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A MOLECULAR MODEL OF ARTIFICIAL GLYCOPROTEIN WITH PREDETERMINED MULTIPLE IMMUNODETERMINANTS FOR GRAM-POSITIVE AND GRAM-NEGATIVE ENCAPSULATED BACTERIA

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Abstract—An artificial molecule was synthesized by covalently linking the oligosaccharide haptens derived frm *Streptococcus pneumoniae* type 6A and *Neisseria meningitidis* group C capsular polysaccharides to the non-toxic mutant protein CRM197, serologically related to diphtheria toxin. Immunochemical analysis using polyclonal and monoclonal antibodies showed in the glycoprotein the presence of specific immunodeterminants of the native polysaccharides and of the carrier protein. The immunological activity of this hybrid molecule tested in two animal models gave evidence for anamnestic induction of serum antibodies specifically directed to the three distinct native molecules. They neutralized the toxicity of diphtheria toxin, recognized the polysaccharide capsule of *S. pneumoniae* type 6A and 6B (group 6) strain and killed the *N. meningitidis* group C bacteria by complement-mediated bacterial lysis. These findings support the possibility of using in humans a multivalent antigen with immunogenic activity for several epidemiologically significant Gram-positive and Gram-negative encapsulated bacterial strains.

INTRODUCTION

Since the pioneer work of Avery and Goebel (1929) the induction of a specific immunological response in some animal species against bacterial oligosaccharides, using carrier proteins for these haptens, is well known. It was later demonstrated that this immunological response involves the stimulation of carrier-specific T-helper cells inducing anti-hapten antibody synthesis (Paul *et al.*, 1971).

This kind of approach to the manipulation of the immunological response of mammalians to polysaccharide antigens has recently been revised by some authors (Schneerson *et al.*, 1980; Jennings and Lugowski, 1981; Anderson, 1983; Porro *et al.*, 1985) in view of the observation that the modern meningococcal and pneumococcal polysaccharide vaccines have shown a low immunogenic potential in inducing protective levels of serum antibodies in infants (Gotschlich, 1975; Austrian, 1979; Peltola, 1983). The protective role of human IgG and IgM anti-meningococcal antibodies was shown to be related to their complement-mediated bacterial lytic activity, whereas that of anti-pneumococcal antibodies appears related to their opsonizing characteristics favouring the phagocytosis by polymorphonuclear leukocytes (Gotschlich *et al.*, 1969; Gotschlich, 1975; Giebink *et al.*, 1980).

We have studied and developed the preparation of a hybrid antigen with the purpose of obtaining a molecule with predetermined multivalent activity as well as to demonstrate the induction of antibodies showing specific immunochemical properties for diphtheria toxin, and for meningococcal and pneumococcal bacterial strains. In previous works, we reported on the bivalent immunogenic efficacy of a semi-synthetic molecule employing a type 6A pneumococcal oligosaccharide hapten carried by the protein CRM197, compared to the low immunogenic potential of a molecule involving the homologous high mol. wt polysaccharide carried by the same protein (Porro *et al.*, 1983*a*; 1985). In this report we show that the molecular model involving the protein CRM197 as a carrier for oligosaccharide haptens derived from their respective homologous polymers is also effective for the induction of a multivalent immunological response specifically directed against Gram-positive and Gram-negative encapsulated bacteria.

MATERIALS AND METHODS

Preparation and characterization of the oligosaccharide haptens

The peparation of the *Streptococcus pneumoniae* type 6A (S. Pneum 6A) oligosaccharide using acid hydrolysis of the native homologous polysaccharide has been previously reported (Porro *et al.*, 1985). The *Neisseria meningitidis* group C (N. Mening C) oligosaccharide was prepared in a similar manner but using a concn of 5.5×10^{-4} *M* acetic acid and 8 hr of hydrolysis. Their native homologous poly-

