On the Antigenic Determinants of the Lipopolysaccharides of Vibrio cholerae O:1, Serotypes Ogawa and Inaba*

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Monoclonal, murine IgG1s S-20-4, A-20-6, and IgA 2D6, directed against Vibrio cholerae O:1 Ogawa-lipopolysaccharide exhibited the same fine specificities and similar affinities for the synthetic methyl α-glycosides of the (oligo)saccharide fragments mimicking the Ogawa O-polyasaccharide (O-PS). They did not react with the corresponding synthetic fragments of Inaba O-PS. IgG1s S-20-4 and A-20-6 have absolute affinity constants for synthetic Ogawa mono- to hexasaccharides of from $10^5$ to $10^6$ M$^{-1}$. For IgG1s S-20-4, A-20-6, and IgA 2D6, the nonreducing terminal residue of Ogawa O-PS is the dominant determinant, accounting for $90\%$ of the maximal binding energy shown by these antibodies. Binding studies of derivatives of the Ogawa monosaccharide and IgGs S-20-4 and A-20-6 revealed that the 2-O-methyl group fits into a somewhat flexible antibody cavity and that hydrogen bonds involving the oxygen and, respectively, the OH at the 2- and 3-position of the sugar moiety as well as the 2'-position in the amide side chain are required.

Monoclonal IgA ZAC-3 and IgG2 I-24-2 are specific for V. cholerae O:1 serotypes Ogawa/Inaba-LPS. The former did not show binding with members of either series of the synthetic ligands related to the O-antigens of the Ogawa or Inaba serotypes, in agreement with its reported specificity for the lipid/core region (1). Inhibition studies revealed that the binding of purified IgG2 I-24-2 to Ogawa-LPS might be mediated by a region in the junction of the OPS to the lip-id-core region of the LPS.

cDNA cloning and analysis of the anti-Ogawa antibodies S-20-4, A-20-6, and 2D6 revealed a very high degree of homology among the heavy chains. Among the light chains, no such homology between S-20-4 and A-20-6 on one hand, and 2D6 on the other hand, exists. For the anti-Inaba/Ogawa antibodies I-24-2 and ZAC-3, their heavy chains are completely different, with some homology among the light chains.

Vibrio cholerae O:1 is subdivided into two serological types, Inaba and Ogawa. The internal part of their O-polyasaccharide chains (O-antigen) are homopolymers of (1→2)-linked linear

4,6-dideoxy-4-(3-deoxy-l-glycero-tetramamido)-o-mannopyranosyl residues. The two types apparently differ by the presence of a 2-O-methyl group in the nonreducing terminal sugar of the Ogawa O-antigen, absent in the Inaba O-antigen (2, 3).

We used synthetic mono- to hexasaccharides that mimic the fragments of the O-antigen of Ogawa and Inaba O-polyasaccharides (2→4), together with certain analogs of their monosaccharides to evaluate specificity. The binding of three immunoglobulins G (two specific for Ogawa and one specific for Ogawa/Inaba) and of two immunoglobulins A (one specific for Ogawa and one specific for Inaba/Ogawa) were characterized by ligand-induced fluorescence titration or ELISA inhibition. The cDNA sequences of these antibodies are also presented in this report.

