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Polysaccharide Capsule-Mediated Resistance to Opsonophagocytosis in *Klebsiella pneumoniae*

PHILIP DOMENICO,[1,2]* RICHARD J. SALO,[3] ALAN S. CROSS,[4] AND BURKE A. CUNHA[1,2]

Infectious Disease Division, Winthrop-University Hospital, Mineola,[1] SUNY School of Medicine, Stony Brook,[2] and Department of Pediatrics, Nassau County Medical Center, East Meadow,[3] New York, and Walter Reed Army Institute of Research, Washington, D.C.[4]

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The polysaccharide capsule of *Klebsiella pneumoniae* is an important virulence factor that confers resistance to phagocytosis. The treatment of encapsulated bacteria with salicylate to inhibit capsule expression was found to enhance the phagocytosis of encapsulated bacteria by human neutrophils only in the presence of cell surface-specific antibodies. Both type-specific rabbit antisera and anticapsular human hyperimmune globulin were employed as opsonins. Salicylate significantly enhanced phagocytosis with homologous, but not heterologous, whole-cell antisera. Antisera, diluted 1:40, no longer opsonized fully encapsulated bacteria but promoted the uptake of multiple salicylate-treated bacteria in >90% of neutrophils. Salicylate (0.25 to 1.0 mM) also enhanced opsonization with globulin against homologous bacteria. Higher salicylate levels (1 to 2.5 mM) enhanced the opsonization of heterologous serotypes with human globulin. The nature of antibody attachment to encapsulated bacteria was determined by immunofluorescence. Even after the addition of purified capsular polysaccharide to prevent phagocytosis, K-specific antibodies attached in large amounts to bacteria. K-specific antibodies reacted with antigens throughout the capsule and showed a predilection for a denser inner layer of the capsule, indicating that many of the K-specific antibodies may be masked underneath the capsule surface. K-specific antibodies can also be rendered nonfunctional by soluble, cell-free capsular antigen. In culture, large quantities of soluble capsular polysaccharide extrude from bacteria after overnight growth. The reduction in capsule expression caused by salicylate largely affected the soluble, cell-free fraction. Purified capsular polysaccharide was shown to retard the opsonophagocytosis of salicylate-treated bacteria in a concentration-dependent manner. However, extensive washing of encapsulated bacteria to remove loosely attached capsular material did not significantly enhance opsonophagocytosis. In conclusion, cell-free capsule and cell-associated capsule are antiphagocytic; both act to neutralize K-specific antibodies by binding or concealment. Salicylate-mediated inhibition of capsule expression, particularly of the cell-free fraction, improved K-specific opsonization dramatically.

Encapsulated gram-negative bacteria, such as *Klebsiella pneumoniae*, are among the leading causes of morbidity and mortality in the hospital environment (16). An important characteristic of *K. pneumoniae* growth is the production of capsular polysaccharide (CPS). A thick CPS layer, referred to as a capsule, envelops these bacteria and thwarts protective host defenses (20, 22, 25, 26). Encapsulated *K. pneumoniae* also produces large quantities of cell-free CPS (12), which is aggregated with toxic lipopolysaccharide (LPS) and contributes to pathogenicity (1, 11, 21). Circulating, cell-free CPS may also bind and neutralize antibodies that would otherwise attach to and opsonize bacteria (5, 18).

The production of opsonizing antibodies is an important aspect of immunity to *K. pneumoniae*. In vitro analysis has identified antibodies to CPS (K-specific antibodies) as important opsonins for phagocytic uptake (19). Thus, vaccine development for encapsulated bacteria has emphasized capsular (K-specific) antigens (3, 6). However, K-specific immunotherapy for *K. pneumoniae* must cover numerous serotypes among the 70+ serologically distinct capsular types identified within this species. A 24-valent, K-specific vaccine, now in clinical trials, covers 60 to 70% of the bacteremic isolates of *K. pneumoniae*. Human hyperimmune globulin has been raised from this vaccine for passive administration and has shown promise in combatting *K. pneumoniae* infections (3, 4).

