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POTENTIATION OF THE SECRETORY IgA RESPONSE BY ORAL AND ENTERIC ADMINISTRATION OF CP 20,961*

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A protective immune response against gastrointestinal pathogens requires that specific secretory IgA antibodies be generated and transported across the appropriate mucosal surfaces.[1] Oral priming has been shown to be superior to parenteral inoculation in generating such a response.[2] With the exception of intraperitoneal priming, immunization by other parenteral routes may cause depression of subsequent IgA responses. In addition, inactivated, attenuated or toxoided antigens often fail to exhibit the essential immunogenic properties needed for protective mucosal immunity. Thus the development of adjuvants to enhance the immunogenicity of enteric vaccines is essential.[3]

We have performed the following studies to determine the effectiveness of the lipoidal amine adjuvant *N*, *N*-dioctadecyl-*N'*, *N'-bis* (2-hydroxyethyl) propanediamine (CP 20,961) in enhancing mucosal immunity against cholera toxin, reovirus serotype 1/Lang, and *S. mutans*. Cholera toxin binds to all intestinal epithelial cells; serotype reovirus 1/Lang binds only to the membranous cells overlying Peyer's patches. *S. mutans* is an oral pathogen responsible for dental caries and binds to teeth and oral mucosal membranes.

Intraduodenal (ID) priming[1] with 10 μg of cholera toxin in a soybean oil lipid emulsion containing 0.3 mg CP 20,961 resulted in fourfold enhancement of toxin-specific IgA in intestinal secretions, as measured by enzyme-linked immunosorbent assay (ELISA). Maximal titer was achieved 14 days following a single ID priming dose of CP 20,961 and toxin (TABLE 1 and FIGURE 1).

Mice were primed ID with live reovirus, 10^8 plaque-forming units (pfu), in the presence or absence of the CP 20,961 adjuvant. The recovery of reovirus in intestinal homogenates from mice receiving virus plus adjuvant or virus alone was similar (1.9×10^6 pfu/ml. and 2.0×10^6 pfu/ml., respectively). Reovirus specific IgA levels present in the intestines were determined by ELISA (TABLE 2). Secretory antibody levels were approximately 2.5 times higher in mice that received reovirus plus adjuvant compared to mice that received reovirus alone. Maximal antibody titers were achieved on day 7 in both groups. The antibody titers decreased by day 14, but this decrease was much less for the adjuvant-treated group than that seen for reovirus alone. It is not known whether the earlier appearance of high antibody titers with reovirus is due to the capacity of reovirus to replicate or its preferential accumulation in Peyer's patches. In both

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TABLE 1

LEVELS OF CHOLERA TOXIN SPECIFIC ANTIBODY IN INTESTINAL SECRETIONS

Experimental Group	Antibody Levels by ELISA Units*					
	IgA			IgG		
	Day 3	Day 7	Day 14	Day 3	Day 7	Day 14
Cholera toxin 10 μg	0	41	187	0	15	0
Cholera toxin + CP 20,961 0.3 mg.	28	165	754	0	9	12
Reovirus 10^{10} particles	0	0	0	0	0	0
CP 20,961 alone	0	0	0	0	0	0

*As determined by ELISA using monospecific antisera to α and γ heavy chains. Values expressed as the mean optical density reading at 405 nm ($\times 10^{-3}$) of duplicate tests per sample (1/20 dilution of original) per group.

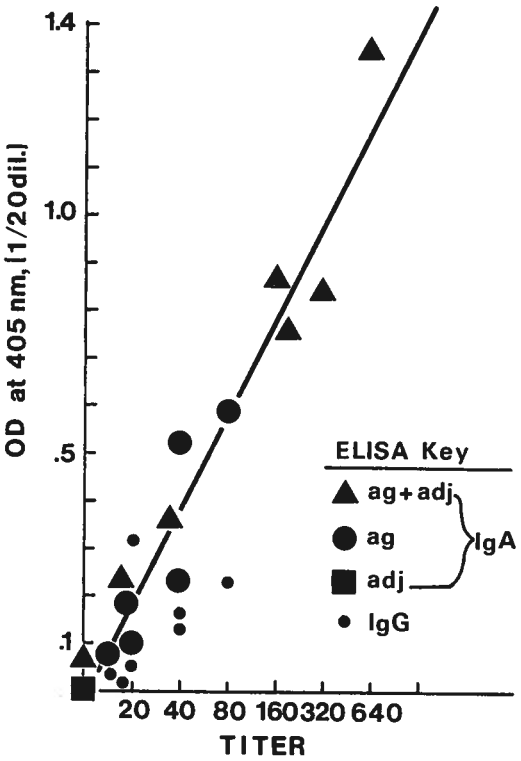

FIGURE 1. There is a direct correlation between the optical density (OD) at 405 nm of ELISA samples diluted 1/20 before incubation with substrate and the antibody titer as measured by dilution to greater than 60% reduction in OD.