MATERIALS AND METHODS

Monoclonal Antibodies—Murine ascites fluids of A-20-6 and S-20-4 both contain vibriocidal IgG1 specific for Ogawa-LPS. 1 I-24-2, in contrast, contains IgG3, specific for both serotypes Ogawa and Inaba-LPS, and it has low vibriocidal activity (5) (clone S-20-4 comes from the same hybridoma cells as clone S-20-3 described in this reference). Murine ascites fluid 2D6 and ZAC-3 contain IgA specific for Ogawa-LPS and IgG specific for Inaba/Ogawa-LPS, respectively. The latter two hybridomas were gifts from Drs. Marian Neutra, Harvard Medical School, and Dr. Richard Weltzin, Oravax, Cambridge, MA (1, 6) and were grown in BALB/c mice. IgGs were purified using ImmunoPure® (G) IgG purification kits (Pierce). Briefly, ascites fluid (2 ml, clarified by centrifugation) was mixed with ImmunoPure® (G) binding buffer (2 ml) and applied to a protein G column. After washing the column with 5 × 2 ml aliquots of the ImmunoPure® (G) binding buffer, the bound IgG was eluted with 6 ml of ImmunoPure® (G) elution buffer, dialyzed against PBS, pH 7.4 (2000 ml) for three changes at 0°C, frozen, and labeled. The purified A-20-6, S-20-4, and I-24-2 showed a single arc of precipitation versus goat anti-mouse IgG, and IgG3, respectively (heavy chain-specific), and goat anti-whole mouse serum (Sigma) by immunoelectrophoresis. IgAs were purified from ascites fluid by 40% ammonium sulfate precipitation and anion-exchange DEAE-Sephadex A-25 chromatography (7). Monomeric IgA was obtained by mild reduction with 5 μl 1,4-dithiothreitol (Sigma) and alkaline with 11 μl 2-iodoacetamide (Sigma), followed by re-adsorption of the sample on DEAE-Sephadex A-25 and elution with PBS, pH 7.4. The purity of IgAs was also verified by immunoelectrophoresis against anti-mouse IgA and serum and SDS-polyacrylamide gel electrophoresis.

