The Mouse Antibody Response to Infection with *Cryptococcus neoformans*: V_H and V_L Usage in Polysaccharide Binding Antibodies

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Summary

Cryptococcus neoformans is a ubiquitous fungus that can cause serious infections in humans. The fungus has a polysaccharide (*C. neoformans* capsular polysaccharide; CNPS) capsule that contributes to its pathogenicity and can elicit an antibody response. Nevertheless, only 4 of 60 BALB/c mice chronically infected with *C. neoformans* had a detectable increase in serum anti-CNPS. The sera of three responder mice contained both IgM and IgG anti-CNPS antibody, and the titers of λ and κ anti-CNPS antibody were approximately equal. Eight IgM and one IgG3 monoclonal antibodies (mAbs) were generated from the spleen of one responder mouse, and one IgA was generated from the spleen of another mouse. Seven of the IgMs, the IgG3, and the IgA mAb had λ light chains and were specific for serotype D CNPS. Molecular analysis confirmed that this was a highly restricted antibody response. All of the D-specific antibodies used V_H441, J_H3, and either V_λ2/J_λ2 or V_λ1/J_λ1, and all had the same heavy chain CDR3 amino acid sequence, even though there were differences in the nucleotide sequence of the N/D segment. One IgM mAb reacted with both serotype A and D CNPS, and this mAb used different V_H and J_H genetic elements and had κ light chains. All the anti-CNPS mAbs used J proximal V_H gene elements that have previously been shown to bind dextran and other polysaccharides. Sequence and Southern blot analysis indicate that the serotype-D CNPS-specific mAbs arose from only a few precursor B cells.

The fungus *Cryptococcus neoformans* can cause serious infection in humans, and immunocompromised individuals are at particular risk (1). *C. neoformans* causes disease in up to 10% of individuals with AIDS (2). In the setting of AIDS, cryptococcal infections are usually incurable and often fatal (3). *C. neoformans* has a large polysaccharide capsule that inhibits phagocytosis by macrophages (4). The capsular polysaccharide is poorly immunogenic and causes the phenomenon of immune paralysis in mice (5–7). Structural differences in the capsular polysaccharides allow the grouping of cryptococcal strains into five serotypes, A, B, C, D, and AD (8, 9). Serotypes A and D cause the majority of infections in AIDS patients (10).

Cellular immunity is believed to provide the primary host defense against cryptococcosis (1). The role of humoral immunity to the *C. neoformans* capsular polysaccharide (CNPS)[1] in protection is uncertain. Favoring an important role for antibodies are the observations that: (*a*) individuals with cryptococcal infection have a better prognosis if they have serum antibodies (11); (*b*) antibody enhances phagocytosis by macrophages (12), mediates fungistasis by NK cells (13), and facilitates leukocyte killing (14, 15); and (*c*) passive administration of an IgG1 murine mAb produced a sixfold increase in the survival of lethally infected complement-deficient mice (16), and potentiated the therapeutic action of Amphotericin B (17). However, certain observations are not consistent with an important role for humoral immunity. For example, B cell–deficient mice are not especially susceptible to cryptococcal infection (18); vaccination with immunogenic polysaccharide glyconjugates has not been protective in mice (19); and passively administered mAbs failed to protect mice in another model (20). Thus, many in vitro observations indicate an important role for antibody by enhancing cellular immunity, whereas some in vivo experiments have confirmed a protective effect and some have not. The finding that AIDS patients lack anti-CNPS IgG (21) raises the possibility that lack of antibody contributes to their marked susceptibility to cryptococcus.

The overall aim of our work has been to find mouse mAbs to CNPS that are protective against cryptococcus. This paper reports the serologic characterization of the serum response to cryptococcal infection and the generation of mAbs to CNPS

[1] *Abbreviation used in this paper:* CNPS, *Cryptococcus neoformans* capsular polysaccharide.

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from these animals. Protection experiments using these mAbs are continuing and the results will be described elsewhere. We have determined the primary structure of the anti-CPNS mAbs from their mRNA sequences and studied the Ig gene rearrangements in the hybridomas to gain a more detailed understanding of the genetic and molecular basis for the responses.

Materials and Methods

C. neoformans. The strain used to infect mice was isolated from an AIDS patient with cryptococcal meningitis at Bronx Hospital Medical Center, and is referred to as "GH". Standard serotype strains A, B, C, and D (nos. 24064, 24065, 24066, and 24067, respectively) were obtained from the American Type Culture Collection (ATCC) (Rockville, MD). The strains were maintained in Saboraud's agar slants at 4°C. CNPS was prepared as described by others (22, 23). The concentration of polysaccharide was determined by the phenol-sulfuric acid method (24).

ELISA. ELISA plates (no. 25801; Corning Glass Works, Corning, NY) were coated with CNPS by incubating 50 μl of a 10 μg/ml solution of CNPS in 0.02 M PBS, pH 7.2, in each well at room temperature overnight. The concentration of protein in GH CNPS was determined with a protein assay (Bio-Rad Laboratories). Plates were blocked with a solution of 1% BSA in PBS. Alkaline phosphatase–conjugated goat anti-mouse IgM, IgG1, IgG3, IgG2a, IgG2b, IgA, κ, and λ reagents (Fisher Biotech) were used to develop the ELISA.

Mice. BALB/c mice were obtained from the National Cancer Institute (Bethesda, MD). The mice were infected with cryptococci intraperitoneally. Before inoculation, the yeast was washed with PBS and counted in a hemocytometer. Mice were bled from the retro-orbital sinus, and sera were separated by centrifugation and stored at −20°C.

Monoclonal Antibodies. mAbs to GH CNPS were made from chronically infected BALB/c mice with high serum titers. The use of spleens from infected mice posed the potential problem of hybridoma cell culture contamination with cryptococci. We avoided this problem by treating the mice with Amphotericin B and the hybridoma cultures with Nystatin. The mice were treated with Amphotericin B intraperitoneally (5–15 mg/kg total dose) during the week before harvesting the spleen to decrease the number of cryptococci in their tissues. We cultured the brain, heart, lungs, liver, and kidney from a mouse that had received 15 mg/kg of Amphotericin B, and found cryptococci only in brain tissue. Hybridomas were made by fusing splenocytes with NSO myeloma cells at a 4:1 ratio with polyethylene glycol by a protocol described previously (25). Nystatin (Gibco Laboratories, Grand Island, NY) was added to the hybridoma cultures at a concentration of 100 U/ml 1 d after the fusion. Hybridomas were screened by ELISA using plates coated with 50 μl of 10 μg/ml GH CNPS. Cells from positive wells were cloned in soft agar.

