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Structural Characteristics of Polysaccharides That Induce Protection against Intra-abdominal Abscess Formation

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***Bacteroides fragilis* is the anaerobe most commonly isolated from clinical cases of intra-abdominal sepsis. In a rodent model of this disease process, intraperitoneal injection of the capsular polysaccharide complex (CPC) from *B. fragilis* provokes abscess formation, while subcutaneous administration of this complex confers protection against *B. fragilis*-induced intra-abdominal abscesses. The CPC consists of two discrete polysaccharides, polysaccharides A and B (PS A and PS B), each possessing oppositely charged structural groups critical to the ability of these carbohydrates to induce the formation of abscesses. Other bacterial polysaccharides that possess oppositely charged groups (such as the group antigen or capsular polysaccharide from *Streptococcus pneumoniae* type 1 strains) also exhibited potent abscess-inducing capabilities. We report here that positively and negatively charged groups on polysaccharides are also essential for inducing protection against abscess formation. Vaccination of rats with *B. fragilis* PS A, PS B, or the *S. pneumoniae* type 1 capsule protected against intra-abdominal abscesses subsequent to intraperitoneal challenge with each of these polysaccharides. Chemical conversion of the free amino or carboxyl groups on PS A to uncharged *N*-acetyl or hydroxymethyl groups, respectively, abrogated the ability of this polymer to confer protection against polysaccharide-mediated abscess formation. Adoptive transfer of splenic T cells from polysaccharide-vaccinated rats to naive animals demonstrated that T cells mediated this protective activity. T cells transferred from animals vaccinated with a polysaccharide repeating unit (*Salmonella typhi* Vi antigen) that normally contains one carboxyl group but was chemically converted to a polymer that possesses both free amino and carboxyl groups (accomplished by de-*N*-acetylating the Vi antigen) protected naive T-cell recipients against polysaccharide-induced abscesses. These results demonstrate that a distinct structural motif associated with the *B. fragilis* polysaccharides is necessary for induction of protective immunity against abscess formation associated with intra-abdominal sepsis. However, protection is not antigen specific in a traditional sense. Rather, the protective ability of these structurally dissimilar polysaccharides is conferred by, and perhaps specific for, a motif of oppositely charged groups.**

Bacteroides fragilis is the most frequently isolated anaerobic species from human infections such as intra-abdominal sepsis and bacteremia (2, 17), despite the fact that this bacterial species constitutes less than 1% of the normal colonic microflora. Abscess formation associated with intra-abdominal sepsis is a unique pathobiologic host response that may serve to localize infecting organisms within the peritoneal cavity. Although this tissue response is initially beneficial to the patient, untreated abscesses cause significant morbidity and can be fatal. Investigations of the pathogenic potential of *B. fragilis* have shown that the *B. fragilis* capsular polysaccharide complex (CPC) is a primary virulence determinant (7, 13). The CPC promotes abscess formation in a rat model of intra-abdominal sepsis, and vaccination with this complex affords protection against abscess induction following challenge with whole organisms (8, 13). The ability to both induce abscesses and confer protection to abscess formation following exposure to the CPC is mediated by T lymphocytes (14). Induction of abscesses requires the presence of T cells with the $CD4^+$ $CD8^+$ phenotype, while immunity to abscesses caused by *B. fragilis* depends on non-H-2-restricted T cells of the $CD8^+$ phenotype (21, 22).