LPS and Synthetic Oligosaccharides—V. cholerae O:1 LPSs were obtained from acetone-treated cells of strain 569B, classical biotype, serotype Inaba, lot VC1219; strain 3083, classical biotype, serotype Ogawa; and V. cholerae O:139 Bengal, strain 4450. Salmonella paratyphi A LPS was a field isolate in Nepal, strain NTP-6. All LPSs were purified as described (8) and at 2 mg/ml showed negative tests (Comassie Blue) for protein. Severely base-degraded V. cholerae O:139 LPS (9) was a gift from Dr. Andrew D. Cox, National Research Council, Ottawa, Canada. De-O-acylated Ogawa-LPS (10) was oxidized in aqueous 0.8% periodate solution for 3 days, in the dark, and freeze-dried and then reduced in aqueous sodium borohydride (11) to convert O-acetyl groups to alcohol groups to give O/R-DeOAc-Ogawa-LPS. As-prepared Ogawa-LPS was oxidized in aqueous 0.8% periodate solution for 3 days in the dark, and freeze-dried and then reduced in aqueous sodium borohydride (11) to convert aldehyde groups to alcohol groups to give O/R-DeOAc-Ogawa-LPS. As-prepared Ogawa-LPS was oxidized in aqueous 0.8% periodate solution for 3 days in the dark, and freeze-dried and then reduced in aqueous sodium borohydride (11) to convert aldehyde groups to alcohol groups to give O/R-DeOAc-Ogawa-LPS.
ple. The synthesis of methyl α- isomaltoside has been described (13). D-Glucose, methyl α-D-glucopyranoside, methyl β-D-glucopyranoside, D-galactose, methyl α-D-galactopyranoside, D-mannose, methyl α-D-mannopyranoside, D-mannohexose, D-glucosamine, hydrochloride, 2-keto-3-deoxy-D-manno-octonate, D-fructose, and dextran 107 were purchased from Sigma. Methyl β-D-glucopyranoside was obtained from the National Institutes of Health Carbohydrate Collection. Synthetic methyl α-glycosides of the mono- to hexaaccharides of the α-antigen of V. cholerae O:1, serotypes Ogawa (15 to 6) (Fig. 1) and Inaba (1 to 6), as well as the preparation of methyl 4-acetamido-4,6-dideoxy-2-O-methyl-α-D-mannopyranoside (15-Ae), methyl 4,6-dideoxy-4,3-deoxy-l-glycero-2-hexulopyranosidido-2-O-ethyl-D-mannopyranoside (15-Pr), methyl 4,6-dideoxy-4,3-deoxy-l-glycero-tetronamido-2-O-propyl-α-D-mannopyranoside (15-Pr), methyl 2,4,6-trideoxy-4,3-deoxy-l-glycero-tetronamido-α-D-mannopyranoside (15-2d), methyl 3,4,6-trideoxy-4,3-deoxy-l-glycero-tetronamido-α-D-mannopyranoside (15-3d), methyl 4,6-dideoxy-4,3-deoxy-l-glycero-tetronamido-2-O-methyl-α-D-mannopyranoside (15-D), methyl 4,6-dideoxy-4-(4-hydroxybutyramido)-2-O- methyl-α-D-mannopyranoside (15-4d), methyl 4,6-dideoxy-4-(3,4-dideoxy-l-glycero-tetronamido)-2-O-methyl-α-D-mannopyranoside (15-3d), and methyl 4,6-dideoxy-4-(2-deoxy-l-glycero-tetronamido)-2-O-methyl-α-D-mannopyranoside (15-2d,3,5-OH) have been described elsewhere (14–20). 3-Deoxy-N-isopropylidene-L-glycero-tetronic acid hydrazide (TAH) was prepared as follows: 3-deoxy-N-isopropylidene-L-glycero-tetronic acid (for the VH) and 3-deoxy-N-isopropylidene-L-glycero-tetronic acid hydrazide (for the VL): a mixture of 3-deoxy-l-glycero-tetronalactone (161) (1 g) and hydrazine hydrate (17 ml) was stirred at room temperature overnight, when TLC (51:1 CHCl 3 :MeOH) showed that all starting lactone was consumed. The solution was dried at 60 °C in vacuum for 15 min. Crystallization from MeOH–EtOAc gave 3-deoxy-l-glycero-tetronic acid hydrazide, m.p. 108–108.5 °C, [α] D 42.2° (c 0.8, H 2O). 3-Deoxy-4-(3-deoxy-L-mannopyranoside (6 ml) at 50 °C for 15 min. Crystaline, NTAH dication were applied using 20 pmol of each flanking primer in a reaction mixture containing the Elongase enzyme mix (Life Technologies, Inc.) at a concentration of 1.5 mM KCl. Thirty cycles of denaturation (94 °C), annealing (55 °C), and extension (72 °C) were done in a thermal cycle (Ericon). Positive PCR products were obtained on 1.0% low melting agarose gels using AgarACE enzyme (Promega) to extract the DNA and ligated to pCRII vectors as described by the manufacturer (Invitrogen). White colonies were selected and screened by restriction analysis for cloned inserts. The isolated V L and V H clones were grown in YT medium to produce phage, and the single-stranded DNA was purified (21). The DNA was then subjected to single-stranded DNA sequencing according to the