The mAb isotype and light chain type were determined using goat anti-mouse isotype and light chain–specific alkaline phosphatase–labeled antibodies. Hybridoma supernatants containing the mAbs were used for binding studies. The mAb concentration was determined by ELISA relative to standards of the same isotype and of known concentration for all antibodies except 4H3. Because the goat anti-IgG3 reagents were of low affinity, the 4H3 mAb was purified using an anti-mouse IgG column, dialyzed against PBS, and its concentration was determined by a protein assay (Bio-Rad Laboratories) using a myeloma IgG3 as a standard rather than by ELISA.

mRNA Sequencing. Total cellular RNA was prepared by the guanidinium method, and poly(A)$^+$ mRNA was isolated using an oligo-dT affinity column. The nucleotide sequence of the antibody mRNAs was determined using a modification of the method of Geleibter et al. (26). The following oligonucleotides were used as primers: TGGATGGTGGGAAGATG (κ), TCTCGCAGGAGACGAGGGGGA (μ), CCTGAGAATCTGACAGGAACA (Vλ), TGTTCTTGGCATTGTCTCTG (V_HGa150.1), TACAGCGTATTTTTGGCGTT (V_H441), CGTGAGGGCAGCCTTGTCTCC (5′ Vλ), GAGTGTCAGTGGGTAGATGGT (Cα), GAGAGCAGAAATAAACTCCC ($V_κ$5.1), TGCACAGGAGAGTTTCAG (5′ V_H441), GGTGAGGGCAGCCTTGTCTCC (5′ Vλ), GACCCCAGAAAATCGGTT (5′ $V_κ$5.1), ACCTAGGACAGTGACCTT ($J_λ$2), CACCAGATTCTTATCAGA (μ), AAGTAGGCCTTTGACAAGGCA (γ3). The oligonucleotide primers were made in the DNA synthesis facility in our institution. For 4H3, in addition to sequencing directly from the mRNA, V_H cDNA was made using the primer AAGTAGCCTTTGACAAGGCA and then amplified by the PCR using that same primer and GAGGTGAAGCTTCTCGAGTCT. The total PCR product was then sequenced using the Sequenace kit (United States Biochemical Corp., Cleveland, OH) to confirm the 4H3 CDR3 sequence.

Southern Blot Hybridization. DNA was extracted from the NSO myeloma, the hybridomas, and BALB/c liver. Restriction enzyme fragments were separated in 0.8–1.0% agarose gels and transferred to modified Nylon membranes (GeneScreen) by blotting using 10× SSC (1× SSC is a 0.15 M NaCl and 0.015 M sodium citrate solution). Blots were prehybridized in a solution of denatured salmon sperm DNA (0.5–1.0 mg/ml) in 5× SSC, 7% SDS, 10× Denhardt's reagent, 10% dextran sulfate, in 20 mM sodium phosphate buffer (pH 7.2) at 65°C. Hybridization was performed under the same conditions with DNA probes labeled with γ-[^{32}P]-dCTP using random primers (Boehringer Mannheim Biochemicals). After hybridization, blots were washed initially with a solution of 3× SSC, 5% SDS, 10× Denhard's Reagent, in 20 mM phosphate buffer, pH 7.2, and finally in a solution of 1× SSC and 1% SDS. Blots were washed at 65°C.

Immunofluorescence. A suspension of yeast (10^6 to 10^7 yeast/ml) in PBS was air dried on glass slides. The slides were first blocked with 1% BSA in PBS, then incubated with cell supernatants containing mAb at a concentration of 1 μg/ml, washed, and then incubated with fluorescein-labeled anti-mouse IgM.

Results

Mouse Antibody Response to Infection. The sera of BALB/c mice infected intraperitoneally with sublethal innocula of 10^4 to 10^6 cryptococci (GH) were assayed for the presence of antibody to GH CNPS. The antibody titer was measured by ELISA after serial dilution on plates coated with GH CNPS. Uninfected animals usually had serum titers of 1:50 to 1:100, which probably reflect the presence of crossreactive antibodies. Of 60 infected mice, only four had titers of anti-CNPS antibody >1:200. Fig. 1 shows the serum titers of IgM, IgG, κ, and λ at several times after infection for three of the mice that had high titers of anti-CNPS. The fourth animal was killed early to fuse its spleen. These data show that: (*a*) antibody titers peak between days 11 and 18 and then slowly decline with time even though these animals are chronically

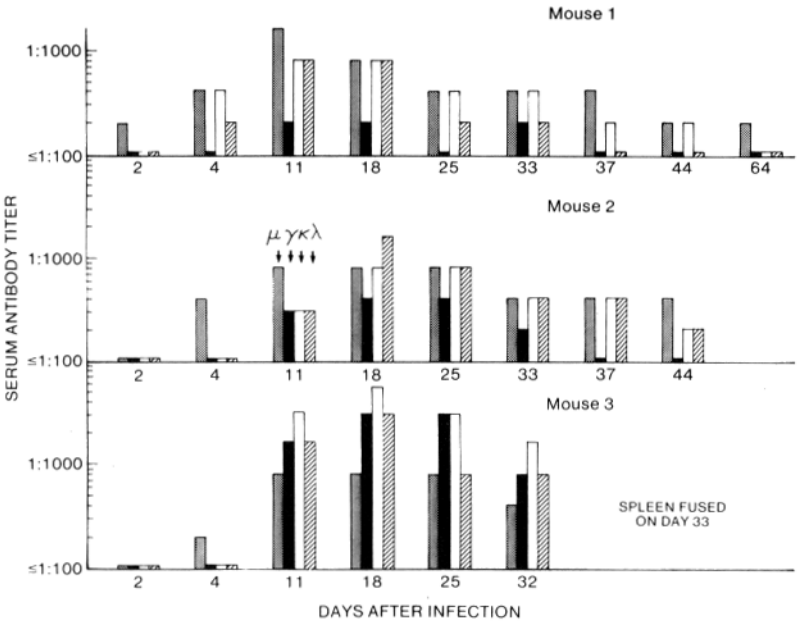

Figure 1. Serum antibody responses of three responder BALB/c mice infected with the GH cryptococcal strain. The bars represent the antibody titer in terms of IgM, total IgG, κ, and λ at various time points after infection. Antibody titer was measured by serial dilutions on ELISA plates coated with 10 μg/ml of GH CNPS. The titer was defined as the serum dilution that gave an optical density at 405 nm, which was at least 1.5 times the background in the ELISA. The three mice (aged 9–12 mo) were infected with sublethal innocula of 10^4 to 10^6 cryptococci intraperitoneally. The IgG fraction of mouse 3 consisted exclusively of IgG1 and IgG3 subtypes.

infected; (*b*) both IgM and IgG are present; (*c*) in many of the bleedings, the titer of λ is roughly equivalent to that of κ.

Monoclonal Antibodies. Spleens from four infected mice were used in this study. Two of the spleens came from the mice with the highest titers of antibody to CNPS. Since it was possible that the low serum titers measured for the majority of mice were the result of antibody sequestration in complexes with polysaccharide, we also fused splenocytes from two mice with low titers of antibody to CNPS (<1:200). Each spleen was handled separately. Hybridomas producing mAb to CNPS were obtained only from the spleens of the animals with high serum titers of anti-CNPS antibody. One of the two productive fusions used a spleen harvested on day 33 of infection (mouse 3 in Fig. 1), and it yielded seven IgM- and one IgG3 mAb-producing hybridomas. The other productive fusion used a spleen harvested on day 17 of infection, and it yielded only one IgA hybridoma–producing mAb.

Serological Characterization of the Monoclonal Antibodies. The mAbs were initially characterized for heavy chain isotype, light chain type, and binding to CNPS from standard ATCC A, B, C, and D serotypes and the GH strain (Table 1). Although the serotype of the GH strain used in this study was not initially known, the reactivity of our panel of mAbs with GH CNPS indicates that GH belongs to either the D or A/D serotypes (9). With the exception of 21D2, all the mAbs had λ light chains and reacted only with serotype D CNPS. The 21D2 mAb had a κ light chain and it reacted with CNPS from both serotypes A and D. This indicates that 21D2 recognizes a different epitope than the other mAbs.

