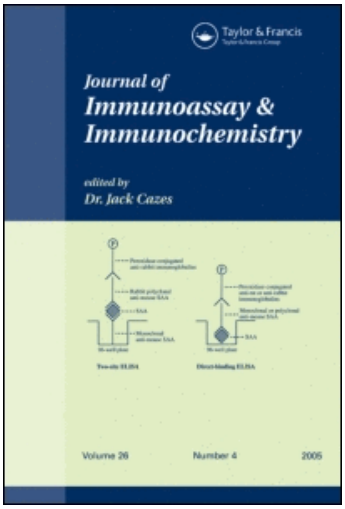

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Monoclonal Antibodies Against two Discrete Determinants on Vi Capsular Polysaccharide

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MONOCLONAL ANTIBODIES AGAINST TWO DISCRETE DETERMINANTS ON Vi CAPSULAR POLYSACCHARIDE.

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ABSTRACT

Vi is a linear homopolymer of 1,4 N-acetyl galactosaminuronic acid. It is present in S.typhi and some other members of Enterobacteriaceae. Vi antigen of S.typhi has been associated with the virulence of the organism and a vaccine based upon this antigen has been found to confer immunity against typhoid. In this paper, we report production and characterization of four hybrid cell clones secreting monoclonal antibodies against Vi capsular polysaccharide. Binding analysis using different derivatives of Vi showed that three monoclonal antibodies reacted with the antigenic determinant constituted by O-acetyl group and one recognised the epitope constituted by N-acetyl and carboxyl groups together. All the antibodies bound to Vi positive strains of S.typhi and did not show any significant reactivity with Vi negative strains of S.typhi, S.paratyphi A, S.paratyphi B and E.coli. Besides their utility in studying the sub-specificity of antibodies produced after vaccination with Vi, these antibodies would be helpful in the diagnosis of typhoid fever. (KEY WORDS : Capsular polysaccharide - CPS, polygalacturonic acid - PGUA, enzyme linked immunosorbent assay - ELISA, monoclonal antibody - MoAb).

INTRODUCTION

The capsular polysaccharide (Vi) of Salmonella typhi is a linear homopolymer of ∝ 1,4 N-acetyl galactosaminuronic acid, variably O-acetylated to about 90% at the C-3 position and is

structurally identical to the CPS of Citrobacter freundii (1-5). Whiteside and Baker (6) have found that purified Vi antigen preparations from Citrobacter ballerup, S.typhi and Escherichia freundii were serologically identical. However, after O-deacetylation minor serological difference between the antigen of S.typhi and C.ballerup was observed (7). Recently Szu et al. (8) have shown by FTIR spectroscopy and ^{13}C.Nuclear Magnetic Resonance that the structures of Vi from WR7011 strain of C.freundii and the Ty2 strain of S.typhi were indistinguishable. Capsular polysaccharides are essential for the invasiveness of capsulated bacteria (9) and vaccines based upon these antigens have been found to induce immunity by eliciting serum antibodies (10-16). The Vi capsular polysaccharide has been found to confer immunity against typhoid fever in 70% of subjects in two clinical trials in areas with high rate of typhoid (17,18). Vi-protein conjugates have been synthesized in order to both enhance the immunogenecity and confer T-dependent properties to Vi (8). This antigen has also been found to be a good candidate for diagnosis of typhoid fever. Patients with typhoid fever are reported to excrete Vi antigen in the urine for which an assay has been proposed (19,20). However, attempts to use Vi-based assays for diagnosis have not been successful mainly due to lack of specific antibodies (21). We report here four hybrid cell clones secreting monoclonal antibodies highly specific for Vi. Their binding pattern further confirms the presence of two antigenic determinants on Vi. The antibodies will be useful in analysing the sub-specificity of protective

antibodies induced by the Vi vaccine and also in the diagnosis of typhoid fever.

MATERIALS AND METHODS

Bacteria

Bacterial strains of S.typhi, S.paratyphi A, S.paratyphi B and E.coli were obtained from the Microbiology departments of Lady Hardinge Medical College and All India Institute of Medical Sciences,New Delhi. The microorganisms were well characterized by biochemical and serological traits. They were grown in trypton-yeast extract (Difco Labs ,Detroit Michigan, USA)-saline broth overnight at 37^0C. Bacterial cells were heat inactivated at 60^0C for 30 min., harvested and washed with saline.

