Prokaryotic Peptides That Block Leukocyte Adherence to Selectins

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Summary

Pertussis toxin binds target cells through the carbohydrate recognition properties of two subunits, S2 and S3, which share amino acid sequence similarity with the lectin domains of the eukaryotic selectin family. Selectins appear on inflammed endothelial cells and promote rolling of leukocytes by reversibly binding carbohydrates. S2, S3, and synthetic peptides representing their carbohydrate recognition domains competitively inhibited adherence of neutrophils to selectin-coated surfaces and to endothelial cells in vitro. These proteins and peptides also rapidly upregulated the function of the leukocyte integrin CD11b/CD18. These findings implicate mimickry of eukaryotic selectins by prokaryotic adhesive ligands and link the mechanisms underlying leukocyte trafficking to microbial pathogenesis.

Proper cell trafficking in eukaryotic systems involves recognition of addresses encoded in cell surface carbohydrates. A cell-cell recognition system similar in principle operates during infections to assure colonization of the host and proper tissue tropism of the pathogen. While most bacteria recognize a highly restricted range of eukaryotic cellular carbohydrates (1), a few display more complex adhesins capable of multivalent binding to carbohydrates or proteins, a motif that is reminiscent of eukaryotic cells (2). Bordetella pertussis, the etiologic agent of whooping cough, attaches to cilia and macrophages of the human respiratory tract via two multivalent proteins, filamentous hemagglutinin (FHA) and pertussis toxin (PT) (3–6). PT has been shown to cooperate with FHA in promoting uptake of bacteria into macrophages (7). This effect is linked to the ability of FHA to recognize the integrin CD11b/CD18 on leukocytes (4) and suggests the interaction of PT with the leukocyte might lead to the upregulation of this integrin. The fact that the CD18 integrins are important for leukocyte trafficking, particularly the process of transmigration from blood into tissues, raised the possibility that PT might also interact with a eukaryotic system of cell-cell adherence proteins.

PT is a hexameric toxin of the classical AB architecture (8). This toxin has two clearly distinguishable physiological effects that appear to involve separate mechanisms. The most well-known effect involves the ADP-ribosylation of G proteins by the A subunit leading to increased intracellular cyclic AMP and eventual cellular dysfunction (8). The physiological hallmarks of this effect are sustained leukocytosis and histamine sensitization. For this effect the B oligomer (subunits S2, S3, S4, S5; 1:1:2:1) serves to confer cellular recognition properties for delivery of the toxic A subunit. In Chinese hamster ovary cells, the B oligomer appears to bind to a 165-kD sialylated glycoprotein, which then slowly (over hours) leads to the ADP-ribosylation by the A subunit of a 41-kD protein and the subsequent morphologic response of cell clustering (9). Distinct from this slow effect on eukaryotic cells is a second set of rapid effects that are attributable to the B oligomer and are completely independent of the A subunit, including mediation of adherence of the intact bacteria to cilia, bacterial adherence and uptake into macrophages, and T cell mitogenicity (3, 7, 10). All known cellular recognition by PT is directed to carbohydrates on eukaryotic cell surface proteins or lipids but the identification of a single receptor to explain the rapid effects of the B oligomer has not been possible. Rather, it may be that there are multiple surface components on eukaryotic cells, that bear sialylated and nonsialylated carbohydrate determinants for binding of the B oligomer (7, 10–12). Binding of the B oligomer to T cells in a sialic acid-dependent fashion mediated by the S3-S4 dimer leads to the rapid downregulation of the TCR CD3 (13). In contrast, binding of the B oligomer by the S2-S4 dimer to T cells in a non-sialic acid-dependent fashion to a 43-kD T cell protein leads within seconds to the generation of inositol triphosphate, diacylglycerol, and increased cytosolic free calcium independent of the TCR CD3 or CD28 (10). These effects suggest that the B oligomer of PT can

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1 Abbreviations used in this paper: FHA, filamentous hemagglutinin; PT, pertussis toxin.
affect cellular signal transduction by a more direct mechanism than inactivation of G proteins and that some of the resulting effects could alter cellular adhesion properties.