sequencing protocol provided by the manufacturer (Sequenase, U. S. Biochemical Corp.). For the hybridoma cells producing A-20-6, S-20-4, and I-24-2 antibodies, the total RNA was extracted by a modification of a known method (22). The reaction mixture (100 μl) containing maximally 10 μg of total RNA, 20 mM mercaptoethanol, 80 units of RNasin (RNase inhibitor, Promega) 1 × first strand buffer (Life Technologies, Inc., Basel, Switzerland), 8 mM dithiothreitol, 0.1 μg/ml primer d(T) 18 (Boehringer Mannheim GmbH, Mannheim, Germany), 500 μM each dNTP, and 400 units of Moloney murine leukemia virus reverse transcriptase (Promega, using their protocols) was incubated for 2 h at 42 °C. The reaction mixture was then used directly for PCR amplification. For that, each cDNA mixture so obtained was divided into, respectively, eight (for the VH) and ten (for the VL) separate mixtures, each one of which was then treated with the appropriate, separate, back primers. A 50-μl reaction mixture containing 2 μl of the reaction mixture from the cDNA synthesis, 30 pmol of one of the back primers, 30 pmol of forward primer, 500 μM dNTP, 1 mmol of MgSO 4, 0.05 × Thermopol buffer, and 1 unit of Vent DNA polymerase (New England Biolabs) or Taq DNA polymerase (U. S. Biochemical Corp.). The reaction mixture was subjected to 40 cycles of amplification, each one 1-min denaturation, 1-min annealing (54 °C), and 2-min extension (72 °C). Products (5 μl) were analyzed on a 2% agarose gel. The back primer that yielded products used in the amplification of the VL of clones A-20-6 and S-20-4 was V H-1-D (5′-GAA GTT ATG CTC GAG CAG TCT GGA GC-3′), and the γ-chain primer (C H) used as forward primer was γ1 (5′-AGG GCT ATT ACA ATA CCT GGG CAC AAT-3′) for the clones A-20-6 and S-20-4. For the VH of I-24-2 the Pharmacia heavy chain primer kit was used to amplify the VH. The primers used for the amplification of each of V L of three clones were the following: for I-24-2 the back primer V L 1 (5′-CAG GAA GTG AGG TCG TGA CCC AGA ATG CTG CA-3′) was used, while VL 10 (5′-GGG AAT TCA TGA CCT GGA (C/T,C,T,G,A/G,C,T,C,A,T,G,A/T) GA,C,T,C,A-3′) TCT-3′) was used as the back primer for A-20-6 and S-20-4. The λ-chain primer (C L) (5′-GAC ACG CTT TGA CTT TGA AAG CTT TGA CAT CTT GCT GAA-3′) was used as forward primer for the clones A-20-4 and S-20-4. The ϵ-chain primer used for the clone I-24-2 was 5′-GGG CCG TCT GAC ATT ACT AAT CCT TGC GTA A-3′ (23, 24). The back primers were designed to hybridize to the partially conserved sequences in the leader or the FR1 regions of the V H or V L. The forward primers corresponded to the N-terminal beginning of the hinge region and the C-terminal part of the C H, region, respectively. Direct sequencing of these PCR products was performed on an Applied Biosystem Apparatus ABI 373A (Genome Express, Grenoble, France) using dye terminators. Sequence data were analyzed and compared, performed with software from the Genetics Computer Group, Inc. (Madison, WI) and the GeneBank™ (Los Alamos, NM) and EBI (Heidelberg, Germany). Fluorescence Titration—Purified immunoglobulins were diluted with PBS, pH 7.4, to solutions having an A 280 of 0.04. Protein solutions (1.1 ml) were added to each of two cuvettes, one for ligand addition, the other a reference. A third cuvette filled with 1.1 ml of ligand solution to the test cell. Affinity constants (K D) for the association between ligands and antibodies were obtained by monitoring the ligand-induced changes in fluorescence of the antibody as a function of ligand concentration (25, 26). We used a Perkin-Elmer LS 50 luminescence spectrophotometer and
to ligand addition, divided by the maximal ligand-induced fluorescence change, \(1 - \nu\) is the fraction of free antibody sites, and \(C_L\) is the concentration of free ligand. A Scatchard plot of \(n/C_L\) versus \(n\) gives a line whose slope is equal to the \(K_a\). Two representative Scatchard plots are shown in Fig. 2: one for the \(K_a\) of IgG, A-20-6 with the Ogawa monosaccharide determinant \(1^0\) and one for the \(K_a\) of IgG, S-20-4 with the Ogawa pentasaccharide \(5^0\). These are expressed either in M or (Table I) as the free energy (kJ) of binding \(\Delta G = -RT\ln K_a\).