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saccharides originated from a bulk powder for the preparation of human vaccines (Sclavo SpA, Siena, Italy) and their characteristics were in accordance with the WHO requirements for these antigens, as reported earlier (Porro *et al.*, 1983*b*). The derived oligosaccharides were purified by molecular sieving on Sephadex G-15 (Pharmacia, Uppsala, Sweden) and appeared on Sephadex G-50 as a symmetrical bell-shaped curve with $K_d = 0.48$ (S. Pneum 6A) and 0.46 (N. Mening C). The eluted fractions were pooled and chemically analyzed for methyl pentose, phosphorus and reducing groups (S. Pneum 6A), or sialic acid and reducing groups (N. Mening C), according to the reported procedures (Kabat, 1964; Chen *et al.*, 1956; Svennerholm, 1957; Porro *et al.*, 1981). Characterization of the physical dimension of the S. Pneum 6A oligosaccharide by ^{13}C NMR spectroscopy has been reported previously (Porro *et al.*, 1985). The average mol. wt of the N. Mening C oligosaccharide appeared to be similar to that determined for the S. Pneum 6A oligosaccharide, based on the observation that the two linear haptens showed a comparable K_d by molecular sieving.

Immunochemical characterization of the oligosaccharide haptens

The specificity of horse and rabbit polyclonal antibodies for the obtained oligosaccharide haptens was analyzed by testing the ability of the oligosaccharide haptens to inhibit their homologous polysaccharide–antibody reactions using differential immunoelectrophoresis under the conditions reported by Porro *et al.* (1985).

Synthesis of the artificial glycoprotein

The adopted procedure of synthesis has been previously described for a similar model of glycoprotein (Porro *et al.*, 1985). Briefly, the procedure involved three main steps:

(1) introduction of a primary amino group by reductive amination in the end-reducing group of the S. Pneum 6A oligosaccharide or in the hemiketal group of the end-reducing sialic acid residue of the N. Mening C oligosaccharide;

(2) chemical derivatization of the amino-activated oligosaccharides to their corresponding active esters; and

(3) coupling of monoester-activated oligosaccharide haptens to the free amino groups of arginine (Arg) and Lysine (Lys) residues present in the structure of the protein carrier CRM197, obtained and purified as reported previously (Porro *et al.* 1980, 1985).

Introduction of a primary amino group in the structure of the oligosaccharide haptens. The procedure of reductive amination adopted for the S. Pneum 6A oligosaccharide was identical to that reported by Porro *et al.* (1985). The N. Mening C oligosaccharide was chemically activated using a similar reaction but keeping the temp of the reaction at 37°C for 1 week and the pH adjusted to 8 by 1 *N* NaOH, in order to prevent the possibility of *O*-deacylation for this oligosaccharide. Both the oligosaccharides were separately purified by gel chromatography on Sephadex G-15 equilibrated in 0.15 *M* NaCl (pH = 7.0). The chromatographic effluents showing $K_d \leq 0.1$ were pooled and the chemical activity detected by methyl pentose (Kabat, 1964), phosphorus (Chen *et al.*, 1956) and amino groups (Habeeb, 1966) in the case of S. Pneum 6A oligosaccharide, or sialic acid (Svennerholm, 1957) and amino groups (Habeeb, 1966) in the case of N. Mening C oligosaccharide.

Conversion of the amino-group-activated oligosaccharides to their corresponding active ester. The purified and analyzed oligosaccharides were concentrated in a vacuum evaporator at room temp and then reacted with the disuccinimidyl ester of adipic acid under the same conditions as reported previously for the S. Pneum 6A oligosaccharide (Porro *et al.*, 1985). Analysis for the disappearance of amino groups was performed after this step.

Coupling of the monoester-activated oligosaccharides to CRM197. Coupling of the two oligosaccharide haptens to CRM197 was achieved by reacting separately each one of the two oligosaccharides with the carrier protein under the conditions described previously (Porro *et al.*, 1985). Purification of the artificial glycoprotein was followed by gel chromatography on Sephadex G-100, equilibrated in 0.2 *M* NaCl (pH = 7.0). The eluted fractions showing chemical activity for protein (Lowry *et al.*, 1951), phosphorus (Chen *et al.*, 1956) and sialic acid (Svennerholm 1957) were pooled, sterile filtered by a 0.22-μm membrane and stored at 4°C.

