ANTI-PHOSPHORYLCHOLINE ANTIBODIES OF THE T15 IDIOTYPE ARE OPTIMALLY PROTECTIVE AGAINST *STREPTOCOCCUS PNEUMONIAE**

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Since their initial discovery (1) immunoglobulin idiotypes have proven to be useful in studies examining antibody diversity, inheritance of variable region expression, regulation of specific antibody responses, and the possible use of antibody heavy chain variable (V_H)[1] regions in T cell antigen receptors. In the present report, we present evidence that there is a close relationship between the idiotype of an anti-phosphorylcholine (PC) antibody and its ability to protect mice from pneumococcal infection. These findings extend idiotypic analysis into the area of microbial pathogenesis and provide added relevance to previous studies of the regulation of idiotype expression, and the evolution of immunoglobulin (Ig) V region genes.

Murine anti-PC antibodies and myelomas fall into three discrete families bearing either the T15, M603, or M511 idiotype (2–4). Antibodies within each of these families have fine specificities for PC analogues and PC-carrier conjugates that are characteristic of their respective families (3, 4). These three idiotype families, however, are very closely related, and their heavy chains are thought to be transcribed from the same rearranged V_H, D, and J region genes (5). Each of the three idiotype families is characterized, however, by a different family of light chains (3, 6, 7). Of the three idiotypic families, the T15 antibodies show the least variation, due to somatic mutation (5), in their V_L and V_H sequences (7). T15 anti-PC antibodies can be made directly from an unmodified V_H (and probably V_L) gene (5, 7) and comprise a large fraction (30–90%) of murine immune and naturally occurring anti-PC antibody (6, 8). It has been suggested that the predominant expression of the T15 idiotype on the present structure of the T15 structural genes may have been the result of selective pressure during murine evolution (4, 9).

Immune responses to PC may indeed have some survival value, as naturally occurring and hybridoma IgM and IgG_3 anti-PC antibody (9–11) as well as PC-binding C-reactive protein (12, 13) can protect mice from fatal infection with virulent

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[1] *Abbreviations used in this paper:* FCS, fetal calf serum; PC, phosphorylcholine; PD_{50}, median protective dose, i.e., dose of antibody at which half of the animals survived; V_H, heavy chain variable region; V_L, light chain variable region.

Streptococcus pneumoniae. Because of the protective effect of anti-PC antibody against pneumococcal infections, it seemed likely that anti-PC V_H and V_L regions might exist in the genome for the express purpose of producing protective antibody reactive with PC-containing teichoic acids. If this were the case, one would expect that T15 anti-PC antibody would be more protective than anti-PC antibody made after more extensive somatic mutation of the V_H region. The data presented below indicate that IgM T15 antibodies are, in fact, more protective than IgM anti-PC antibodies of other idiotypes that have been shown to express more frequently somatic variation in their light and heavy chains (5, 7).

Materials and Methods

Mice. All mice used in these studies were (CBA/N × DBA/2)F_1 males, obtained from the Rodent and Rabbit Production Unit of the National Institutes of Health, through the courtesy of Dr. Kenneth Schroer, Laboratory of Pathology, National Cancer Institute.

Bacteria. S. pneumoniae type 3, strain WU2, was grown from frozen stock cultures in Todd-Hewitt broth (Difco Laboratories, Detroit, MI) supplemented with 0.5% yeast extract (Difco Laboratories) and 2% heparinized human blood (9). One subsequent passage was made in medium without blood, and early log phase pneumococci were harvested. Bacteria were enumerated by optical density and plating, and were injected intravenously in 2% heat-inactivated fetal calf serum-Ringer's lactate (FCS-Ringer's) as described (9).

