

LACK OF EXPRESSION OF THE  $V_{H}S107$  GENE FAMILY IN  
THE LIPOPOLYSACCHARIDE-SENSITIVE B CELL SUBSET  
OF X-LINKED IMMUNODEFICIENCY-DEFECTIVE MICE

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CBA/N mice possess an X-linked immunodeficiency (xid) defect which has been attributed to the absence of the Lyb-5<sup>+</sup> B cell subset (1-3). Since the B cell precursors that give rise to the T15<sup>+</sup>, IgM anti-PC antibodies are largely restricted to the Lyb-5<sup>+</sup> B cell subset (4-9), defective F1 male xid mice produce mainly IgG T15<sup>-</sup> anti-PC antibodies in both their primary and secondary immune responses (9, 10). Although this altered response of CBA/N mice to PC has been directly linked to the loss of the Lyb-5<sup>+</sup> B cell subsets (4-9), it remains to be established why Lyb-5<sup>-</sup> cells cannot effectively express a normal Ig repertoire. We addressed this problem by analyzing, in male and female (CBA/N × B6)F<sub>1</sub>, the total expression of the V<sub>H</sub>T15 gene product independently from its property to impart anti-PC activity. Our results, obtained both at the protein and at the RNA level, clearly show that, contrary to (CBA/N × B6)F<sub>1</sub> females, (CBA/N × B6)F<sub>1</sub> males fail to express the V<sub>H</sub>T15 segment even on Ig devoid of PC activity.

**Materials and Methods**

*Mitogenic Activation of Splenic B Cells with Bacterial LPS.* Spleen cells from 8-wk-old male and female (CBA/N × C57BL/6)F<sub>1</sub> mice (kindly donated by Dr. P. Truffa-Bachi, Institut Pasteur) were treated with anti- $\theta$  and C' and cultured at  $3 \times 10^4$ /ml in RPMI medium supplemented with glutamine, antibiotics, 10% FCS, and  $2 \times 10^{-5}$  2-ME in the presence of either 50  $\mu$ g/ml of LPS from *Salmonella typhimurium* (Difco Laboratories Inc., Detroit, MI) or 50  $\mu$ g/ml of LPS and 10  $\mu$ g/ml of Dextran Sulfate (Pharmacia Fine Chemicals, Uppsala, Sweden). B cell blasts and culture supernatants were collected at day 4 and 8 of cultures, respectively.

*mAbs and RIA.* The H106-131 mAb anti-V<sub>k</sub>21 D-E was a kind gift Dr. M. Pierres (CIML, Marseille) and was obtained as previously described (11-12). The TC54 anti-V<sub>H</sub>T15 mAb was kindly provided by Professor M. Scharff (Albert Einstein Medical College, Bronx, NY) and has been previously described (13). The characteristics of the MS40 anti- $\lambda$ 1 monoclonal protein have been detailed elsewhere (14). The RIA used in these studies consisted of a radioactive binding inhibition and was used to quantify the levels of V<sub>H</sub>T15, V<sub>k</sub>21 D-E, and  $\lambda$ 1<sup>+</sup> Ig in sera and in cultures supernatants. Briefly, serial dilutions of the sample to be tested were added to MOPC511 (V<sub>H</sub>15<sup>+</sup>) (1  $\mu$ g/ml), 14.4.4 (V<sub>k</sub>21 E) (1  $\mu$ g/ml), or H15 ( $\lambda$ 1/ $\mu$ ) (1  $\mu$ g/ml) precoated plastic wells together with <sup>125</sup>I-labeled TC54, H106-131, or MS40 proteins. After an overnight incubation the plates were washed and bound radioactivity was measured in a gamma counter.

The level of anti-PC antibodies in various sera was analyzed by a direct binding test.

This work was supported by Université Pierre and Marie Curie and by the Fondation pour la Recherche Médicale. Address correspondence to Daniele Primi, Unité d'Immunochimie Analytique, Département d'Immunologie, Institut Pasteur, 25 Rue du Dr. Roux, 75724 Paris Cedex 15, France.

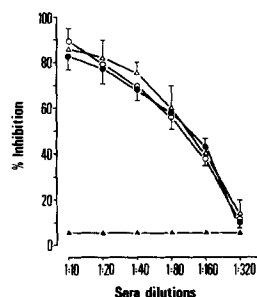


FIGURE 1. Analysis of the property of normal sera of the indicated mouse strains to inhibit the M511 ( $V_H T15^+$ ) – TC54 ( $\alpha V_H T15$ ) interaction. Results represent the means + SD of four individual sera per group. ( $\Delta$ ) CBA/N  $\times$  C57BL/6 females, ( $\blacktriangle$ ) CBA/N  $\times$  C57BL/6 males, ( $\circ$ ) C57BL/6 females, ( $\bullet$ ) C57BL/6 males.

