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Intranasal or Intragastric Immunization with Proteosome-*Shigella* Lipopolysaccharide Vaccines Protects against Lethal Pneumonia in a Murine Model of *Shigella* Infection

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Mice immunized intranasally or intragastrically with proteosome vaccines containing either *Shigella sonnei* or *S. flexneri* 2a lipopolysaccharide were protected against lethal pneumonia caused by homologous organisms in an experimental murine intranasal challenge model of *Shigella* infection. Histopathological analysis demonstrated that immunization also protected against the progressive lesions resulting from invasion of the pulmonary mucosa by *S. sonnei*. These data show that mucosal proteosome-lipopolysaccharide vaccines can protect against lethal bacterial pneumonia and indicate that such vaccines are promising candidates for protection against intestinal shigellosis.

Shigella sonnei and *S. flexneri* are major causes of bacillary dysentery and diarrhea worldwide (15, 30). These organisms elicit inflammation by invading the colonic mucosa (14). Group-specific antibody recognizing the lipopolysaccharide (LPS) somatic antigen is associated with protection against shigellosis (3, 4, 31). Several vaccine approaches are under active investigation (10), including two types of parenteral subunit O-antigen vaccines (16, 34). We have described subunit *Shigella* vaccines designed for mucosal delivery consisting of either *S. sonnei* or *S. flexneri* LPS complexed by hydrophobic interactions with meningococcal outer membrane protein proteosomes (25, 26). Intranasal or intragastric immunization with these proteosome-LPS vaccines induces secretory immunoglobulin A (IgA) and serum IgG in mice (25, 26) and protects guinea pigs against keratoconjunctivitis shigellosa (26). In the current study, we demonstrated the efficacy of the proteosome-LPS vaccines in a murine intranasal challenge model (23, 35, 36) of *Shigella* infection. As confirmed by histopathologic analyses, intranasal or intragastric immunization with the proteosome-LPS vaccines protected mice against fatal suppurative pneumonia evoked by invasion of pulmonary mucosa by shigellae.

LPS was purified by phenol extraction (37) from clinical isolates of *S. sonnei* and *S. flexneri* serotype 2a. Outer membrane protein proteosomes were prepared from group B serotype 2b *Neisseria meningitidis* by using modifications of previously described methods (19, 22, 25). Briefly, phenol-killed bacterial paste was extracted with a solution of 6% Empigen BB (Albright and Wilson, Whitehaven, Cumbria, United Kingdom) in 1 M calcium chloride. Following removal of debris with 20% ethanol, outer membrane protein complex vesicles were precipitated with 45% ethanol. This precipitate was solubilized by homogenization and sonication in a 1.0% Empigen BB–Tris–EDTA–saline buffer, and proteosomes were isolated from meningococcal LPS by precipitation with ammonium sulfate (500 to 600 g/liter) three times. Following solubilization of the precipitate in the 1.0% Empigen BB buffer, the proteosomes were dialyzed against Tris-EDTA-saline containing 0.1% Empigen BB and stored at −70°C. Shigella LPS and proteosomes were hydrophobically complexed at a 1:1 (wt/wt) ratio by removal of solubilizing detergent using exhaustive dialysis, as previously described (19, 22, 25). Mice were anesthetized with a mixture of xylazine (0.3 mg/25 g) and ketamine HCl (1.0 mg/25 g) or with methoxyflurane and immunized intranasally or intragastrically with the proteosome-*S. sonnei* LPS vaccine or the proteosome-*S. flexneri* 2a LPS vaccine. Intranasal immunization involved delivery of 20 μl of vaccine in four to six drops applied to the external nares with a Hamilton syringe, whereas intragastric immunization involved delivery of 100 μl of vaccine through a polyethylene feeding tube inserted into the esophagus. In protection studies, vaccine efficacy was determined after intranasal challenge of anesthetized mice with approximately 5×10^6 CFU of *S. sonnei* 53G (13) or with approximately 6×10^6 CFU of *S. flexneri* 2a strain 2457T (6) suspended in 30 μl of saline. The mice were observed daily for 14 days after challenge, and mortality resulting from the ensuing pulmonary disease was recorded.