Binding of 14A12 (μλ) or 21D2 (μκ) mAbs to cryptococci, followed by staining with fluorescein-conjugated anti-mouse IgM, produced capsular fluorescence like those reported previously for both immune polyclonal sera (19) and for other anti-CNPS mAbs (23) (data not shown). This confirms that these mAbs bind to the cryptococcal capsule.

Fig. 2 shows binding curves of 14A12 (μλ) and 21D2 (μκ) to GH CNPS. The binding curves of the other IgMλ antibodies, 7B13, 11E2, 12G5, 20B5, and 20C5, are indistinguishable from those of 14A12 and are not shown here. Since we found no significant differences in the binding of these IgMλ mAbs to GH CNPS or in their mRNA nucleotide sequences (see below), 14A12 was designated as the prototype for the IgMλ group. The binding curves of 14A12 and 21D2 are

Table 1. *Characteristics of CNPS Binding Antibodies*

		Serotype polysaccharide								
mAb	Class	A	B	C	D	GH	V_H	J_H	V_L	J_L
21D2	IgMκ	+	−	−	+	+	50.1	2	$V_κ$5.1	$J_κ$1
14A12	IgMλ	−	−	−	+	+	V_H441	3	$V_λ$2	$J_λ$2
11E2	IgMλ	−	−	−	+	+	V_H441	3	$V_λ$2	$J_λ$2
7B13	IgMλ	−	−	−	+	+	V_H441	3	$V_λ$2	$J_λ$2
12G5	IgMλ	−	−	−	+	+	V_H441	3	$V_λ$2	$J_λ$2
20C5	IgMλ	−	−	−	+	+	V_H441	3	$V_λ$2	$J_λ$2
20B5	IgMλ	−	−	−	+	+	V_H441	3	$V_λ$2	$J_λ$2
4H3	IgG3λ	−	−	−	+	+	V_H441	3	$V_λ$1	$J_λ$1
15C6	IgAλ	−	−	−	+	+	V_H441	3	$V_λ$2	$J_λ$2

Class, light chain usage, reactivity with CNPS of serotypes A, B, C, D, and GH, and the V_H and V_L usage for the anti-CNPS mAbs. The symbols + and − denote binding and lack of binding, respectively, to ELISA plates coated with 10 μg/ml of CNPS from the different serotypes. The 15C6 mAb is separated from the others by a space because it was generated from the spleen of a different mouse. The V_H, J_H, V_L, and J_L were determined from the Ig mRNA sequences. The V_H50.1 and V_H441 are gene elements belonging to the 7183 and X-24 gene families, respectively (29). $V_κ$5.1 has been reported in antiprenolol mAbs (32). The N/D segment sequences are shown in Fig. 4.

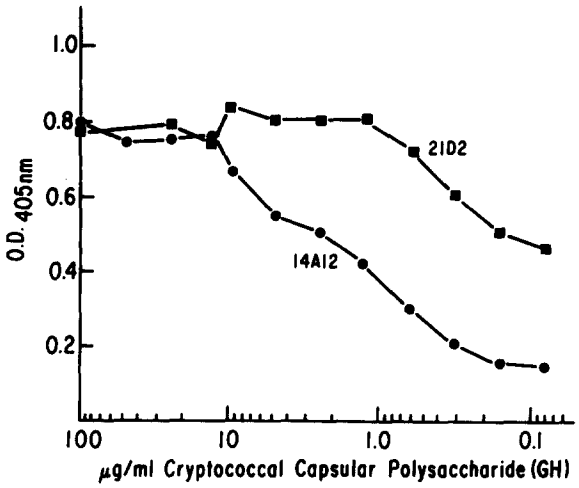

Figure 2. ELISA binding data of the 14A12 and 21D2 mAbs to GH CNPS. The graph shows a plot of OD_{405} vs. GH CNPS concentration, where the mAb concentration is kept constant at 1 μg/ml and the CNPS concentration is varied. The binding curves of the other IgMλ antibodies, 7B13, 11E2, 12G5, 20B5, and 20C5, were like that of the 14A12 mAb and are not shown here. Note that the hybridoma supernatants were screened using plates coated with 10 μg/ml of GH CNPS.

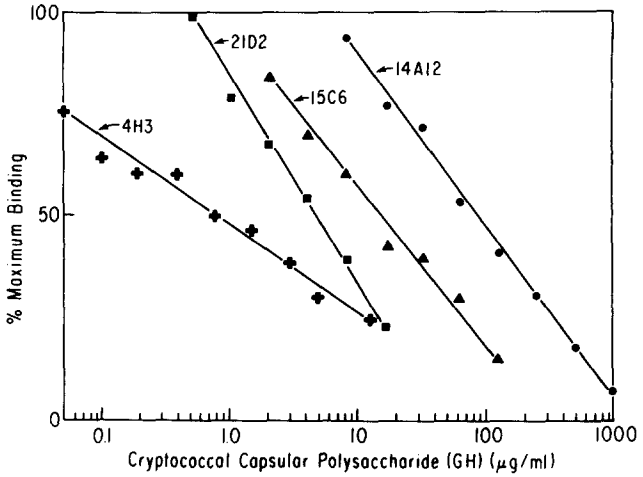

Figure 3. Inhibition of mAb binding to CNPS-coated ELISA plates by soluble CNPS. The plates were coated with a solution of 10 μg/ml of GH CNPS. The antibody and CNPS were incubated for 1.5 h at 37°C. We calculated apparent binding constants (ak_a) of 8×10^8 M^{-1}, 2×10^8 M^{-1}, 7×10^7 M^{-1}, and 8×10^6 M^{-1} for the 4H3, 21D2, 15C6, and 14A12 mAbs, respectively, using the method described by Nieto et al. (27). For the ak_a calculation, we assumed a molecular mass of 800,000 daltons for the polysaccharide (41). For 4H3, an IgG3 mAb, we observed considerable variability in the shape of the curves, and suspect that this variability reflects cooperative binding effects that can occur with this subclass (26). The significance of the different slopes is not understood.

different for CNPS concentrations of <10 μg/ml (Fig. 2). Since the 14A12 (μλ) and 21D2 (μκ) mAbs bind to different epitopes (Table 1), the differences in binding shown in Fig. 2 could reflect variation in epitope density, a higher intrinsic affinity for the 21D2 mAb, or both.

In addition to the seven IgM mAbs described, we also obtained an IgG3λ (4H3) and an IgAλ (15C6) mAb. These two mAbs have the same serotype specificity as those of the 14A12 class, binding only to serotype D and GH CNPS (Table 1). Soluble CNPS inhibited mAb binding to CNPS-coated ELISA plates, as shown in Fig. 3 for 21D2 (μκ), 14A12 (μλ), 4H3 ($\gamma_3\lambda$), and 15C6 (αλ). The binding curves are not directly comparable since the two IgMs, 21D2 and 14A12, bind to different epitopes, and since the 14A12, 4H3, and 15C6 mAbs are of different isotypes that differ in avidity (Table 1). Of the two IgMs, 21D2 requires 10^2 less CNPS to inhibit its binding than 14A12, and hence, it has higher apparent affinity. The 4H3 ($\gamma_3\lambda$) mAb is inhibited by the lowest concentration of soluble CNPS, and hence, this mAb has the highest apparent affinity of the four antibodies. Using the method of Nieto et al. (27), we have calculated apparent binding constants (aK_as) in the range of 10^6 to 10^8 M^{-1} for these four mAbs (see Fig. 3 legend). The three isotypes isolated in this study, namely IgM, IgG3, and IgA, all can bind antigen more strongly than expected from the intrinsic binding constants of their individual binding sites, as a result of either higher avidity due to polymer formation (IgM and IgA) or because of cooperative binding through F_c interactions (IgG3) (28).