For *K. pneumoniae* and related bacteria, the quantity of CPS made is directly related to virulence (8, 11, 14). Salicylate has been used in vitro to reduce CPS expression by several serotypes of *K. pneumoniae* (10). It was shown recently that the inhibition of *K. pneumoniae* capsule expression with salicylate enhanced the phagocytic uptake of an O1:K2 strain, especially in the presence of antiserum raised in rabbits against homologous, formalinized whole cells (9). This report evaluates the opsonic potential of K-specific antibodies against a number of *K. pneumoniae* serotypes after capsule reduction.

MATERIALS AND METHODS

Bacteria and media. *K. pneumoniae* KP1-LC (O1:K1), 52145 (O1:K2), and C3 (O1:K66) were employed in opsonophagocytosis studies. *K. pneumoniae* B5055 (O1:K2) (the reference strain for capsular type 2), originally obtained from Stanley Cryz, Jr. (Swiss Serum Vaccine Institute, Bern, Switzerland), was used for animal studies. Bacteria were subcultured on nutrient or sheep blood agar with careful selection and maintenance of mucoid colonies. A defined minimal salts liquid broth medium with a high glucose concentration and low nitrogen concentration was prepared to promote capsule production (10, 12).

CPS. Purified CPS was obtained from gel filtration chromatography of culture supernatants (7). Briefly, CPS from the

* Corresponding author. Phone: (516) 663-2654. Fax: (516) 663-3886.

60-ml culture supernatant was precipitated in ethanol, redissolved in 10 ml of 1% Zwittergent in 100 mM citric acid, and loaded on a Sepharose 6B column (2.5 by 45 cm) equilibrated with 0.05% Zwittergent in 20 mM sodium citrate (pH 4). Fractions were collected and assessed for their content of CPS, LPS (15), and protein (17). CPS was measured by uronic acid determinations (2). Total CPS was measured after mild, quantitative extraction of bacteria with Zwittergent 3-14 (Calbiochem, La Jolla, Calif.) in citric acid (7, 10).

Opsonophagocytosis. The growth of bacteria with or without salicylate treatment and the purification of human polymorphonuclear leukocytes (PMNL) have been described previously (9). Bacteria and PMNL were suspended in Hanks' balanced salt solution (HBSS) containing 0.1% gelatin. Opsonophagocytic mixtures contained 160 μl of bacteria (2×10^7 CFU/ml), 160 μl of PMNL (2×10^6 cells per ml), 40 μl of normal rabbit serum (Sigma Chemical Co., St. Louis, Mo.) as a source of complement, and 40 μl of antiserum. All antisera were used undiluted except for K2 antiserum, which was diluted 1:40 in HBSS to minimize aggregation of bacteria. Mixtures were incubated for 30 min at 37°C in a shaking water bath and then diluted to 10 ml with cold HBSS. After centrifugation at $400 \times g$ to separate PMNL and extracellular bacteria, the pelleted cells were resuspended in 1 ml of HBSS. Smears were prepared by centrifuging 125 μl onto glass slides with a Cytospin 2 centrifuge (Shandon, Pittsburgh, Pa.). Cells were stained with LeukoStat stain (Fisher Diagnostics, Orangeburg, N.Y.). One hundred PMNL each on duplicate slides were examined to determine the percent phagocytosis (%P) [(number of PMNL containing bacteria/total number of PMNL) × 100] and phagocytic index (PI) (number of intracellular bacteria in 100 PMNL).

Antisera. Serotype-specific, polyclonal antisera were raised in rabbits with formalin-treated, whole-cell encapsulated bacteria (13). K66 antiserum was a gift from Juan M. Tomàs. Nosocuman, a human intravenous immunoglobulin (IVIG) preparation, enriched in antibodies to the *Klebsiella* K2 CPS, was used both for in vivo studies and for in vitro studies.

Immunofluorescence. Smears were prepared from opsonophagocytic mixtures to demonstrate rabbit K2 antibodies bound to O1:K2. Goat anti-rabbit immunoglobulin G (IgG) conjugated with rhodamine (Sigma), diluted 1:100, was incubated with smears for 30 min at 37°C and examined and photographed by using a Labphot microscope with episcopic fluorescence and Microflex AFX photographic attachments (Nikon Corp.).

