V_H Gene Family Expression in Mice with the *xid* Defect

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Summary

Preferential use of particular V_H gene families in the response to specific antigens has been demonstrated in several systems. The lack of responses to certain types of antigens, therefore, could be the result of deletion of or failure to express some V_H genes. Because CBA/N mice, which carry the X-linked immunodeficiency (*xid*) gene defect, have been shown to be unresponsive to thymus-independent polysaccharide antigens, it was of interest to examine if this unresponsiveness could be accounted for by abnormal expression of particular V_H gene families. Using in situ hybridization on B cell colonies, we determined the expression of nine V_H gene families in CBA/CaHN females (genotypically normal), CBA/N males (*xid*) and females (*xid*), and (CBA/N × CBA/CaHN)F_1 males (*xid*) and females (phenotypically normal). Our results indicate that V_H gene family expression, including the S107 family, in CBA/N males and F_1 males, is similar to that of CBA/CaHN and F_1 females with predominant expression of J558, the largest gene family, in all individuals. Interestingly, CBA/N female mice, which carry two defective X chromosomes, as a group expressed significantly reduced levels of the J558 gene family, and as individuals showed variation in which family was predominantly expressed. We conclude that the unresponsiveness of mice with the *xid* defect to polysaccharide antigens can not be attributed to a failure to express the nine V_H gene families that we examined. Our findings do not support previous studies (Primi, D., and P.-A. Cazenave 1986. *J. Exp. Med.* 165:357), which found an absence of expression of the S107 family in *xid* mice.

The CBA/N mouse strain, which carries the *xid* gene, has been used extensively to study the lineage and functional heterogeneity of B lymphocytes (1–3). The defect in CBA/N mice, a subline of CBA/Ca mice, was discovered as a result of unresponsiveness to type III pneumococcal polysaccharide (4). The *xid* defect appears to affect the B cell population predominantly because mice with this defect lack a late-developing B cell subset (5, 6) and are unresponsive to a number of thymus-independent antigens (2, 7), including virtually all polysaccharides. It has been demonstrated that preferential use of particular V_H families occurs in the response to polysaccharide antigens; for example, the use of a particular V_H X-24 gene by antibodies specific for galactans (8) and 3-fucosyl lactosamine (9) and certain antibacterial levan antibodies (10) and the dominance of an S107 V_H gene in the response to the phosphorylcholine hapten of pneumococcal polysaccharide (11). It was thus of interest to ask if the general unresponsiveness to polysaccharide antigens in mice with the *xid* defect could be accounted for by lack of expression of particular Ig variable region genes.

Primi and Cazenave (12) examined the expression of V_H gene families in LPS-stimulated splenocytes from mice with the *xid* defect using a lysate hybridization technique and found a complete absence of expression of the S107 family. Their studies were limited to three V_H gene families and, more importantly, they did not control for the amount of Ig mRNA per sample in their analysis. Thus, it is difficult to interpret a negative result in their studies. The murine V_H region gene locus has recently been shown to be comprised of 12 major families of various sizes, as determined by Southern blot analysis of genomic DNA (13). We have compared the expression of nine V_H gene families by the in situ hybridization method of Schulze and Kelsoe (14). An important feature of this method is that the expression of each V_H gene family can be determined independently as a percent of the total IgM expressing cells, detected with a probe for the μ heavy chain constant region. In contrast to Primi and Cazenave (12), we have found expression of nine V_H gene families, including S107, in mice with the *xid* defect to be comparable with that in normal CBA/CaHN mice.

Materials and Methods

Animals. CBA/CaHN and CBA/N mice were purchased from the Biological Testing Branch, (National Cancer Institute [NCI], Bethesda, MD). All mice were 4–8 wk of age at the time of use.

$(CBA/N \times CBA/CaHN)F_1$ mice were bred in our animal facility. All mice were maintained under pathogen-free conditions in our animal room. Sentinel mice are tested periodically for antibodies to a panel of 19 mouse viral pathogens by Microbiological Assoc. Inc. (Rockville, MD). Experimental mice had been tested periodically, but since April 1990, all mice used in these experiments (including CBA/N female mice nos. 9–16, all males, and all $(CBA/N \times CBA/CaHN)F_1$ mice) have been tested for antibodies to mouse hepatitis virus and been found to be negative.