Traditionally, bacterial polysaccharides have been classified as T-cell-independent antigens (3, 5). We undertook immunochemical and chemical studies of the *B. fragilis* CPC to establish the structural basis for the unusual biologic properties associated with this polysaccharide complex (9, 10, 15). The CPC consists of two discrete, high-molecular-weight component polysaccharides, termed PS A and PS B. Each polysaccharide is composed of distinct oligosaccharide repeating units possessing uncommon constituent sugars with free amino, carboxyl, and phosphonate groups (1) (Fig. 1). PS A is a tetrasaccharide repeating unit that has a balanced positively charged amino group and a negatively charged carboxyl group (Fig. 1A). PS B has a hexasaccharide repeating unit, including an unusual 2-aminoethylphosphonate substituent containing a free amino group and a negatively charged phosphonate group. The galacturonic acid residue contains an additional negatively charged carboxyl group (Fig. 1B). We have shown that ionic interactions between the two saccharide chains tightly link PS A and PS B together to form a high-molecular-weight complex, and immunoelectron microscopy demonstrated that each polysaccharide is coexpressed on the surface of the bacterial cell (25). The elaboration of a complex capsular motif is a conserved trait for all the strains of *B. fragilis* that we have examined (16).

Recently, we have shown in a rat model that particular structural features on PS A and PS B mediate its ability to induce intra-abdominal abscesses (24). Chemical neutralization or removal of the charged amino or carboxyl group

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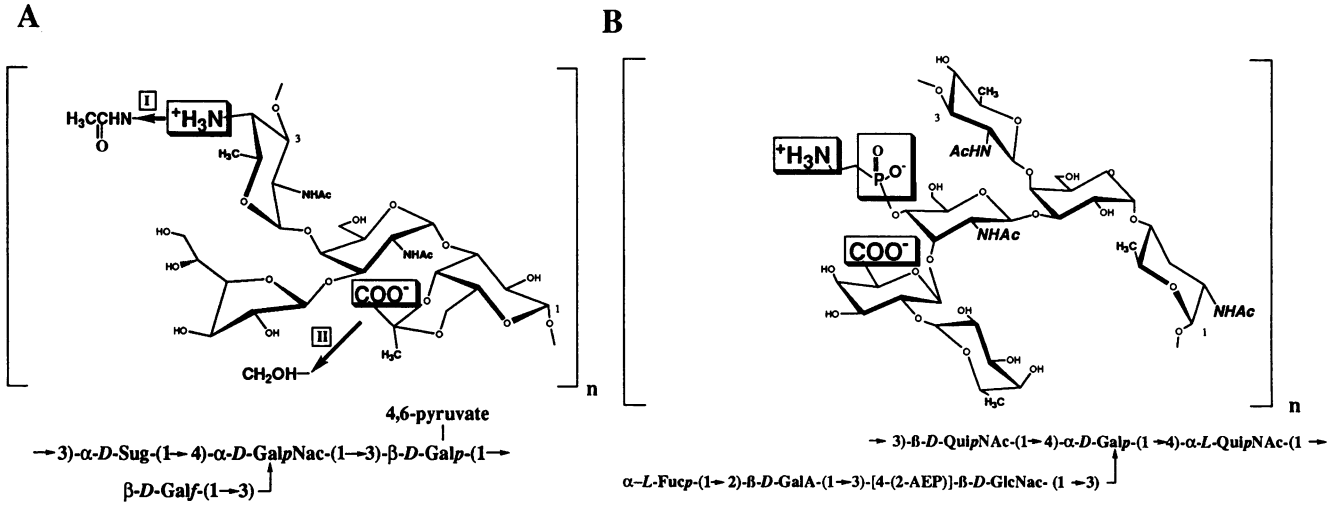

FIG. 1. The fine structures of the *B. fragilis* polysaccharide repeating units were chemically modified (boxed roman numerals) to eliminate or create charged groups. (A) PS A has a tetrasaccharide repeating unit with a balanced positively charged amino group and negatively charged carboxyl group. This polymer was modified as follows: modification I, *N*-acetylation of the free amino group on the 2,4-dideoxy-4-amino-fucosamine (Sug); modification II, carbodiimide reduction of the negatively charged carboxyl group to a hydroxymethyl group. Large boxes indicate charged groups on the repeating unit. (B) PS B has a hexasaccharide repeating unit, including an unusual 2-aminoethylphosphonate (2-AEP) substituent containing a free amino group and a negatively charged phosphonate group. The galacturonic acid residue contains an additional negatively charged carboxyl group. Large boxes indicate charged groups on the repeating unit.