RESULTS

The reduced expression of capsule by all *K. pneumoniae* serotypes was achieved by culturing bacteria in the presence of salicylate. Overnight growth in defined medium containing 2.5 mM salicylate reduced CPS expression by 70 to 80% for all strains (10). In the presence of fresh complement, but in the absence of specific antisera, salicylate treatment increased the %P and the PI only marginally. The results with K-specific antiserum as the opsonin are shown in Fig. 1. Homologous K-specific antiserum increased the %P and PI for salicylate-treated bacteria markedly, especially for O1:K2. K2 antiserum diluted 1:40 no longer promoted bacterial aggregation and showed virtually no opsonization of fully encapsulated O1:K2, yet it promoted a pronounced uptake of salicylate-treated O1:K2 bacteria by PMNL ($P < 0.001$). The absorption of anti-K2 antibody with a noncapsular variant strain of O1:K2 did not appreciably affect the results, and the antisera retained

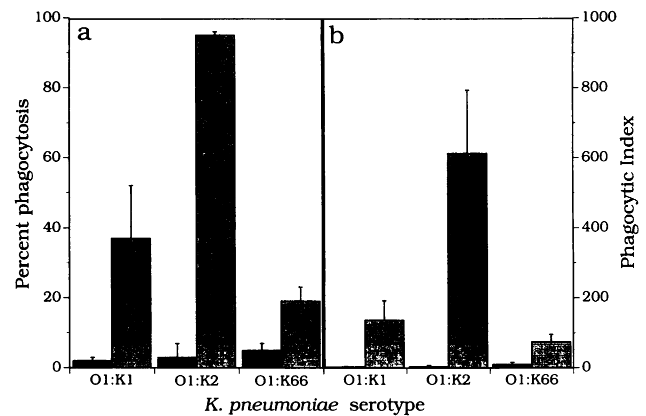

FIG. 1. Effects of salicylate and homologous, K-specific antiserum on opsonophagocytosis. *K. pneumoniae* serotypes O1:K1, O1:K2, and O1:K66 were cultured overnight with (gray bars) or without (black bars) 2.5 mM salicylate, washed, and incubated with human PMNL in 10% fresh, normal serum and 10% K-specific antiserum for 30 min at 37°C. (a) %P; (b) PI. Each error bar represents 1 standard deviation ($n \geq 3$).

serotype specificity even after salicylate treatment of bacteria (data not shown).

Similar results were obtained with IVIG, a hyperimmune globulin produced in humans from a 24-valent K-specific vaccine (Nosocuman). This IVIG is reactive with serotype 2 but not with serotype 1 *K. pneumoniae*. In phagocytic assays employing fully encapsulated O1:K1 or O1:K2 bacteria and 2.5 mg of IVIG per ml, minimal phagocytosis was observed, as shown in Fig. 2. Phagocytic uptake for both serotypes was enhanced by salicylate pretreatment in a concentration-dependent manner. A marked increase in opsonization of O1:K2 occurred at relatively low concentrations of salicylate (≤1 mM), while opsonization of O1:K1 occurred gradually and