Cell Lines. Myeloma and hybridoma cells producing mRNA representative of different V_H gene families were grown in DMEM (Whittaker Bioproducts, Inc., Walkersville, MD) supplemented with 10% calf serum (HyClone Laboratories, Logan, UT), glutamine, oxalacetic acid, pyruvic acid, bovine insulin, hypoxanthine, thymidine, and gentamicin. Myeloma and hybridoma lines, and the V_H gene families they express, indicated in parentheses, were myeloma lines XPRC-24 (V_HX24), J606 (V_HJ606), MOPC-315 (V_H36-60), J558 (V_HJ558), and TEPC-15 (V_HS107), provided by Dr. Michael Potter (NCI); hybridoma lines Nab1 (V_H7183), Nab2 (V_H3609), and Nab4 (V_HQ52), provided by Dr. Corey Mallet (NCI); hybridoma line HPC M2 (V_HS107), from Dr. Patricia Gearhart (15) via Dr. James Kenny (NCI); and hybridoma line BBLC 113.1 (VGam3-8), produced in our laboratory.

Probes. The hybridization probes used in this study were purified by electroelution of agarose gels containing the following DNA fragments: V_H7183, 600-bp EcoRI-HindIII unrearranged genomic fragment of V_H37 subcloned into pGEM-3Z (16); V_HQ52, 300-bp BamHI-EcoRI fragment of pV_HQ52 (17); V_HS107, 450-bp PstI fragment from pV_HS107 (17); V_HX24, 500-bp EcoRI fragment from p17 (8); VGam3-8, 450-bp EcoRI-PstI fragment (18); V_H36-60, 700-bp EcoRI-HindIII fragment from pRN5.15 (19); V_HJ606, 600-bp EcoRI-BamHI fragment from pBV14RB7 (20); V_H3609, 400-bp PstI fragment from pVh3635BR1.6 (21); V_HJ558, 750-bp EcoRI-HindIII fragment of pV_HJ558 subcloned into pGEM-3Z (17); C_μ, 800-bp plus 400-bp PstI fragments from pABμ_8 (22). Plasmids for isolating probes for VGam3-8, X24, 3609, J606, S107, and 36-60 were provided by Dr. Roy Riblet (Medical Biology Institute, La Jolla, CA), Q52 by Dr. Roger Perlmutter (University of Washington School of Medicine, Seattle, WA), J558 by Dr. Corey Mallet (NCI), 7183 by Dr. Daniel Kastner (NCI), and C_μ, by Dr. Barbara Birshtein (Albert Einstein College of Medicine, Bronx, NY).

Dot Blot. To examine the specificities of the probes, total RNA was isolated from cells expressing each V_H gene family using the method described previously by Feng et al. (23). The cells were lysed in guanidine thiocyanate, and the lysate was layered onto a 5.7-M cushion of CsCl and centrifuged at 174,000 *g* for 21 h. The RNA was recovered by redissolving the pellet in diethyl pyrocarbonate (Sigma Chemical Co., St. Louis, MO)-treated H_2O. 20 μg of total RNA from each sample was heated at 55°C in a solution containing 5% formamide, 0.1× MOPS buffer, and 1.75% formaldehyde for 15 min. An equal volume of 20× SSC was added to the RNA mixture before RNA was applied to the nitrocellulose filter on a Minifold I (TM) apparatus (Schleicher & Schuell, Inc., Keene, NH). The filters were air dried and baked at 80°C for 2 h, then prehybridized for 4 h at 42°C in a solution containing 50% formamide, 5× SSC, 50 mM sodium phosphate, pH 6.5, 1× Denhardt's solution, and 200 μg/ml salmon sperm DNA (Boehringer Mannheim Corp., Indianapolis, IN). DNA probes were radiolabeled by the random primer technique using kits purchased from Amersham Corp. (Arlington Heights, IL). ^{32}P-labeled V_H family–specific DNA probes were added to the solution to a final concentration of 10^6 cpm/ml. Hybridization was carried out for 20 h at 42°C. The filters were washed in a final solution of 0.1× SSC and 0.1% SDS for 15 min at 68°C, and then exposed to XAR-5 film (Eastman Kodak, Rochester, NY) at −80°C with double intensifying screens for 2 h. In one instance, ^{32}P was quantitated by counting the ^{32}P in each dot with an AMBIS radioanalytic imaging system (AMBIS System Inc., San Diego, CA).