In two representative protection experiments, 86 and 100% of control mice died within 6 days after challenge with *S. sonnei* or *S. flexneri* 2a, respectively, whereas 93 and 75% of mice immunized intranasally with the proteosome-*S. sonnei* or proteosome-*S. flexneri* 2a LPS vaccines, respectively, survived homologous challenge (Fig. 1). The efficacy of the proteosome-LPS vaccines was confirmed in additional experiments employing either the intranasal or the intragastric route of administration (Table 1). The group-specific nature of this protection was demonstrated by a lack of significant protection after immunization with serologically heterologous LPS complexed with proteosomes (Table 1). Thus, mice immunized intranasally or intragastrically with the proteosome-*S. flexneri* 2a LPS vaccine were not protected against *S. sonnei* challenge.

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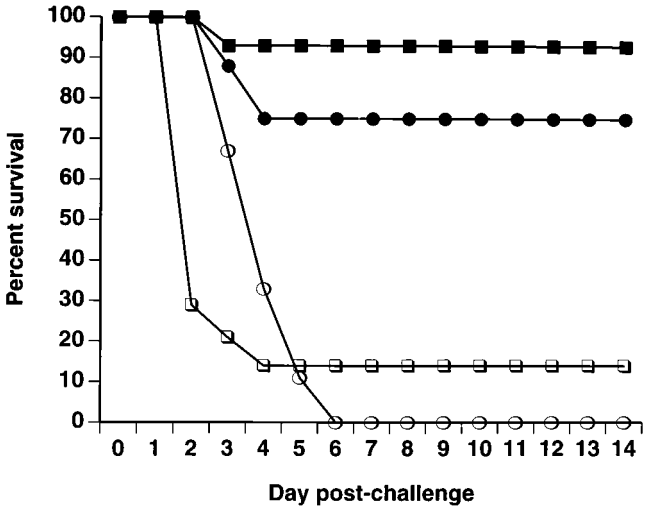

FIG. 1. Intranasally administered proteosome-shigella LPS vaccines elicit homologous protection against lethal pneumonia in Swiss Webster mice after intranasal challenge with virulent *S. sonnei* or *S. flexneri* 2a. In one experiment, a group of mice was immunized with the proteosome-*S. sonnei* LPS vaccine (10 μg each of proteosomes and LPS) (■) and another group was given saline (□) on days 0 and 21. All mice (15 vaccinees and 14 controls) were then challenged with *S. sonnei* on day 49. In the other experiment, a group of mice was immunized with the proteosome-*S. flexneri* 2a LPS vaccine (40 μg of proteosomes and 20 μg of LPS) (●) and another group was given saline (○) on days 0 and 36. All mice (eight vaccinees and nine controls) were then challenged with *S. flexneri* 2a on day 64.

TABLE 1. Homologous protection against *S. sonnei* or *S. flexneri* lethal pneumonia in mice immunized intranasally or intragastrically with proteosome-LPS vaccines

Vaccine (route of immunization)	Days of immunization[a]	Challenge organism[b]	% Protection[c]	*P* value[d]
Proteosome-*S. sonnei* LPS (i.n.)	0, 28	*S. sonnei*	88	0.006
Proteosome-*S. sonnei* LPS (i.n.)	0, 21	*S. sonnei*	92	<0.001
Proteosome-*S. sonnei* LPS[e] (i.n.)	0, 21	*S. sonnei*	100	<0.001
S. sonnei LPS (i.n.)	0, 28	*S. sonnei*	37	0.370
Proteosome-*S. flexneri* 2a LPS (i.n.)	0, 28	*S. sonnei*	17	0.675
Proteosome-*S. sonnei* LPS (i.g.)	0, 21	*S. sonnei*	72	0.041
Proteosome-*S. sonnei* LPS (i.g.)	0, 28	*S. sonnei*	86	0.020
S. sonnei LPS[f] (i.g.)	0, 21	*S. sonnei*	6	1.000
Proteosome-*S. flexneri* 2a LPS (i.g.)	0, 28	*S. sonnei*	29	0.415
Proteosome-*S. flexneri* 2a LPS (i.n.)	0, 21	*S. flexneri* 2a	63	0.087
Proteosome-*S. flexneri* 2a LPS (i.n.)	0, 36	*S. flexneri* 2a	75	0.002
S. flexneri 2a LPS[f] (i.n.)	0, 21	*S. flexneri* 2a	25	0.622

[a] The proteosome-*S. sonnei* LPS vaccines contained 10 μg each of proteosomes and LPS for intranasal (i.n.) delivery and 100 μg each of proteosomes and LPS for intragastric (i.g.) delivery. The proteosome-*S. flexneri* 2a LPS vaccines contained 40 μg of proteosomes and 20 μg of LPS for intranasal delivery and 100 μg of proteosomes and 50 μg of LPS for intragastric delivery. The following amounts of uncomplexed LPS were given: 10 μg (intranasal) or 100 μg (intragastric) of *S. sonnei* LPS and 20 μg (intranasal) of *S. flexneri* 2a LPS.