V Region Use and Antibody Sequences. The mAb mRNAs were sequenced to determine antibody structure and variable gene usage. Based on the nucleotide sequence data, our mAb set can be classified into two groups that correlate with their serotype specificity (Table 1). One group consists of the 14A12 class (μλ), 4H3 ($\gamma_3\lambda$), and 15C6 (αλ), which bind only serotype D CNPS. The second group consists of 21D2 (μκ), which binds both A and D CNPS.

All of the serotype D–specific λ mAbs have a heavy chain variable region (V_H), encoded by V_H441 (V_HGal39.1) (29), a small "diversity" segment consisting of four codons, and J_H3 (Table 1; Fig. 4). The light chain variable region (V_L) is encoded by Vλ2/Jλ2 for the 14A12 class and 15C6 and by Vλ1/Jλ1 for 4H3. The fact that all of these mAbs have a variable region structure that is identical or nearly identical indicates that all recognize the same epitope. No somatic mutations were found in the sequences of the V_H441, J_H3, Vλ2, and Jλ2 genetic elements used in the mAbs of the 14A12 class (μλ) (data not shown). The members of the 14A12 class also have the same sequences in their diversity segments (Fig. 4). We could not identify the D segments in 14A12, 4H3, or 15C6 among reported germline D sequences even if we looked for unusual reading frames, inversions, or deletions. Since these sequences contain many G residues, they may be largely or completely N-sequence, and hence, we refer to this segment in our mAbs as N/D.

Five differences distinguish 4H3 ($\gamma_3\lambda$) from the 14A12 (μλ) group: (*a*) the presence of a silent mutation in codon 64 (CTA to CTT) of the V_H441 (data not shown); (*b*) a replacement mutation in codon 104 (GCT to TCT) resulting in a valine to serine change in J_H3 (Fig. 4); (*c*) the codon GGT instead of GGG at position 97 (in the N/D sequence), which represents either a somatic mutation of the 14A12 N/D sequence or a different N/D segment reflecting a different

	V_H441	N/D			J_H3																	
		arg	gly	gly	tyr																	
V_H14A12	AGA	CGT	GGG	GGG	TAC	TTT	GCT	TAC	TGG	GGC	CAA	GGG	ACT	CTG	GTC	ACT	GTC	TCT	GCA			
V_H7B13	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---			
V_H11E2	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---			
V_H12G5	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---			
V_H20B5	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---			
V_H20C5	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---			
V_H4H3	---	---	---	--t	---	---	T--	---	---	---	---	---	---	---	---	---	---	---	---	GCT		
V_H15C6	---	--g	--t	--t	---	--c	---	---	---	---	---	---	---	---	---	---	---	---	---			

Figure 4. VDJ joining region for the 14A12 group, 4H3, and 15C6 mAbs. Comparison of the 14A12 and 15C6 CDR3 sequence reveals four base differences in this region that result in the same amino acid sequence because of the degeneracy of the code. Dashed lines indicate identity. Replacement mutations are in capital letters, whereas silent mutations are in lower case letters. 4H3 has a replacement mutation in the second codon of the J, leading to the replacement of valine for serine. The 4H3 mAb also has an extra codon GCT at the junction between J_H3 and $C_\gamma3$.

clonal origin of the B cell encoding 4H3 (Fig. 4); (*d*) an additional codon at the 3′ end of the J_H3, which is not encoded by either J_H3 or $C_\gamma3$ (30) (Fig. 4). The origin of this extra codon is not known. One possibility is that somatic mutation occurred in the intervening sequence (31) resulting in altered RNA splicing. The first three nucleotides in the intervening sequence downstream of J_H3 are GGT (30), and a single somatic mutation could have changed this codon to GCT; and (*e*) the 4H3 V_L uses $V\lambda1/J\lambda1$ instead of $V\lambda2/J\lambda2$ (Table 1). The $V\lambda1$ of 4H3 has two somatic mutations, one resulting in replacement of alanine by valine at position 57 (GCT to GTT), and one silent mutation in the $J\lambda1$ at position 107 (CTC to CTG) (data not shown).

The 15C6 ($\alpha\lambda$) mRNA was generated from a different mouse than the other anti-CNPS mAbs. The construction of 15C6 used the same gene elements as used in the 14A12 group, namely V_H441, J_H3, $V\lambda2$, and $J\lambda2$. Remarkably, 15C6 has the same CDR3 protein sequence as the mAbs in the 14A12 class and 4H3, even though its mRNA sequence has three base differences in the N/D segment (Fig. 4). No somatic mutations were found in the 15C6 V_H441, $V\lambda2$, $J\lambda2$ genetic elements, but one silent mutation occurred in the first codon of J_H3 (Fig. 4).

As expected from its different serotype specificity, the construction of the 21D2 A-D–reactive V_H and V_L used different genetic elements than those found in the serotype D–specific mAbs (Table 1). The V_H of 21D2 is composed of $V_H50.1$ (29) (a member of the 7183 gene family), an unidentified D, and

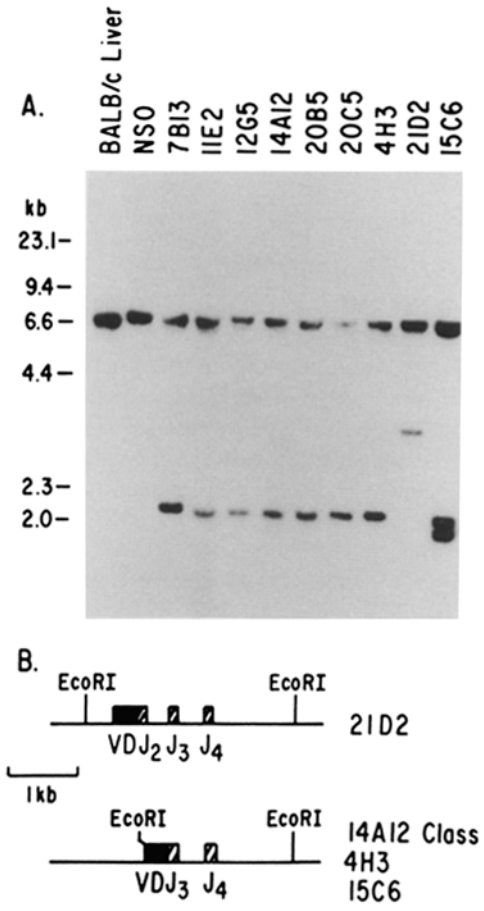

Figure 5. Autoradiograph of Southern blot containing EcoR1 digests of DNA from BALB/c liver, NSO myeloma, and the anti-CNPS hybridomas probed with the BamH1-Eco R_1 J_H3-J_H (J11) probe. (*B*) A map of the rearranged restriction fragments.