Filter Paper Disk Culture. Previous studies (24) indicated that splenocytes of *xid* mice respond poorly to LPS, particularly phenol-extracted LPS. Preliminary studies using [^{3}H]thymidine showed comparable responses to phenol and TCA-purified LPS (data not shown). Phenol-purified LPS was chosen for these studies because of its greater purity. The filter paper disk culture was performed using the procedures described by Schulze and Kelsoe (14), with some modifications. Spleen cells were teased into single cell suspensions using sterile 20-gauge needles and were suspended in RPMI 1640 containing 10% FBS (HyClone Laboratories), penicillin-streptomycin, 5×10^{-5} M 2-ME, glutamine, and 10 μg/ml dextran sulfate (Pharmacia Fine Chemicals, Piscataway, NJ). For CBA/CaHN and $(CBA/N \times CBA/CaHN)F_1$ females, 5 ml of cell suspension containing 3×10^5 splenocytes, and for CBA/N and $(CBA/N \times CBA/CaHN)F_1$ males, 5 ml of cell suspension containing 6×10^5 cells, were added evenly onto an 8.2-cm diameter filter paper disk (No. 54: Whatman Inc., Clifton, NJ) in a petri dish. Cells were allowed to settle for 30 min. Then, another 5 ml of culture medium containing 1–2 × 10^7 autologous thymocytes (teased into a single cell suspension, washed, and resuspended in culture medium) and 200 μg of LPS (*Escherichia coli* 0111:B4, phenol-extracted; Sigma Chemical Co.) were gently added and the dishes were incubated at 37°C for 5 d in 7% CO_2. Contaminating B cells in the thymocyte preparation were eliminated by irradiation with 2,500 rad to inhibit cell growth. This method was used routinely after determining that these cells did not form colonies on filter paper disks as detected by staining or by hybridization with the C_μ probe, and that they were comparable with those prepared by the original method of Schulze and Kelsoe (14), that of anti-Ig plus complement depletion, in supporting B cell growth.

For the filter paper disk culture of hybridoma cells, 8 ml of culture medium (RPMI 1640 containing 10% FCS, glutamine, penicillin, streptomycin, dextran sulfate, and 2-ME) containing 3×10^3 HPC M2 cells, a hybridoma line expressing the S107 gene family, was spread evenly over the filter paper disk and cultured for 5 d.

In Situ Hybridization to B Cell or Hybridoma Colonies. Medium was removed from each petri dish on day 5 of culture, and 7 ml of 0.75 M phosphate buffer containing 10% formaldehyde was added. Disks were fixed for 5 min at room temperature and then washed twice with PBS diluted 1:10 with distilled water. Washed disks were air dried and immediately prehybridized for at least 3 h at 43°C in 7 ml of solution containing 50% formamide, 5× SSC, 5× Denhardt's solution, 50 mM phosphate buffer (pH 7.4), 1% glycine, and 250 μg/ml of salmon sperm DNA. The prehybridization solution was removed and replaced with 7 ml hybridization solution (as above, but with 20 mM phosphate buffer and no glycine) containing 10^7 cpm of ^{32}P-labeled V_H family– or C_μ-specific DNA probe. Disks were hybridized for 2 d at 43°C, then washed three times for 5 min in 2 × SSC containing 0.1% SDS at room temperature and once in 0.1× SSC/0.1% SDS at 43°C for 20 min. After washing, disks were air dried and exposed to XAR-5 film for 5 d without an intensifying screen or 1 d with an intensifying screen at −80°C. Disks were first hybridized to V_H probes and then rehybridized directly to the C_μ probe. The percentage of each of the nine V_H gene families was determined independently from each of nine filter paper disks cultured from individual mice.