**ELISA for Inhibition of Antibody Binding**—The binding constants for the antibodies that did not show a significant change in fluorescence following addition of ligand were determined by ELISA inhibition. For anti-Ogawa IgA 2D6 (6), Ogawa-LPS was the capturing reagent. In the case of anti Inaba-Ogawa IgA ZAC-3 and IgG3 I-24-2, both the Ogawa- and Inaba-LPS were used as capturing reagents in screening with \(1^0\) to \(4^0/4^1\) to \(4^2\), and \(1^0\) to \(6^0/6^1\) to \(6^2\), respectively. In screening of IgG3 I-24-2 with core-related haptens (see Table II), Ogawa-LPS was the capturing reagent.

For IgA 2D6/Ogawa-LPS, the amount of ligand required for 50% inhibition in the ELISA was determined. Since \(K_a = n(1 - \nu) C_L\) (see “Fluorescence Titration” section), at 50% inhibition, assuming solution behavior in ELISA, we have that \(\nu = (1 - \nu)\). Thus, the reciprocal of the molar concentration causing 50% inhibition was taken to be the (relative) \(K_a\). Microtiter plates (96-well; Maxisorb, Nunc, Denmark) were coated with 100 µl of either Ogawa- or Inaba-LPS (10 µg/ml) in 10 mM MgCl2 PBS solution at room temperature overnight. Ascites fluids (1:4000 dilution), purified 2D6, ZAC-3 (1:200 dilution), and purified I-24-2 (1:800 dilution) were incubated in quadruplicate with inhibitors in various concentrations (1–2500 µg/ml PBS) or with PBS for 1 h at 37 °C, followed by 4 °C overnight. Samples were centrifuged and supernatants (100 µl) delivered to microtiter plates. ELISA was performed as described (27). A standard curve for \(A_{405}\) versus antibody concentration added without inhibitor was fitted to the quadratic equation \(aX^2 + bX + c\), where \(X\) is the optical density observed. Correlation factors \((r)\) of 0.999 were routinely obtained. Based on that standard curve, residual, bound antibody in the presence of inhibitor was determined (\(A_i\), and
RESULTS AND DISCUSSION

Antigenic Epitopes on Ogawa-LPS—IGG1, A-20-6 and S-20-4, specific for Ogawa-LPS (vibriocidal titer: 1280), protect mice from mortal challenge with three times the LD50 of V. cholerae O:1 serotype Ogawa strain 920139 (5). Thus, a study of their combining sites is of interest, as oligosaccharide fragments of the O-antigen that bind immunoglobulins maximally will be more likely, when linked to a carrier protein, to elicit antibodies reactive with the parent polysaccharides (28). Each synthetic mono- to hexasaccharide fragment of the Ogawa O-antigen bound the two antibodies, with binding constants of from $-10^3$ to $-10^6$ m$^{-1}$ as measured by fluorescence titration (Table I). The free energies of association for oligosaccharide with each of the two antibodies were close, indicating that these two vibriocidal antibodies have the same fine specificity for the epitope on the Ogawa O-PS. The terminal, nonreducing Ogawa-monomosaccharide (1$^o$) contributed $-90\%$ of the maximal binding energy shown by the entire hexasaccharide. This finding differs from those in some other (homo)polysaccharide-antibody systems (29), where monosaccharide binding accounts for only 50–60% of the maximum binding energy that is shown by the binding of four to six sugars. Methyl 4,6-dideoxy-4-(3-deoxy-1-glycero-tetronamido)-2-O-ethyl-a-D-mannopyranoside (1$^o$-Et) showed an affinity constant for both these antibodies that was an order of magnitude less than that of the Ogawa monosaccharide. Neither the corresponding monosaccharide with a free OH group at C-2 (1$^o$), nor its 2-deoxy derivative (1$^o$-2d), 3-deoxy derivative (1$^o$-3d), 2-O-propyl derivative (1$^o$. Pr), or the one deoxygenated at the 2’-position in the tetronic acid moiety (1$^o$.2’d), exhibited binding. Removal of the primary hydroxyl group in the tetronic acid group gave (1$^o$.4’d), which bound to IgGs A-20-6 and S-20-4 with higher affinity than did 1$^o$ itself (Table I). A hydrophobic pocket of defined size in the antibody might interact with the 2-O-methyl group of the terminus of the Ogawa O-PS. Alternatively, the presence of an O-methyl group at C-2 could dictate a particular conformation due to a pattern of hydrogen bonding that is absent when C-2 bears an OH group. Similarly, increased hydrophobicity, or differently routed, multiple hydrogen bond interactions might also explain why 1$^o$.4’d could have a higher affinity than does 1$^o$. Neither N-isopropylidene-l-tetronic acid hydrazide (TAH) nor methyl 4-acetamido-4,6-dideoxy-2-O-methyl-a-D-mannopyranoside (1$^o$.Ac) showed measurable binding. The monosaccharide corresponding to 1$^o$, but bearing a N-tetronic group instead of the natural l-isomer (1$^o$.D), bound to both IgGs A-20-6 and S-20-4, albeit with an affinity 2 orders of magnitude less ($K_a = -10^3$ m$^{-1}$) than did 1$^o$. None of the synthesized O-antigen saccharidic fragments of the Inaba series, from mono- to hexasaccharides, i.e. those that lack the 2-O-methyl group in the upstream terminal monosaccharide, bound to either of the two IgGs specific for the Ogawa-LPS. Therefore, the 2-O-methylated, terminal monosaccharide seems to be the dominant serotype-specific determinant for Ogawa strains. Furthermore, the Ogawa-specific antibodies do not show any appreciable affinity for internal residues, shared by the O-poly saccharides of both Inaba and Ogawa strains.