Briefly, 100  $\mu$ l of serial dilutions of sera were added to PC-BSA precoated plastic wells. After an overnight incubation the plates were washed and bound material was revealed with  $^{125}$ I-labeled rat anti-mouse  $\kappa$  mAb.

**DNA Probes and Hybridization.** The 70Z V and the 38C V probes, respectively specific for the J558 and S107 families, are agarose gel-purified restriction fragments of the plasmid subclone of the 70Z  $H^+$  gene and the 38C  $H^+$  recombinant phage, respectively, and were kindly provided by Dr. R. Perry (Institute for Cancer Research, Philadelphia, Pennsylvania) (15). The pB V14 J606 is a 600 bp restriction fragment of the plasmid subclone of the J606 gene kindly provided by Dr. D. Holmberg (Institut Pasteur, Paris). Hybridization was carried out by a slight modification of the method described by Manser and Gefer (16). 4-d blasts were washed twice in balanced salt solutions (BSS) and the desired cell concentrations were dispensed in a 96-well microtiter dish in 0.1 ml of RPMI medium containing 10% FCS. After replicate plating each well received 100  $\mu$ l of lysis buffer (0.125% Sarcosyl (ICN), 12.5 mM sodium citrate (7.0) 2.5M KSCN (Mallinckrodt), and 50% DMSO ( $Me_2SO$ ) (Mallinckrodt) was added just before use. After a half-hour incubation in lysate buffer, samples were filtered with a filtration manifold (Schleicher & Schuell, Inc. Keene, NH) on nitrocellulose membrane wetted first in  $H_2O$ , and then in  $20 \times NaCl/Cit$  ( $1 \times NaCl/Cit$  is 0.15M NaCl/0.015M sodium citrate, pH 7).

The filters were then backed at 80°C for 2 h, hybridized in formamide buffer at 48°C for 48 h with  $^{32}P$ -labeled probes, washed, and exposed to x-ray film at  $-80^\circ C$ .

## Results

**Absence of  $V_H T15^+$ Igs in Sera of XID Mice.** We have recently observed that a large component of  $V_H T15^+$  Igs in the sera of nonimmunized C57BL/6 mice is devoid of anti-PC activity (Primi, D., P.-A. Cazenave, unpublished observations), and therefore we analyzed the level of  $V_H T15$  Ig in the sera of normal female and defective male (CBA/N  $\times$  C57BL/6) $F_1$  animals. To this end we used a mAb (TC54) that reacts with a common  $V_H$  determinant present on isolated heavy chains of TEPC15, MOPC167, and MOPC511 proteins. The results in Fig. 1 show that defective males, contrary to normal females, do not express detectable levels of  $V_H T15^+$  Igs in their sera. Analysis of  $V_H T15^+$  Igs isolated from the sera of normal females, moreover, showed that a large component of these molecules are devoid of PC-binding activity (data not shown). These results, therefore, strongly argue against the possibility that the absence of  $V_H T15^+$  Ig expression in defective males is the consequence of these animals' inability to produce anti-PC antibodies, but rather suggest that the xid phenotype affects the overall expression of a given V gene independently from the antigen specificity of the antibody that encodes.

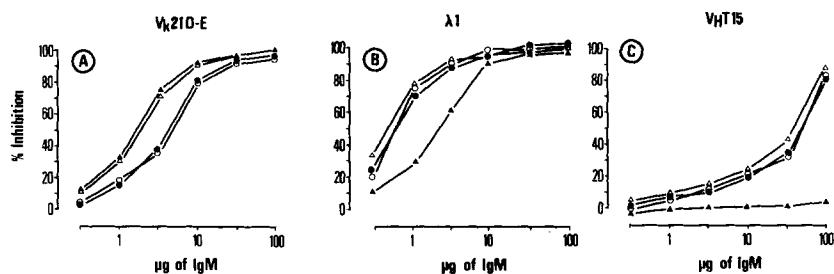


FIGURE 2. Analysis of the level of  $V_{\kappa}210$ -E,  $\lambda 1$ , and  $V_{H}T15^{+}$  Igs in the concentrated supernatants of polyclonally activated B cells of the indicated mouse strains. The results were normalized to the total level of IgM in the various samples. (○) C57BL/6 males, (●) C57BL/6 females, (Δ) (C57BL/6 × CBA/N) $F_1$  females, (▲) (C57BL/6 × CBA/N) $F_1$  males.