Antigens

Purified Vi antigen was a kind gift of Dr.John B. Robbins, National Institutes of Health,Bethesda. It was prepared from Citrobacter freundii. Polygalacturonic acid (Sodium salt) was obtained from Sigma Chemical Company. Lipopolysaccharide isolated from S.enteritidis was from Difco Labs.

Chemical modifications of Vi and PGUA

Carboxyl-reduced Vi antigen was prepared by the method of Taylor and Conrad (22). Deacetylated derivatives of this compound and the native Vi were prepared by treatment with sodium methoxide and methanol as described by Thompson et al. (23). Polygalacturonic acid was acetylated by the method described by Szewczyk & Taylor (24).

Antiserum:

Commercially available monospecific rabbit anti-S.typhi Vi antiserum was obtained from Central Research Institute, Kasauli, India.

Production and Characterization of Monoclonal Antibodies:

BALB/c By J.Nii mice were immunized subcutaneously with 1 μg of Vi antigen emulsified with complete Freund's adjuvant. After 4 weeks, mice were boosted intravenously with 1-2 μg of Vi in saline on three consecutive days before fusion with SP-2/0 Ag 14 myeloma cells, essentially by the method of Kohler and Milstein (25). Hybrids secreting antibodies were cloned repeatedly by limiting dilution. Hybrid cells were grown as ascites in the peritoneal cavity of Pristane (Aldrich Chemicals) primed mice.

Enzyme linked immunosorbent assay

The reactivity of the monoclonal antibodies was determined by an enzyme linked immunosorbent assay. Briefly 96-well polyvinyl microtitration plates (Flow labs.,Irvine, Scotland, U.K) were coated with 10 μg/ml of Vi, 50 μg/ml of PGUA and Ac-PGUA and 10 μg/ml LPS, all diluted in carbonate buffer (50mM; pH9.6). Different derivatives of Vi were also coated at a concentration of 10 μg/ml. Antigen coating was performed at 37^0C overnight. In another protocol plates were coated with whole bacteria at a concentration of $15x10^6$/well and dried at 37^0C. Plates were washed with PBS-Tween (50mM Phosphate buffer pH 7.4, containing 0.05% Tween 20) and the

non-specific sites were saturated with 1% bovine serum albumin (BSA). In the case of whole cell-ELISA, BSA was added without prior washing. After subsequent washing, plates were incubated with tissue culture supernatants for 1hr at 37^0C. Control wells had supernatants either from SP2/0 or from unrelated hybrid cell clone. Rabbit anti-Vi antibody obtained from Central Research Institute, Kasauli, India, was used as a positive control. Plates were washed with PBS-Tween and incubated with sheep anti-mouse Ig (H+L) coupled to horse radish peroxidase (goat anti-rabbit IgG.HRP was used for rabbit antibody) for 1hr at 37^0C. After washing, the enzyme activity was determined by adding freshly prepared substrate solution (0.5mg/ml orthophenylene diamine dissolved in citrate phosphate buffer, pH 5.6, containing 0.03% hydrogen peroxide). The reaction was stopped with 5N H2SO4 and the absorbance was read at 490nm in a Biotek ELISA Reader.

Competitive ELISA

Monoclonal antibodies were competed with monospecific rabbit anti-Vi antibody for binding to Vi antigen. Vi was coated on the plate. Ascitic fluids of MoAbs were taken at a dilution of 1:200 and the rabbit antibody at 1:400. The antibodies were coincubated in the antigen coated plate for 1 hr at 37^0C. Control wells had ascitic fluid from an unrelated monoclonal antibody. After washing with PBS-Tween, plates were incubated with goat anti-rabbit IgG.HRP and enzyme reaction revealed as described above.

Isotyping:

Heavy chain specificity of the antibodies was determined by double immunodiffusion (Ouchterlony) using goat anti-mouse μ, G1, G2a, G2b and G3 antibodies obtained from Sigma Chemical Co.