Are the fine specificities of the IgA 2D6 (6) and IgGs A-20-6 and S-20-4 for the same epitope? Since IgA 2D6 did not show ligand induced fluorescence change, this was investigated by ELISA inhibition of binding of the IgA to Ogawa-LPS. In Fig. 3 it can be seen that LPS (molecular weight ~8000 daltons), as well as 1$^O$ to 4$^O$ inhibit the binding of IgA 2D6 to Ogawa-LPS.

**TABLE I**

The binding constants ($K_a$, m$^{-1}$) and free energy of association ($-\Delta G^o$, kl/mol) for vibriocidal immunoglobulins G specific for LPS of serotype Ogawa, V. cholerae O1 with synthetic Ogawa fragments and monosaccharide derivatives

| IgG1 | Synthetic fragments of Ogawa O-antigen | 2$^o$ | 3$^o$ | 4$^o$ | 5$^o$ | 6$^o$ |
|------|--------------------------------------|------|------|------|------|------|
| A-20-6 | $-\Delta G^O$ | 34.2 | 34.2 | 35.2 | 33.6 | 32.9 | 30.1 |
| | $K_a$ | $1.0 \times 10^5$ | $9.7 \times 10^5$ | $1.5 \times 10^6$ | $7.7 \times 10^5$ | $5.8 \times 10^5$ | $1.9 \times 10^5$ |
| S-20-4 | $-\Delta G^O$ | 34.9 | 34.9 | 35.4 | 33.8 | 34.7 | 31.9 |
| | $K_a$ | $1.3 \times 10^6$ | $1.1 \times 10^6$ | $1.6 \times 10^6$ | $8.5 \times 10^5$ | $1.2 \times 10^6$ | $3.9 \times 10^5$ |

| Synthetic derivatives of Ogawa monosaccharide | 1$^o$.Ac | TAH |
|-----------------------------------------------|--------|-----|
| S-20-4 | $-\Delta G^O$ | 0 | 26.0 | 0 | 19.4 | 0 | 0 | 0 | 36.2 | 19.4 | 0 | 0 |
| | $K_a$ | 3.6 $\times 10^4$ | 2.5 $\times 10^3$ | 0 | 0 | 2.2 $\times 10^5$ | 2.5 $\times 10^3$ | 0 | 0 |
| A-20-6 | $-\Delta G^O$ | 0 | 25.3 | 0 | 20.7 | 0 | 0 | 0 | 33.1 | 18.0 | 0 | 0 |
| | $K_a$ | 2.7 $\times 10^4$ | 4.3 $\times 10^3$ | 0 | 0 | 6.3 $\times 10^5$ | 1.4 $\times 10^3$ | 0 | 0 |