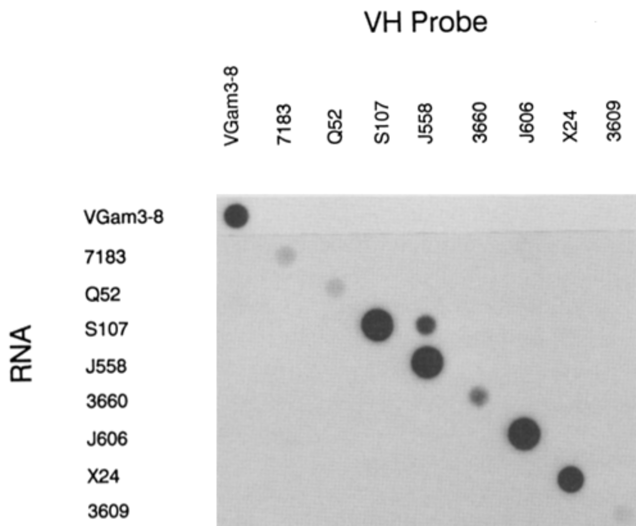

Figure 1. Specificity of nine V_H probes examined by dot blot analysis. RNA representing each family was isolated from BBLC 113.1 hybridoma cells (VGam3-8), Nab1 Hybridoma cells (7183), Nab4 hybridoma cells (Q52), TEPC-15 myeloma cells (S107), J558 myeloma cells (J558), MOPC-315 myeloma cells (36-60), J606 myeloma cells (J606), XRPC-24 myeloma cells (X24), and Nab2 hybridoma cells (3609).

Results

To carry out an analysis of V_H gene family usage in LPS-stimulated splenocytes from various mouse strains, including mice with the *xid* defect, which are known to have reduced responsiveness to LPS, the method of colony hybridization (14) was chosen. The advantage of this method is that it permits analysis of the frequency of expression of each V_H gene family as a fraction of the responding cells rather than the number of input cells, and eliminates error due to variation in the response to the LPS or ability to form colonies.

Specificity of V_H Probes. The specificity of each of the nine V_H probes was examined by dot blot analysis using RNA isolated from myeloma or hybridoma cells. Fig. 1 shows that by dot blot analysis, all of the V_H probes are specific, except for the J558 probe, which crosshybridized with the S107 RNA isolated from TEPC-15 myeloma cells. Quantitation revealed that the ratio of cpm in the J558 dot to the cpm in the TEPC-15 dot was 10:1. This crosshybridization was further investigated by in situ hybridization using an S107-expressing hybridoma cell line (HPC M2). Fig. 2 shows that the HPC M2 cell colonies that were cultured and fixed onto filter paper disks were recognized by the S107 probe but not by the J558 probe or by the 7183 probe. Rehybridization of the same disks with the C_μ probe revealed the presence of colonies on all three disks, demonstrating that crosshybridization of the J558 probe with cells expressing V_H S107 did not occur under the conditions used for in situ hybridization.

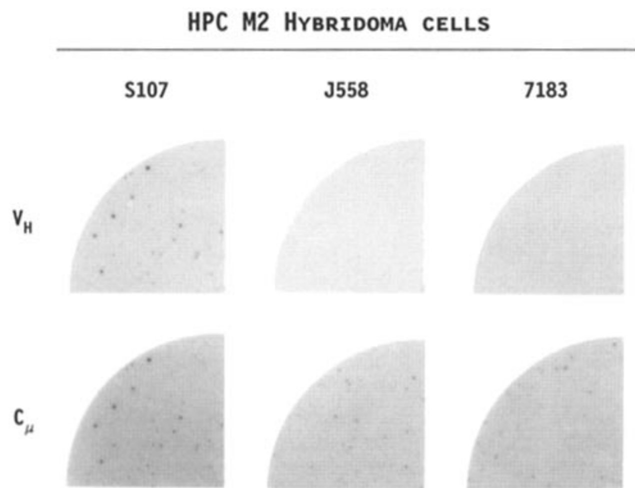

Figure 2. Specificity of the J558 probe demonstrated by in situ hybridization. Filter paper disks containing HPC M2 hybridoma cell colonies, expressing S107 and C_μ genes, were hybridized with the ^{32}P-labeled S107 probe, the J558 probe, and the 7183 probe after fixation. These filter paper disks were then rehybridized with the $C\mu$ probe.

