Brief Definitive Report

IMMUNOGLOBULIN SUBCLASS-SPECIFIC IMMUNODEFICIENCY IN MICE WITH AN X-LINKED B-LYMPHOCYTE DEFECT*

By ROGER M. PERLMUTTER,‡ MOON NAHM,§ KATHRYN E. STEIN,‖ JOHN SLACK,¶ IAN ZITRON, WILLIAM E. PAUL, and JOSEPH M. DAVIE

From The Department of Microbiology and Immunology and Department of Pathology, Washington University School of Medicine, Division of Biology and Biomedical Sciences, St. Louis, Missouri 63110; and The Laboratory of Immunology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20014

Downloaded from http://rupress.org/jem/article-pdf/149/4/993/1089873/993.pdf by guest on 25 September 2022

CBA/N mice express a complex, X-linked immunodeficiency that is manifested primarily in the function of thymus-independent (B) lymphocytes (1, 2). Animals inheriting this defect fail to respond to a number of thymus-independent (TI) antigens, called TI-2 antigens (3), that include haptenated derivatives of Ficoll (4), type III pneumococcal polysaccharide (2), polyinosinic-polycytidilic acid (5), phosphocholine (PC; 6, 7), dextran (8), and levan. The CBA/N defect is not restricted to TI antigens, however, because PC fails to elicit a response in CBA/N mice even when presented as a thymus-dependent protein conjugate (7). In addition, the CBA/N defect is selective with respect to TI antigens because trinitrophenyl (TNP)-derivatized lipopolysaccharide (LPS; 9), TNP-*Streptococcus pneumoniae* (6) and TNP-*Brucella abortus* (10), called TI-1 antigens (3), effectively stimulate TNP-specific responses in mice bearing the immunodeficiency.

It is of interest that many of the antigens to which CBA/N mice appear unresponsive are bacterial carbohydrates. Antigens of this type, in particular PC and dextrans, have been shown recently to elicit antibody responses largely restricted in mice to IgM and the rare IgG3 subclass (11). For this reason we examined immunoglobulin isotype concentrations in sera from mice with the CBA/N phenotype and found preferential deficiencies of IgG3 and IgM which were only partially corrected by polyclonal activation with LPS. These results suggest that CBA/N mice may lack a population of B lymphocytes that contain most of the IgG3 precursors. The absence of this cell population may be the cause of the observed immunodeficiency or, alternatively, the absence of IgG3 precursor cells may be the result of their immunodeficiency.

Materials and Methods

Animals and Immunizations. CBA/N mice of both sexes, (CBA/N × DBA/2)F_1 and (DBA/2 × CBA/N)F_1 male mice were obtained from the Division of Research Services, National

* Supported in part by U. S. Public Health Service grant A1-11635, and by National Science Foundation grant PCM7815318.

‡ Supported by Medical Scientist Training Program grant GM-02016.

§ Supported by U. S. Public Health Service Training Program grant GM-07157.

‖ Supported by U. S. Public Health Service National Research Service Award 7 F32 CA-05328.

¶ Supported by U. S. Public Health Service Training Program grant CA-09118.

Institutes of Health. DBA/2 mice were obtained from The Jackson Laboratory, Bar Harbor, Maine. Serum samples were obtained immediately before and 6 d after intraperitoneal administration of 50 μg LPS (lipopolysaccharide B, *Escherichia coli* 0111: B4; Difco Laboratories, Detroit, Mich.).

Serum Isotype Analysis. Levels of IgM, IgA, IgG1, IgG2a, IgG2b, and IgG3 immunoglobulins in individual mouse sera were determined by using a solid-phase inhibition type radioimmunoassay performed in microtiter plates (Cooke Laboratory Products Div., Dynatech Laboratories, Alexandria, Va.) as previously described (11). Specific antisera used in these assays were prepared in rabbits by immunization with mouse myeloma proteins and were rendered at least 100-fold specific by appropriate absorption with myeloma proteins of other isotypes as described before (11).