Hybridoma Antibodies. The isolated IgM hybridoma antibodies to PC that were used in this study have been described (3, 4). All hybridoma antibodies were made by fusion of spleen cells with the nonsecreting plasmacytoma fusion line SP2/0-Ag14, except 137.2D3 and 140.7C6, which were generated with the nonsecreting line X63-Ag8.653. The idiotypes of these anti-PC hybridoma antibodies have been characterized by a solid-phase radioimmunoassay using heterologous anti-idiotypic antisera to T15, M603, and M511 (6). We also used hybridoma anti-idiotypic antibodies AB1-2 and GB4-10 (obtained courtesy of Dr. John F. Kearney, University of Alabama, Birmingham, AL), which react with T15 but not M511 or M603 idiotypes (14).

Mouse Protection with Hybridoma Antibodies. Mice were injected intraperitoneally with either 100, 20, 2, or 0.2 μg of hybridoma antibody in FCS-Ringer's 1–2 h before the intravenous injection of 100 colony-forming units of *S. pneumoniae.* Mice were observed for 10 d, and deaths were recorded at 24-h intervals. The rank order statistical test was used to compare the death order of mice in the different groups. For groups in which over one-half of the mice died within the 10-d period, the median survival time was estimated by calculating the reciprocal mean survival time for the mice in each group. In groups where less than one-half of the mice died in 10 d, the median survival time was simply listed as >10 d. The median protective dose for each of the hybridoma antibodies was calculated by the method of Reed and Muench (15).

Results

In this study, we tested a panel of 10 hybridoma IgM anti-PC antibodies (3, 4) of the T15, M603, and M511 idiotype families for their efficiency in protecting mice from *S. pneumoniae.* Table I lists the reactivities of these hybridoma antibodies with heterologous anti-idiotypic sera and with two monoclonal anti-idiotypic antibodies AB1-2 and GB4-10 (14).

In the experiments described below, we have used (CBA/N × DBA/2)F_1 male mice. Because these mice express the *xid* defect of CBA/N mice (16) and have little or no detectable anti-PC antibody in their normal serum (9, 17, 18), they are ideal animals in which to study the effects of passive anti-PC antibody (9–11).

When we examined the relative ability of these 10 hybridoma anti-PC antibodies to protect mice, we observed that the 3 antibodies of the T15 idiotype were the most

TABLE I
Idiotype of Anti-PC Hybridomas and Myelomas

	Idiotypic specificities*				
	T15	AB1-2	GB4-10	M603	M511
IgM anti-PC hybridomas					
55.2D3	+	+	+	−	−
22.1A4	+	+	−	−	−
140.7C6	+	+	−	−	−
55.6F3	−	−	−	+	−
100.6F9.1	−	−	−	+	−
101.3C2.4	−	−	−	−	+
101.3G8.4	−	ND‡	ND	−	+
137.2D3	−	−	−	−	+
101.6G6.4	−	−	−	−	+
100.1C11.5	−	−	−	−	+
IgA anti-PC myelomas					
T15	+	+	+	−	−
M603	−	−	−	+	−
M511	−	−	−	−	+

* Detected by ability to inhibit reaction of appropriate antisera with ^{125}I-labeled M511, M603, or T15.
‡ Not done.

protective against fatal infection with *S. pneumoniae* (Table II). M511 antibodies were found to be the least protective, and M603 antibodies showed an intermediate level of protection. The pooled data for the hybridoma antibodies of the three different idiotypes have been plotted in Fig. 1.

T15 antibodies were statistically more protective than the M603 and M511 antibodies, based on two sample rank test comparisons of the mortality data of mice given the 20 μg of each hybridoma with the pooled data for mice given 20 μg of T15 antibody (Table III). Because this calculation fails to take into account the better protection seen with 2 and 100 μg of T15 than with the same amounts of M511 or M603 antibody (Table II), the real statistical differences are even greater than those calculated.

The calculated median protective doses (PD_{50}) for each of the hybridoma antibodies at 2, 3, and 10 d post-infection are shown in Table III. Although the T15 antibodies were found to be more protective than the others at all three time points, the greatest difference was seen at day 2, when the T15 antibodies were ~8 and 28 times as effective as the M603 and M511 idiotype antibodies, respectively. The observation that the difference in protective ability of T15 and non-T15 antibodies decreased slightly after 2 d could be an artifact caused by the fact that antibody was given only once, thus allowing any bacteria that survived to eventually kill the mice.