[b] All mice were challenged intranasally 4 weeks after the last immunization.

[c] Each percent protection value refers to protection against lethal pneumonia for a group of 8 to 15 (mean, 11) immunized mice that was compared with a group of 8 to 14 (mean, 11) control mice given saline intranasally and challenged on the same day as the vaccinees. Percent protection was calculated with the following formula: [(% death in controls) − (% death in vaccinees) × 100]/% death in controls.

[d] *P* values were calculated by using the Fisher exact test (two tailed [9]).

[e] This experiment was performed with BALB/cByJ mice; all other experiments were performed with Swiss Webster mice.

[f] Six mice were challenged in these vaccine groups. In the group that received uncomplexed *S. sonnei* LPS, four mice died because of technical problems associated with experimental procedures. In the group that received uncomplexed *S. flexneri* 2a LPS, five mice died 3 to 5 days after the first immunization, independent of technical manipulation.

These results also emphasize that there is no evidence that proteosomes per se induce nonspecific protection. Intranasal or intragastric immunization with homologous uncomplexed LPS was also ineffective (Table 1). Moreover, intranasal delivery of uncomplexed *S. flexneri* 2a LPS (20 μg) resulted in several deaths (5 of 12 mice; $P = 0.037$ [Fisher exact test, two tailed; reference 9]) after the first immunization. In contrast, an equal amount of LPS complexed with proteosomes caused no deaths, suggesting that this vaccine formulation is well tolerated.

To confirm the immunogenicity of the proteosome-LPS vaccines by serum enzyme-linked immunosorbent assay (25, 26), Swiss Webster mice were immunized intranasally (10 μg each of proteosomes and LPS) or intragastrically (100 μg each of proteosomes and LPS) with the proteosome-*S. sonnei* LPS vaccine on days 0 and 28 and then bled on day 42. An additional group of Swiss Webster mice was immunized intranasally with uncomplexed *S. sonnei* LPS (10 μg) and bled on the same schedule. The proteosome-LPS vaccine delivered intranasally induced group-specific anti-LPS IgG and IgA with reciprocal geometric mean titers of 14,700 and 260, respectively, whereas uncomplexed *S. sonnei* LPS delivered intranasally induced detectable IgG titers in only four of nine mice (geometric mean titer, 280) and no detectable IgA. Two intragastric immunizations with the proteosome-*S. sonnei* LPS vaccine induced IgG and IgA geometric mean titers of 1,700 and 150, respectively. Swiss Webster mice immunized with the proteosome-*S. flexneri* 2a LPS vaccine also had high group-specific serum antibody titers consistent with the responses previously reported for BALB/c mice after immunization with the proteosome-*S. sonnei* or the proteosome-*S. flexneri* 2a LPS vaccines (25, 26). Furthermore, mice immunized with the proteosome-*S. sonnei* LPS vaccine did not produce anti-*S. flexneri* 2a LPS antibodies (unpublished data), which is consistent with the lack of heterologous protection. In addition, we have shown that the proteosome-LPS vaccines induce group-specific anti-LPS IgA in mouse lung and intestinal lavage fluids (25, 26).

To determine the extent to which these vaccines protect against pulmonary inflammation in addition to protecting against death, anesthetized mice were immunized intranasally with the proteosome-*S. sonnei* LPS vaccine (10 μg each of proteosomes and LPS) or given saline on days 0 and 21 and then challenged intranasally with 4×10^6 CFU of *S. sonnei* 53G on day 49. Three immunized and three saline control mice were then euthanatized 0, 6, 24, and 48 h postchallenge for histopathological evaluation of the lungs. Unchallenged mice were given saline intranasally on the same treatment schedule and were also euthanatized at the same time points for histological evaluation. The lungs were removed and slowly filled with approximately 2.0 ml of 10% buffered formalin phosphate via intratracheal infusion. Sections (2 μm thick) were cut trans-