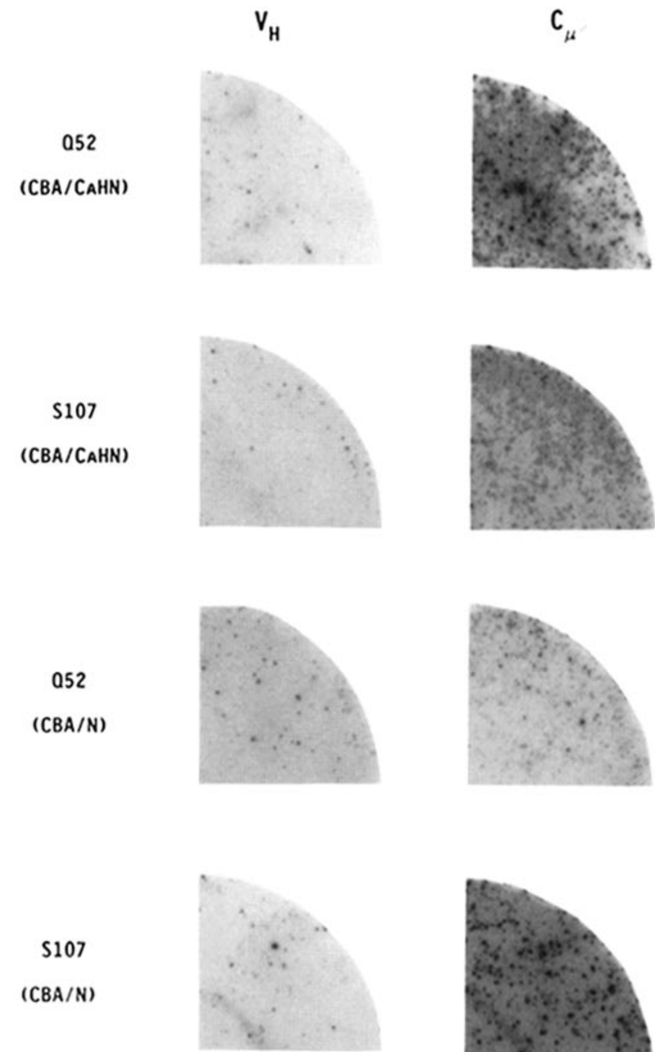

Figure 3. Sequential hybridization of LPS-stimulated B cell colonies with the V_H and $C\mu$ probes. 3×10^5 splenocytes of CBA/CaHN or 6×10^5 splenocytes of CBA/N female mice were stimulated with LPS and cultured for 5 d on filter paper disks. After fixation, disks containing CBA/CaHN (*top two pairs*) or CBA/N (*bottom two pairs*) B cell colonies were hybridized with the Q52 and the S107 probes, then subjected to autoradiography. The same disks were rehybridized with the $C\mu$ probe and again subjected to autoradiography. Only a quarter of each disk is shown.

Efficiency of Colony Formation of LPS-stimulated Splenocytes. CBA/N splenocytes were examined for their ability to form B cell colonies on filter paper disks and to be hybridized with different probes. Fig. 3 shows examples of V_H-hybridized filter paper disk segments containing stimulated B cell colonies derived from CBA/CaHN and CBA/N mice. Each V_H-hybridized disk was rehybridized with the C_μ probe in order to calculate the percent expression for each V_H gene family. The data show that splenocytes from CBA/N mice could be stimulated by LPS to form cell colonies that were of comparable size with colonies from CBA/CaHN mice and were readily detectable by V_H and C_μ probes. Table 1 shows the average numbers of $C_\mu{}^+$ colonies derived from 10^5 LPS-stimulated splenocytes of *xid* and non-*xid* mice on filter paper disks. The results indicate that mice with the *xid* defect have reduced efficiency of colony formation. By applying twice the number of splenocytes per filter paper disk for defective strains compared with normal, similar numbers of colonies were obtained for V_H analysis of both groups (~1,000 colonies per disk).