*Correlation factors ($R^2$) for the Scatchard plots of the affinity constants are between 0.990 and 0.911 except where noted, when it is 0.877.
while 1^1 and 1^1-2d show (weak) nonspecific inhibition. The inhibition curves of the Ogawa tetra- and trisaccharide are identical. There was little difference in slope between the mono-, di-, and trisaccharide. For 50% inhibition of the binding of the antibody to Ogawa-LPS, mono-, di-, and trisaccharides were required in concentrations of 34, 12.5, 5, and 5 μM, respectively. Their relative affinities were therefore 3 x 10^4, 8 x 10^4, 2 x 10^5, and 2 x 10^6 M^-1, respectively. So, this antibody also shows a (relative) free energy of binding for the monosaccharide 1^1 that is quite dominant (some 85% of the free energy of binding shown by the trisaccharide 4^1). Thus, our results for the binding of two vibriocidal IgGs A-20-6 and S-20-4 as well as IgA 2D6 to a series of saccharides mimicking the Ogawa O-antigen have provided strong evidence that these anti-Ogawa-LPS IgGs and the IgA are specific for the same antigenic epitope. The terminal monosaccharide, bearing the 2-O-methyl group, in the O-antigen of Ogawa-LPS is most likely the serotype-specific determinant for the Ogawa strain. It is not unusual that small determinants can dictate an immune response in mice (30).

**Antigenic Epitopes on Inaba-LPS—Synthetic oligosaccharide fragments reflecting the structural difference between Ogawa and Inaba O-antigens were used to study the specificity of monoclonal IgA ZAC-3, obtained from a lymphocyte of the mouse’s Peyer’s patches following immunization with V. cholerae O:1 serotype Inaba (1), and monoclonal IgG3 1-24-2, obtained following immunization of mice with a lysate of V. cholerae O:1 serotype Inaba (5). Others showed (1) that the IgA ZAC-3 dimer and polymer bound all the fragments (including the 3–4-kDa fragment) of V. cholerae LPS (Inaba), indicating that the determinant epitope for this IgA is located in the lipid A or core region, and not in the O-specific side chain. These workers also showed by microcalorimetric measurements that the Kd for IgA ZAC-3 and detergent-solubilized LPS was ~6 x 10^6 M^-1. Their proposed specificity of ZAC-3 for the lipid/core (1) would accommodate our results described below, namely that none of our Ogawa or Inaba (oligo)saccharides show binding to that IgA.

IgG3 1-24-2 is reported to possess weak agglutination titers against Ogawa and Inaba organisms and a weak vibriocidal titer against either organism (5). No ligand-induced fluorescence change was observed with antibody IgG3 I-24-2 and either 1O to 6O or 1O to 4O with or without ZAC-3 and either 1O to 6O or 1O to 4O. The absence of binding of these ligands to these two systems (ZAC-3 and 1-24-2) purified antibodies was verified by ELISA inhibition systems (antibody/Ogawa- or Inaba-LPS). Indeed, these saccharides also failed to show interaction with either antibody in the ELISA system employing either LPS-Ogawa or LPS-Inaba as the capturing agent, except for Inaba monosaccharide 1^1, which could moderately inhibit (50%) only the IgG3 1-24-2/Ogawa-LPS system (see Table II), but not the ZAC-3/Ogawa-LPS or ZAC-3/Inaba-LPS ELISA system. (It is puzzling to us why only the mono- and not the higher oligosaccharides of the Inaba series is able to inhibit the IgG3 1-24-2/Ogawa-LPS system.) The binding of either IgA ZAC-3 or 1-24-2 to ELISA plates, using either the LPS-Ogawa or LPS-Inaba as the capturing agent, could be inhibited by either LPS.