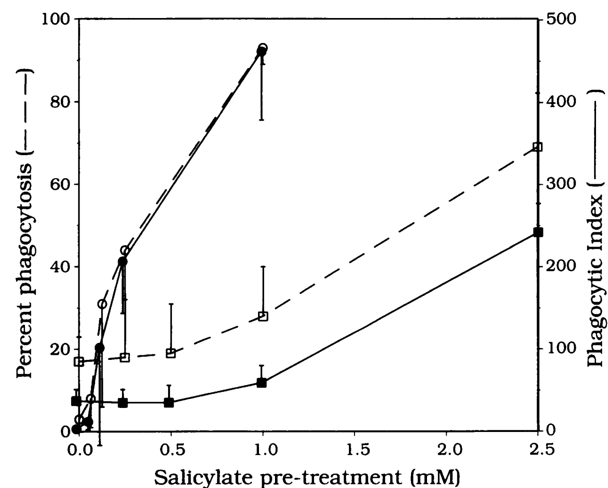

FIG. 2. Effects of salicylate concentration on opsonophagocytosis of *K. pneumoniae* in the presence of K-specific IVIG. *K. pneumoniae* serotypes O1:K1 and O1:K2 were cultured overnight in various concentrations of salicylate and incubated with human PMNL in 10% fresh, normal serum and 2.5 mg of IVIG per ml for 30 min at 37°C. The %P of strains O1:K1 (□) and O1:K2 (○) and the PI of strains O1:K1 (■) and O1:K2 (●) are shown. Each error bar represents 1 standard deviation ($n \geq 3$).

TABLE 1. Effects of the removal of loosely adherent CPS on opsonophagocytosis

Salicylate pretreatment concn (mM)[a]	No. of washes[b]	Cell-free CPS (ng/ml)[c]	%P[d]	PI[d]
0	0	4,800 ± 700	ND[e]	ND
0	1	120 ± 70	0 ± 0	0 ± 0
0	2	20 ± 10	0.5 ± 0.5	0.5 ± 0.5
0	3	10 ± 4	2.5 ± 2.5	3.0 ± 3.0
1	0	950 ± 470	ND	ND
1	1	12 ± 7	97.5 ± 2.5	581 ± 140

[a] *K. pneumoniae* O1:K2 was cultured overnight in defined medium with salicylate added as indicated.

[b] One milliliter of an overnight bacterial culture was washed with 1 ml of HBSS to remove loosely adherent CPS. Washed bacteria were placed immediately into opsonophagocytic assays.

[c] Estimate of the cell-free CPS concentration carried over to the phagocytic mixture. CPS concentrations were determined from washes of overnight cultures (10^9 CFU/ml) that were diluted to final concentrations of 8×10^6 CFU/ml in phagocytic assays. CPS concentrations were factored from uronic acid determinations (2) multiplied by 3.78 (uronic acid is 26.4% of the weight of CPS).

[d] Determined after a 30-min incubation at 37°C in the presence of complement, PMNL, and homologous, K-specific antiserum.

[e] ND, not determined.

only after pretreatment with high concentrations of salicylate (>1 mM). Both the %P and the PI increased in proportion to the salicylate pretreatment concentration. Thus, opsonization by K-specific, human hyperimmune globulin was enhanced substantially by the treatment of bacteria, particularly the homologous O1:K2 serotype, with salicylate.

An understanding of how decapsulation enhances K-specific opsonophagocytosis may lie in the nature of antibody attachment. It is widely held that bacteria neutralize anticapsular antibodies by producing large quantities of cell-free CPS. Loosely adherent CPS that detaches from bacteria (antigen sloughing) may bind to an opsonic antibody, thus making it unavailable to promote phagocytosis. The influence of antigen sloughing was assessed by thorough removal of loosely attached CPS from bacteria. Three washes of fully encapsulated O1:K2 reduced the quantity of cell-free CPS in samples by several orders of magnitude, as summarized in Table 1. However, when washed cells were immediately placed into phagocytic assays, the %P and PI in the presence of K-specific antibody increased only slightly. Thus, extensive washing of bacteria to remove cell-free capsule and loosely attached capsule had little influence on the poor opsonization of fully encapsulated bacteria with K-specific antibodies. Compare this with the extensive opsonophagocytosis of O1:K2 pretreated with 1 mM salicylate, which was used as a positive control (Table 1).

Salicylate also had a significant effect on the proportion of CPS that became cell free during culture. Overnight cultures of *K. pneumoniae* O1:K2 contained large amounts of cell-free capsular material, which fractionated into the supernatant upon centrifugation. The influence of salicylate concentration on overall CPS production and capsule shedding from serotype O1:K2 is shown in Fig. 3. Cultures showed salicylate concentration-dependent decreases in both cell-associated CPS and cell-free CPS. However, with increasing amounts of salicylate, there was a disproportionately greater decrease in cell-free CPS. At 2.5 mM salicylate, the percentage of CPS in supernatants was 35.9% ± 8.2% versus 53.6% ± 7.9% for cultures not treated with salicylate ($P < 0.005$).