V_H Expression in xid and Non-xid Mice. V_H gene expression was examined in CBA/CaHN, CBA/N females and males, and (CBA/N × CBA/CaHN)F_1 females and males. The average percent expression of each of the nine V_H gene families, and the numbers of colonies counted from which the percents are calculated, are shown in Table 2. CBA/CaHN showed random V_H expression with the largest gene family, J558, expressed in the greatest frequency, however, two families, 3609 and S107, differed from that expected based on the family sizes in BALB/c mice (17). The 3609 family, which has a complexity of 15 by Southern analysis, was used by only 2% of the CBA/CaHN B cell colonies. In contrast, the S107 family, which has only three functional genes, was expressed by 11% of the CBA/CaHN B cell colonies. The data in Table 2 show that there was little difference between the defective and nondefective mice, except that CBA/N females used J558 to a significantly lesser degree when compared with the other mice (18.4% in CBA/N females vs. 27.8–33.9% in all the others, $p < 0.005$). Furthermore, whereas in every individual mouse of all other groups examined, J558 was the predominantly expressed family, in the CBA/N females, J558 was the predominantly expressed family in only 5 of 16 individuals (data not shown). In addition, the total percent of B cell colonies that used these nine V_H gene families was reduced in cells from CBA/N females compared with CBA/CaHN mice (73% vs. 84%).

Discussion

The X chromosome of mice and humans is known to have significant influence on the immunological function of these species. In humans, mutations on certain genes of the X chromosome result in a number of immunological diseases, such as the Bruton's X-linked agammaglobulinemia (25), the Wiskott-Aldrich syndrome (26), and an X-linked form of severe combined immunodeficiency disease (27). Studies of X-linked immunodeficiency diseases in inbred mice gained attention in the 1970s, when the CBA/N subline of CBA/CaHN was found to carry an X-linked defect (4). Since that time, detailed investigations of various aspects of the *xid* defect of CBA/N mice or the F_1 male hybrids derived by crossing CBA/N females to normal inbred male mice have been widely reported.

We are interested in mice with the *xid* defect because of their inability to respond to thymus-independent polysaccharide antigens and their poor response to thymus-dependent forms of polysaccharides (28). The purpose of our study was to investigate whether the defect in responsiveness to polysaccharide antigens in *xid* mice could be accounted for by an abnormality in V_H gene family expression. The method chosen to address this question was in situ hybridization on LPS-stimulated B cell colonies grown on filter paper disks. With this technique it is possible to analyze large numbers of B cell clones and independently determine the expression of each V_H gene family. To perform the in situ hybridization assay, splenocytes were stimulated with LPS for 5 d to induce differentiation into Ig-producing cells and colony formation. In our study, CBA/N splenocytes, which have been shown previously to fail to form colonies in soft agar (29), proliferated and formed considerable numbers of colonies on filter paper disks after stimulation with phenol-extracted LPS. It is possible, however, that the thymocyte feeder layer used in the filter paper disk method, or the growth on filter paper, itself, provided a better environment for growth of *xid* splenocytes than the agar method. Splenocytes from *xid* mice have also been shown to respond poorly to phenol-extracted LPS (24). Using [^{3}H]thymidine incorporation into DNA as a measure of stimulation, both phenol- and TCA-extracted LPS were equivalent as mitogens for the splenocytes of mice with the *xid* defect, although the extent of proliferation of CBA/N splenocytes was about half that of CBA/CaHN splenocytes (data not shown). The reason for the discrepancy between the two studies is not clear, however, our thymidine incorporation data correlated well with the difference in the effi-

Table 1. *Efficiency of Colony Formation of LPS-stimulated Splenocytes Cultured on Filter Paper Disks in CBA/CaHN Females, CBA/N Females and Males, and F1 Females and Males*

	CBA/CaHN females	CBA/N females	CBA/N males	F_1 females	F_1 males
No. of colonies formed per 10^5 splenocytes	421 ± 171*	232 ± 98	212 ± 43	612 ± 133	336 ± 103
Efficiency of colony formation	1	0.6	0.5	1.5	0.8

Results represent mean ± SD of at least four experiments for each group of mice.