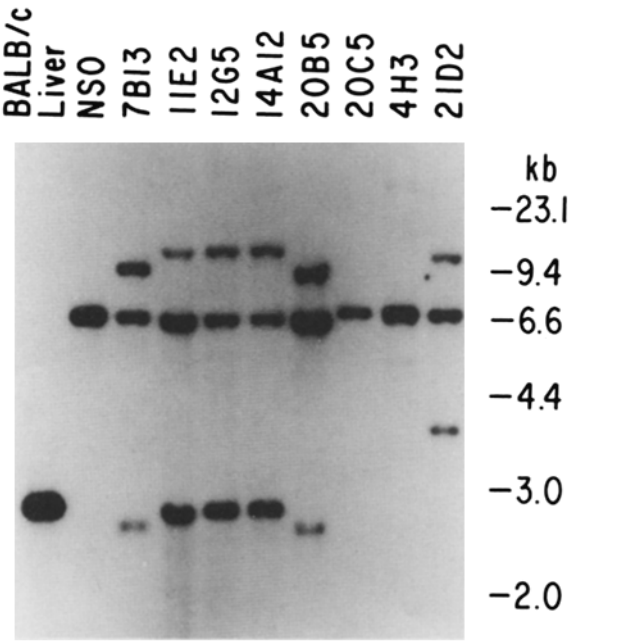

Figure 6. Autoradiograph of Southern blot containing HindIII digest of DNA from BALB/c liver, NSO myeloma, and the anti-CNPS hybridomas probed with the 2.7-kb HindIII fragment J_K1-5 probe.

J_H2. The D segment of 21D2 has seven codons (CGGGAC-AGCTCGGGCCAGTAC), and thus is larger than that found in the serotype D–specific mAbs. The light chain of 21D2 is composed of the $V_K5.1$ (32) and J_K2 gene elements (data not shown). Although no somatic mutations were identified in the genetic elements used in the 21D2 mAb, the last codon of the $V_K5.1$ sequence (at the VJ junction) was CCA instead of CCT (32), resulting in a silent substitution.

In summary, the sequence data revealed: (*a*) a marked restriction in variable region gene usage in the serotype D–specific set of mAbs; (*b*) a few somatic mutations in the IgG3 and IgA mAbs; (*c*) the use of V_H gene elements from 3′ proximal gene families that have previously been reported in antipolysaccharide antibodies (29); and (*d*) N/D segments with different base sequences but identical amino acid sequences in the serotype D–specific mAbs.

Southern Blot Analysis of the Ig Gene Rearrangements. The sequence data suggested that the antibodies from the first fusion were the products of at least three different B cell clones that served as precursors to the 21D2 ($\mu\kappa$), the 4H3 ($\gamma_3\lambda$), and the 14A12 group ($\mu\lambda$) hybridomas. To further examine the clonal relationship of the members of the 14A12 class and 4H3, we studied the restriction patterns of their productive and nonproductive alleles. Fig. 5 shows an autoradiograph of a Southern blot with EcoR1 fragments of DNA from BALB/c liver, the NSO fusion partner, and the various anti-CNPS hybridomas after hybridization with the J_{11} probe for the J_H3-J_H4 segment. A single band of 6.6 kb is present in liver DNA corresponding to the germline size of the EcoR1 fragment containing J_H3-J_H4. A single band is also present in the NSO DNA, but it migrates slightly slower (6.8 kb) than the liver band. The restriction patterns obtained with J_{11} for the serotype D–specific anti-CNPS hybridomas (7B13, 11E2, 12G5, 14A12, 20B5, 20C5, 4H3, and 15C6) all show the 6.8-kb fragment from the NSO fusion partner, and a new rearranged band at 2.1 kb. Some of the hybridomas also have a weak band of 6.6 kb directly below the 6.8-kb band, which presumably corresponds to either an unrearranged allele or to another rearrangement. The size of the 2.1-kb fragment is consistent with that expected from rearrangement of the V_H441 gene to J_H3 (29) (Fig. 3 *B*). These Southern blot data strongly suggest that the 14A12 group ($\mu\lambda$) and the 4H3 ($\gamma_3\lambda$) hybridomas have identical V_H-J_H rearrangements. The DNA from 15C6 (which originates from the spleen of a different mouse) shows three bands; the 6.8-kb band from the fusion partner, and bands at 2.1 and 1.9 kb, which presumably correspond to the productive and nonproductive rearrangements, respectively. The 21D2 hybridoma has the NSO band at 6.8 kb, a band at 6.6 kb, and a new band at 3.3 kb, which presumably corresponds to its productive rearrangement (Fig. 5). The size of the 21D2 band at 3.3 kb is consistent with rearrangement of the $V_H50.1$ to J_H2 (29).

Since J_κ rearrangements usually precede λ rearrangements (33), we also analyzed the J_κ locus rearrangements of the 14A12 ($\mu\lambda$) group and 4H3 ($\gamma_3\lambda$). Fig. 6 shows an autoradiograph of a Southern blot of HindIII DNA restriction fragments probed with the J_K1-5 probe. All hybridomas have a band at 6.6 kb that comigrates with the single band in NSO DNA, and presumably comes from the fusion partner. In addition, the blot reveals three types of J_κ locus rearrangements in the 14A12 group: (*a*) 7B13 and 20B5 have two bands each at 12 and 2.5 kb; (*b*) 11E2, 12G5, and 14A12 have two bands each at 2.7 kb (suggesting that at least one allele is in the germline configuration) and at 9.4 kb; and (*c*) 20C5 has only the band at 6.6 kb corresponding to that of NSO, indicating that the J_κ locus was lost either in the precursor B cell or after hybridoma formation, making it impossible to assign to either group. The 4H3 ($\gamma_3\lambda$) hybridoma has different J_κ locus rearrangements than those found in the 14A12 group, having one band at 5.2 kb and another band >23 kb. Also shown in Fig. 6 is the 21D2 ($\mu\kappa$) hybridoma, which has two bands at 4.0 and 9.4 kb in addition to the NSO fusion partner band.

Discussion

The decision to generate anti-CNPS mAbs from cryptococcus-infected mice was made with the premise that such antibodies would be elicited by biologically relevant epitopes. Eight mAbs (seven IgM, one IgG3) were generated from the fusion of one spleen, and one IgA mAb was generated from another spleen. The serum antibody titers and the isotype distribution of the mAbs generated suggests that the hybridomas obtained generally reflect the animals total B cell response. Since IgM has an intravascular half life ($t_{1/2}$) of 8.5 h, whereas both IgG1 and IgG3 have $t_{1/2}$ of ~200 h (34), the ratio of IgM to IgG hybridomas obtained in the first fusion is consistent with the IgM and IgG anti-CNPS serum titers of 1:400 and 1:800, respectively, measured on the day before the fusion. Most of the mAbs had λ light chains, consistent with the λ-rich serum antibody response. However, despite the fact that the serum titers of κ and λ anti-CNPS antibodies were roughly equal, only one κ mAb (21D2) was isolated from two protective fusions. The paucity of κ anti-CNPS hybridomas might be explained by either a preferential association of κ light chains with IgG (which have much longer serum $t_{1/2}$) or the absence of κ anti-CNPS B cells in the spleen lymphoid compartment.