Anti-γ3 was prepared by immunizing a rabbit with 250 μg of FLOPC-21 protein (γ3, K; Litton Bionetics, Inc., Kensington, Md.) in complete Freund's adjuvant (CFA) i.p.; 1 mo later the animal was boosted with 600 μg of W5606 (γ3, λ) in CFA and bled 1 mo later. The serum was absorbed with a Sepharose conjugate of the globulin fraction of serum from an MPC-11 (γ2b, K) bearing mouse. When used in the radioimmunoassay at 1:8,000 dilution, maximum binding of ^{125}I-J606 (γ3, K) was 15–30%. Inhibition of binding by myeloma proteins showed at least 1,000-fold specificity for IgG3 compared to all other isotypes.

Isotype-Specific Plaque-Forming Cell (PFC) Enumeration. Spleen cells secreting IgM and IgG3 immunoglobulins were detected by a modified hemolytic plaque assay (13) by using staphylococcal protein A-derivatized sheep erythrocytes (SRBC) and our isotype-specific rabbit antisera. Specificity of this assay system was verified by using a panel of mouse myeloma cells secreting each of the major mouse isotypes. Up to 60% of J606 and M104 myeloma cells formed plaques when tested with anti-γ3 and anti-μ antisera, respectively. However, essentially no plaques were observed with these reagents when tested against myelomas secreting any other isotype, even at cell densities of 1×10^5 per slide.

Results and Discussion

Table I shows the IgG3 concentrations in nonimmune sera derived from CBA/N, DBA/2, and their hybrids. Both CBA/N male and female mice show exceedingly low circulating levels of IgG3, near the limits of detection. In contrast, DBA/2 and (DBA/2 × CBA/N)F_1 males, both of which possess the DBA/2 X chromosome and which do not therefore express the CBA/N defect, have relatively high levels of IgG3, approaching 200 μg/ml in 14-mo-old mice. (CBA/N × DBA/2)F_1 males, that are hemizygous for the defective CBA/N × chromosome, behave much like the homozygous CBA/N mice and show low base-line levels of circulating IgG3. Even older individuals fail to express more than 20 μg/ml of IgG3 antibodies.

It has not been possible thus far to generate a radioimmunoassay for mouse IgG3 with >1,000-fold specificity. Thus, serum levels of IgG3 less than 5 μg/ml cannot be measured because of cross-reactivity of other isotypes with the anti-γ3 reagent. To distinguish between low levels of IgG3 and a total absence of IgG3 in mice bearing the CBA/N defect, stimulation of IgG3 secretion was attempted by using LPS immunization. LPS is believed to stimulate B-cell proliferation and antibody secretion relatively nonspecifically (14).

Fig. 1 shows the serum IgM, IgA, IgG1, IgG2a, IgG2b, and IgG3 concentrations as determined in nonimmune animals and in animals 6 d after LPS stimulation. (CBA/N × DBA/2)F_1 defective males show relatively normal serum levels of IgA, IgG1, IgG2a, and IgG2b immunoglobulins (left upper panel), but are strikingly deficient in IgM and IgG3 isotypes (right upper panels). In addition, the serum IgM levels in both defective and nondefective males are stimulated about 10-fold by LPS immunization, IgG3 concentrations increase approximately threefold in both groups of

Downloaded from http://rupress.org/jem/article-pdf/149/4/993/1089873/993.pdf by guest on 25 September 2022

TABLE I

IgG3 Concentration in Normal Serum

Strain	Sex	Genotype*	Phenotype‡	Number	Age	IgG3 concentration§
					mo	*μg/ml*
CBA/N	Male	xid/Y	Defective	12	2–3	6.0 ± 0.5
CBA/N	Female	xid/xid	Defective	11	2–3	7.8 ± 0.8
DBA/2	Male	+/Y	Normal	10	2–3	66.6 ± 2.6
(CBA/N × DBA/2)F_1	Male	xid/Y	Defective	17	2–3	5.1 ± 0.5
(DBA/2 × CBA/N)F_1	Male	+/Y	Normal	17	2–3	36.8 ± 2.3
CBA/N	Male	xid/Y	Defective	10	9	5.5 ± 1.4
(CBA/N × DBA/2)F_1	Male	xid/Y	Defective	4	14	17.7 ± 7.0
(DBA/2 × CBA/N)F_1	Male	+/Y	Normal	4	14	180.5 ± 45.0

* CBA/N mice possess an X-chromosomal gene, xid, which when present in the homozygous or hemizygous form leads to the characteristic immune defect.