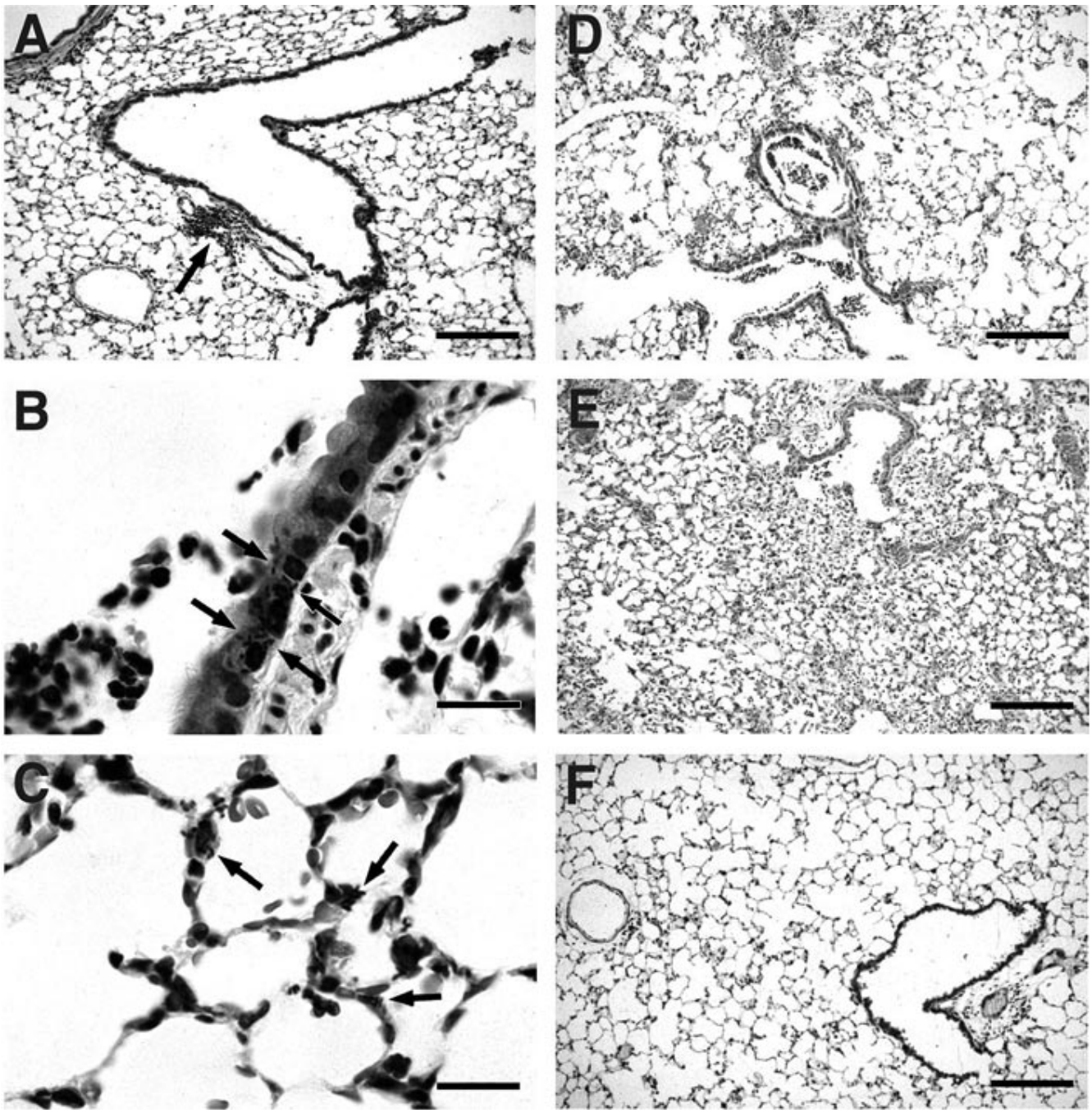

FIG. 2. Photomicrographs of mouse lung tissue after intranasal challenge with *S. sonnei*. Groups of 12 Swiss Webster mice were immunized intranasally with the proteosome-*S. sonnei* LPS vaccine (10 μg each of proteosomes and LPS) or saline on days 0 and 21 and then challenged with *S. sonnei* on day 49. Three immunized and three saline control mice were euthanatized 0, 6, 24, and 48 h postchallenge for histopathological analyses. (A) Sample taken at time zero postchallenge from a mouse immunized with the proteosome-LPS vaccine. The arrow indicates a prominent lymphoid aggregate. Such aggregates were present in immunized but not unimmunized mice. Hematoxylin-eosin stain was used. (B) Bronchiolar epithelium of a saline control mouse 6 h postchallenge. The arrows indicate bacteria within the cytoplasm of the bronchiolar epithelial cells. Giemsa stain was used. (C) Alveolar sacs of a saline control mouse 6 h postchallenge. The arrows indicate bacteria within the cytoplasm of the squamous epithelium. Giemsa stain was used. (D) Sample taken from a saline control mouse 24 h postchallenge demonstrating acute suppurative bronchiolitis and pneumonia. Hematoxylin-eosin stain was used. (E) Sample taken from a saline control mouse 48 h postchallenge showing extensive pneumonia. Hematoxylin-eosin stain was used. (F) Sample taken from a proteosome-LPS-immunized mouse 48 h postchallenge showing relatively normal pulmonary tissue. Hematoxylin-eosin stain was used. The bars represent 60 μm (A and D to F) or 10 μm (B and C). The figure was generated with Adobe Photoshop (Version 2.5.1 for Windows; Adobe Systems Inc., Mountain View, Calif.) after the microscopic images from Ektachrome slides were scanned into an Omniplex 566 computer (Dell Computer Corp., Austin, Tex.) by using a Coolscan LS-10 (Nikon Inc., Melville, N.Y.).

versely from paraffin-embedded tissue and stained with hematoxylin-eosin or Giemsa stain. At time zero, immediately (45 ± 5 s) after challenge, no bacteria or accompanying pathological lesions were found in either immunized or unimmunized mice. At this time, however, perivascular and peribronchiolar lymphoid aggregates were prominent in the lungs of proteosome-LPS-immunized