All three T15 positive anti-PC hybridoma antibodies protected mice to a similar degree, even though one of them reacted with the GB4-10 anti-idiotype and the other two did not. Thus, the idiotypic differences among T15 antibodies detected by GB4-10 (14) appears not to be associated with different protective abilities.

Discussion

These results demonstrate that IgM anti-PC antibody of the T15 idiotype is more protective against pneumococcal infection than anti-PC antibody of the other two

TABLE II

Protection of Mice from Type 3 S. Pneumoniae with IgM Hybridoma Anti-PC Antibody

Idiotype	Tumor	Dose	Number of mice alive at indicated day								Median‡ days alive
			0	1	2	3	4	5	6	>10	
		μg/mouse									
T15	55.2D3	100	2	2	2	2	2	2	2	2	>10*§
		20	4	4	4	4	4	4	4	4	>10**
		2	2	2	0	0	0	0	0	0	1.0
	22.1A4	100	5	5	5	5	5	5	5	5	>10***
		20	9	9	9	9	9	9	9	9	>10***
		2	9	9	6	4	3	2	2	2	2.0**
	140.7C6	100	4	4	4	3	3	3	3	3	>10**
		20	11	11	11	8	8	8	8	8	>10***
		2	6	6	2	1	1	1	1	1	1.3
		.2	4	4	1	0	0	0	0	0	1.1
M603	55.6F3	100	10	10	10	10	10	10	10	10	>10***
		20	10	10	7	4	4	3	3	3	2.1**
		2	4	4	0	0	0	0	0	0	1.0
	100.6F9.1	100	4	4	4	4	4	4	4	4	>10**
		20	6	6	2	1	1	1	1	1	1.3
		2	2	2	0	0	0	0	0	0	1.0
M511	101.3C2.4	100	4	4	4	3	3	3	2	2	5.7**
		20	4	4	2	1	1	1	1	1	1.6
	101.3G8.4	100	4	4	3	3	3	3	3	3	>10*
		20	4	4	1	1	1	1	1	1	1.3
	137.2D3	100	6	6	5	4	4	4	4	4	>10*
		20	6	6	0	0	0	0	0	0	1.0
	101.6G6.4	100	6	6	3	2	2	2	2	2	1.7
		20	8	8	1	1	1	1	1	1	1.1
		2	4	4	0	0	0	0	0	0	1.0
	100.1C11.5	100	4	4	0	0	0	0	0	0	1.0
		20	4	4	1	0	0	0	0	0	1.1
FCS (diluent)			25	25	1	0	0	0	0	0	1.02

‡ For values <10, the median days alive is the reciprocal mean days alive for each mouse in the group. That is, median days alive $= 1 \Big/ \left(\left(\sum_{i=1}^{n} \frac{1}{xi} \right) \Big/ n \right)$, where x is the number of days that mouse i lived.

§ Statistically different from diluent control at $P < 0.05$ (*); $P < 0.01$ (**); $P < 0.001$ (***); by two-sample rank test.

major idiotypic families of mouse anti-PC antibodies. Recent evidence at the molecular level indicates that much of the variability in Ig structure is the result of somatic mutation of recombined germ line V_H, J, and D segments (5, 19–21). In the case of anti-PC antibody, it is known that although the same V_H appears to code for the V_H regions of virtually all murine anti-PC antibody, the exact translation of this gene yields an amino acid sequence characteristic of the heavy chains of T15 anti-PC antibodies (5, 7). On the average, T15 anti-PC hybridoma and myeloma antibodies differ from this sequence by only one amino acid substitution (7). The V_H regions of M603 and M511 heavy chains, which are thought to be coded for by the T15 gene, differ on the average by five amino acids from the germ line sequence (7). Thus, the direct translation of the germ line gene results in anti-PC V_H regions characteristic of those antibodies (T15) that show optimal protective ability against the pneumococcus.