The following lines of evidence indicate that our mAbs recognize polysaccharide epitopes. (*a*) The mAbs are serotype specific and serotype specificity has been assigned to polysaccharide structural determinants (9, 35–37); (*b*) immunofluorescence shows that 14A12 and 21D2 bind to the polysaccharide capsule; (*c*) the polysaccharide preparations used in the ELISAs had an extremely low level of protein relative to polysaccharide concentration (<2 ng/ml, or <1:5000 ratio of protein/polysaccharide by weight), and this amount of protein is below the usual detection threshold in the ELISA (38); and (*d*) all the mAbs use gene elements that have been associated with antipolysaccharide antibodies such as V_H441 and $V_HGa150.1$ (28, 39, 40).

mAbs to CNPS have been generated by three other groups using animals immunized with either CNPS (22), CNPS con-

jugated to sheep erythrocytes (41), or CNPS conjugated to BSA (35). Our mAbs came from B cells stimulated during cryptococcal infection. They are different from previously described mAbs in that most are serotype D–specific antibodies that use λ light chains. Using polyclonal antisera and sequential absorbtions, Ikeda et al. (9) developed a numerical taxonomy system for categorizing CNPS serotype according to eight antigenic factors, and concluded that serotype was determined by only a few antigenic determinants. In the Ikeda scheme, antigenic factor 8 is a D-specific antigen, and factor 3 is found shared by the A and D serotypes. Since the 14A12 group, 4H3, and 15C6 bind only serotype D, they probably recognize antigenic factor 8. Since the 21D2 mAb binds to both serotypes A and D, it probably recognizes antigenic factor 3. The specificities of our mAbs are thus consistent with and support the Ikeda (9) classification.

The molecular characterization of the mAbs revealed a striking degree of restriction in variable gene element usage in the serotype D–specific mAbs. In fact, the large proportion of λ antibodies in the serum antibody response to GH CNPS suggests a restricted response since λ antibodies are relatively rare in the mouse and comprise only 3–5% of the total antibody pool. There are only two V_λ genes and they share a high degree of homology (33). All of our λ antibodies were specific for serotype D CNPS. Restriction in variable gene element usage has been reported in responses to some carbohydrates such as 3-fucosyllamine (40). However, other carbohydrates such as group A streptococcal polysaccharide (42) and dextran (43) elicit antibodies using a variety of variable gene elements.

The V_H of all of the serotype D–specific antibodies used V_H441, an N/D segment consisting of four codons, and J_H3. The V_H441 is a member of the X-24 V_H family, which is the smallest V_H family and consists of only two gene elements (44). V_H441 has a potential glycosylation site in the CDR2 domain. V_H glycosylation has recently been shown to increase the affinity of an antidextran antibody (45) and may be important in the binding to CNPS. V_H441 usage has been reported in several anticarbohydrate mAbs that recognize very different carbohydrate structures (29, 40, 45). Since most of these mAbs use V_H441 in its germline sequence, it has been proposed that the fine specificity of these mAbs for carbohydrate epitopes resides in their CDR3 and/or light chains (39, 40). The fact that the N/D segments of all serotype D–specific mAbs, including antibodies from two different animals, encode the same protein sequence suggests that the structure of CDR3 is important for binding. In addition, serotype D epitope specificity may require the use of λ light chains.

Since VDJ recombination and the introduction of N sequence usually occur before light chain rearrangements (46), the most conservative interpretation of the sequence and Southern blot data is that there was a single pre-B cell precursor for all members of the 14A12 group ($\mu\lambda$) and for 4H3, which assembled its heavy chain gene and underwent subsequent cell divisions. Two such subclones then carried out different nonproductive κ rearrangements and went on to use the same V_λ and J_λ elements to form identical productive light chains resulting in the 14A12 group. Since 4H3 has different $J\kappa$ rearrangements and uses different V_λ/J_λ gene elements than the 14A12 group, its ancestor may have been a third subclone of the original B cell precursor. In this interpretation, the one base difference in the N/D segment of 4H3 compared with those of the 14A12 group is attributed to somatic mutation. It is noteworthy that the response that generated the 14A12 group (and possibly 4H3) appears to be a second example where a single pre-B cell gave rise to antigen-specific B cells with different light chain gene rearrangements (47). However, if one base difference in the 4H3 N/D sequence arose during VDJ formation rather than as a result of somatic mutation, then this fact, along with the differences in V_λ/J_λ gene usage and in κ rearrangements, would suggest that 4H3 arose from a completely different B cell precursor. Thus, the B cell precursors for the hybridoma group specific for the D serotype comprised at least two, probably three, and possibly five different pre-B cell clones, all with the same N/D amino acid sequence.

We found only a few mice (4/60) that responded to infection with high circulating anti-CNPS titer. The lack of serum antibody in most mice is consistent with an earlier observation that anti-CNPS antibodies are not detectable in infected mice (48). A similar phenomenon has been described in humans, where most individuals with cryptococcal disease lack circulating antibody (11). This lack of response may be due to the immunological paralysis that has been reported to follow immunization with CNPS (5–7). CNPS has properties of a T cell–independent (TI) type 2 antigen (49). There is evidence that the antibody response to CNPS in mice, like the response to pneumococcal polysaccharide (50), is regulated by suppressor T cells (7). Additional studies have shown that cryptococcal antigen preparations can induce suppressor T cells (51). Our inability to obtain hybridomas producing anti-CNPS mAbs from the spleens of two infected animals with low serum titers to CNPS (1:200 or less) is consistent with few antibody-producing cells in those spleens at the time fusion.

Given that CNPS has only a few immunogenic determinants (9) and that the antibody response to some of these determinants is highly restricted, one explanation for the paucity of responder mice is that only occasional mice have precursor B cells that can respond to CNPS. However, this is unlikely since most animals do respond to optimal doses of polysaccharide immunization (49) and to conjugates of CNPS and protein (19). This leads us to believe that the few animals that responded did so because, before infection, they had expanded the number of B cells that could produce CNPS-specific antibodies. It is possible that such an expanded pool of B cells could escape from immunological paralysis either because of the greater number of B cells or because the expanded pool contained cells that could no longer be suppressed. The fact that we were able to recover six IgM hybridomas with identical CDR3 sequences from one animal certainly indicates that this particular B cell clone was greatly expanded at the time of the fusion, and raises the possibility that it might have been expanded before infection. This could have

resulted indirectly from an idiotype-antiidiotype network or directly from exposure to a crossreacting antigen. This latter possibility is supported by the fact that most of the responders were older mice: 3 of 12 9–12-mo-old mice had a high titer response, whereas only 1 of 48 adult mice that were <6 mo old responded. Another study that showed unresponsiveness to infection used 3–4-mo-old mice (48).

If the responder mice were able to make an antibody response because they had prior exposure to a crossreactive antigen, that antigen could have been another polysaccharide (52), or even a protein, since protein epitopes can resemble carbohydrate (53). Since CNPS is a TI antigen (49), it is presumably unable to induce a secondary immune response (54). However, TI antigens are capable of activating pre-existing memory cells (54–56). The IgM hybridomas lack somatic mutations and reflect a TI response to a D-specific epitope in CNPS. However, the 4H3 hybridoma has somatic mutations in both V_H and V_L, which may have occurred in the response to a T cell–dependent (TD) stimulus prior to memory B cell activation by CNPS. In this scenario, CNPS stimulates memory cells and produces an antibody response with characteristics of both TI and TD antigens.