To elucidate the inhibitory effect by various other potential inhibitors in the 1-24-2/Ogawa-LPS ELISA system we examined the ligands shown in Table II. Using whole ascites fluid, Inaba- and Ogawa-LPS were the most effective inhibitors (90% inhibition). V. cholerae O:139 LPS, 2-glucose, Inaba monosaccharide, and 2-galactose all showed moderate inhibition. The V. cholerae O:139 LPS has a very short O-PS that is structurally unrelated to the O-PS of V. cholerae O:1 (31–35) and has its core region in common with that of V. cholerae O:1 Ogawa and Inaba (9), save for the fact that the O:139 has a branch of isosmaltose instead of just glucose attached to its core. The O:139 LPS did weakly inhibit the interaction of both ascites fluid and purified IgG3 1-24-2 with Ogawa-LPS as the capturing reagent. Severely base-degraded O:139 LPS (9) consists of the core region that is flanked at the upstream end by the base-stable remnant of its short O-PS, namely two carbohydrate residues: a threo-hex-4-enuronopyranosyl residue linked to 2-amino-2,6-dideoxy glucosyl residue, while at the downstream end of the core two phosphorylated glucosaminyl residues remain. That product did not react with ascites fluid, but did weakly inhibit the interaction of purified IgG3 1-24-2 with
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Ogawa-LPS as the capturing reagent. O/R-DeOac-Ogawa-LPS is a substance that has only its core destroyed by the periodate oxidation (borohydride reduction was executed to render the aldehyde groups formed chemically unreactive), since the core has vicinal hydroxyl groups (33). Its O-PS is unaffected by the periodate oxidation (borohydride reduction was executed to render the aldehyde groups formed chemically unreactive), since the core is a substance that has only its core destroyed by the periodate oxidation, as we showed by measuring its \( k \) values these antibodies have with the hexameric O-PS saccharide \( 6^6 \). This O/R-DeOac-Ogawa-LPS was able to inhibit IgG3 I-24-2 on ELISA (see Table II) only 70\%, whereas the LPS itself could do so nearly completely (90\%). Since it is unlikely that the lipid plays a role, this could indicate a partial specificity of IgG3 I-24-2 for the core, as that is the only part of the molecule that would be destroyed by periodate oxidation. Dextran 10T, D-glucose, methyl \( \alpha \)-D-glucopyranoside, \( \alpha \)-mannotriose, and methyl \( \alpha \)-L-mannopyranoside (the latter two are structurally related to the heptose in the core region) showed moderate to significant (50 or 70\%) inhibition. The inhibition by \( \beta \)-galactose and methyl \( \beta \)-galactopyranoside also remains unexplained. Although from the above it does appear to recognize determinants in the core region of the LPS, an immunoblotting experiment (5), shows IgG3 I-24-2 to bind only to the high molecular weight LPS from both Inaba and Ogawa V. cholerae O:1.


d-together with the same family, as they show a high degree of homology. It is interesting that IgGs A-20-6 and S-20-4 have identical (\( \lambda \)) V\( \lambda \) regions, whereas IgA 2D6, possessing a k L-chain, has a V\( \lambda \) sequence that is therefore quite different. Since they show identical specificity patterns, that may be dictated more by the H- than by the L-chain. It is noteworthy that the IgGs A-20-6 and S-20-4 show significant ligand-induced tryptophan fluorescence change, while IgA 2D6 does not. The former two IgGs both possess a tryptophan residue at position L-91, while the latter (IgA) carries a glycine at that position. Tryptophan at that position of the L-chain has been correlated before with identical specificity patterns, that may be dictated more by the hypervariable regions of the VL.


cDNA-derived amino acid sequences of the anti-Ogawa immunoglobulins IgG3(s) A-20-6 and S-20-4, as well as IgA(s) 2D6.

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