Physico-chemical analysis of the artificial glycoprotein

The molecule was analyzed for content of protein, phosphorus and sialic acid using the above-mentioned analysis. The stoichiometry of the glycoprotein, in terms of the determination of the substitution degree (sd) as moles of S. Pneum 6A and N. Mening C oligosaccharides bound per mole of carrier protein, was calculated according to the method reported by Porro *et al.* (1985) on the basis of the physical dimensions of the oligosaccharides as estimated by ^{13}C NMR spectroscopy and gel chromatography. SDS–PAGE analysis was performed in reducing conditions by the Laemmli (1970) technique, using an acrylamide gradient [3–9% (w/v)] and silver staining of the gel (Porro *et al.*, 1982). All the analyses were performed in comparison with the native CRM197 protein. Comparison between the average mol. wt estimated by SDS–PAGE and that calculated by determining the SD value then followed.

Molecular mapping of the immunodeterminants present in the artificial glycoprotein

Monoclonal antibodies to the main epitopes of CRM197 (Zucker and Murphy, 1984) and rabbit and

horse polyclonal antisera to S. Pneum 6A and N. Mening C capsular polysaccharides (Statens Serum Institute, Copenhagen, Denmark, and Office of Biologics, FDA, Bethesda, MD), were used under the previously reported conditions in an ELISA test (Zucker and Murphy 1984) and in differential immunoelectrophoresis (Porro *et al.*, 1985), respectively, to determine their specificity for the immunodeterminants present in the artificial glycoprotein antigen.

Immunization of animal models and immunological analysis

Two groups of 10 albino rabbits each (av. wt = 2 g) and one group of 10 guinea-pigs (av. wt = 350 g) were inoculated subcutaneously with two doses of the glycoprotein antigen adsorbed to the mineral adjuvant $AlPO_4$ (1.5 mg/dose). The booster dose was injected 28 days after the administration of the first dose. Bleedings were performed just before the first or the booster dose was injected and 11 days after the booster dose. Each dose contained 12.5 Lf (flocculation unit) of CRM197 (Porro *et al.*, 1980) corresponding to 31.2 μg of protein, 4.8 μg of S. Pneum 6A oligosaccharide and 3 μg of N. Mening C oligosaccharide.

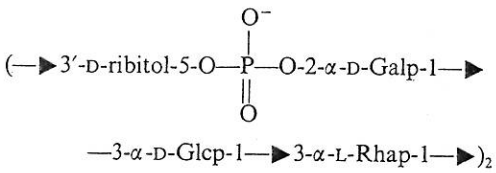
Rabbit antisera were analyzed for the content of IgG isotype antibody to S. Pneum 6A and N. Mening C capsular polysaccharides by an ELISA method using the conditions previously described for the S. Pneum 6A carbohydrate (Porro *et al.*, 1985). The Quellung reaction on living *S. pneumoniae* type 6A and 6B strains was performed according to Austrian (1976), using both reference and glycoprotein-induced rabbit antisera.

The complement-mediated bacterial lytic activity of rabbit antibodies was tested on live strains of *N. meningitidis* group C according to the method described by Wong *et al.* (1977). Guinea-pig antisera were analyzed for their content of antitoxin according to the U.S. Pharmacopea. A reference horse antiserum to diphtheria toxin (Sclavo SpA) was employed in the titration of diphtheria toxin used in the biological assay. The absence of significant titers in specific antibodies was detected in all groups of animals before immunization. Control immunizations using only oligosaccharide haptens or polysaccharides were omitted based on our previous observations (Porro *et al.*, 1983*a*) and on the large experience of several laboratories concerning the absence of a significant immunogenicity of highly purified oligo- and polysaccharides in rabbits.