mice (Fig. 2A). Lymphoid aggregates were minimal in distribution and size in the lungs of both saline control mice and unchallenged mice at time zero and were considered to be within normal limits (data not shown). Six hours postchallenge, bacteria were found within the cytoplasm of bronchiolar and alveolar epithelial cells in several microscopic fields of saline control mice (Fig. 2B and C). In contrast, bacteria were not detected in the tissues of either proteosome-LPS-immunized mice or unchallenged mice (data not shown). Acute suppurative bronchiolitis and pneumonia were clearly evident 24 h postchallenge in the saline control mice (Fig. 2D), but pneumonia was the more prominent lesion at 48 h postchallenge (Fig. 2E). In proteosome-LPS-immunized mice, minimal to mild suppurative bronchiolitis and pneumonia were evident 24 h postchallenge (data not shown). However, the most dramatic finding of these studies was the virtual lack of pneumonia or bronchiolitis in proteosome-LPS-immunized mice 48 h postchallenge (Fig. 2F).

The protective immune response elicited by intranasal immunization with the proteosome-*S. sonnei* LPS vaccine decreased bacterial subsistence in the tissues to levels below the limit of detection by histological analysis. The proteosome-LPS vaccines were effective only against homologous challenge, strongly suggesting that group-specific antibody recognizing the O polysaccharide of LPS is the protective modality. Nonetheless, LPS antibody does not inhibit invasion of cultured epithelial cells by shigellae in vitro (11), and the transient inflammatory response seen in mice immunized with the proteosome-LPS vaccine suggests that some bacterial invasion also occurred in the lungs of these animals. Hence, it is reasonable to postulate that antibody-mediated host defense mechanisms, such as opsonization (27) and antibody-dependent, cell-mediated antibacterial activity (20, 33) or antibody-dependent cellular cytotoxicity (32), are responsible for truncating the *Shigella* infection before progressive clinical disease ensues. It is noteworthy in this regard that IgA (32, 33) can participate in these cell-mediated effector mechanisms with mucosal lymphocytes (32) or monocytes (21).