*Polyclonal Activation Does Not Result in  $V_{H}T15$  Expression.* To study whether the data obtained with normal serum are a direct consequence of the impairment of xid mice to respond to certain TI-2 antigens, we analyzed the level of  $V_{H}T15$ , as well as of  $V\lambda 1$  and  $V_{\kappa}21$  D-E segments as controls, in the supernatants of polyclonally activated B cells of normal females and immuno-defective males. Fig. 2 A–C shows the levels of  $V_{\kappa}21$  D-E,  $\lambda 1$ , and  $V_{H}T15^{+}$  Igs in the supernatants of normal female and defective male B cells. Although we constantly observed a fivefold reduction of the level of  $\lambda 1$  bearing Igs in cultures of defective male B cells, the amount of  $V_{\kappa}21$  D-E $^{+}$  Igs was similar in all supernatants tested. The  $V_{H}T15$  segment however was completely absent only in the supernatant of polyclonally activated B cells of defective mice.

*Lack of Expression of the Entire S107  $V_H$  Family in Defective Male B Cells.* Analysis of available proteins and nucleic acid sequences data has made it possible to group murine  $V_H$  gene segments into discrete families (17). In addition, recombination studies suggest that the different  $V_H$  families are grouped discretely and not interspersed along the chromosome (18). The lack of expression of  $V_{H}T15^{+}$  gene segments in xid defective animals raises the interesting possibility that this defect can influence the expression of entire families of  $V_H$  genes. To study this possibility we tested LPS or LPS- and dextran sulfate-induced B cell blast for the productive rearrangement of V gene segments by analyzing the presence of mRNA homologous to DNA probes specific for three well-defined  $V_H$  families. Fig. 3 shows an autoradiographic exposure of one filter from such a screen after hybridization with three probes specific for the S107, J606, and J558 families. Of the three  $V_H$  families analyzed, one, the J558, seems to be equally well expressed in normal females and defective males, while  $V_H$  genes belonging to the S107 family are rearranged only in normal females. It is difficult to discriminate whether the low signal obtained with the J606-specific probe on defective male blasts represents specific hybridization or simply background noise. It is clear however that the xid defect also has strong influence on the expression of members of this family.

### Discussion

The results presented here establish that the LPS-sensitive B cell populations of mice expressing the X-linked CBA/N xid gene fail to express one, and possibly more, entire families of  $V_H$  genes. The expression of  $V_L$  genes, on the other

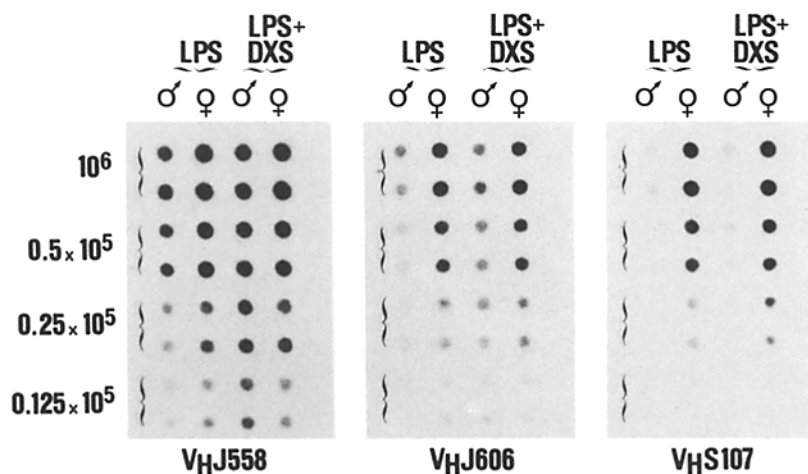


FIGURE 3. Expression of  $V_H$  families in polyclonally activated B cells of (CBA/N  $\times$  C57BL/6) $F_1$  males and females. Spleen cells were cultured at  $3 \times 10^5$ /ml either with 50  $\mu$ g/ml of LPS or with 50  $\mu$ g/ml of LPS and 10  $\mu$ g/ml of DXS. After 4 d of culture, the cells were washed and viable blasts were plated in 96-microwell plates at the indicated numbers. Lysates were separately hybridized with either 70Z, pBV14 J606, or 38C probes. Autoradiographic exposure of the filters was for 24 h with intensifiers.

hand, appears to be less affected in these animals since we found normal levels of  $V_k21$  D-E<sup>+</sup> Igs and only a slight reduction of  $\lambda 1$  molecules. Although the S107 probe hybridized with mRNA of normal female no signal could be detected with mRNA of defective male B cell blasts. In addition to this, since the cells studied in the present report were polyclonally activated, it seems likely that the inability of xid-defective mice to produce anti-PC antibody is the consequence, not the cause, of the lack of expression of the  $V_H T15$  gene.