RESULTS

Physico-chemical and immunochemical characteristics of the oligosaccharide haptens

The physical structure of the S. Pneum 6A oligosaccharide, as estimated by ^{13}C NMR spectroscopy (Porro *et al.*, 1985), was consistent with about 10 monose residues ($\overline{MW} = 1800$), with the end-reducing group localized at the level of the rhamnose unit:



Chemical analysis of this oligosaccharide showed a phosphorus/rhamnose ratio of 1 (moles/mole) and a rhamnose/reducing group ratio of 3 (moles/mole). The immunochemical specificity of reference rabbit polyclonal antibodies for this oligosaccharide hapten as well as for the immunodominant sugar galactose, in comparison with that determined for the native capsular polysaccharide, is reported in Table 1.

The structural formula for N. Mening C oligosaccharide was deduced as similar to

Table 1. Immunochemical specificity of reference polyclonal antibodies for their respective homologous bacterial capsular polysacccharides compared to that observed for the derived haptens in unconjugated or conjugated form with the carrier protein CRM197

Reference antiserum	Specificity for			
	Native polysaccharide	Derived hapten	Immunodominant sugar	Conjugate hapten
Horse meningococcal group C antiserum	1	1.06×10^{-3}	1.18×10^{-5} (NANA)	1.06×10^{-2}
Rabbit pneumococcal type 6A antiserum	1	1.08×10^{-3}	4.22×10^{-5} (Gal)	1.09×10^{-2}

The values were detected by differential immunoelectrophoresis under earlier described conditions (Porro *et al.*, 1985), using the oligosaccharide haptens or the artificial glycoprotein as inhibitors of the respective homologous polysaccharide–antibody immunoprecipitation reactions. Specificity expresses the ratio between the minimal inhibitory concn experimentally observed for the native polysaccharides and that observed for their homologous oligosaccharide haptens as well as for the identified immunodominant sugar in the *N. meningitidis* group C polysaccharide [*N*-acetylneuraminic acid (NANA)] and the *S. pneumoniae* type 6A polysaccharide [galactose (Gal)].

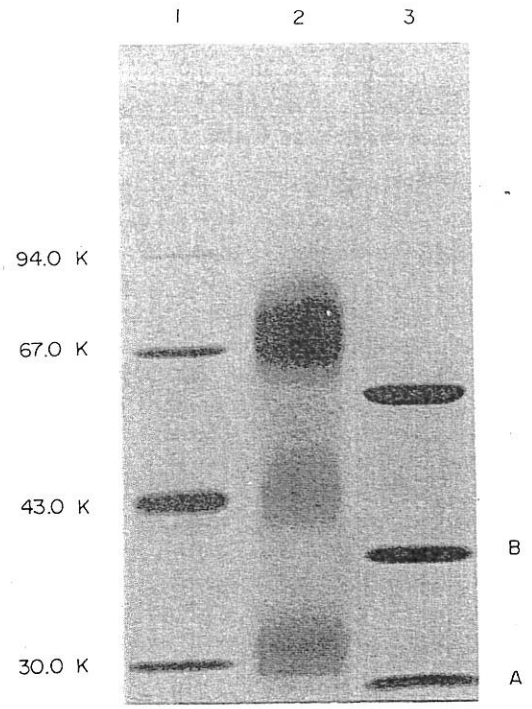


Fig. 1. Silver-stained acrylamide gel (Porro *et al.*, 1982) showing the electrophoretic behavior of the artificial glycoprotein antigen [5 μg (lane 2)] compared to the native protein CRM197 [5 μg (lane 3)] using SDS–PAGE in reducing conditions according to the Laemmli (1970) procedure and an acrylamide gradient [3–9% (w/v)]. The chemical glycosylation appears to be distributed on both fragment A and fragment B of the carrier protein CRM197 in partial "nicked" form [Pappenheimer *et al.*, 1972). Molecular-wt references (lane 1): 94.0 K, phosphorylase b; 67.0 K, BSA; 43.0 K, ovalbumin; 30.0 K, carbonic anhydrase.