RESULTS

Four stable hybrid cell clones secreting antibodies to Vi were obtained from a single fusion. Three of them P6D6A3/D3, P2C2D5/H5 and P5B2D8/A9 were IgM type and the fourth P2B1G2/A9 was IgG1. Table 1 gives the reactivities of these antibodies with Vi and its different analogs. Three monoclonal antibodies reacted equally well with the carboxyl reduced derivative of Vi. However, when this derivative was deacetylated, none of them exhibited reactivity. These three MoAbs also reacted with 0-acetylated polygalacturonic acid. P5B2D8/A9 on the other hand had negligible reactivity with carboxyl reduced Vi and its deacetylated derivative and did not bind to Ac-PGUA. None of the four MoAbs reacted with PGUA.

The binding of these antibodies with various bacteria was investigated by ELISA in which intact bacteria were coated on the solid support. Results are given in Table 2. All MoAbs reacted with S.typhi.They were devoid of significant cross reaction with Vi negative strains of S.typhi, S.paratyphi A, S.paratyphi B and E.coli.

The specificity of the antibodies was further studied by a competitive ELISA. MoAbs were allowed to compete with a well

TABLE 1

Reactivity of monoclonal anti-Vi antibodies[a] with various antigens, as determined by ELISA.

Antigen	Rabbit anti-Vi	A_{490} with various MoAbs			
		P6D6A3/D3 (IgM)	P2B1G2/A9 (IgG1)	P2C2D5/H5 (IgM)	P5B2D8/A9 (IgM)
Vi	1.399	1.485	2.088	1.822	1.628
Deacetylated Vi	N.D	0.087	0.080	0.060	0.100
Carboxyl reduced Vi	1.165	1.539	2.065	2.065	0.103
Carboxyl reduced deacetylated Vi	0.194	0.073	0.097	0.097	0.105
PGUA	0.055	0.148	0	0	0
Ac-PGUA	0.788	1.501	1.669	1.669	0.004
LPS	0	0	0	0	0

[a]The assay is described in Materials and Methods. Values are the averages of three sets of duplicate determinations.

TABLE 2

Binding pattern of monoclonal anti-Vi antibodies with different bacteria, as seen in ELISA[a].

Bacterial species	Rabbit anti-Vi	A_{490} with various MoAbs			
		P6D6A3/D3	P2B1G2/A9	P2C2D5/H5	P5B2D8/A9
S.typhi (9,12;Vi +)[b]	1.018	1.214	1.406	0.851	1.168
S.typhi (9,12;Vi -)	0.129	0.159	0	0	0
S.paratyphi A (1,2,12)	0.134	0.203	0	0	0
S.paratyphi B (1,4,5,12)	0.214	0.186	0	0	0
E.coli	0.085	0.205	0	0	0

[a]The assay is described in Materials and Methods. Values are the averages of three sets of duplicate determinations. [b]Numbers within the parentheses are the O antigens expressed

TABLE 3

Competitive enzyme linked immunosorbent assay[a] using rabbit anti-Vi and monoclonal antibodies.

Antibody	A_{490}	Percent inhibition
Rabbit anti-serum alone	0.571	-
Rabbit anti-Vi plus P6D6A3/D3	0.117	79.5
Rabbit anti-Vi plus P2B1G2/A9	0.135	76.3
Rabbit anti-Vi plus P2C2D5/H5	0.191	67.0
Rabbit anti-Vi plus P5B2D8/A9	0.585	0
Rabbit anti-Vi plus unrelated MoAb	0.577	0

[a]The assay is described in Materials and Methods. Values are the averages of three sets of duplicate determinations.

defined commercially available rabbit anti-S.typhiVi antibody. One MoAb did not compete with the polyclonal antibody. An unrelated antibody also did not show any inhibition (Table 3).