The influence of antigen sloughing on opsonophagocytosis was also tested by adding purified K2 CPS to salicylate-treated

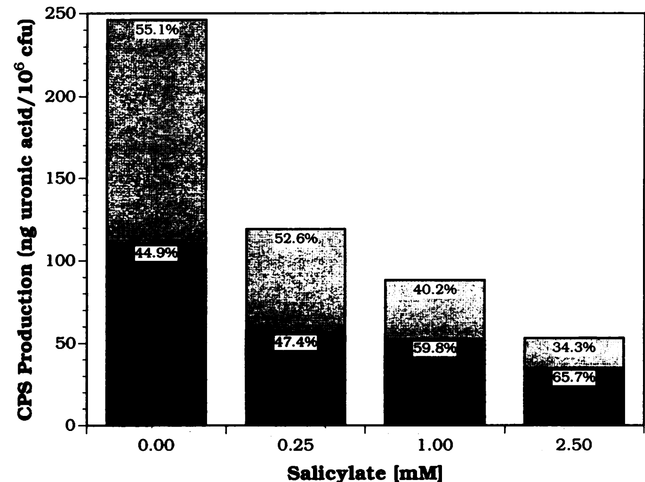

FIG. 3. Influence of salicylate concentration on CPS production and shedding. *K. pneumoniae* serotype O1:K2 was cultured in defined medium containing various salicylate concentrations. Cultures were centrifuged, and CPS was extracted from the bacterial pellet (black bars) and culture supernatants (gray bars). CPS is expressed as the quantity (in nanograms) of uronic acid recovered per 10^6 CFU. Each bar represents the mean of at least three trials.

O1:K2 bacteria in phagocytic assays. Added CPS produced a concentration-dependent decrease in %P for decapsulated O1:K2 in the presence of PMNL and K-specific rabbit polyclonal antiserum diluted 1:40. The %P was reduced by 23% ± 15%, 73% ± 29%, and 97% ± 5% with 0.025, 0.25, and 2.5 μg of added CPS per ml, respectively. The 50% inhibitory concentration of CPS was 0.145 μg/ml. Immunofluorescence microscopy showed that even at 2.5 μg of added CPS per ml, K-specific antibodies attached in apparently large amounts to bacteria, as illustrated in Fig. 4a, yet virtually no phagocytosis was observed. In Fig. 4b, K-specific antibodies are shown attached to fully encapsulated O1:K2 bacteria which are bound to PMNL. Though the intensity of fluorescence was high, again no phagocytosis was observed.

DISCUSSION

Promoting phagocytosis with decapsulating agents represents a novel approach to immunotherapy as well as a novel form of immune modulation. It is widely assumed that K-specific antibodies can be neutralized and rendered nonfunctional by copious production of CPS. If copious capsule production and sloughing play an important role in pathogenesis, then salicylate may curtail this virulence parameter and allow K-specific opsonophagocytosis to occur efficiently.

The potential importance of cell-free CPS in pathogenicity warranted a closer look at the conditions that influence its production. Several investigators (11, 12, 14) have observed that cell-free CPS elaborated by *K. pneumoniae* increases in proportion to the size of the cell-associated capsule. The shedding of capsule is influenced by a number of factors, including pH and cation content (7, 23). It is now evident that salicylate diminishes the sloughing of cell-free CPS to a disproportionately greater degree than expected. The effects of salicylate on pH or chelation of cations could possibly affect the degree of sloughing. Since multivalent cations can act to stabilize capsule structures, copious capsule production can act