(α-D-*N*,*O*-acetylneuraminic acid-2—▶9-α-D-*N*,*O*-acetylneuraminic acid)$_3$ (MW = 2100) based on the known structure of the native homologous capsular polysaccharide (Jennings *et al.*, 1977) and on the observation that the linear oligosaccharide hapten showed a K_d value of 0.46, comparable with that of the S. Pneum 6A oligosaccharide (K_d = 0.48). The immunochemical specificity of reference horse polyclonal antibodies for this oligosaccharide and for the immunodominant sugar *N*-acetyl neuraminic acid, in comparison with that determined for the native capsular polysaccharide, is shown in Table 1.

Physico-chemical and immunochemical characteristics of the artificial glycoprotein

The stoichiometry of the glycoprotein was consistent with 87% (w/w) of protein, 8% (w/w) of S. Pneum type 6A oligosaccharide and 5% (w/w) of N. Mening group C oligosaccharide. Transferred on molar basis, these values corresponded to four S. Pneum 6A oligosaccharide haptens and two N. Mening C oligosaccharide haptens per mole of protein carrier (MW = 6.2×10^4). The electrophoretic pattern of the glycoprotein antigen is shown in Fig. 1, referred to the native protein CRM197 in partial "nicked" form (Pappenheimer *et al.*, 1972). The estimated av. MW of the glycoprotein was 7.5×10^4 as compared to the calc. value of 7.3×10^4 and the glycosylation appeared to be distributed on both fragment A and fragment B of the carrier protein CRM197.

The molecular mapping for the immunodeterminants present in the structure of the artificial glycoprotein is reported in Tables 1 and 2. The specificity of polyclonal antibodies for their respective oligosaccharide haptens was 10 times higher than that estimated for the respective oligosaccharides in unconjugated form. These findings gave evidence for the exposure of the oligosaccharide haptens on the surface of the protein carrier. The increased specificity shown by homologous polyclonal antibodies can be explained with the observation that the specificity of polyclonal antibodies for carbohydrates, as the amount of antibodies recognizing the antigen (Berzofsky and Schechter, 1981) is related to the mol. wt of the carbohydrate (Bishop and Jennings, 1982; Porro *et al.*, 1983*b*, 1985). Thus, in the case of the glycoprotein described, the carrier

Table 2. Immunochemical specificity of monoclonal antibodies for different epitopes of protein CRM197 compared to that observed for the artificial glycoprotein antigen involving CRM197 as protein carrier[a]

Monoclonal antibody	Epitope recognized (approx. aa sequence in CRM197)	Number of basic aa residues in the epitopes		Specificity (%) for	
		Lys	Arg	CRM197	Glycoprotein antigen
Group I (clone 6B12)	1–156	14	2	100	31
Group IIb (clone 2A5)	157–193	2	5	100	10
Group IIIa (clone 2A7)	293–345	1	—	100	21
Group IVcl (clone 2E10)	465–535	6	2	100	95

[a]The values, obtained in triplicate experiments, were detected using an ELISA technique basically performed as described by Zucker and Murphy (1984). The percentage specificity of the monoclonal antibodies for the glycoprotein antigen was calculated assuming as 100 the specificity observed by measuring the absorbance values in the ELISA assay for CRM197.

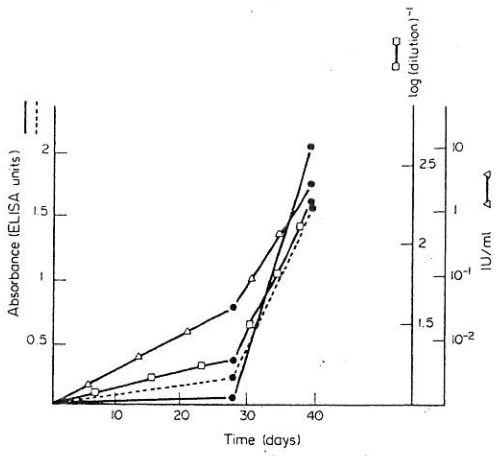


Fig. 2. Immunological characteristics of animal sera obtained after immunization by the artificial glycoprotein antigen. Each solid point shown in the graph represents the geometric mean of the titers obtained in each group of the animals who received the antigen as reported in Materials and Methods. (——) Rabbit IgG antibody specific for the capsular polysaccharide of *S. pneumoniae* type 6A, (– – – –) rabbit IgG antibody specific for the capsular polysaccharide of *N. meningitidis* group C, (□——□) rabbit antisera with complement-dependent bactericidal activity for *N. meningitidis* group C strain, and (△——△) guinea-pig antisera specific for diphtheria toxin.