abrogated abscess induction by these polysaccharides. Polysaccharides from other organisms, such as the group antigen or capsular polysaccharide (CP) from *Streptococcus pneumoniae* type 1 strains, that had different repeating unit structures but the same charged structural groups also promoted abscess formation (24). Presently, we have investigated the structural basis for polysaccharide-induced immunity to abscess formation in the rat model of sepsis. These studies show that like abscess induction, the ability of PS A and PS B to induce protection against abscess formation is dependent on the presence of charged groups on polysaccharides and is mediated by T cells.

MATERIALS AND METHODS

Bacterial strain and isolation of *B. fragilis* polysaccharides. *B. fragilis* NCTC 9343 and ATCC 23745 were originally obtained from the National Collection of Type Cultures (London, England) and the American Type Culture Collection (Bethesda, Md.), stored at −80°C in peptone-yeast broth until used, and grown anaerobically as described previously (15). The CPCs from *B. fragilis* NCTC 9343 and ATCC 23745 were isolated by hot phenol-water extraction, and subsequent purification of PS A and PS B was performed as described previously (15, 25).

Polysaccharides with structural features related to PS A and PS B. The *S. pneumoniae* type 1 CP possesses one free amino group and two carboxyl groups per repeating unit (Fig. 2A) (11) and was obtained from the American Type Culture Collection. Previously, this polysaccharide was shown to possess abscess-inducing properties in the rat model of sepsis (24).

Polysaccharide repeating units that lack charges or have negative charges. Polysaccharides that lack charges or have only negative charges were used as controls for these experiments. The capsules from type III group B *Streptococcus* sp. (GBS) (26) and type 3 *S. pneumoniae* each have one negative charge per repeating unit (18), while the capsule from type 14 *S. pneumoniae* lacks charged groups (12). The pneumococcal polysaccharides were obtained from the American Type Culture Collection.

Chemical modifications to polysaccharides. Two specific chemical alterations were introduced to modify charged groups on PS A (24). Modification I was *N*-acetylation of PS A and was achieved by treating the sample with acetic anhydride in

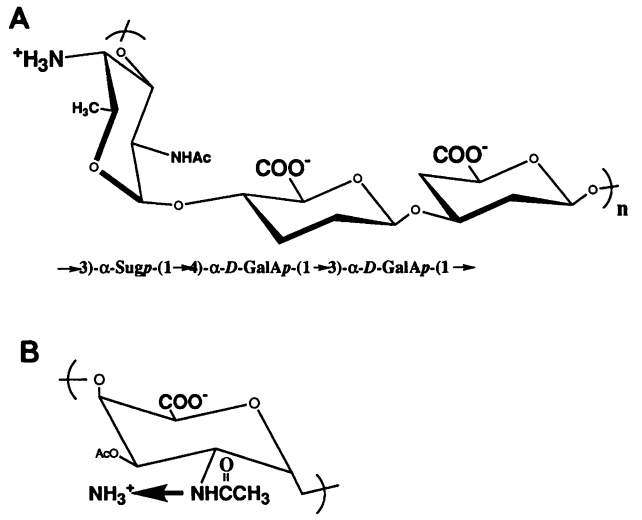

FIG. 2. Repeating unit structures of bacterial polysaccharides tested in the rat model. (A) The type 1 capsule from *S. pneumoniae* has one positive and two negative charges per repeating unit. This polysaccharide is a potent inducer of abscesses in the rat model of sepsis (24). Sug, 2,4-dideoxy-4-amino-fucosamine. (B) The Vi polysaccharide from *S. typhi* is a homopolymer of galactaminuronic acid. This polysaccharide was selected for use because removal of the acetyl group after alkali treatment leaves a free amino group and gives this repeating unit one positively charged group and one negatively charged group (large arrow).