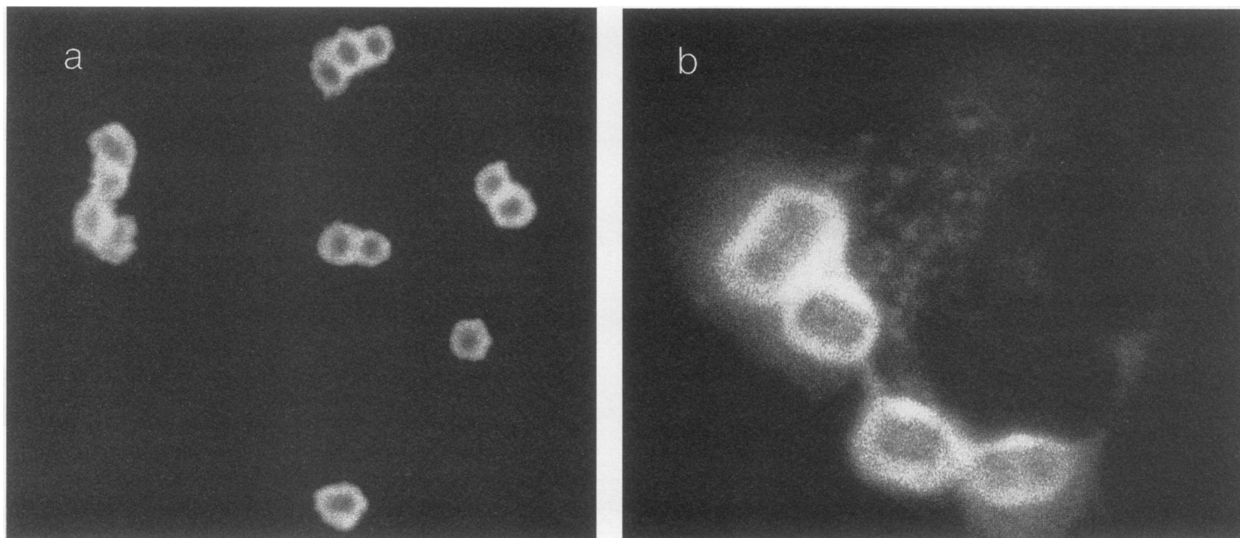

FIG. 4. Immunofluorescence. Samples of opsonophagocytic mixtures of *K. pneumoniae* serotype O1:K2 and rabbit anti-K2 serum were stained with goat anti-rabbit IgG conjugated with rhodamine. (a) Salicylate-treated bacteria in the presence of cell-free CPS (2.5 μg/ml). Magnification, ×19,278. (b) Fully encapsulated bacteria. Magnification, ×49,140.

as a cation sink, thus depleting the "cement" that keeps the capsule together.

By limiting the expression of both cell-associated CPS and cell-free CPS, salicylate rendered bacteria susceptible to phagocytosis in the presence of anticapsular antibodies. Our evidence indicates that even the smallest amount of cell-free CPS carried over from sample preparation can inhibit opsonophagocytosis. The quantity of cell-free CPS in phagocytic assays after one wash of fully encapsulated O1:K2 (120 ng/ml; Table 1) approximated the 50% inhibitory concentration for K2 CPS on anti-K2 opsonization (145 ng/ml). Thus, in the standard phagocytosis procedure, enough cell-free CPS is carried over after the preparation of bacteria to inhibit opsonophagocytosis by nearly 50%. However, a more thorough washing of encapsulated O1:K2 did not enhance opsonophagocytosis to any great extent. Though much antibody was bound to the capsule, as seen by immunofluorescence, such attachment did not promote phagocytosis. The nature of antibody attachment in general suggested a concentration gradient, whereby most of the antibody bound to proximal sites within the capsule. A similar immunofluorescence pattern with K-specific antibodies has been reported previously for this serotype (24).