DISCUSSION

The data presented here show that at least two types of monoclonal antibodies were produced against the Vi antigen. Vi is a linear homopolysaccharide of galactosaminuronic acid

with an N-acetyl at carbon-2 and O-acetyl at carbon-3 (Fig 1). The presence of two antigenic determinants on this antigen has been suggested by Szewczyk and Taylor (24). One determinant is constituted by the O-acetyl group and the other by N-acetyl and carboxyl groups together. In the present study three out of four monoclonal antibodies reacted with Vi as well as O-acetylated polygalacturonic acid. The fourth one did not bind to Ac-PGUA. The latter antibody did not also react with carboxyl-reduced Vi or its deacetylated derivative, thereby suggesting that carboxyl as well as N-acetyl groups are involved in the binding of this antibody to Vi. The three MoAbs reacted with the carboxyl reduced Vi but not with its deacetylated derivative or deacetylated native Vi. Thus this set of antibodies is recognizing antigenic determinant constituted by the O-acetyl group. The reactivity of the antibodies is shown diagrammatically in Fig 1. The predominant IgM response could be due to short term immunization schedule or to the carbohydrate nature of the antigen.

The data given in Table 2 show that all the monoclonal antibodies raised against Vi extracted and purified from C.freundii react with S.typhi,demonstrating that Vi antigens isolated from these two bacteria had similar immunologic properties. This is in agreement with earlier studies of Whiteside and Baker (6,7). The weak reaction of P6D6A3/D3 with Vi negative bacteria seems to be a non-specific interaction, since at high antibody concentration P2C2D5/H5

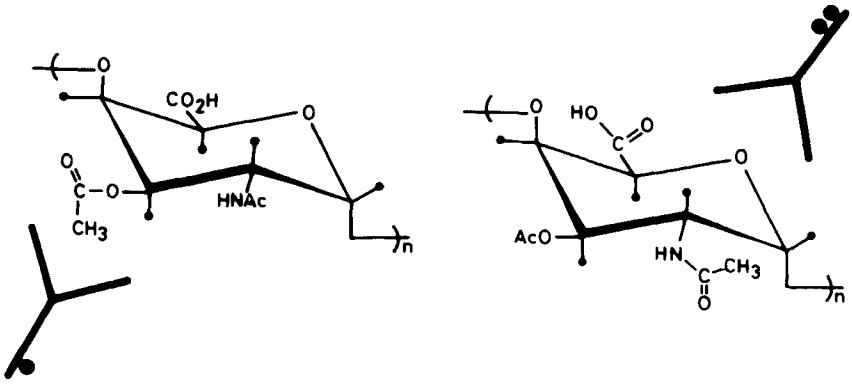

FIGURE 1: Schematic representation of reactivity of anti-Vi monoclonal antibodies.

$Y_{\bullet}$ - Antibodies reacting with the antigenic determinant constituted by O-acetyl group.

$Y_{\bullet\bullet}$ - Antibody binding to the determinant constituted by N-acetyl and carboxyl groups together.

had similar properties (less than 10% binding with Vi negative bacteria).

In the competitive ELISA, only three of the four antibodies could inhibit the binding of a well defined rabbit anti-Vi to Vi. This correlates well with the direct binding ELISA.The MoAb which does not compete with rabbit anti-Vi recognizes the antigenic determinant constituted by N-acetyl and carboxyl groups together. As can be seen from Table 1, the reactivity of polyclonal anti-Vi is not drastically changed

when the carboxyl group of Vi is reduced, thereby suggesting that the antiserum has either very little or no antibodies directed against the determinant constituted by N-acetyl and carboxyl. Furthermore low binding of rabbit antiserum with Ac-PGUA as compared to the whole Vi is suggestive of antibody response against determinants other than the two major ones described above. Antibody response to Vi antigen has been found to vary with the antigenic preparation used for immunization. Felix and Bhatnagar (26) have presented evidence which could be interpreted to mean that treatment of Vi antigen with formalin inactivated the part responsible for protective antibodies. Differences in the antibody response to Vi in rabbits immunized either with S.typhi or C.ballerup has been demonstrated by Szewczyk and Taylor (24). Tsang and Chau (27) have shown that in mice Vi antigen present on whole bacterial cells is less immunogenic as compared to the purified one which seems to be in conformity with the fact that only a small percentage of typhoid patients elicit antibodies to Vi during illness (28,29).