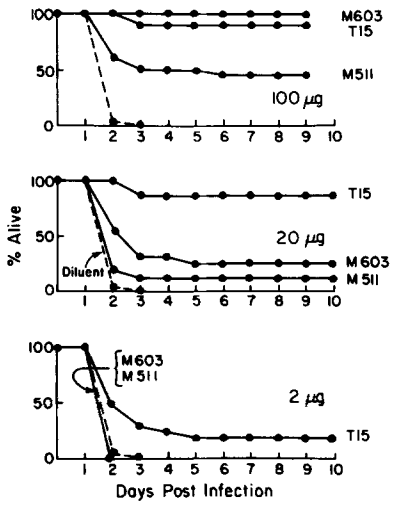

FIG. 1. Survival curves of C × D male mice protected from type 3 *S. pneumoniae* with hybridoma anti-PC antibodies. This figure depicts the data in Table II pooled according to the idiotype of the hybridomas used.

TABLE III

Relative Capacity of Anti-PC Hybridomas to Protect Against S. Pneumoniae

Idiotype	Hybridoma antibody	PD_{50} on day*			*P* vs. T15‡
		2	3	10	
		μg/mouse			
T15	55.2D3	6	6	6	—
	22.1A4	<2	2.5	4.6	—
	140.7C6	2.7	9.5	9.5	—
	A11 T15	2.0	5.3	6.4	—
M603	55.6F3	10	26	32	<0.01
	100.6F9.1	30	38	38	<0.01
	A11 M603	16	31	34	<0.001
M511	101.3C2.4	20	45	69	<0.05
	101.3G8.4	45	45	45	<0.05
	137.2D3	67	67	67	<0.001
	101.6G6.4	79	>100	>100	<0.001
	100.1C11.5	>100	>100	>100	<0.01
	A11 M511	57	83	93	$<10^{-5}$

* PD_{50} doses were calculated at 2, 3, and 10 d post-infection from the data in Table II.

‡ Comparison of the 20-μg data for each hybridoma antibody with the pooled T15 data using the two-sample rank test.

It is likely that the differences in idiotype, specificity, and protection among different mouse anti-PC antibodies are even more heavily affected by the variation observed in the light chain than that in the heavy chain (5).[2] The light chains of the T15, M603, and M511 antibodies form three distinct families as judged by their isoelectric focusing spectra (6) and amino acid sequences (7). Thus there exists a close

[2] S. H. Clarke, J. L. Claflin, M. Potter, and S. Rudikoff. Evolution of gene families encoding anti-phosphorylcholine antibodies. Manuscript submitted for publication.

association between protection against *S. pneumoniae* and the presence of particular L chains: T15, V_κ-22 family > M603, V_κ-8 family > M511, V_κ-24 family. The L chain sequence differences among the antibodies in the different families are great enough to suggest that they are the result of at least three different germ line V_κ genes (5, 7, 19, 21).

It is of interest that, as with the heavy chains, there is much less sequence variation within the T15 light chain V_κ sequences than within the V_κ sequences of M603 and M511 anti-PC antibodies and myelomas (7). The uniformity of T15 light chain sequences suggests that few if any somatic mutations are required to produce a suitable anti-PC light chain from the T15 V_κ gene and that T15 anti-PC antibody can result from the direct translation of the germ line T15 V_H and V_κ genes (plus the appropriate J and D region segments).

The fact that T15 antibody provides optimal protection against pneumococci raises the possibility that the structure of the T15 genes (4), and perhaps most germ line V_H and V_L genes, may have evolved to provide antibody to one or another common antigen of pathogens. PC, for example, has been found on many microorganisms including species of fungi, nematodes, and gram-negative and -positive bacteria (22). The preexistence of germ line genes for antibody to common antigens might allow for a more rapid initial antibody response. Antibodies to antigens either not associated or less commonly associated with pathogens would be expected, in general, to require somatic mutation of the V region genes to produce suitable binding sites.