protein CRM197 can play the role of a spatial support for several oligosaccharide haptens, mimicking for the carbohydrate moiety a higher mol. wt than that evidenced for the unconjugated oligosaccharides. We have reported a similar result for a previous model of an artificial glycoprotein (Porro *et al.*, 1985). The carrier protein CRM197 retained, after the procedure of coupling, the reactivity of the epitope localized in the 8000-dalton carboxy terminal region of the protein [approx. amino-acid (aa) sequence 465–535] as detected by the specific monoclonal antibody belonging to the collection of four main groups of antidiphtheria toxin monoclonal antibodies recently developed (Zucker and Murphy, 1984).

Immunological properties of the artificial glycoprotein

The immunogenicity of the described model of glycoprotein antigen is summarized in Fig. 2. Specific IgG isotype antibodies directed towards the native capsular polysaccharides of *S. pneumoniae* group 6A and *N. meningitidis* group C, as detected by an ELISA assay, were induced in rabbits. After injection of the booster dose, the levels of IgG serum antibodies increased exponentially, evidencing the induction of an immunologic memory for the carbohydrate moiety of the artificial glycoprotein. A parallel phenomenon was shown by the complement-dependent bactericidal titers of the rabbit antisera, for the *N. meningitidis* group C living bacteria which, after the booster dose, reached values of positivity at the serum dilution 1:180. The Quellung reaction of the rabbit antisera for the *S. pneumoniae* type 6A and 6B (group 6) living bacteria gave the same positive results as previously reported (Porro *et al.*, 1985). The carrier protein CRM197 induced in guinea-pigs antidiphtheria toxin titers that, after the booster dose, exceeded 3 IU/ml serum, which means more than 300 times the minimum protective level estimated in man for antidiphtheria activity (Wilson and Miles, 1975).

DISCUSSION

Despite the introduction of a new generation of polysaccharide vaccines in the last decade (Gotschlich *et al.*, 1969; Austrian, 1979), the prevention of the infant population getting meningitis by encapsulated bacteria is still an unsolved problem (Makela *et al.*, 1977, 1981; Peltola, 1983). This could also be related to the "T-independent" immunological properties of the polysaccharide antigens (Paul *et al.*, 1971) which do not express in human infants the high level of immunogenicity demonstrated in adults (Gotschlich, 1975; Austrian, 1979). The well-known approach of using the "T-dependent" antigens (mainly proteins) as carriers for oligo- and polysaccharides in animals, appears advantageous to also overcome similar problems in humans (Lepow *et al.*, 1984). However, the use of this kind of molecule as immunogens would require a molecular characterization of the antigen in order to better evaluate the induced immunological response and to facilitate their "*in vitro*" control. In particular it would be possible to map the presence of the desired antigenic sites by determining the specificity of monoclonal and/or polyclonal antibodies towards definite epitopes of the carrier protein as well as the immunodeterminants of the carried carbohydrate hapten(s). Once a given artificial glycoprotein has shown its "*in vivo*" immunogenic potential, the "*in vitro*" molecular mapping by specific antibodies could be predictive for the immunogenicity of a new generation of artificial antigens synthesized using the same model tested. We have applied this concept in synthesizing a glycoprotein of predetermined multivalent antigenicity, based on the results recently obtained with a previous generation of artificial glycoprotein antigen (Porro *et al.*, 1985).