Since mRNA of xid blasts did not give any specific signal with the specific DNA probe, it is likely that the xid defect abrogates the expression of all the members of this family (19) in the B cell population studied. Although the J558 family appears to be equally well expressed in the two B cell populations studied, we cannot exclude the possibility that this observation is caused by the well-documented difference in size of the families. There are in fact at least 10 times more genes in the  $V_H J558$  than in the S107 group, and consequently, a reduction in the expression of members of the J558 family may be less easily detectable than in a family consisting of few members only.

The data concerning the S107 group of V genes are quite surprising since CBA/N mice not only contain the allelic form of the T15 family but they also can express them, as shown by Clarke et al. (20) who isolated CBA/N hybridomas that use a  $V_H$  segment identical to C3 (the allelic form of T15 found in C57BL [21]). Since these hybridomas have been obtained by immunizations with a thymus-dependent antigen (PC-KLH), it appears likely that the members of the S107 and J606  $V_H$  families are selectively not expressed in the LPS-sensitive B cell subset studied here. Thus, our results support the notion that different B cell subsets express different functional B cell repertoires. An important implication of this conclusion is that, in the course of ontogenesis, B cells develop along alternative functional pathways which selectively rearrange different  $V_H$

families. The existence of asymmetric  $V_H$  expression among B cell populations was originally postulated by Slack et al. (22) who showed that the IgG3 component of the response to T1-1 antigens is absent in mice expressing the CBA/N phenotype. Furthermore, this conclusion would be consistent with the findings that the T15 idiotype family is functionally skewed in the Lyb-5<sup>+</sup> B cell subset (9) and that the Ly-1 B cell subset expresses a rather homogeneous Ig repertoire selectively directed against self antigens (23). The possibility that  $V_H$  genes rearranged nonrandomly has also already been inferred by recent studies showing a developmentally controlled sequence of  $V_H$  rearrangements (24).

In light of the results presented here we propose that during ontogenesis B cells of different lineage, and possibly with different functions and triggering requirements, are expressed sequentially and that each of these lineages preferentially rearranges one or more  $V_H$  families. This hypothesis provides direct explanation for the many examples in the literature showing compartmentalization of  $V_H$  gene expression among B cell subsets.

### Summary

The expression of the  $V_H$ T15 gene product was analyzed in the sera and in the supernatants of polyclonally activated B cells of X-linked immunodeficient (xid) mice. We found that defective males, contrary to normal females, do not express the  $V_H$ T15 gene product even on antibodies devoid of anti-PC specificity. RNA analysis of polyclonally activated cells with  $V_H$  specific probes revealed that xid-defective B cells do not express the entire S107 or part of the J606  $V_H$  family. Members of the J558 family, on the other hand, are equally well expressed among defective males and normal female B cells. These results strongly suggest that  $V_H$  families are asymmetrically represented among B cell subsets.

We thank Dr. M. Pierres and Professor M. D. Scharff for the H106-131 and TC54 mAbs; and Ms. C. Gris and M. Berson for their technical and secretarial assistance.

*Received for publication 12 March 1986.*

### References

1. Scher, I. 1981. B lymphocyte development and heterogeneity: analysis with the immune-defective CBA/N mouse strain. *In* Immune Defects in Laboratory Animals. E. Gershium and B. Merchant, editors. Plenum Publishing Corp., New York. 163.
2. Scher, I. 1982. The CBA/N mouse strain: an experimental model illustrating the influence of the X-chromosome on immunity. *Adv. Immunol.* 33:2.
3. Scher, I. 1982. CBA/N immune defective mice: evidence for the failure of a B cell subpopulation to be expressed. *Immunol. Rev.* 64:117.
4. Wickler, L. S., G. Guelde, I. Scher, and J. J. Kenny. 1982. Antibodies from the Lyb 5<sup>-</sup> B cell subset predominate in the secondary IgG response to phosphorylcholine. *J. Immunol.* 129:950.
5. Scher, A., and M. Cohn. 1972. Inheritance of an idiotype associated with the immune response of inbred mice to phosphorylcholine. *Eur. J. Immunol.* 2:319.
6. Cosenza, H., and M. Kohler. 1972. Specific inhibition of plaque formation to phosphorylcholine by antibody against antibody. *Science (Wash. DC)* 176:1027.
7. Claflin, J. L., R. Liberman, and J. M. Davie. 1974. Clonal nature of the immune