Our results demonstrating differential protective effects of anti-PC antibodies of different idiotypes may also have important implications for the observations that the expression of idiotypes in immune responses appears to be regulated (23). Evidence from a number of laboratories indicates that the maintenance of T15 antibodies as the dominant anti-PC idiotype is under strict regulation. Effector mechanisms involving helper T cells, suppressor T cells, and auto-anti-idiotype have all been shown to be able to effect idiotype regulation in this system (23–27). Our results, demonstrating better antipneumococcal protection with T15 antibodies than with anti-PC antibodies of other idiotypes, suggest that the mechanisms regulating idiotype expression may play important roles in the maintenance of antibody populations that are optimally protective against various infectious agents.

However, our conclusions about the role of anti-PC antibody in the defense against natural murine infections must remain somewhat speculative. It is known that PC is present on a wide variety of microbes, including certain lactobacilli and murine pinworms, both of which are part of the natural murine flora (22). It is not known, however, whether or not PC-containing pathogenic microbes represent a frequent enough threat to mice to have had an effect on the evolution of anti-PC immune responses. It is possible that the selective pressures leading to the present anti-PC immune responsiveness could have been present in the evolutionary past, but absent at the present. In this connection, it is of interest that pneumococcal pneumonia is a common epidemic in rat colonies (28), but has not been reported to affect mice in natural outbreaks (Russell Linday, personal communication). Alternatively, the lack of natural pneumococcal infections in mice is due in part to the anti-PC antibody in their serum. This supposition is supported by our observation that *xid* mice, which lack serum anti-PC antibody (9, 17), are 1,000–10,000 times more susceptible to pneumococcal infection than normal mice (9, 11). Furthermore, in unpublished

studies we have shown that, whereas intranasal inoculation of pneumococci seldom leads to fatal disease in normal mice (28), as few as 100 colony-forming units of type 3 *S. pneumoniae* inoculated intranasally readily cause death in *xid* mice.

Our finding that antibodies with the T15 idiotype were more protective than those with M603 or M511 idiotypes is not consistent with previous studies by others (7) and ourselves that show that antibodies of T15, M603, and M511 idiotypes all have similar affinities for PC as a hapten.[2] However, because anti-PC antibodies of the three idiotype families are known to differ in their fine specificity for different PC analogues and PC-carrier complexes (3, 4), it seems likely that the affinity of these antibodies for the PC hapten does not accurately reflect their ability to bind pneumococcal-PC.

Our earlier observations that anti-PC antibodies are protective against pneumococcal infection (9–11) emphasized the importance that species-specific antibodies could have the protection against *S. pneumoniae* and possibly other pathogens. The results of the present study emphasize the fact that, at least in this system, there is a clear correlation between the idiotypic family of antibody produced and its protective effect. This type of information could be used to advantage in the development of human vaccines where a prior knowledge of the most protective idiotype could assist the development of a vaccine that primarily elicits that idiotype and not idiotypes associated with antibodies of little protective value. Studies have shown (29–31) that the idiotype and specificity of murine antibodies to carbohydrates can be greatly affected by the carrier that is used to make them immunogenic.

Summary

In the mouse, most anti-PC antibody is found in one of the three murine anti-PC idiotype families: T15, M603, or M511. The antibodies within each of these idioytpic families have characteristic fine specificities for phosphorylcholine (PC)-analogues. In this paper we compare the ability of hybridoma IgM anti-PC antibodies of the three idiotype families to protect mice from fatal infection with *S. pneumoniae*. Antibody bearing the T15 idiotype was ~8 times as effective as antibody with the M603 idiotype and ~30 times as protective as antibody with the M511 idiotype. Reports by others have shown that the heavy chains of virtually all mouse anti-PC antibodies are produced by translocation of a single variable region gene and that the direct translation of this gene (in the absence of somatic mutations) results in heavy chains characteristic of the T15 idiotype. Thus, our findings suggest that the T15 germ line heavy chain variable region gene may have been selected through evolution to code for antibody binding PC-containing pathogens such as *S. pneumoniae*. Our observations may also explain the existence of regulatory mechanisms that result in maintenance of T15 idiotype expression in murine anti-PC immune responses.

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