The data suggests that the antigenic determinants constituted by O-acetyl and N-acetyl-carboxyl groups together,are the immunodominant epitopes on the Vi antigen. While Tsang and Chau (27) have raised monoclonal antibodies against Vi , all of them seem to be recognizing the same determinant. The present study is thus the first report where monoclonal antibodies have been obtained against two discrete determinants present on Vi. With the help of these antibodies it should be possible to study the fine specificity of

protective antibodies induced by Vi. This has importance in view of the fact that Vi is undergoing clinical trials as a vaccine (17). These antibodies will also be highly useful for developing an assay for the detection of Vi antigen and alongwith anti-O (30) and anti-H (31) MoAbs, for the detection of S.typhi in clinical specimens (32).

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REFERENCES

1. Clark,W,R., McLaughlin,J. and Webster,M.E. An aminohexuronic acid as the principal hydrolytic component of the Vi antigen. J.Biol.Chem. 1958; 230: 81-89

2. Heyns,K., Kiessling,G., Lindberg,W., Laulsen,H. and Webster,M.E. D-Galaktosaminuronsaure (2-amino 2-deoxy- D - galakturonsaure) als baustein des Vi-antigens. Chem. Ber. 1959; 92: 2435-2438.

3. Heyns,K., and Kiessling,G. Structurafklarung des Vi antigens aus Citrobacter freundii (E.coli)5396/38. Carbohydr.Res. 1967; 3: 340-353.

4. Webster,M.E., Clark,W.R.,and Freeman,M.E. Evidence for an aminohexuronic acid as hydrolytic product of Vi antigen. Arch. Biochem.Biophys. 1954; 50: 223-224.

5. Webster,M.E., Sagin,J.F., Anderson,P.R., Breeze,S.S., Freeman,M.E. and Landy,M. Physico-chemical characterization of Vi antigens isolated from V form of enterobacteriaceae. J. Immunol. 1954; 73: 16-22.

6. Whiteside,R.E. and Baker,E.E. The Vi antigens of the enterobacteriaceae. II Immunologic and biologic properties. J.Immunol. 1959; 83: 687-696.

7. Whiteside,R.E. and Baker,E.E. The Vi antigens of the enterobacteriaceae. V Serologic differences of Vi antigens revealed by deacetylation. J.Immunol. 1961; 86: 538-542.

8. Szu,S.C., Stone,A.L., Robbins,J.D., Schneerson,R. and Robbins,J.B. Vi capsular polysaccharide-protein conjugates for prevention of typhoid fever. J. Exp. Med. 1987; 166: 1510-1524.

9. Jann,K. and Jann,B. Cell surface components and virulence: Escherichia coli O and K antigens in relation to virulence and pathogenecity. The virulence of Escherichia coli.(Edited by M.Sussman) 1985, p. 156-176.Academic press.Inc. New York.

10. Gotschlich,E.C., Rey,M., Sanborn,W.R., Triau,R. and Cjetanovic,B. The immunological responses observed in field studies in Africa with group A menningococcal vaccines. Prog. Immunobiol. Stand. 1972; 129: 485-492.

11. Jennings,H.J. Capsular polysaccharides as human vaccines. Adv. Carbohydr. Chem. Biochem. 1983; 41: 155-190.

12. Kahty,H., Karano,V., Peltola,H. and Makela,P.H. Serum antibodies after vaccination with Haemophilus influenzae type b capsular polysaccharide and response to reimmunization: no evidence of immunological tolerance or memory. Pediatrics 1981; 74: 857-865.

13. Peltola,H., Kahty,H., Sivonen,A. and Makela,P.H. The Haemophilus influenzae type b vaccine in children. A double blind feild study of 100,000 vaccinees 3 months to 5 years of age in Finland. Pediatrics 1977; 60: 730-737.

14. Robbins,J.B. Vaccines for prevention of encapsulated bacterial diseases: current status, problems and prospects for future. Immunochemistry 1978; 25: 839-854.

15. Robbins,J.B., Austrian,R., Lee,C.J., Rastogi,S.C., Schiffman,G., Hendrichson,J., Makela,P.H. and Parke,Jr.,J.C. Considerations for formulating the second generation pneumococcal capsular polysaccharide vaccine with emphasis on the cross-reactive types within groups. J. Infect. Dis. 1983; 148; 1136-1159.

