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EFFECT OF ENTERIC PRIMING WITH REOVIRUS AND LIPOIDAL AMINE ADJUVANT ON MUCOSAL LYMPHATIC TISSUE AND ANTI-VIRAL IgA SECRETION*

Arthur O. Anderson, Alan Plotner, and Donald H. Rubin

University of Pennsylvania
Philadelphia, Pennsylvania 19104

Priming by a mucosal route has been shown to be superior to parenteral inoculation in eliciting a protective secretory IgA response.[1] Indeed, parenteral priming often depresses subsequent IgA responses elicited by mucosal challenge and vice versa.[2] In addition, inactivated, attenuated, or toxoided antigens often fail to retain the essential immunogenic properties needed for an IgA response, and unmodified pathogens carry with them unacceptable toxicity. We explored the feasibility of using a mucosal adjuvant to potentiate the intestinal secretory IgA response to Reovirus serotype 1/Lang. The lipoidal amine *N*, *N*-dioctadecyl-*N'*, *N'*-*bis*(2-hydroxyethyl) propanediamine (CP 20,961) (FIGURE 1) was used, because previous studies documented its effectiveness in potentiating an antiviral IgG response when used for parenteral priming.[3] CP 20,961 also appeared to spontaneously form antigen-bearing liposomes when mixed with nonpolar soybean oil lipids (FIGURE 2). The reovirus system has been utilized as a model for viral gastrointestinal disease.[4,5] Reovirus serotype 1/Lang binds specifically to membraneous (M)[6] cells, and it can be recovered in high titers from Peyers patches and intestinal secretions in adult mice (D. Rubin, unpublished results). Fifty-one BALB/c mice were given a single intraduodenal (ID) priming dose of live or partially UV inactivated reovirus (10^{10} particles), reovirus plus CP 20,961 (0.3mg) in soybean lipid, or adjuvant alone. Virus-specific IgA levels in intestinal secretions were measured by ELISA from 1-14 days following priming. In addition, mucosal-associated lymphatic tissues were excised, weighed, and examined histologically. The use of CP 20,961 increased reovirus specific IgA in secretions threefold on day 7 and nearly fourfold (TABLE 1) on day 14 after a single ID dose compared to mice inoculated with reovirus alone. The lymphoid compartments of the spleen and Peyer's patches (PP) were markedly enlarged at all time points after use of combinations of antigen and adjuvant (TABLES 2 & 3 and FIGURE 3a to e). In the absence of antigen, CP 20,961 appeared to increase extramedullary hematopoiesis (EMH) in the splenic red pulp without affecting follicle formation, whereas adjuvant plus virus was not associated with significant EMH stimulation. In PP, the antigen and [^{3}H]CP 20,961 accumulated as multilamellar liposome-like bodies within macrophages under the dome epithelium (FIGURE 2). There was an initial increase in small B-cells in the corona that was followed by expansion of the follicles up to twice their size (FIGURES 3d and e). Reovirus in the presence of CP 20,961 mimicked chronic antigen exposure by inducing the development of additional PP between day 7 and 14. Studies of the biological effects of parenterally administered immunological adjuvants indi-

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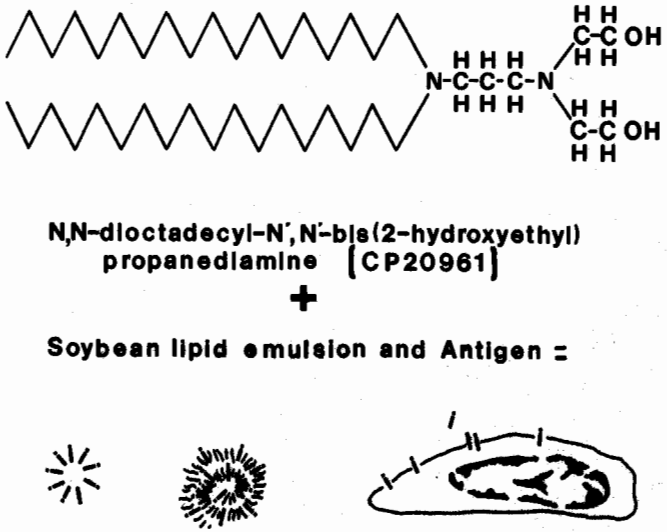

FIGURE 1. The structure of the lipoidal amine adjuvant resembles polar molecules that form lipid components of cell membranes. This might contribute to its affinity of membranes and its capacity to generate liposomes on aggitation with nonpolar lipids.