Mucosal vaccine studies using other delivery systems have focused on protein antigens (5, 12, 28) or intact pathogens (1, 2, 7, 8, 24), and proteosomes can also effectively confer mucosal immunogenicity upon peptides and proteins (17). Furthermore, the ability of the proteosome-LPS vaccines to elicit protective serum IgG and mucosal IgA antibodies that recognize *Shigella* O polysaccharide indicates that the noncovalent association of LPS with proteosomes can change the character of a host's immune response to bacterial polysaccharide antigens. LPS is a T-cell-independent antigen (29), but LPS (25, 26) and other bacterial polysaccharides (38) complexed with proteosomes induce secondary IgG (25, 26, 38) and IgA (25, 26) immune responses characteristic of T-cell-dependent antigens. Although they may participate as classic protein carriers in eliciting T-cell help, the ability of proteosomes to mitogenically activate B cells (18) suggests that other mechanisms are involved. For example, neisserial porin proteosomes have recently been shown to upregulate expression of major histocompatibility complex class II and the B7.2 costimulatory ligand on mouse B cells in vitro (39). In additional experiments, these proteosome-activated B cells were shown to induce proliferation of naive $CD4^+$ helper T cells. Thus, proteosomes may enhance antibody responses by facilitating B-cell-mediated T-helper cell activation.

The results reported here, showing protection against suppurative pneumonia, suggest that immunization with the proteosome-LPS vaccines could also protect against the inflammatory colitis that characterizes shigellosis. Therefore, vaccine trials with humans using the proteosome-*Shigella* LPS complexes are planned. Furthermore, T-cell-dependent potentiation of the immune response against polysaccharide antigens by mucosally administered proteosome vaccines may be broadly applicable for immunization against a variety of respiratory pathogens, including *Haemophilus influenzae*, *Bordetella pertussis*, and *Streptococcus pneumoniae.*

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ADDENDUM IN PROOF

It was recently demonstrated (C. J. Chelen, Y. Fang, G. J. Freeman, H. Secrist, J. D. Marshall, P. T. Hwang, L. R. Frankel, R. H. DeKruyff, and D. T. Umetsu, J. Clin. Invest. **95:**1415–1421, 1995) that alveolar macrophages present antigen ineffectively to $CD4^+$ T cells due to defective expression of B7 costimulatory cell surface molecules. Proteosome-mediated upregulation of B7.2 (39) may therefore be particularly relevant to the mechanism whereby proteosome vaccines enhance respiratory and other mucosal immune responses.

REFERENCES

1. **Black, R. E., M. M. Levine, M. L. Clements, C. R. Young, A.-M. Svennerholm, and J. Holmgren.** 1987. Protective efficacy in humans of killed whole-vibrio oral cholera vaccine with and without the B subunit of cholera toxin. Infect. Immun. **55:**1116–1120.
2. **Chen, K.-S., D. B. Burlington, and G. V. Quinnan, Jr.** 1987. Active synthesis of hemagglutinin-specific immunoglobulin A by lung cells of mice that were immunized intragastrically with inactivated influenza virus vaccine. J. Virol. **61:**2150–2154.
3. **Cohen, D., M. S. Green, C. Block, T. Rouach, and I. Ofek.** 1988. Serum antibodies to lipopolysaccharide and natural immunity to shigellosis in an Israeli military population. J. Infect. Dis. **157:**1068–1071.
4. **Cohen, D., M. S. Green, C. Block, R. Slepon, and I. Ofek.** 1991. Prospective study of the association between serum antibodies to lipopolysaccharide O antigen and the attack rate of shigellosis. J. Clin. Microbiol. **29:**386–389.
5. **Eldridge, J. H., R. M. Gilley, J. K. Staas, Z. Moldoveanu, J. A. Meulbroek, and T. R. Tice.** 1989. Biodegradable microspheres: vaccine delivery system for oral immunization. Curr. Top. Microbiol. Immunol. **146:**59–66.
6. **Formal, S. B., G. J. Dammin, E. H. LaBrec, and H. Schneider.** 1958. Experimental shigella infections: characteristics of a fatal infection produced in guinea pigs. J. Bacteriol. **75:**604–610.
7. **Forrest, B. D., J. T. LaBrooy, P. Robinson, C. E. Dearlove, and D. J. C. Shearman.** 1991. Specific immune response in the human respiratory tract following oral immunization with live typhoid vaccine. Infect. Immun. **59:** 1206–1209.
8. **Freihorst, J., J. M. Merrick, and P. L. Ogra.** 1989. Effect of oral immunization with *Pseudomonas aeruginosa* on the development of specific antibacterial immunity in the lungs. Infect. Immun. **57:**235–238.
9. **Glantz, S.** 1992. Primer of biostatistics. McGraw-Hill Book Co., New York. (MacIntosh Version 3.0 written by B. Peterson.)
10. **Hale, T. L.** 1995. *Shigella* vaccines, p. 179–204. *In* D. A. A. Ala'Aldeen and C. E. Hormaeche (ed.), Molecular and clinical aspects of bacterial vaccine development. John Wiley & Sons Ltd., London.
11. **Hale, T. L., and P. F. Bonventre.** 1979. Shigella infection of Henle intestinal epithelial cells: role of the bacterium. Infect. Immun. **24:**879–886.
12. **Kimura, A., K. T. Mountzouros, D. A. Relman, S. Falkow, and J. L. Cowell.** 1990. *Bordetella pertussis* filamentous hemagglutinin: evaluation as a protective antigen and colonization factor in a mouse respiratory infection model. Infect. Immun. **58:**7–16.
13. **Kopecko, D. J., O. Washington, and S. B. Formal.** 1980. Genetic and physical evidence for plasmid control of *Shigella sonnei* form I cell surface antigen. Infect. Immun. **29:**207–214.
14. **LaBrec, E. H., H. Schneider, T. J. Magnani, and S. B. Formal.** 1964. Epithelial cell penetration as an essential step in the pathogenesis of bacillary dysentery. J. Bacteriol. **88:**1503–1518.