‡ Defective denotes failure to respond to TI-2 antigen stimulation.

§ Minimum value of IgG3 is 5 μg/ml because of cross-reactivity of anti-γ3 with other isotypes. Values shown have not been corrected for this cross-reactivity.

animals. The relative proportions of each isotype in defective compared to nondefective sera are shown in the lower panel of Fig. 1. Clearly mice bearing the CBA/N defect differ little from phenotypically normal reciprocal males except with respect to the serum concentrations of IgM and IgG3 immunoglobulins. Decreased levels of serum IgM have been previously reported in CBA/N mice (2, 15).

A more precise method of evaluating immunoglobulin production is to determine the number of cells secreting the various isotypes. Fig. 2 shows the number of spleen cells secreting IgM and IgG3 in unstimulated and LPS-stimulated defective and nondefective mice. The deficiencies seen before in serum levels of these isotypes are reflected by comparable deficiencies of isotype-secreting cells. Although there are no detectable IgG3-secreting cells in unstimulated CBA/N or (CBA/N × DBA/2)F_1 male mouse spleens, LPS does stimulate significant responses. However, although the anti-γ3 facilitating serum is highly specific for isotype in the reverse plaque assay, it is more difficult to evaluate specificity for variable region determinants, because the panel of myelomas used to evaluate specificity must of necessity express limited variable region determinants.

Restriction of the CBA/N defect to IgM and IgG3 isotypes might be explained by arguing that the environmental antigens that are normally responsible for stimulation of persistent serum IgM and IgG3 titers are mainly bacterial carbohydrates, antigens to which CBA/N mice are unresponsive. Thus, the CBA/N immunodeficiency may not directly affect the appearance of IgG3-bearing B lymphocytes; the observed low circulating levels of IgM and IgG3 might simply reflect the range of antigens to which the CBA/N mouse is unresponsive. Alternatively the central defect in the CBA/N mouse strain might be a selective deficiency in stimulable cells committed to the production of IgM and IgG3.

On the other hand, the fact that LPS treatment of mice with the CBA/N defect does not completely repair their deficiency in IgG3 is not evidence that the rate of appearance of lymphocytes that are precursors of IgG3 secreting cells is abnormal in these mice. It is quite likely that environmental stimulation with polysaccharide antigens normally causes an expansion in IgG3 precursors, whereas cells from immune

Downloaded from http://rupress.org/jem/article-pdf/149/4/993/1089873/993.pdf by guest on 25 September 2022