cated that they enhance lymphoid cell migration into regional lymphatic tissues,[3] increase the burst size of expanding clones of primed lymphocytes, and increase the output of accessory cell types (macrophages and dendritic cells) in thoracic duct lymph.[7,8] The local and systemic effects of CP 20,961 suggest that it might enhance mucosal immunity by producing similar effects on populations of lymphoid cells in the spleen and PP that are responsible for IgA-committed responses.[1] Localization of the adjuvant and antigen by macrophages in the dome

TABLE 1

LEVELS OF REOVIRUS-SPECIFIC ANTIBODY IN INTESTINAL SECRETIONS AFTER PRIMARY IMMUNIZATION

	Antibody Levels by Elisa Units*					
	IgA			IgG		
Experimental Group	Day 3	Day 7	Day 14	Day 3	Day 7	Day 14
Reovirus 10^{10} p† (1/Lang)	529	588	234	60	0	15
Reovirus 10^{10} p + CP 20,961 0.3 mg	63	1,352	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 mcg	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.

†p – particles

‡PFU – plaque-forming units

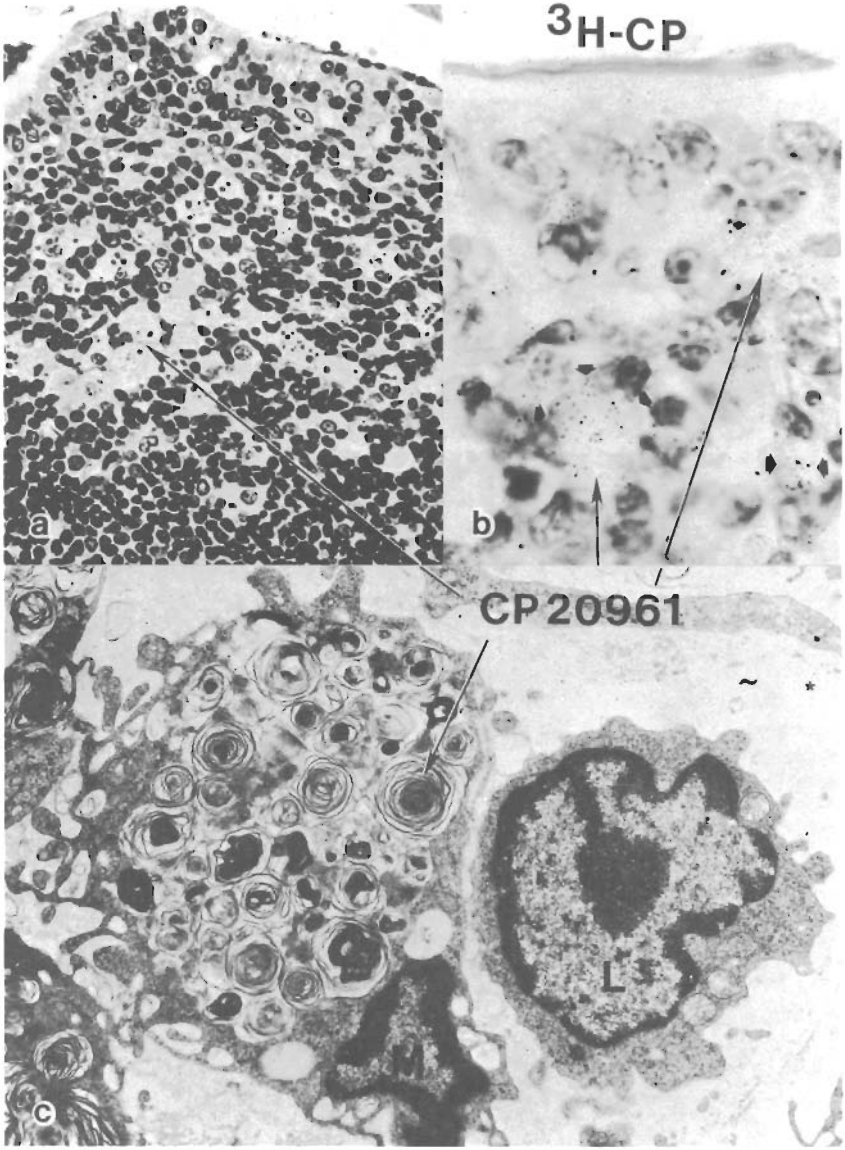

FIGURE 2. Tritiated CP 20,961 was administered enterically as described and radioautography showed that the label accumulated in swollen macrophage-like cells under the Peyers patch dome epithelium. This figure shows this histologically (a), radioautographically (b), and ultrastructurally (c).