Since CPS is exposed on the surface of encapsulated *K. pneumoniae*, it is not immediately apparent why the inhibition of CPS enhances opsonization by anticapsular antibodies. The data presented here point to at least three aspects of capsule-mediated resistance to K-specific phagocytosis: (i) neutralization of antibody by cell-free CPS, (ii) blocking of phagocytic receptors on PMNL by immune complexes of cell-free CPS and antibodies, and (iii) masking of antibody by the capsule. The first aspect is probably important in situations involving limited amounts of antibody. The blocking of phagocytic receptors, especially those for IgG molecules, by immune complexes may also mediate resistance. Our evidence for this is that added cell-free CPS inhibited the opsonophagocytosis of salicylate-treated bacteria even when bacteria contained bound antibody, as determined by immunofluorescence. A similar phenomenon may occur with fully encapsulated bacteria, in that antibody-coated bacteria may bind to PMNL and then detach because of the loosely adherent nature of CPS. Thirdly, the capsule may act as a large antigenic sink, capable of absorbing large quantities of anticapsular antibody. Inhibiting capsule expression limits capsule shedding, allowing more antibodies to reach their intended targets, and may also unmask many of the antibodies attached to inner layers of the capsule. Much of the antibody fluorescence emanated from interior sections of the capsule, indicating that considerable quantities of IgG were masked below the capsule surface. Such antibodies may not be opsonic unless they are exposed on the bacterial surface.

With such a pronounced reduction of capsule by salicylate, perhaps other non-K opsonins may also play a role. Apparently, certain non-K antibodies are present in IVIG since the O1:K1 strain was opsonized after high-dose salicylate treatment of bacteria, even though the K1 serotype is not represented in this IVIG preparation. Apparently, antibodies directed at targets masked by the capsule (i.e., LPS) were present at low levels and became functional opsonins only after severe repression of CPS production (25). After salicylate treatment of bacteria, IVIG not only became a better opsonin but also exhibited a broader range of activity. Thus, salicylate treatment may improve both efficiency and coverage in immunotherapy.

Whether salicylate actually inhibits capsule production in vivo is still not known. Preliminary data indicate that while salicylate allowed the reduction of the IVIG (Nosocuman) protective dose under certain conditions, the data were mixed. IVIG has been shown to protect mice against lethal *K. pneumoniae* challenge (3, 4). Mice challenged with *K. pneumoniae* (25 50% lethal doses) received IVIG and salicylate at the time of challenge. At subtherapeutic levels of anti-K2 IgG, salicylate (~1 mM peak serum concentration) improved survival, perhaps by facilitating interaction between bacteria and host cells. However, at doses of IVIG capable of mediating protection, the addition of salicylate decreased survival, perhaps by altering an important host response. There are likely pleiotropic effects of salicylate on both host and target bacteria that are not well understood.