Table 2. *V_H Gene Expression of LPS-stimulated Splenocytes in CBA/CaHN Females, CBA/N Females and Males, and F_1 Females and Males*

V_H gene family [complexity]*	CBA/CaHN females	CBA/N females	CBA/N males	F_1 females	F_1 males
J558 [60]	28.7 ± 8.2 (8)[‡]	18.4 ± 6.8 (16)[§]	33.9 ± 5.3 (4)	33.4 ± 6.6 (5)	27.8 ± 9.0 (7)
	{11,475}[∥]	{22,398}	{5,858}	{9,124}	{14,893}
3609 [15]	2.2 ± 1.0 (8)	1.4 ± 1.0 (16)	1.5 ± 0.8 (4)	1.4 ± 0.5 (5)	0.9 ± 0.2 (7)
	{11,334}	{19,752}	{5,150}	{8,582}	{14,213}
J606 [10]	6.5 ± 1.9 (8)	6.0 ± 5.0 (16)	4.7 ± 1.4 (4)	4.7 ± 1.4 (5)	4.3 ± 1.7 (7)
	{9,725}	{23,156}	{5,192}	{9,268}	13,877}
VGam3-8 [5]	2.3 ± 2.3 (8)	1.8 ± 1.1 (16)	2.6 ± 1.3 (4)	2.5 ± 1.6 (5)	3.7 ± 1.6 (7)
	{12,829}	{22,179}	{5,214}	{8,456}	{12,811}
36-60 [5]	3.3 ± 1.5 (7)	2.0 ± 1.4 (16)	2.4 ± 0.9 (4)	2.0 ± 0.6 (5)	2.5 ± 1.7 (7)
	{11,418}	{19,802}	{5,188}	{9,176}	{14,517}
X24 [2]	1.6 ± 1.0 (6)	0.6 ± 0.3 (16)	0.7 ± 0.2 (4)	0.6 ± 0.3 (5)	0.5 ± 0.1 (7)
	{9,653}	{20,367}	{4,322}	{9,556}	{14,621}
S107 [4]	11.5 ± 3.1 (9)	12.8 ± 6.0 (16)	12.6 ± 1.8 (4)	13.4 ± 2.2 (5)	10.5 ± 2.1 (7)
	{12,093}	{19,933}	{4,904}	{9,778}	{13,851}
Q52 [15]	13.2 ± 2.0 (8)	13.5 ± 6.9 (16)	9.4 ± 1.9 (4)	11.9 ± 3.0 (5)	12.9 ± 3.4 (7)
	{11,415}	{21,834}	{5,230}	{9,282}	{13,818}
7183 [12]	15.4 ± 1.9 (5)	17.0 ± 4.9 (14)	15.9 ± 2.0 (4)	12.9 ± 2.3 (5)	14.4 ± 2.6 (7)
	{8,262}	{18,255}	{5,594}	{9,460}	{14,353}
	84.7[¶]	73.5	83.7	82.8	78.7

* Complexity was determined by the number of hybridizing restriction fragments resolved on Southern blots and serves as estimate of the number of V_H genes as determined for BALB/c mice (17).
Results were calculated as percent of $C\mu^+$ colonies expressing a given V_H family, and represent mean ± SD.
‡ Numbers in parentheses indicate the number of mice used. Each mouse was studied in a separate experiment.
§ The difference in the usage of the J558 family between CBA/CaHN and CBA/N females is significant ($p < 0.005$).
∥ Numbers in braces represent the total number of $C\mu^+$ colonies screened to determine each V_H gene family per strain.
¶ Total percent.

ciency of colony formation between CBA/N and CBA/CaHN splenocytes (0.55 vs. 1.0 in Table 1). (CBA/N × CBA/CaHN)F_1 females also had an efficiency twice that of F_1 males. It is interesting to note that F_1 females had an higher efficiency than CBA/CaHN, and F_1 males were higher than CBA/N, but all the *xid* mice were consistently lower than the normal mice.

Table 2 indicates that the V_H expression of splenocytes in CBA/CaHN female mice appeared to be random with the J558 family expressed predominantly. Each V_H family was expressed to the extent corresponding to the size of its family, except for the S107 and 3609 families. The S107 family is a small family with only three functional genes in BALB/c and CBA/J mice (30, 31), however, it was used more frequently than expected (11% of the B cells) in CBA/CaHN. In contrast, the 3609 family has a complexity of 15, but only 2% of the B cells use this family; the number of pseudogenes present in this family, however, is unclear. It was also noted that the 7183 family usage by these mice was slightly higher than expected (13–17% expression for a family with a complexity of 12). We think these data reflect the strain-dependent variation reported in V_H gene usage (32). Jeong et al. (32) have reported previously V_H gene usage in CBA/J by in situ hybridization examining single LPS-stimulated splenocytes. Our data from CBA/CaHN mice were quite comparable with theirs, indicating that the two methods have little variation in determining the V_H gene usage and confirming that the differences between CBA/Ca and BALB/c are likely due to strain variation and not methodology. The data further indicate that while there may be significant differences between strains, these two sublines of the CBA strain do not differ. Jeong et al. (32) did not examine the expression of the 3609 and VGam3-8 families, thus, our results provide new data on the expression of these V_H gene families in CBA mice.