FIGURE 2. Germfree rats were treated as above. Please note that each rat was exposed to *S. mutans* antigens five times in addition to being infected with *S. mutans* six days after initial priming with antigen. CP 20,961 was added to the three booster doses that were given 21, 14, and 7 days before measuring titers in saliva.

TABLE 2

LEVELS OF REOVIRUS SPECIFIC ANTIBODY IN INTESTINAL SECRETIONS AFTER PRIMARY IMMUNIZATION

Experimental Group	Antibody Levels by ELISA Units*					
	IgA			IgG		
	Day 3	Day 7	Day 14	Day 3	Day 7	Day 14
Reovirus 10^{10} particles (p) (1/Lang)	529	588	234	60	0	15
Reovirus 10^{10} p + CP 20,961 0.3 mg	63	1352	849	55	78	0
Reovirus 10^{10} p, 10^4 pfu (UV† inac)	0	0	67	0	0	0
Reovirus (as above) + CP 20,961	345	160	220	0	0	100
Cholera toxin 10 μg	0	0	0	0	0	0
CP 20,961 alone	ND	0	0	0	0	0

*As determined by ELISA using monospecific heavy chain antisera. Values expressed as the mean optical density reading at 405nm ($\times 10^{-3}$) of duplicate tests per sample (1/20 dilution of original) per group after two hours of incubation with substrate.

†UV – ultraviolet light at 14 cm for 30 minutes.

TABLE 3

ENHANCEMENT OF *S. MUTANS* SPECIFIC sIgA IN SALIVA BY CP 20,961

Experimental Group	Level of anti-*S. mutans* Antibodies (EU)*				
	Saliva		Serum		
	IgA	IgG	IgM	IgG	IgA
Infected only	6.5	<5	<5	<5	<5
S. mutans 6715 WC	25.2	7.5	6.1	35.2	8.5
S. mutans 6715 WC + CP 20,961	80.0	30.0	5.3	105.0	10.0
S. mutans 6715 WC + Liposomes†	320.0	80.0	7.9	55.0	20.0
CP 20,961 only	<5	<5	<5	<5	<5

*As determined by ELISA using monospecific anti-rat α, γ, and μ. Values expressed as ELISA units (EU), where EU equal the mean reciprocal of the dilution per sample per group giving an optical density reading at 405 nm of 0.1 after 1.5 hours of incubation with substrate.

†The liposomes contained CP 20,961.

TABLE 4

REDUCTION OF DENTAL PLAQUE IN *S. MUTANS* IMMUNE GNOTOBIOTIC RATS

Experimental Group	Plaque Score*	Number of Viable *S. mutans* (CFU × 10^6)†
Infected only	15.3 ± 0.4	6.40 ± 0.35
S. mutans 6715 WC	9.2 ± 0.6	3.96 ± 0.53
S. mutans 6715 WC + CP 20,961	7.3 ± 0.4	2.96 ± 0.21
S. mutans 6715 WC Liposomes‡	6.1 ± 0.3	1.88 ± 0.09
CP 20,961 only	16.0 ± 0.9	6.75 ± 0.48

*Represents mean value of smooth surface plaque on one mandible per rat per group ± SEM.

†Represents mean number of CFU (colony-forming unit approximately equal to number of bacteria recovered) in plaque from one mandible per rat per group as determined on blood and Mitis-Salivarius agar ± SEM.

‡The liposomes contained CP 20,961.

cases the CP 20,961 increased the magnitude but not the kinetics of the antibody and tissue response (TABLE 1 and 2).

In the *S. mutans* and dental caries study, groups of germfree rats were orally immunized with *S. mutans* 6715 WC alone or in combination with CP 20,961, according to the protocol depicted in FIGURE 2. *S. mutans* specific antibodies in serum and saliva samples from the five groups of animals were assessed by ELISA. Approximately threefold higher salivary IgA- and IgG-antibody levels were seen in rats treated with antigen and CP 20,961 compared to rats given *S. mutans* alone (TABLE 3). When liposomes prepared from CP 20,961 were used, the salivary IgG and IgA levels were enhanced tenfold. CP 20,961 did not enhance serum antibody levels when used orally. Plaque scores and microbiology are presented in TABLE 4. It is clear that oral immunization with *S. mutans* 6715 WC results in reduced molar plaque and microbial counts when compared with infected controls. Administration of CP 20,961 with antigen resulted in a further reduction in plaque levels and organisms recoverable.

In summary, the results indicate that the lipoidal amine CP 20,961 elicited an increase in specific IgA in secretions for all three antigens studied.

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