Thus, the analysis of the antibodies produced by the responder mice suggests explanations for why some animals were able to respond. They also suggest that priming either with a glycoconjugate (56, 57), an antiidiotype, or other TD crossreactive antigens could increase the number of responder mice in infection. It is interesting to note that dextran, which elicits antibody responses in mice that use some of the same genetic elements that we have described above (58), has been used by AIDS patients as an antiviral agent (59). Given that the ability to mount an antibody response to CNPS in infection may depend on prior exposures to other antigens, it is conceivable that the administration of dextran to AIDS patients could influence their ability to make antibody to CNPS.

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References

1. Diamond, R.D. 1985. *Cryptococcus neoformans*. *In* Principles and Practice of Infectious Disease. G.L. Mandell, R.G. Gordon, Jr., and J.E. Bennett, editors. John Wiley and Sons, New York. 1460–1468.
2. Zuger, A., E. Louie, R.S. Holtzman, M.S. Simberkoff, and J.J. Rahal. 1986. Cryptococcal disease in patients with the acquired immunodeficiency syndrome: diagnostic features and outcome of treatment. *Ann. Intern. Med.* 104:240.
3. Chuck, S.L., and M.A. Sande. 1989. Infections with *Cryptococcus neoformans* in the acquired immunodeficiency syndrome. *N. Engl. J. Med.* 321:321.
4. Kozel, T.R., and E.C. Gotschlich. 1982. The capsule of *Cryptococcus neoformans* passively inhibits phagocytosis of the yeast by macrophages. *J. Immunol.* 129:1675.
5. Kozel, T.R., and J. Cazin, Jr. 1972. Immune responses to cryptococcus soluble polysaccharide. *Infect. Immun.* 5:33.
6. Murphy, W.J., and G. Cozad. 1972. Immunological unresponsiveness induced by cryptococcal polysaccharide assayed by the hemolytic plaque technique. *Infect. Immun.* 5:896.
7. Breen, J.F., I.C. Lee, F.R. Vogel, and H. Friedman. 1982. Cryptococcal capsular polysaccharide-induced modulation of murine immune responses. *Infect. Immun.* 36:47.
8. Wilson, D.E., J.E. Bennett, and J.W. Bailey. 1968. Serologic grouping of *Cryptococcus neoformans*. *Proc. Soc. Exp. Biol. Med.* 127:820.
9. Ikeda, R., T. Shinoda, Y. Fukazawa, and L. Kaufman. 1982. Antigenic characterization of *Cryptococcus neoformans* serotypes and its application to serotyping of clinical isolates. *J. Clin. Microbiol.* 16:22.
10. Rinaldi, M.G., D.J. Drutz, A. Howell, M.E. Sande, C.B. Wofsy, and W.K. Hadley. 1986. Serotypes of *Cryptococcus neoformans* in patients with AIDS. *J. Infect. Dis.* 153:642.
11. Diamond, R.D., and J.E. Bennett. 1974. Prognostic factors in crytococcal meningitis. A study of 111 cases. *Ann. Intern. Med.* 80:176.
12. Kozel, T.R., and T.G. McGaw. 1979. Opsonization of *Cryptococcus neoformans* by human immunoglobulin G: role of immunoglobulin G in phagocytosis by macrophages. *Infect. Immun.* 25:255.
13. Nabavi, N., and J.W. Murphy. 1986. Antibody-dependent natural killer cell-mediated growth inhibition of *Cryptococcus neoformans*. *Infect. Immun.* 51:556.

