaerts, P., Iacomini, J., Johnson, R. S., Herrup, K., Tonegawa, S. & Papaioannou, V. E. (1992) *Cell* **68,** 869–877.
19. Shinkai, Y., Rathbun, G., Lam, K.-P., Oltz, E. M., Stewart, V., Mendelsohn, M., Charron, J., Datta, M., Young, F., Stall, A. M. & Alt, F. W. (1992) *Cell* **68,** 855–867.
20. Kitamura, D., Kudo, A., Schaal, S., Muller, W., Melchers, F. & Rajewsky, K. (1992) *Cell* **69,** 823–831.
21. Bosma, G. C., Custer, R. P. & Bosma, M. J. (1983) *Nature (London)* **301,** 527–530.
22. Vasicek, T. J., Levinson, D. A., Schmidt, E. V., Campos-Torres, J. & Leder, P. (1992) *J. Exp. Med.* **175,** 1169–1180.
23. Osmond, D. G. (1993) *Immunol. Today* **14,** 34–37.
24. Bishop, G. A. & Haughton, G. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 7410–7414.
25. Cambier, J. C. & Lehmann, K. R. (1989) *J. Exp. Med.* **170,** 877–886.
26. Forsgren, S., Pobor, G., Coutinho, A. & Pierres, M. (1984) *J. Immunol.* **133,** 2104–2110.
27. Smiley, S., Laufer, T. M., Lo, D., Glimcher, L. H. & Grusby, M. J. (1995) *Int. Immunol.* **7,** 665–677.
28. Tepper, R. I., Levinson, D. A., Stanger, B. Z., Campos-Torres, J., Abbas, A. K. & Leder, P. (1990) *Cell* **62,** 457–467.
29. Scher, I. (1982) *Immunol. Rev.* **64,** 117–136.
30. Kitamura, D., Roes, J., Kuhn, R. & Rajewsky, K. (1991) *Nature (London)* **350,** 423–426.
31. Nitschke, L., Kosco, M. H., Kohler, G. & Lamers, M. C. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 1887–1891.
32. Jakobovits, A., Vergara, G. J., Kennedy, J. L., Hales, J. F., McGuinness, R. P., Casentini-Borocz, D. E., Brenner, D. G. & Otten, G. R. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 2551–2555.
33. Chen, J., Troustine, M., Alt, F. W., Young, F., Kurahara, C., Loring, J. F. & Huszar, D. (1993) *Int. Immunol.* **5,** 647–656.
34. Murray, J., Madri, J., Tite, J., Carding, S. & Bottomly, K. (1989) *J. Exp. Med.* **170,** 2135–2140.
35. Gajewski, T. F., Pinnas, M., Wong, T. & Fitch, F. W. (1991) *J. Immunol.* **146,** 1750–1758.
36. DeKruyff, R. H., Fang, Y. & Umetsu, D. T. (1992) *J. Immunol.* **149,** 3468–3476.
37. Bottomly, K. (1988) *Immunol. Today* **9,** 268–274.
38. June, C. H., Ledbetter, J. A., Linsley, P. S. & Thompson, C. B. (1990) *Immunol. Today* **11,** 211–216.
39. Finkelman, F. D., Snapper, C. M., Mountz, J. D. & Katona, I. M. (1987) *J. Immunol.* **138,** 2826–2830.