5% (wt/vol) $NaHCO_3$ (1). Modification II was carbodiimide reduction of the negatively charged carboxyl group on PS A, achieved by the method of Taylor and Conrad (23). The CP from *Salmonella typhi*, a homopolymer of galactaminuronic acid, termed Vi antigen (Fig. 2B), was also used in these studies (4). This polymer was de-*N*-acetylated to create a polymer with a positively charged free amino group and a negatively charged carboxyl group as described previously (24). Modifications to polysaccharide structures have been confirmed by nuclear magnetic resonance spectroscopy (24).

Animal model of intra-abdominal abscess formation. A rat model of intra-abdominal sepsis was used in this study (13). Briefly, male Wistar rats (180 to 200 g; Charles River Laboratories, Wilmington, Mass.) were used for all experiments. Animals were housed separately and received chow (Ralston Purina, St. Louis, Mo.) and water ad libitum. Animals were anesthetized with a single intraperitoneal injection of 0.15 ml of pentobarbital sodium (Nembutal; Abbott Laboratories, North Chicago, Ill.; 50 mg/ml), and their abdomens were shaved and swabbed with tincture of iodine. An anterior midline incision (1 cm) was made through the abdominal wall and peritoneum, and a gelatin capsule with 0.5 ml of inoculum containing 10^8 CFU of *B. fragilis* or 200 μg of polysaccharide was inserted into the pelvis. The incisions were closed with interrupted 3.0 silk sutures, and the animals were returned to the cages. The inoculum contained a 1:1 mixture of the test polysaccharide in phosphate-buffered saline (PBS) and an adjuvant solution containing sterile rat cecal contents and 10% (wt/vol) barium sulfate. Six days later, animals were necropsied in a blinded fashion and examined for intra-abdominal abscesses.

Vaccination with bacterial polysaccharides. Animals were vaccinated with bacterial polysaccharides (21, 22) by subcutaneous injection of 10 μg of polysaccharide in 0.1 ml of PBS three times a week for 3 weeks. Animals received a booster injection on week 5 and were available for challenge or adoptive transfer experiments on week 6.

Cell transfer experiments. Cell transfer experiments were performed as described previously (21, 22). Spleens were removed from vaccinated or naive rats and gently teased into RPMI medium supplemented with 5% fetal calf serum. Cells were counted with a Coulter FN counter (Coulter Electronics, Inc., Hialeah, Fla.) and were examined for viability by trypan blue exclusion. The preparation was enriched for T cells by passage over nylon wool columns. Purified T cells were then counted and adjusted to the appropriate cell number (10^7 per animal) prior to intracardiac transfer to animals (0.2-ml volume).

Statistical analyses. Fisher's exact test was used to calculate differences in abscess-inducing potential between experimental and control polysaccharide groups.

RESULTS

Polysaccharide-mediated protection against abscesses induced by heterologous *B. fragilis* strains. Previous studies have shown that the NCTC 9343 CPC and ATCC 23745 CPC are distinct polysaccharide complexes (16). Although the fine structure of the NCTC 9343 CPC has been elucidated, structural analysis of the ATCC 23745 CPC has not been completed. Immunochemical studies have demonstrated that like the NCTC 9343 CPC, the ATCC 23745 CPC consists of at least two distinct polysaccharides (16) possessing positively and negatively charged groups, although the constituent monosaccharides of the CPCs found on the two strains are distinct (10). Heterologous and homologous *B. fragilis* species were used to challenge rats previously vaccinated with either purified NCTC 9343 CPC or ATCC 23745 CPC. In both cases, vaccination with CPC protected rats against abscess formation following challenge with either *B. fragilis* NCTC 9343 or ATCC 23745 (Table 1).