14. Diamond, R.D. 1974. Antibody-dependent killing of *Cryptococcus neoformans* by human peripheral blood mononuclear cells. *Nature (Lond.).* 247:148.
15. Diamond, R.D., and A.C. Allison. 1976. Nature of the effector cells responsible for the cell-mediated killing of *Cryptococcus neoformans*. *Infect. Immun.* 14:716.
16. Dromer, F., J. Charriere, A. Contrepois, C. Carbon, and P. Yeni. 1987. Protection of mice against experimental cryptococcus infection by anti-*C. neoformans* monoclonal antibodies. *Infect. Immun.* 55:749.
17. Dromer, F., and J. Charreire. 1990. Improved Amphotericin B activity by a monoclonal anti-*Cryptococcus neoformans* antibody, E_1; *in vivo* and *in vitro* studies. Thirtieth Interscience Conference on Antimicrobial Agents and Chemotherapy. Atlanta, GA. Abstract 484, pg. 167.
18. Monga, D.P., R. Kumar, L.N. Mohapatra, and A.N. Malaviya. 1979. Experimental cryptococcosis in normal and B-deficient mice. *Infect. Immun.* 26:1.
19. Goren, M.B., and G.M. Middlebrook. 1967. Experimental murine cryptococcosis: effect of hyperimmunization to capsular polysaccharide. *J. Immunol.* 98:914.
20. Sanford, J.E., D.M. Lupan, A.M. Schlageter, and T.R. Kozel. 1990. Passive immunization against *Cryptococcus neoformans* with an isotype-switch family of monoclonal antibodies reactive with capsular polysaccharide. *Infec. Immun.* 58:1919.
21. Dromer, F., P. Aucuoturier, J.-P. Clauvel, G. Saimot, and P. Yeni. 1988. *Cryptococcus neoformans* antibody levels in patients with AIDS. *Scand. J. Infect. Dis.* 20:283.
22. Kozel, T.R., and R. Cazin. 1971. Nonencapsulated variant of *Cryptococcus neoformans*. *Infect. Immun.* 3:287.
23. Dromer, F., J. Salamero, A. Contrepois, C. Carbon, and P. Yeni. 1987. Production, characterization and antibody specificity of a mouse monoclonal antibody reactive with *Cryptococcus neoformans* capsular polysaccharide. *Infect. Immun.* 55:742.
24. Dubois, M., R.A. Gilles, J.K. Hamilton, P.A. Rebens, and F. Smith. 1956. Colorimetric method for determination of sugars and related substances. *Anal. Chem.* 28:350.
25. Fazekas de St. Groth, S., and D. Scheidegger. 1980. Production of monoclonal antibodies: strategy and tactics. *J. Immunol. Methods.* 35:1.
26. Gelibeter, J., R.A. Zeff, R.W. Melvold, and S.G. Nathenson. 1986. Mitotic recombination in germ cells generated two major histocompatibility complex mutant genes shown to be identical by RNA sequence analysis: K^{bm9} and K^{bm6}. *Proc. Natl. Acad. Sci. USA.* 85:2298.
27. Nieto, A., A. Goya, M. Jansa, C. Moreno, and J. Vives. 1984. Direct measurement of antibody affinity distribution by hapten-inhibition enzyme immunoassay. *Methods Immunol.* 21:537.
28. Greenspan, N.S., D.A. Dacek, and L.J.N. Cooper. 1989. F_c region dependence of IgG_3 anti-streptococcal antibody functional affinity. I. The effect of temperature. *J. Immunol.* 141:4276.
29. Hartman, A.B., and S. Rudikoff. 1984. V_H genes encoding the immune responses to beta-(1,6) galactan: mutation in IgM molecules. *EMBO (Eur. Mol. Biol. Organ.) J.* 3:3023.
30. Kabat, E.A., T.T. Wu, M. Reid-Miller, H.M. Perry, and K. Gottesman. 1987. Sequences of proteins of immunological interest. U.S. Department of Health and Human Services. Public Health Service, National Institute of Health.
31. French, D.L., R. Laskov, and M.D. Scharff. 1989. The role of somatic hypermutation in the generation of the antibody diversity. *Science (Wash. DC).* 244:1152.
32. Nahmias, C., A.D. Strosberg, and L.J. Emorine. 1988. The immune response toward beta-adrenergic ligands and their receptors. VIII Extensive diversity of V_H and V_L genes encoding anti-protenolol antibodies. *J. Immunol.* 140:1304.
33. Selsing, E., J. Durdick, M.W. Noore, and D.M. Persiani. 1989. Immunoglobulin λ genes. *In* Immunoglobulin Genes. T. Honjo, F.W. Alt, and T.H. Rabbitts, editors. Academic Press Inc., San Diego. 111.
34. Pollack, R.R., D.L. French, J.P. Metlay, B.K. Birshtein, and M.D. Scharff. 1990. Intravascular metabolism of normal and mutant mouse immunoglobulin molecules. *Eur. J. Immunol.* 20:2021.
35. Todaro-Luck, F., E. Reiss, R. Cherniak, and L. Kaufman. 1989. Characterization of *Cryptococcus neoformans* capsular glucoronoxylomannan polysaccharide with monoclonal antibodies. *Infect. Immun.* 57:3882.
36. Bhattacharje, A.K., J.E. Bennett, and C.P.J. Glaudemans. 1984. Capsular polysaccharides of *Cryptococcus neoformans*. *Rev. Infect. Dis.* 6:619.
37. Cherniak, R., E. Reiss, and S.H. Turner. 1982. A Galactoxylomannan antigen of *Cryptococcus neoformans* serotype A. *Carbohydr. Res.* 103:239.
38. Spira, G., H.L. Aguila, and M.D. Scharff. 1987. T15 PC binding monoclonal antibodies retain specificity when they switch from IgM to IgG. *J. Immunol.* 140:2675.
39. Kimura, H., R. Cook, K. Meek, M. Umeda, E. Ball, J.D. Capra, and D.M. Marcus. 1988. Sequences of the V_H and V_L regions of murine monoclonal antibodies against 3-fucosyllactosamine. *J. Immunol.* 140:1212.
40. Kimura, H., E.S. Buescher, E.D. Ball, and D.M. Marcus. 1989. Restricted usage of V_H and V_K genes by murine monoclonal antibodies against 3-fucosyllactosamine. *Eur. J. Immunol.* 19:1741.
41. Eckert, T.F., and T.R. Kozel. 1987. Production and characterization of monoclonal antibodies specific for *Cryptococcus neoformans* capsular polysaccharide. *Infect. Immun.* 55:1895.
42. Lutz, C.T., T.L. Bartholow, N.S. Greenspan, R.J. Fulton, W.J. Monojo, R.M. Perlmutter, H.V. Huang, and J.M. Davie. 1987. Molecular dissection of the murine antibody response to streptococcal group A carbohydrate. *J. Exp. Med.* 165:531.
43. Akolkar, P.N., S.K. Sikder, S.B. Bhattacharya, J. Liao, F. Grueza, S.L. Morrison, and E.A. Kabat. 1987. Different V_H and V_L germ line genes are used to produce similar combining sites with specificity for alpha(1-6) dextrans. *J. Immunol.* 38:4472.
44. 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.* 14:1922.
45. Wallick, S.C., E.A. Kabat, and S.L. Morrison. 1988. Glycosylation of a V_H residue of a monoclonal antibody against alpha (1-6) dextran increases its affinity for antigen. *J. Exp. Med.* 168:1099.
46. Yancopoulos, G.C., and F.W Alt. 1986. Regulation of the assembly and expression of variable region genes. *Annu. Rev. Immunol.* 4:339.
47. Caton, A.J. 1990. A single pre-B cell can give rise to antigen-specific B cells that utilize distinct immunoglobulin gene rearrangements. *J. Exp. Med.* 172:815.
48. Lim, T.S., J.W. Murphy, and L.K. Cauley. 1980. Host-etiological agent interactions in intranasal and intraperitoneal induced cryptococcosis in mice. *Infect. Immun.* 29:633.
49. Dromer, F., P. Yeni, and J. Charriere. 1988. Genetic control of the humoral response to cryptococcal antigen polysaccharide in mice. *Immunogenetics.* 28:417.

50. Baker, P.J. 1990. Regulation of magnitude of antibody response to bacterial polysaccharide antigens by thymus-derived lymphocytes. *Infect. Immun.* 58:3465.
51. Murphy, J.W., and R.A. Cox. 1988. Induction of antigen-specific suppression by circulating *Cryptococcus neoformans* antigen. *Clin. Exp. Immunol.* 73:174.
52. Baker, C.J., D.L. Kasper, M.S. Edwards, and G. Schiffman. 1980. Influence of preimmunization antibody levels on the specificity of the immune response to related polysaccharide antigens. *N. Engl. J. Med.* 303:173.
53. Geysen, H.M., R. Macfarlan, S.J. Rodda, G. Tribbick, T.J. Mason, and P. Schoofs. 1987. Peptides which mimic carbohydrate antigens. *In* Towards Better Carbohydrate Vaccines. R. Bell and G. Torrigiani, editors. John Wiley & Sons Ltd., Chichester, England. 103–118.
54. Mosier, D.E., and B. Subbarao. 1982. Thymus-independent antigens: complexity of B-lymphocyte activation revealed. *Immunol. Today.* 3:217.
55. Tittle, T.V., and M.B. Rittenberg. 1980. IgG B memory cell subpopulations: differences in susceptibility to stimulation by TI-1 and TI-2 antigens. *J. Immunol.* 124:202.
56. Insel, R.A., and P.W. Anderson. 1986. Oligosaccharide-protein conjugate vaccines induce and prime oligoclonal IgG antibody responses to the *hemophilus influenza* b capsular polysaccharide in human infants. *J. Exp. Med.* 163:262.
57. Devi, S.J.N., R. Schneerson, J.E. Bennett, and J.B. Robbins. 1990. A glucuronoxylomannan-tetanus toxoid conjugate vaccine against *Cryptococcus neoformans*. Thirtieth Interscience Conference on Antimicrobial Agents and Chemotherapy. Atlanta, GA. Abstract 70, pg. 98.
58. Borden, P., and E.A. Kabat. 1987. Nucleotide sequence of the cDNAs encoding the variable region heavy and light chains of a myeloma protein specific for the terminal nonreducing end of alpha (1-6) dextran. *Proc. Natl. Acad. Sci. USA.* 84:2440.
59. Abram, D.E., S. Kuno, R. Wong, K. Jeffords, M. Nosh, J.B. Malaghan, R. Gorter, and R. Veno. 1989. Oral dextran sulfate (VA 001) in the treatment of the Acquired Immunodeficiency Syndrome (AIDS) and AIDS-related complex. *Ann. Intern. Med.* 110:183.