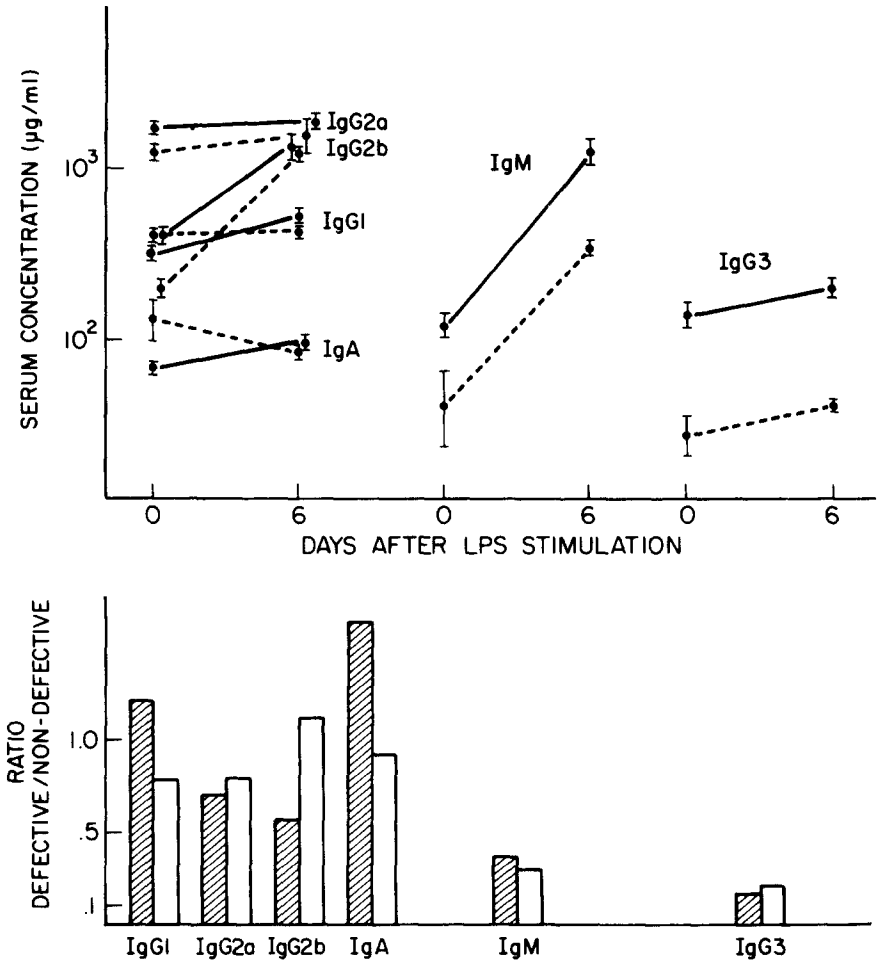

FIG. 1. Serum isotype levels in (DBA/2 × CBA/N)F_1 and (CBA/N × DBA/2)F_1 male mice. Serum was collected before and 6 d after LPS stimulation of groups of four to six defective and nondefective mice. Upper panels: isotype levels in serum as determined by radioimmunoassay. Lower panels: relative concentration (defective compared to nondefective mice) of each isotype before and after LPS stimulation. The relative amounts of IgM and IgG3 in the serum of normal and defective mice 4 and 8 d after LPS treatment were similar to those at 0 and 6 d. It should be noted that the IgG3 levels in unstimulated mice of both phenotypes are higher here than those shown in Table I. We have found a consistent difference in IgG3 levels in animals maintained in different facilities, which probably reflects different environmental stimulants. ●——●, nondefective (DXC); ●----●, defective (CXD); ■, before LPS, □, 6 d after LPS.

defective mice, because they are unresponsive to such antigens, fail to undergo a similar environmental antigen-driven expansion. Indeed, contact of IgG3 precursors from defective mice with environmental polysaccharides may actually induce tolerance in these cells (16). In either case, mice with the CBA/N defect would appear to have diminished numbers of precursors of IgG3-secreting cells when compared to normal individuals. LPS stimulation, although it would increase their absolute IgG3 levels should not change their expression of this isotype relative to similarly treated normal controls.

Finally, it is interesting to speculate on the mechanism by which the responses to bacterial polysaccharides and certain related antigens might be largely confined to

Downloaded from http://rupress.org/jem/article-pdf/149/4/993/1089873/993.pdf by guest on 25 September 2022