REFERENCES

1. **Batshon, B. A., H. Baer, and M. F. Schaffer.** 1963. Immunologic paralysis in mice by *Klebsiella pneumoniae* type 2 polysaccharide. J. Immunol. **90:**121–126.
2. **Blumenkrantz, N., and G. Asboe-Hansen.** 1973. New method for quantitative determination of uronic acids. Anal. Biochem. **54:** 484–489.
3. **Cross, A. S., J. C. Sadoff, E. Furer, and S. J. Cryz, Jr.** 1990. *Escherichia coli* and *Klebsiella* vaccines and immunotherapy. Infect. Dis. Clin. N. Am. **4:**271–282.
4. **Cryz, S. J., Jr.** 1990. *Klebsiella* polysaccharide vaccines. Adv. Biotechnol. Processes **13:**87–103.
5. **Cryz, S. J., Jr., A. S. Cross, E. Fürer, N. Chariatte, J. C. Sadoff, and R. Germanier.** 1986. Activity of intravenous immune globulins against *Klebsiella*. J. Lab. Clin. Med. **108:**182–189.
6. **Cryz, S. J., Jr., E. Fürer, and R. Germanier.** 1984. Protection against fatal *Klebsiella pneumoniae* burn wound sepsis by passive transfer of anticapsular polysaccharide. Infect. Immun. **45:**139–142.
7. **Domenico, P., D. L. Diedrich, and B. A. Cunha.** 1989. Quantitative extraction and purification of exopolysaccharides from *Klebsiella pneumoniae*. J. Microbiol. Methods **9:**211–219.
8. **Domenico, P., W. G. Johanson, Jr., and D. C. Straus.** 1982. Lobar pneumonia in rats produced by clinical isolates of *Klebsiella pneumoniae*. Infect. Immun. **37:**327–335.
9. **Domenico, P., R. J. Salo, D. C. Straus, J. C. Hutson, and B. A. Cunha.** 1992. Salicylate or bismuth salts enhance opsonophagocytosis of *Klebsiella pneumoniae*. Infection **20:**66–72.
10. **Domenico, P., S. Schwartz, and B. A. Cunha.** 1989. Reduction of capsular polysaccharide production in *Klebsiella pneumoniae* by sodium salicylate. Infect. Immun. **57:**3778–3782.
11. **Domenico, P., and D. C. Straus.** 1985. Extracellular polysaccharide production by *Klebsiella pneumoniae* and its relation to virulence. Can. J. Microbiol. **32:**472–478.
12. **Duguid, J. P., and J. F. Wilkinson.** 1953. The influence of culture conditions on polysaccharide production by *Aerobacter aerogenes*. J. Gen. Microbiol. **9:**174–189.
13. **Edmondson, A. S., and E. M. Cooke.** 1979. The production of antisera to the *Klebsiella* capsular antigens. J. Appl. Bacteriol. **46:**579–584.
14. **Ehrenworth, L., and H. Baer.** 1956. The pathogenicity of *Klebsiella pneumoniae* for mice: the relationship to the quantity and rate of production of type-specific capsular polysaccharide. J. Bacteriol. **72:**713–717.
15. **Karkhanis, Y. D., J. Y. Zeltner, J. J. Jackson, and D. J. Carlo.** 1978. A new and improved microassay to determine 2-keto-3-deoxyoctonate in lipopolysaccharides of gram-negative bacteria. Anal. Biochem. **85:**595–601.
16. **Lee, C.-J.** 1987. Bacterial capsular polysaccharide—biochemistry, immunity and vaccine. Mol. Immunol. **24:**1005–1019.
17. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193:**265–275.
18. **Pollack, M.** 1976. Significance of circulating capsular antigen in klebsiella infections. Infect. Immun. **13:**1543–1548.
19. **Robbins, J. B., R. Schneerson, W. B. Egan, W. Vann, and D. T. Liu.** 1980. Virulence properties of bacterial capsular polysaccharides—unanswered questions, p. 115–132. *In* H. Smith, J. J. Skehel, and M. J. Turner (ed.), The molecular basis of microbial pathogenicity, Dahlem Konferenzen. Verlag Chemie GmbH, Weinheim, Federal Republic of Germany.
20. **Simoons-Smit, A. M., J. J. Verweij-van Vught, and D. M. Maclaren.** 1986. The role of K antigens as virulence factors in *Klebsiella*. J. Med. Microbiol. **21:**133–137.
21. **Straus, D. C.** 1987. Production of an extracellular toxic complex by various strains of *Klebsiella pneumoniae*. Infect. Immun. **55:**44–48.
22. **Tomás, J. M., V. J. Benedí, B. Ciurana, and J. Jofre.** 1986. Role of capsule and O antigen in resistance of *Klebsiella pneumoniae* to serum bactericidal activity. Infect. Immun. **54:**85–89.
23. **Vermeulen, C., A. Cross, W. R. Byrne, and W. Zollinger.** 1988. Quantitative relationship between capsular content and killing of K1-encapsulated *Escherichia coli*. Infect. Immun. **56:**2723–2730.
24. **Wacharotayankun, R., Y. Arakawa, M. Ohta, K. Tanaka, T. Akashi, M. Mori, and N. Kato.** 1993. Enhancement of extracapsular polysaccharide synthesis in *Klebsiella pneumoniae* by RmpA2, which shows homology to NtrC and FixJ. Infect. Immun. **61:**3164–3174.
25. **Williams, P., P. A. Lambert, M. R. W. Brown, and R. J. Jones.** 1983. The role of the O and K antigens in determining the resistance of *Klebsiella aerogenes* to serum killing and phagocytosis. J. Gen. Microbiol. **129:**2181–2191.
26. **Williams, P., and J. M. Tomás.** 1990. The pathogenicity of Klebsiella pneumoniae. Rev. Med. Microbiol. **1:**196–204.