15. **Lee, L. A., C. N. Shapiro, N. Hargrett-Bean, and R. V. Tauxe.** 1991. Hyperendemic shigellosis in the United States: a review of surveillance data for 1967–1988. J. Infect. Dis. **164:**894–900.
16. **Levenson, V. I., T. P. Egorova, Z. P. Belkin, V. G. Fedosova, J. L. Subbotina, E. Z. Rukhadze, E. K. Dzhikidze, and Z. K. Stassilevich.** 1991. Protective ribosomal preparation from *Shigella sonnei* as a parenteral candidate vaccine. Infect. Immun. **59:**3610–3618.
17. **Lowell, G., R. Kaminski, C. Colleton, C. Mallett, N. Orr, S. Amselem, J. Estep, L. Pitt, K. Kersey, M. Hughes, T. Vancott, W. Baker, D. Frost, R. Hunt, D. Cohen, R. Arnon, G. Smith, R. Redfield, D. Birx, T. Hale, and W. Baze.** 1994. Proteosomes and PA adjuvants enhance mucosal immunogenicity and efficacy of HIV, Shigella, and staphylococcal enterotoxin B vaccines, abstr. 71, p. 71. *In* F. Brown, R. Chanock, H. S. Ginsberg, and E. Norrby (ed.), Abstracts of the Meeting on Molecular Approaches to the Control of Infectious Diseases. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
18. **Lowell, G. H.** 1990. Proteosomes, hydrophobic anchors, iscoms, and liposomes for improved presentation of peptide and protein vaccines, p. 141–160. *In* G. C. Woodrow and M. M. Levine (ed.), New generation vaccines. Marcel Dekker, Inc., New York.
19. **Lowell, G. H., W. R. Ballou, L. F. Smith, R. A. Wirtz, W. D. Zollinger, and W. T. Hockmeyer.** 1988. Proteosome-lipopeptide vaccines: enhancement of immunogenicity for malaria CS peptides. Science (Washington, D.C.) **240:** 800–802.
20. **Lowell, G. H., L. F. Smith, M. S. Artenstein, G. S. Nash, and R. P. MacDermott, Jr.** 1979. Antibody-dependent cell-mediated antibacterial activity of human mononuclear cells. I. K lymphocytes and monocytes are effective against meningococci in cooperation with human immune sera. J. Exp. Med. **150:**127–137.
21. **Lowell, G. H., L. F. Smith, J. M. Griffiss, and B. L. Brandt.** 1980. IgA-dependent, monocyte-mediated, antibacterial activity. J. Exp. Med. **152:**452–457.
22. **Lowell, G. H., L. F. Smith, R. C. Seid, and W. D. Zollinger.** 1988. Peptides bound to proteosomes via hydrophobic feet become highly immunogenic without adjuvants. J. Exp. Med. **167:**658–663.
23. **Mallett, C. P., L. Van De Verg, H. H. Collins, and T. L. Hale.** 1993. Evaluation of *Shigella* vaccine safety and efficacy in an intranasally challenged mouse model. Vaccine **11:**190–196.
24. **Nedrud, J. G., X. Liang, N. Hague, and M. E. Lamm.** 1987. Combined oral/nasal immunization protects mice from Sendai virus infection. J. Immunol. **139:**3484–3492.
25. **Orr, N., R. Arnon, G. Robin, D. Cohen, H. Bercovier, and G. H. Lowell.** 1994. Enhancement of anti-*Shigella* lipopolysaccharide (LPS) response by addition of the cholera toxin B subunit to oral and intranasal proteosome-*Shigella flexneri* 2a LPS vaccines. Infect. Immun. **62:**5198–5200.