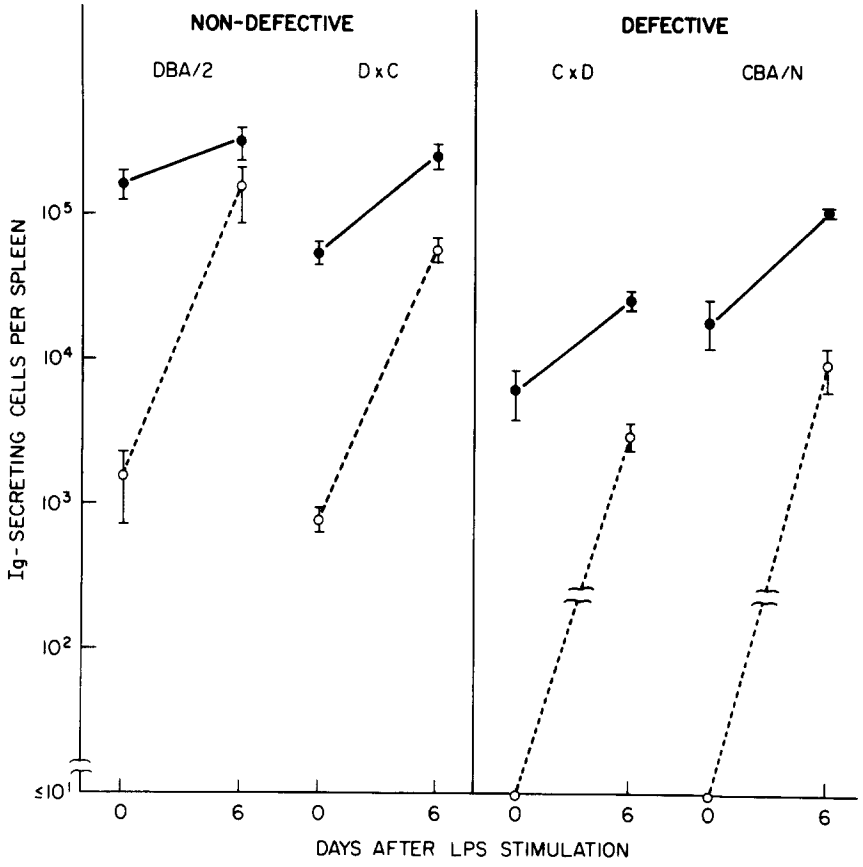

FIG. 2. IgM- and IgG3-secreting cells in spleens of defective and nondefective mice. Spleens of defective and nondefective mice (in groups of three to nine per point) were assayed by reversed plaque assay for IgM and IgG3 secretion before and 6 d after LPS stimulation. ●, IgM; ○, IgG3.

the IgM and IgG3 classes. We have previously demonstrated that many bacterial carbohydrate antigens elicit antibody responses in the mouse largely restricted to IgG3 (11). Our attempts to alter this subclass-restriction by carrier manipulation have been unsuccessful (11). This suggests that certain V_H regions are only expressed in the context of certain C_H regions. Inability to elaborate IgG3, perhaps a subclass (as yet unidentified) of IgM, and the V_H regions that are ordinarily linked to these C_H genes might either explain the complex immunodeficiency of the CBA/N mouse or be an important consequence of a primary inability to respond to polysaccharide antigens.

Summary

CBA/N mice express an X-linked deficiency in their antibody response to many bacterial carbohydrates; we have shown recently that these antigens normally elicit antibody responses predominantly of the IgM and IgG3 isotypes. Here we demonstrate that mice with the CBA/N phenotype have preferential deficiencies of IgM and IgG3 immunoglobulin expression, both when measured in serum and in cells secreting these isotypes, and that this deficiency is only partially corrected by polyclonal activation of B cells. This suggests that CBA/N mice may lack a subpopulation of B cells that contains most of the IgG3 precursors.

Downloaded from http://rupress.org/jem/article-pdf/149/4/993/1089873/993.pdf by guest on 25 September 2022

We thank Donna Venturini for superb technical assistance, and Dr. David Briles and Dr. Daniel Hansburg for their advice and encouragement.

Received for publication 23 January 1979.