TABLE 1. Protection against abscess formation by viable *B. fragilis* NCTC 9343 and ATCC 23745

Vaccine[a]	Challenge organism (10^8 CFU/animal)	No. of rats with abscesses/ total no. of rats
Saline (control)	NCTC 9343	4/4
	ATCC 23745	3/3
NCTC 9343 CPC	NCTC 9343	1/10
	ATCC 23745	1/10
ATCC 23745 CPC	NCTC 9343	1/10
	ATCC 23745	1/10

[a] Each animal received 10 μg of vaccine.

Protection against abscesses induced by heterologous bacterial polysaccharides. We have recently established that particular structural features (free amino and negatively charged groups) on polysaccharides mediate abscess formation in the rat model (24). To test whether polysaccharides possessing these charged groups also confer protection against polysaccharide-mediated abscess induction, animals were vaccinated with either PS A, PS B, or the *S. pneumoniae* type 1 CP and challenged with each of these abscess-inducing polysaccharides. In each case, protection against abscess formation was afforded by the homologous or heterologous polymer (Table 2; $P < 0.05$).

Effect of chemical modification of PS A on protection against abscess formation. To determine if the charged groups

TABLE 2. Polysaccharide-mediated cross-protection against abscess formation by *B. fragilis* polysaccharides

Vaccine[a]	Challenge polysaccharide[b]	No. of rats with abscesses/ total no. of rats	P^c
B. fragilis NCTC 9343	*B. fragilis* NCTC 9343	1/8	
Saline (control)	PS A	8/8	
	PS B	9/9	
	S. pneumoniae type 1 PS	8/9	
PS A	PS A	3/10	<0.005
	PS B	1/10	<0.005
	S. pneumoniae type 1 PS	3/10	<0.05
PS B	PS A	1/8	<0.005
	PS B	2/10	<0.005
	S. pneumoniae type 1 PS	4/10	<0.05
S. pneumoniae type 1 CP	PS A	2/10	<0.005
	PS B	2/10	<0.005
	S. pneumoniae type 1 PS	0/10	<0.005

[a] Each animal received 10 μg of vaccine.
[b] Challenge polysaccharide was administered in a 200-μg amount.
[c] Each group was compared with saline-immunized rats challenged with the homologous polysaccharide type.

TABLE 3. Effect of chemical modifications to PS A on induction of protection against abscesses caused by *B. fragilis* polysaccharides

Polysaccharide vaccine[a]	Challenge polysaccharide[b]	No. of rats with abscesses/total no. of rats	P[c]
PS A	PS A	1/8	
N-Acetylated PS A (eliminates positive charge)	PS A	8/10	<0.005
Reduced PS A (eliminates negative charge)	PS A	7/10	<0.05
GBS type 3 CP	PS A	7/9	<0.05
	PS B	8/9	
	S. pneumoniae type 1 CP	7/9	
S. pneumoniae type 3 CP	PS A	6/9	
S. pneumoniae type 14 CP	PS A	6/7	

[a] Each animal received 10 μg of vaccine.
[b] Challenge polysaccharide was administered in a 200-μg amount.
[c] Each group was compared with animals immunized with unmodified PS A and challenged with this same polysaccharide.

TABLE 4. T-cell-mediated protection against abscess formation by native and chemically modified polysaccharides

Polysaccharide vaccine[a]	Challenge polysaccharide[b]	No. of rats with abscesses/total no. of rats	P
Saline (control)	PS A	7/8	
Type III GBS CP	PS A	4/5	
PS A	PS A	0/8	<0.05[c]
	PS B	1/8	<0.05[c]
	S. pneumoniae type 1 PS	0/7	<0.05[c]
N-Acetylated PS A	PS A	7/9	<0.005[d]
Reduced PS A	PS A	6/9	<0.05[d]
Vi	PS A	7/9	
De-*N*-acetylated Vi	PS A	2/8	<0.05[e]