- response to phosphorylcholine. I. Specificity, class, and idiotype of phosphorylcholine-binding receptors on lymphoid cells. *J. Exp. Med.* 139:58.
8. Clafin, J. L., and J. M. Davie. 1975. Clonal nature of the immune response to phosphorylcholine (PC). V. Cross idiotypic specificity among heavy chain of murine anti-PC antibodies and PC-binding myeloma proteins. *J. Exp. Med.* 141:1073.
  9. Kenny, J. J., L. J. Yaffe, A. Ahmed, and E. S. Metcalf. 1983. Contribution of Lyb5<sup>+</sup> and Lyb5<sup>-</sup> B cells to the primary and secondary phosphocholine-specific antibody response. *J. Immunol.* 130:2574.
  10. Kohler, H., S. Smyk, and J. Fung. 1981. Immune response to phosphocholine. VIII. The response of CBA/N mice to PC-LPS. *J. Immunol.* 126:1790.
  11. Devaux, C. A., C. Cabit, S. Marchetto, and M. Pierres. 1984. Shared idiotope on monoclonal anti-Ia7 antibodies reactive with determinants in a structural domain of the I-E molecule. *J. Immunol.* 132:1353.
  12. Devaux, C., D. Moinier, G. Mazza, X. Guo, S. Marchetto, M. Fougereau, and M. Pierres. 1985. Preferential expression of V<sub>k</sub>21E light chains on IdX Ia.7 positive monoclonal anti-I-E antibodies. *J. Immunol.* 134:4024.
  13. Desaymard, C., A. M. Giusti, and M. D. Scharff. 1984. Rat anti-T15 monoclonal antibodies with specificity for V<sub>H</sub> and V<sub>H</sub>-V<sub>L</sub>. *Mol. Immunol.* 21:961.
  14. Sanchez, P., D. Primi, M. Levi-Strauss, and P.-A. Cazenave. 1985. The regulatory locus rλ1 affects the level of λ1 light chain synthesis in lipopolysaccharide-activated lymphocytes but not the frequency of λ1-positive B cell precursors. *Eur. J. Immunol.* 15:66.
  15. Nelson, K. J., J. Haimovich, and R. P. Perry. 1983. Characterization of productive and sterile transcripts from the immunoglobulin heavy-chain locus: processing of μm and μs mRNA. *Mol. and Cell. Biol.* 3:1317.
  16. Manser, T., and M. Gefter. 1984. Isolation of hybridomas expressing a specific heavy chain variable region gene segment by using a screening technique that detects mRNA sequences in whole cell lysates. *Proc. Natl. Acad. Sci. USA.* 81:2470.
  17. Brodeur, P. H., and R. Riblet. 1984. The immunoglobulin heavy chain variable region (Igh-V) locus in the mouse. I. One hundred Igh-V genes comprise seven families of homologous genes. *Eur. J. Immunol.* 14:922.
  18. Dildrop, R. 1984. A new classification of mouse V<sub>H</sub> sequences. *Immunol. Today.* 5:85.
  19. Crews, S., J. Griffin, H. Huang, K. Calame, and L. Hood. 1981. A single V<sub>H</sub> gene segment encodes the immune response to phosphorylcholine: somatic mutation is correlated with the class of the antibody. *Cell.* 25:59.
  20. Clarke, S. H., J. J. Kenny, D. G. Sieckmann, and S. Rudikoff. 1984. Amino acid sequence of a phosphocholine-binding antibody from an immune defective CBA/N mouse employing the T15 V<sub>H</sub> region associated with unusual D<sub>H</sub>, J<sub>H</sub> and V<sub>k</sub> segments. *J. Immunol.* 3:1544.
  21. Rudikoff, S., and M. Potter. 1980. Allelic forms of the immunoglobulin heavy chain variable region. *J. Immunol.* 124:2089.
  22. Slack, J., G. P. Der-Balian, M. Nahn, and J. M. Davie. 1980. Subclass restriction of murine antibodies. II. The IgG plaque-forming cell response of thymus-independent type 1 and type 2 antigens in normal mice and mice expressing an x-linked immunodeficiency. *J. Exp. Med.* 151:853.
  23. Hayakawa, K., R. R. Hardy, D. R. Parks, and L. A. Herzenberg. 1983. The "Ly-1 B" cell subpopulation in normal, immunodeficient, and autoimmune mice. *J. Exp. Med.* 157:202.
  24. Jancoupolous, G. D., S. V. Desiderio, M. Paskind, J. F. Kearney, D. Baltimore, and F. W. Alt. 1984. Preferential utilization of the most JH-proximal V<sub>H</sub> gene segments in pre-B cell lines. *Nature (Lond.)* 311:727.