The non-toxic mutant protein CRM197 as a carrier for the oligosaccharide haptens has been already described as a potential antidiphtheria antigen (Pappenheimer *et al.*, 1972; Porro *et al.*, 1980). The use of this protein was also stimulated by the fact that the genetic map of the tox-gene carried by the β-corynephage encoding for the aa sequence of CRM197 in the host *Corynebacterium diphtheriae* strain C7 (Uchida *et al.*, 1973), has been elucidated and the full aa sequence (535 aa) of this cross-reactive antigen deduced (Giannini *et al.*, 1984). The aa sequence of CRM197 appeared identical to that of diphtheria toxin except for the substitution of a Gly residue at position 52 by a Glu residue. Furthermore, the localization of the basic aas Lys and Arg became

possible. Because the adopted procedure of coupling involves the primary amino groups of some of these basic aas, their localization represented an excellent opportunity of investigating the immunochemical characteristics retained by CRM197 after its involvement as a carrier protein for oligosaccharide haptens. In fact, a collection of monoclonal antibodies to diphtheria toxin and CRM197 has recently been developed and at least four main antigenic regions have been identified in these protein structures (Zucker and Murphy, 1984). The antibody directed towards the epitope identified in the 8000-dalton carboxy terminal region of the toxin [approx. aa sequence 465–535) showed one of the strongest antitoxic activity by inhibition of the toxin binding to the cell membrane. This important region of the protein CRM197 contains the 14.5% of the full basic aa content (39 Lys and 16 Arg) and was still reactive in the artificial glycoprotein antigen when tested with the specific group IV monoclonal antibody. In contrast, the other three groups of monoclonal antibodies directed towards their respective epitopes in the protein structure lost more than 50% of their immunochemical specificity when compared to that observed for the native antigen. An attempt to explain these observations was done by considering the presence of Lys and Arg residues in particularly hydrophilic (potentially exposed) regions of the carrier protein, theoretically involved in the glycosylation reaction. According to the reported aa sequence of the protein, predictions of the most hydrophilic regions were done (data not shown) using the Hopp and Woods (1981) analysis. Four regions in the fragment A and seven regions in the fragment B of the protein (Pappenheimer *et al.*, 1972) showing hydrophilicity values higher than 1 were predicted. These regions contained altogether eleven Lys and five Arg residues (respectively 28.2 and 31.2% of the total Lys and Arg content). In the 8000-dalton carboxy terminal region of CRM197 (aas 465–535), only one hydrophilic sequence with a value higher than 1 was predicted [Ser-Ser-Ser-Glu-Lys-Ile (aas 494–499)]. In this hydrophilic sequence only one Lys residue was present, accounting for no more than 6% of the theoretically exposed basic aas. Thus the probability for this region to be involved in the glycosylation reaction is quite low and, as a result, the retained specificity of the group IV monoclonal antibody for this epitope in the artificial glycoprotein should not be surprising. These findings showed that the adopted procedure of coupling did not significantly affect one of the main epitopes of the carrier protein involved in the induction of specific antibodies to diphtheria toxin and gave evidence for CRM197 as a very suitable carrier protein, being *per se* non-toxic and able to elicit anti-hapten-specific antibodies.

The T-dependent immunological response to the homologous bacterial capsular polysaccharides was evidenced in the rabbit by induction of specific IgG immunoglobulins which exponentially increased after that the booster dose of the glycoprotein antigen was administered to the animals (Beuvery *et al.*, 1982). It is well known that the purified polysaccharides do not show a similar immunological property when injected in rabbits (Kabat, 1964; Paul *et al.*, 1971). In addition, rabbit antisera showed a positive "Quellung reaction" (Austrian, 1976) on the polysaccharide capsule of the live strain of *S. pneumoniae* type 6A and 6B (group 6) which have been shown to be immunochemically cross-reactive (Rebers and Heidelberger, 1961; Kenne *et al.*, 1979; Zon *et al.*, 1982) and a complement-mediated bacterial lysis on the live strain of *N. meningitidis* group C.

In conclusion, the model here reported appears consistent for a wide antigenic potential and implies the possibility of developing similar molecules as a new generation of multivalent antigens able to induce a specific T-dependent immunological response towards epidemiologically significant encapsulated bacteria.

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