References

1. Paul, W. E., B. Subbarao, J. J. Mond, D. G. Sieckmann, I. Zitron, A. Ahmed, D. E. Mosier, and I. Scher. B lymphocyte development and activation: analysis with a mutant mouse strain. *In* Cells of Immunoglobulin Synthesis. B. Pernis and H. J. Vogel, editors. Academic Press, Inc., New York. In press.
2. Amsbaugh, D. F., C. T. Hansen, B. Prescott, P. W. Stashak, D. R. Barthold, and P. J. Baker. 1972. Genetic control of the antibody response to Type III pneumococcal polysaccharide in mice. I. Evidence that an X-linked gene plays a decisive role in determining responsiveness. *J. Exp. Med.* **136:**931.
3. Mosier, D. E., I. M. Zitron, J. J. Mond, A. Ahmed, I. Scher, and W. E. Paul. 1977. Surface immunoglobulin D as a functional receptor for a subclass of B lymphocytes. *Immunol. Rev.* **37:**89.
4. Cohen, P. L., I. Scher, and D. E. Mosier. 1976. *In vitro* studies of the genetically determined unresponsiveness to thymus-independent antigens in CBA/N mice. *J. Immunol.* **116:**300.
5. Scher, I., M. Frantz, and A. D. Steinberg. 1973. The genetics of the immune response to a synthetic double-stranded RNA in a mutant CBA mouse strain. *J. Immunol.* **110:**1396.
6. Quintans, J. 1977. The "patchy" immunodeficiency of CBA/N mice. *Eur. J. Immunol.* **7:** 749.
7. Mond, J. J., R. Lieberman, J. K. Inman, D. E. Mosier, and W. E. Paul. 1977. Inability of mice with a defect in B-lymphocyte maturation to respond to phosphorylcholine on immunogenic carriers. *J. Exp. Med.* **146:**1138.
8. Fernandez, C., and G. Moller. 1977. Immunological unresponsiveness to thymus-independent antigens: two fundamentally different genetic mechanisms of B cell unresponsiveness to dextran. *J. Exp. Med.* **146:**1663.
9. Mosier, D. E., I. Scher, and W. E. Paul. 1976. *In vitro* responses of CBA/N mice: spleen cells of mice with an X-linked defect that precludes immune responses to several thymus-independent antigens can respond to TNP-lipopolysaccharide. *J. Immunol.* **117:**1363.
10. Mond, J. J., I. Scher, D. E. Mosier, M. Blaese, and W. E. Paul. 1978. T-independent responses in B cell-defective CBA/N mice to *Brucella abortus* and to trinitrophenyl (TNP) conjugates of *Brucella abortus*. *Eur. J. Immunol.* **8:**459.
11. Perlmutter, R. M., D. Hansburg, D. E. Briles, R. A. Nicolotti, and J. M. Davie. 1978. Subclass restriction of murine anti-carbohydrate antibodies. *J. Immunol.* **121:**566.
12. Hansburg, D., R. M. Perlmutter, D. E. Briles, and J. M. Davie. 1978. Analysis of the diversity of murine antibodies to dextran B1355. III. Idiotypic and spectrotypic correlations. *Eur. J. Immunol.* **8:**352.
13. Gronowicz, E., A. Coutinho, and F. Melchers. 1976. A plaque assay for all cells secreting Ig of a given type or class. *Eur. J. Immunol.* **6:**588.
14. Kearney, J. F., and A. R. Lawton. 1975. B lymphocyte differentiation induced by lipopolysaccharide. I. Generation of cells synthesizing four major immunoglobulin classes. *J. Immunol.* **115:**671.
15. Amsbaugh, D. F., C. T. Hansen, B. Prescott, P. W. Stashak, R. Asofsky, and P. J. Baker. 1974. Genetic control of the antibody response to Type III pneumococcal polysaccharide in mice. II. Relationship between IgM immunoglobulin levels and the ability to give an IgM antibody response. *J. Exp. Med.* **139:**1499.
16. Merchant, B., H. Snippe, E. F. Lizzio, and J. K. Inman. 1978. Cellular and molecular requirements for X-linked, hapten-specific B-cell blockade in CBA/N mice. *J. Exp. Med.* **147:**1755.

Downloaded from http://rupress.org/jem/article-pdf/149/4/993/1089873/993.pdf by guest on 25 September 2022