Table 2 lists the average V_H gene expression in *xid* and non-*xid* mice. A significant difference was found in the usage of the J558 family by comparing the CBA/N female with CBA/CaHN female mice. The lower percentage in J558 usage also accounts for the lower total percent of B cells expressing the nine V_H gene families in CBA/N females. It indicates

that ~30% of the B cells in CBA/N females use V_H10, V_H11, V_H12, or other unknown V_H gene families.

The variation in V_H gene expression by CBA/N females was magnified when individual mice were considered. Analysis of individuals (data not shown) indicated that whereas J558 was the only family predominantly expressed in CBA/CaHN mice, 11 of 16 CBA/N females used J606 (n = 1), S107 (n = 4), Q52 (n = 3), or 7183 (n = 3) alternatively as the predominantly expressed V_H gene family. This variation, however, does not correlate directly with the presence of the *xid* defect since CBA/N males and F_1 males did not exhibit biased V_H gene usage. Among the four CBA/N male individuals and seven F_1 male individuals, all have a V_H expression pattern similar to that of CBA/CaHN, with J558 being the only predominantly expressed family. One explanation for the individual variation observed in CBA/N females is that an interaction between the *xid* defect and female hormones, such as estrogen, has a significant influence on V_H gene rearrangement and results in a nonrandom V_H expression. It is also possible that because CBA/N females, but none of the other mice studied, have two copies of the *xid* gene, there is a gene dosage effect of the *xid* gene on V_H gene rearrangement. Possibly other factors could compensate for the products of one defective *xid* gene but not of two. The actual products of the *xid* gene and its normal counterpart are unknown. We plan to investigate the gene dosage hypothesis using other strains of inbred mice homozygous for the *xid* gene.

Our data clearly demonstrate that *xid* mice are able to express all the V_H gene families that were examined, therefore, the unresponsiveness of these mice to polysaccharide antigens can not be attributed to a failure to express these V_H gene families. We cannot rule out by this analysis, however, that specific genes within these families fail to be expressed. We think it unlikely because of the overall similarity of V_H gene expression between *xid* and CBA/CaHN mice. Primi and Cazenave (12) reported previously that the S107 family was not expressed in LPS-stimulated B cells of *xid* mice. In contrast, we found that *xid* mice were able to express the S107 gene family and, in some CBA/N females, S107 was even the predominantly expressed family. We believe that the reason they did not detect the S107 family may be due to the fact that they did not standardize the amount of C_μ^+ mRNA applied to their filters before hybridization with the V_H probes.

Dighiero et al. (33) reported that by examining Ig-secreting hybridomas, F_1 male *xid* mice express with high frequency 36-60 and J606 V_H gene families when compared with non-*xid* F_1 females of the same matings. In our study, no significant differences in the expression of 36-60 or J606 were found between *xid* and non-*xid* mice. The discrepancy is probably due to a difference in the sample sizes between the two studies since the sample size of hybridoma analysis is relatively limited when compared with the tens of thousands of colonies in our analysis.

In conclusion, we have found that mice with the *xid* defect can express the nine V_H gene families we examined at levels comparable with the CBA/CaHN control strain; thus, altered V_H gene family expression cannot explain their failure to respond to polysaccharide antigens. In the course of these studies, significant variation in the usage of V_H genes by individual CBA/N female mice was observed. This variation was not found either in the normal mice or in other *xid* mice with only one copy of the *xid* gene, namely, CBA/N males and F_1 males. The mechanism underlying this phenomenon is unclear, however, it is possible that two defective X chromosomes are magnifying a defect not normally observed in mice with only one defective X. Further studies will be required in order to determine where the defect lies.

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