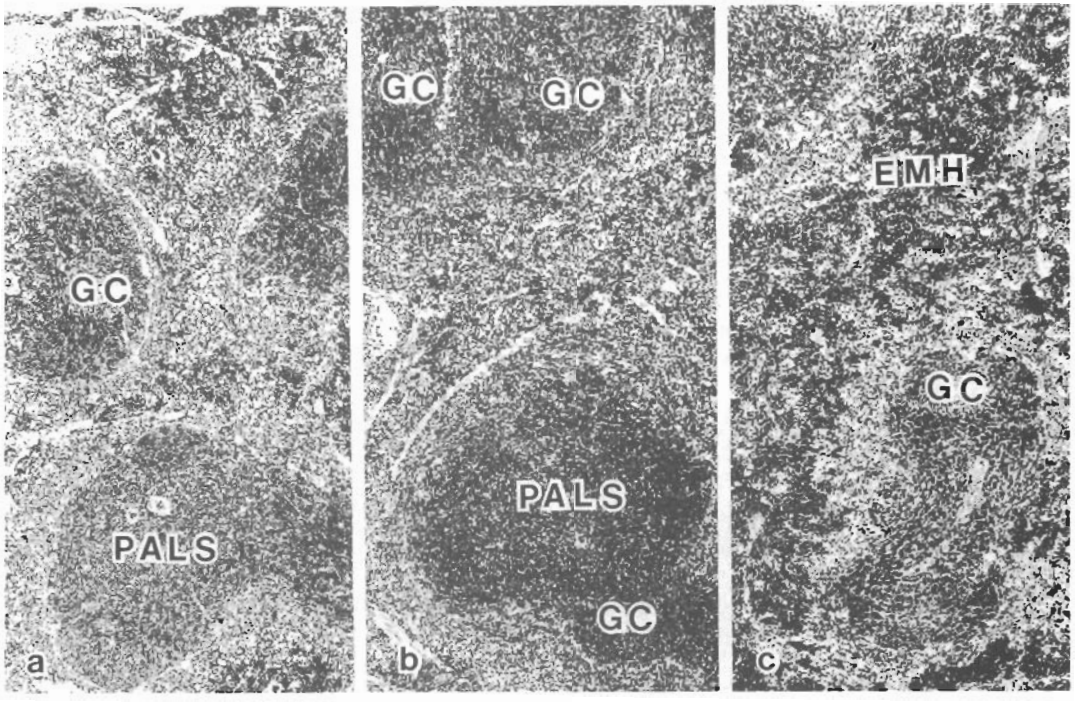

FIGURE 3. Reovirus infection following enteric inoculation appears to cause sequestration of small lymphoid cells in the periarteriolar lymphatic sheath (PALS) of the spleen (a) and the corona of Peyer's patches.[2] Use of adjuvant with reovirus results in early enlargement of the PALS followed by increased germinal center formation in the spleen (b) and PP (c) by the seventh day after priming. Adjuvant alone causes some increase in follicles, but most of the spleen enlargement is due to increased extramedullary hematopoeisis (c).