[a] Each animal received 10 μg of vaccine.
[b] Challenge polysaccharide was administered in a 200-μg amount.
[c] Compared with animals given T cells from saline-immunized rats and then challenged with PS A.
[d] Compared with animals given T cells from PS A-immunized rats and then challenged with PS A.
[e] Compared with animals given T cells from Vi polysaccharide-immunized rats and then challenged with PS A.

on these polysaccharides were responsible for mediating protection against abscess formation, we performed chemical modifications of PS A. This polysaccharide repeating unit has a balance of one positive and one negative charge conferred by free amino and carboxyl groups, respectively (Fig. 1A). Specific chemical modifications to PS A were performed to neutralize each of these charged groups, and the modified polysaccharides were used to vaccinate animals for protection studies. *N*-Acetylation of the free amino groups on PS A converts these groups to uncharged *N*-acetyl substituents (Fig. 1A, modification I). Carbodiimide reduction reduces the carboxyl group to a hydroxymethyl group, eliminating its negative charge (Fig. 1A, modification II) (24). Animals were vaccinated with either *N*-acetylated PS A or reduced PS A and challenged with the native, unmodified PS A. In each case, the chemically modified polysaccharides failed to protect animals against polysaccharide-induced abscess formation (Table 3; $P < 0.05$ compared with animals vaccinated with native PS A and challenged with PS A). This experiment demonstrated that the free amino and carboxyl groups on PS A are essential for polysaccharide-mediated protection against abscess formation.

Another experiment testing bacterial polysaccharides that either completely lacked charged groups or have only negatively charged substituents was performed. Animals were vaccinated with the type III GBS CP and challenged with either PS A, PS B, or the *S. pneumoniae* type 1 CP. The type III GBS CP, which has one negatively charged group per repeating unit in a terminal sialic acid residue, failed to protect animals against challenge with each of these abscess-inducing polymers (Table 3). Animals vaccinated with the type 3 *S. pneumoniae* CP (one negatively charged group per repeating unit) or the type 14 *S. pneumoniae* CP (no charged substituents in its repeating unit) also failed to protect against abscess formation induced by PS A (Table 3).

T-cell-mediated protection against polysaccharide-induced abscesses. Previously, we have demonstrated that vaccination with the purified CPC protects against abscess formation following challenge with viable *B. fragilis* (8) by a T-cell-dependent mechanism (14, 21, 22). In the present experiments, we tested whether protection against polysaccharide-induced abscesses is also T lymphocyte dependent. Naive rats were administered purified T cells (10^7 cells per animal) obtained from animals previously vaccinated with PS A. T-cell recipients were then challenged with abscess-inducing polysaccharides (PS A, PS B, or the type 1 *S. pneumoniae* CP). In each case, T cells from PS A-vaccinated animals protected naive animals against abscesses formed subsequent to homologous and heterologous polysaccharide challenge (Table 4; $P < 0.05$ compared with animals given T cells from saline-treated animals and challenged with native PS A). Rats receiving T cells obtained from animals immunized with type III GBS CP were not protected against abscesses following challenge with PS A (Table 4).

Chemical modification to eliminate oppositely charged groups on polysaccharides that induce T-cell-dependent protection against abscess induction. To assess the role of the oppositely charged groups on PS A in T-cell-mediated protection against abscess induction, animals were vaccinated with either *N*-acetylated PS A or carbodiimide-reduced PS A, and T cells from these animals were administered to naive rats. T cells taken from animals vaccinated with the chemically modified versions of PS A failed to confer protection against challenge (Table 4; $P < 0.05$). Another group of rats receiving adoptively transferred T cells taken from rats previously immunized with the native PS A were protected from polysaccharide-induced abscesses (Table 4).