16. Schneerson,R., Rodrigues,P., Parke,J.C. and Robbins,J.B. Immunity to disease caused by Haemophilus influenzae type b.II.Specificity and some biological characteristics of "natural", infection-acquired and immunization antibodies to the capsular polysaccharide of Haemophilus influenzae type b. J.Immunol. 1971; 107: 1081-1090.

17. Acharya,I.L., Tapa,R., Gurbacharya,V.L., Shrestha,M.B., Lowe,C.U., Bryla,D.D., Schneerson,R., Robbins,J.B., Crampton,T., Trollfors,B., Cadoz,M., Schulz,D. and Armand,J. Prevention of typhoid fever in Nepal with the Vi capsular polysaccharide of Salmonella typhi: a preliminary report. N.Engl. J. Med. 1983; 317: 1101-1104.

18. Klugman,K.P., Gilbertson,I., Korhof,H.J., Robbins,J.B., Schneerson,R., Scjhluz,D., Sadoz,M. and Armand,J. Protective effect of Vi capsular polysaccharide vaccine against typhoid fever. Lancet 1987; ii: 1165-1169.

19. Rockhill,R.C., Rumans,L.W., Lesmana,M.,and Dennis,D.T. Detection of Salmonella typhi D,Vi and d antigens by slide agglutination in urine from patients with typhoid fever. J. Clin. Microbiol. 1980; 11: 213-216.

20. Barrett,T.J., Snyder,J.D., Blake,P.A.and Feeley,J.C. Enzyme linked immunosorbent assay for detection of Salmonella typhi Vi antigen on urine from typhoid patients. J.Clin. Microbiol. 1982; 15: 235-237.

21. Taylor,D.N., Harris,J.R., Barrett,T.J., Hargrett,N.T., Prentzell,I., Valdivieso,C., Palomino,C., Levine,M.M. and Blake,P.A. Detection of urinary Vi as a diagnostic test for tryphoid fever. J. Clin. Microbiol. 1983; 18: 872-876.

22. Taylor,R.L. and Conrad,H.E. Stoichiometric depolymerization of polyuronides glycosaminoglycuronans to monosaccharides following reduction of their carbodiimide activated carboxyl groups. Biochemistry 1972; 11: 1383-1388.

23. Thompson,A and Wolform,M.L. Deacetylation. Methods in Carbohydrate chemistry 1963; Vol II: 215-220.

24. Szewczyk,B. and Taylor,A. Immunochemical properties of Vi antigen from Salmonella typhi Ty 2. Presence of two antigenic determinants. Inf. Immun. 1980; 29: 539-544.

25. Kohler,G. and Milstein,C. Continuous cultures of fused cells secreting antibodies of predicted specificity. Nature (London) 1975; 256: 495-497.

26. Felix,A and Bhatnagar,S.S. Further studies on the properties of Vi antigen of B.typhosus and its corresponding antibody. Br.J.Exp.Pathol. 1935; 16: 422-434.

27. Tsang,M.S.W. and Chau,P.Y. Production of Vi monoclonal antibodies and their application as diagnostic reagents. J. Clin. Microbiol. 1987; 25: 531-535.

28. Felix,A., Krikorian,A.S.,and Rettler,R. The occurence of typhoid bacilli containing Vi antigens in case of typhoid fever abd vi antibodies in their sera. J.Hyg. 1935; 35: 421-427.

29. Lanata,C.F., Ristori,C., Jimenez,L., Garcia,J., Levine,M.M., Black,R.E., Saleedo,M. and Sotomayor,V. Vi serology in detection of chronic Salmonella typhi carriers in an endemic area. Lancet 1983; ii: 441-443.

30. Qadri,A., Gupta,S.K. and Talwar,G.P. Monoclonal antibodies delineate multiple epitopes on the O-antigens of Salmonella typhi lipopolysaccharide. J. Clin. Microbiol. 1988; 26: 2292-2296.

31. Qadri,A., Ghosh,S., Upadhyay,S. and Talwar,G.P. Monoclonal antibodies against flagellar antigen of Salmonella typhi. Hybridoma 1989; 8: 353-359.

32. Qadri,A., Ghosh,S., Prakash,K., Kumar,R., Moudgil,K and Talwar,G.P. Sandwich enzyme immunoassays for detection of Salmonella typhi. Submitted for publication.

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