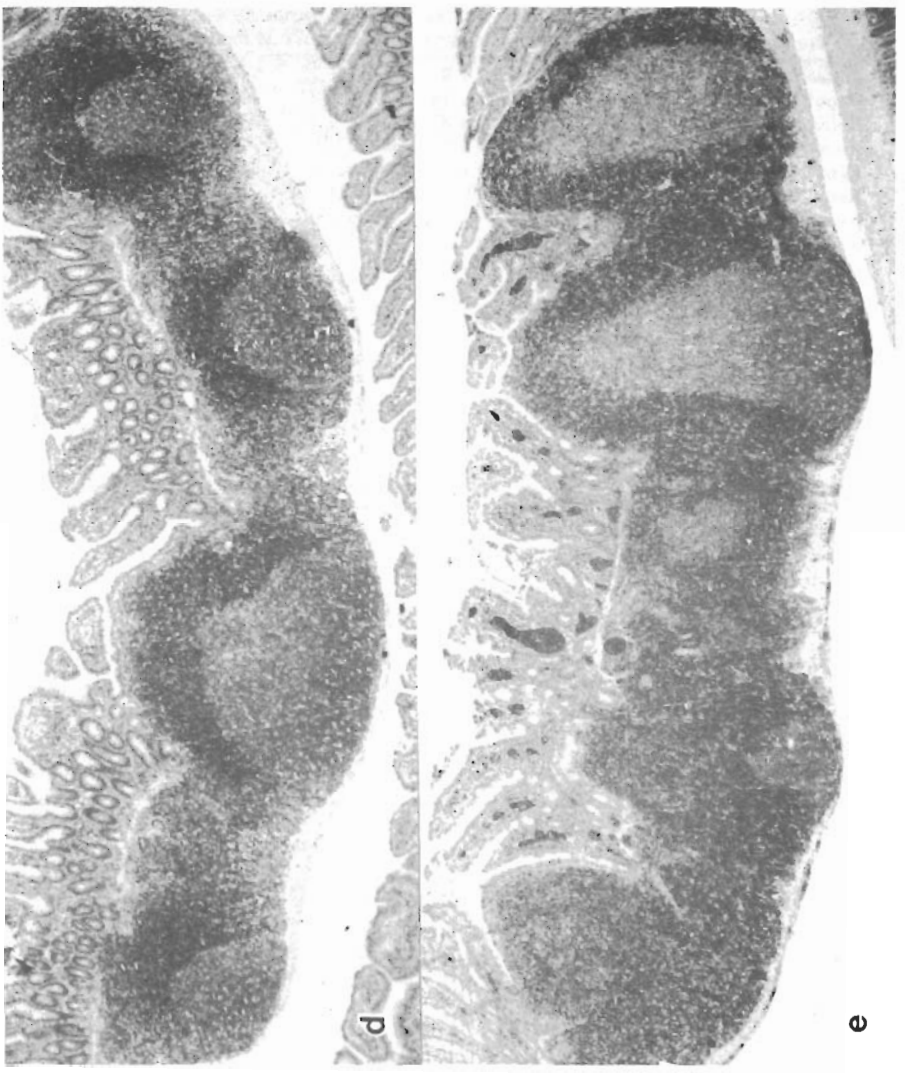

FIGURE 3. Continued.

TABLE 2

RESPONSE OF LYMPHATIC TISSUES TO ENTERIC IMMUNIZATION WITH REOVIRUS 10^{10} P PLUS 0.3MG CP 20,961 (PFIZER)*

Treatment ID†		Wet weight in mg ± 1 SD Spleen	Mesenteric L.N.	Peyers Patches	Number PP
Untreated		97.30 ± 9	53.73 ± 13	43.68 ± 12	7.33 ± 1
Reovirus + CP 20,961	day 1	110.95 ± 16	60.15 ± 3	68.52 ± 8	8.00 ± 1
Reovirus + CP 20,961	day 3	164.12 ± 35	57.98 ± 11	68.33 ± 6	8.67 ± 1
Reovirus + CP 20,961	day 7	126.73 ± 12	61.12 ± 9	74.25 ± 21	9.67 ± 1
Reovirus + CP 20,961	day 14	130.05 ± 21	60.00 ± 7	67.84 ± 4	12.33 ± 1
Reovirus alone	day 1	N.D.	N.D.	N.D.	N.D.
Reovirus alone	day 3	148.60 ± 30	53.94 ± 3	58.13 ± 5	8.00 ± 2
Reovirus alone	day 7	123.56 ± 56	55.90 ± 17	53.87 ± 6	8.33 ± 1
Reovirus alone	day 14	113.86 ± 9	50.06 ± 5	75.83 ± 9	11.33 ± 1
CP 20,961 alone	day 1	N.D.	N.D.	N.D.	N.D.
CP 20,961 alone	day 3	112.32 ± 9	63.12 ± 3	61.44 ± 7	7.54 ± 1
CP 20,961 alone	day 7	126.65 ± 8	61.93 ± 6	54.64 ± 10	6.30 ± 1
CP 20,961 alone	day 14	120.84 ± 5	79.96 ± 8	52.73 ± 9	6.88 ± 1

*All tissues were maintained at uniform hydration by fixation in 10% buffered formalin.

†Three mice per group were immunized by direct ID inoculation of the respective agents; the control was sham incised. All mice were of identical age at the time of sacrifice.

region of PP may be responsible for the restricted stimulation of mucosal-associated lymphatic tissues.

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TABLE 3

ALTERATION OF SPLENIC LYMPHOID COMPARTMENTS INDUCED BY ENTERIC IMMUNIZATION

Experimental Group	Mass of Lymphatic Tissue (mg)* Germinal Center	PALS†	Marginal Zone	Extramedullary Hematopoiesis
Untreated control	1.94	14.69	14.68	6.81
Reovirus + CP 20,961 day 1	2.09	58.47	24.74	2.50
Reovirus + CP 20,961 day 3	4.43	86.49	37.25	4.10
Reovirus + CP 20,961 day 7	8.87	39.53	28.76	10.13
Reovirus + CP 20,961 day 14	8.71	42.78	27.44	9.90
Reovirus alone day 7	3.58	35.46	35.95	10.37
CP 20,961 alone day 7	3.67	21.02	19.25	31.66

*The mass of lymphocytes constituting each anatomic compartment was calculated by measuring the percent volume (by planimetry) and multiplying it by the wet weight. Because the lymphoid mass is directly proportional to the number of lymphocytes present (i.e. a 20 mg lymph node contains 10^9 cells), it is clear to see that substantial redistribution and/or proliferation of lymphocytes occurred.

†PALS – periarteriolar lymphatic sheath.

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