26. **Orr, N., G. Robin, D. Cohen, R. Arnon, and G. H. Lowell.** 1993. Immunogenicity and efficacy of oral or intranasal *Shigella flexneri* 2a and *Shigella sonnei* proteosome-lipopolysaccharide vaccines in animal models. Infect. Immun. **61:**2390–2395.
27. **Reed, W. P.** 1975. Serum factors capable of opsonizing *Shigella* for phagocytosis by polymorphonuclear neutrophils. Immunology **28:**1051–1059.
28. **Shahin, R. D., D. F. Amsbaugh, and M. F. Leef.** 1992. Mucosal immunization with filamentous hemagglutinin protects against *Bordetella pertussis* respiratory infection. Infect. Immun. **60:**1482–1488.
29. **Stein, K. E.** 1992. Thymus-independent and thymus-dependent responses to polysaccharide antigens. J. Infect. Dis. **165:**S49–S52.
30. **Stoll, B. J., R. I. Glass, M. I. Huq, M. U. Khan, H. Banu, and J. Holt.** 1982. Epidemiologic and clinical features of patients infected with *Shigella* who attended a diarrheal disease hospital in Bangladesh. J. Infect. Dis. **146:**177–183.
31. **Tacket, C. O., S. B. Binion, E. Bostwick, G. Losonsky, M. J. Roy, and R. Edelman.** 1992. Efficacy of bovine milk immunoglobulin concentrate in preventing illness after *Shigella flexneri* challenge. Am. J. Trop. Med. Hyg. **47:**276–283.
32. **Tagliabue, A., D. Boraschi, L. Villa, D. F. Keren, G. H. Lowell, R. Rappuoli, and L. Nencioni.** 1984. IgA-dependent cell-mediated activity against enteropathogenic bacteria: distribution, specificity, and characterization of the effector cells. J. Immunol. **133:**988–992.
33. **Tagliabue, A., L. Nencioni, L. Villa, D. F. Keren, G. H. Lowell, and D. Boraschi.** 1983. Antibody-dependent cell-mediated antibacterial activity of intestinal lymphocytes with secretory IgA. Nature (London) **306:**184–186.
34. **Taylor, D. N., A. C. Trofa, J. Sadoff, C. Chu, D. Bryla, J. Shiloach, D. Cohen, S. Ashkenazi, Y. Lerman, W. Egan, R. Schneerson, and J. B. Robbins.** 1993. Synthesis, characterization, and clinical evaluation of conjugate vaccines composed of the O-specific polysaccharides of *Shigella dysenteriae* type I, *Shigella flexneri* type 2a, and *Shigella sonnei* (*Plesiomonas shigelloides*) bound to bacterial toxoids. Infect. Immun. **61:**3678–3687.
35. **Van De Verg, L. L., C. P. Mallett, H. H. Collins, T. Larsen, C. Hammack, and T. L. Hale.** 1995. Antibody and cytokine responses in a mouse pulmonary model of *Shigella flexneri* 2a infection. Infect. Immun. **63:**1947–1954.
36. **Voino-Yasenetsky, M. V., and M. K. Voino-Yasenetskaya.** 1962. Experimental pneumonia caused by bacteria of the Shigella group. Acta Morphol. Acad. Sci. Hung. **11:**439–454.
37. **Westphal, O., and K. Jann.** 1965. Bacterial lipopolysaccharides: extraction with phenol-water and further applications of the procedure. Methods Carbohydr. Chem. **V:**83–91.
38. **Wetzler, L. M.** 1994. Immunopotentiating ability of neisserial major outer membrane proteins: use as an adjuvant for poorly immunogenic substances and potential use in vaccines. Ann. N. Y. Acad. Sci. **730:**367–370.
39. **Wetzler, L. M. (Boston City Hospital).** 1994. Personal communication.