T-cell-mediated protection by a polysaccharide created to contain oppositely charged groups. We performed an additional adoptive T-cell transfer experiment to confirm that the presence of both positively and negatively charged groups on polysaccharides is required for protection against polysaccharide-mediated abscess formation. Chemical de-*N*-acetylation of the Vi capsular polysaccharide of *S. typhi*, a homopolymer of galactaminuronic acid, was employed to convert this polysaccharide from a polymer that possessed one negatively charged carboxyl group per repeating unit to a saccharide that possessed one positively charged free amino group and one negatively charged carboxyl group per repeating unit. In this experiment, T cells were harvested from the spleens of animals

vaccinated with the unmodified or de-*N*-acetylated Vi polysaccharides and transferred to separate groups of naive rats. Each group of rats that received T cells was then challenged with *B. fragilis* PS A. Rats receiving T cells from animals vaccinated with the unmodified Vi polysaccharide were not protected against abscess induction by PS A, while animals receiving T cells from rats vaccinated with de-*N*-acetylated Vi polysaccharide were protected against abscess induction by PS A (Table 4; $P < 0.05$).

DISCUSSION

Abscess formation is a classic immunologic host response to particular microorganisms. Recently, we reported that distinct charged structural features associated with the component polysaccharides of the *B. fragilis* CPC as well as other bacterial polysaccharides mediated intra-abdominal abscess induction in a rodent model by this organism (24). In the present study, we examined whether these same oppositely charged groups were requisite physical properties for polysaccharides to confer protection against abscess formation.

The experiments performed demonstrated that vaccination with either of two antigenically distinct *B. fragilis* polysaccharides afforded protection against abscesses induced by challenge with viable bacteria of each strain. Vaccination with the CPC from either *B. fragilis* NCTC 9343 or *B. fragilis* ATCC 23745 protected animals against abscess formation following challenge with the intact homologous or heterologous bacterial strain. These results supported the concept that positively and negatively charged groups on these polysaccharides may be responsible for this protective activity. Further animal experiments with purified polysaccharides were conducted to test this hypothesis.

We sought to determine whether the component polysaccharides of the *B. fragilis* NCTC 9343 CPC conferred protection against polysaccharide-stimulated (rather than bacterial cell-stimulated) abscesses. Each of the *B. fragilis* component polysaccharides is structurally distinct but possesses similar charged groups. Vaccination with PS A or PS B protected against challenge with each of the abscess-inducing polymers (PS A, PS B, or the *S. pneumoniae* type 1 CP) tested. Furthermore, vaccination with the *S. pneumoniae* type 1 CP protected rats against abscess formation following challenge with these same abscess-inducing polysaccharides. These experiments indicated that the heterologous protective nature of the different polysaccharides tested was due to the presence of oppositely charged groups on these polymers.

To address the issue of the role of these charged groups in polysaccharide-mediated protection, chemical modifications were introduced into the repeating unit structure of PS A. These modifications were performed such that the free amino or carboxyl groups on this polymer were converted to uncharged *N*-acetyl and hydroxymethyl groups, respectively. Previously, we have performed similar modifications to the structures of both PS A and PS B to demonstrate the essential role of these oppositely charged groups in conferring abscess-inducing potential to these polymers (24). In the present experiments, vaccination with *N*-acetylated PS A or carbodiimide-reduced PS A failed to induce protection against abscess formation following challenge with the abscess-inducing native PS A. Vaccination with bacterial polysaccharides that possessed one negative charge per repeating unit (type III GBS CP and *S. pneumoniae* type 3 CP) or lacked any charged groups (*S. pneumoniae* type 14 CP) also failed to protect against challenge with the unmodified PS A. The chemical modifications of PS A demonstrated that positively charged free amino groups and negatively charged carboxyl groups on this polysaccharide are critical to the protective activity exhibited by these polysaccharides.

Previously, protection conferred by the *B. fragilis* CPC against abscesses formed in response to challenge with intact *B. fragilis* was shown to be mediated by T cells but not B cells (14, 21, 22). To establish that T cells also controlled protective activity exhibited by the *B. fragilis* component polysaccharides following challenge with abscess-inducing polysaccharides, we performed adoptive transfer experiments. T lymphocytes from PS A-vaccinated rats transferred the ability to protect naive rats against challenge with the abscess-inducing polysaccharides (PS A, PS B, and *S. pneumoniae* type 1 CP). However, transfer of T cells from rats vaccinated with *N*-acetylated or reduced PS A did not confer this protective activity. An important T-cell transfer experiment was performed in which animals were vaccinated with either a polysaccharide that has one negatively charged carboxyl group per repeating unit (the Vi polysaccharide) or the same polysaccharide chemically converted to possess both a positively charged amino group and a negatively charged carboxyl group (the de-*N*-acetylated Vi polysaccharide). The ability of T cells from animals vaccinated with the de-*N*-acetylated polymer to confer protection against abscess induction confirmed the importance of the free amino and carboxyl groups on abscess-inducing polysaccharides in conferring T-cell-mediated protection against abscess formation.

The role of specific T-cell phenotypes in abscess induction and protection against abscess formation has been established by using adoptive transfer experiments in nude mice (21, 22). In these earlier studies, abscess induction by *B. fragilis* or the CPC was dependent on a $CD4^+$ $CD8^+$ inducer T cell, while two distinct T cells of the $CD8^+$ phenotype were responsible for conferring immunity to abscess formation. Immunity to abscesses in this system was non-major histocompatibility complex restricted. In the present study, we show that the unusual ability of the *B. fragilis* CPC to evoke T-cell-dependent protection is attributable to the distinctive structural motif associated with the component polysaccharides of this complex. The ability of polysaccharides to provide protection to abscess induction following challenge with different abscess-inducing polysaccharides and the fact that elimination of either of the charged groups on polysaccharides abrogates this protective activity suggest that these polymers act in a similar manner on T cells. These data also suggest that the oppositely charged groups on these polysaccharides are somehow responsible for this interaction. Recently, we have demonstrated that abscess-inducing polysaccharides require free amino groups as the specific type of positive charge on these repeating units, while different negatively charged groups (such as carboxyl or phosphate groups) can serve as the source of negatively charged groups on these polymers (24a). This was an interesting finding that prompted us to consider that direct interaction of these polysaccharides with T cells is most likely facilitated through the amino functions on these polymers since these structural groups can readily participate in chemical reactions that lead to covalent bonding. A number of published reports support this concept. Investigators have recently demonstrated that free amino groups are capable of forming transient covalent bonds with T-cell surfaces as a preliminary step to antigen-specific T-cell mitogenesis (19, 20, 27). It is thought that this interaction serves to bring T cells and antigen-presenting cells in close proximity to facilitate T-cell activation. These data indicate that free amino groups on polysaccharides may participate in similar interactions that function to stimulate a distinct T-cell activity that eventually leads to abscess

induction or confers protection against abscess formation in the host.

This hypothesis raises another question concerning the type of T cell that is targeted during "immunization" with these polysaccharides. Previously, we have suggested the possibility that a T-cell circuit controls abscess induction and immunity to abscess formation (6). In this system, two discrete suppressor T cells ($CD8^+$) downregulate the ability of $CD4^+$ $CD8^+$ inducer T cells to form abscesses. We can now speculate that perhaps repeated immunization with abscess-inducing polysaccharides actually serves to activate a suppressor cell subset that downregulates specific T-cell function responsible for abscess formation. We are currently investigating the cell type(s) involved and the possible existence of this downregulatory activity.

In summary, these data demonstrate that distinct structural features on polysaccharides that have been shown to induce abscesses in a rat model of sepsis also mediate the ability of these polymers to confer protection against abscess induction. Adoptive transfer studies showed that this protective activity is conferred by T cells. This work forms the basis for our understanding of the unusual biologic properties associated with the *B. fragilis* CPC and provides an opportunity to study in greater detail the polysaccharide–T-cell interaction that mediates this pathobiologic host response.

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