Recognition of eukaryotic cells by the B oligomer is mediated by the carbohydrate recognition domains of subunit S2 (21,925 daltons) and the 80% homologous subunit S3 (21,873 daltons). Localization and structural requirements for ligand binding by these lectin domains have been accomplished using recombinant subunits, extensive site-directed mutagenesis, and antibody mapping studies (6, 14–18). Although the precise residues that have various carbohydrate binding properties differ between each assay in each laboratory, a common theme has emerged implicating the 50 NH2-terminal residues and a region roughly spanning residues 90–120 as having strong effects on carbohydrate recognition. All carbohydrate recognition can be abolished by mutation of S2 residues Arg92, Asn93, or Asn105 or S3 Lys105 (6, 18). Mutation of a second region of the subunits extending between residues 37 and 52 causes alteration of carbohydrate specificity between lactose or sialic acid (6). We have focused on this latter region for two reasons: a) loss of carbohydrate binding as shown for other regions can be a nonspecific effect due to alteration of tertiary structure, while changing of carbohydrate target specificity is a property compatible with a region at or very near an actual binding site, and (b) this region shows sequence similarity with the eukaryotic C-type lectin family (6). We reasoned that the coincidence of this structural similarity with the rapid effects of the B oligomer on leukocytes might indicate that PT subunits have properties of the selectins, the subset of C-type lectins that mediate leukocyte-endothelial cell interactions (19).

Three selectins have been characterized and all share a terminal lectin domain. P-Selectin (GMP-140) and E-selectin (ELAM-1) appear on activated endothelial cells and mediate adherence of leukocytes by recognition of sialylated and nonsialylated determinants (20–24). L-Selectin (human leukocyte homing receptor; hLHR) is present on lymphocytes and mediates homing to lymphoid tissue bearing sialylated glycoconjugates (25). Selectins play an important role in promoting the margination and reversible rolling of leukocytes at sites of tissue inflammation. Upon ligation of selectins, neutrophils rapidly upregulate the integrin CD11b/CD18, which then promotes the transition from leukocyte rolling to transmigration (24, 26). Since FHA binds to CD11b/CD18 (4) and the B oligomer of PT rapidly promotes FHA-mediated bacterial uptake by this receptor (7), it appeared reasonable to investigate if PT subunits had selectin-like properties. Furthering the rationale to focus this line of investigation on the carbohydrate recognition domain in the NH2-terminal region of S2 and S3 was the observation that carbohydrate binding properties have recently been attributed to the NH2-terminal region of E-selectin (27), and peptides patterned after residues 23–63 of P-selectin inhibit leukocyte adherence to P-selectin–coated substrates (28, 29). We present evidence herein that the lectin domains of S2 and S3 of pertussis toxin are structural and functional analogues of the lectin domains of eukaryotic selectins.

Materials and Methods

Recombinant Subunits and Peptides. PT and B oligomer (List Biologicals, Campbell, CA) were tested as purified proteins. Recombinant subunits S2, S3, S4, and S2 subunit analogues with site-specific amino acid substitutions and chimeric subunits with S2 containing S3 residues 31–54 (S2/S3) or S3 containing S2 residues 31–61 (S3/S2) were generated as described previously (6, 30, 31) and tested as inclusion body preparations standardized to 50 µg/ml of the desired protein.

12 peptides derived from the region of sequence similarity shown in Fig. 1 were synthesized. Using residue sequences as shown in Fig. 1, these peptides were: P-selectin 24–38 and 27–46; E-selectin 24–38, 27–45, and 32–45; L-selectin 24–38 and 27–45; S2 28–45, 32–53, and 39–53; S3 28–45 and 39–53. A peptide of scrambled S2 sequence SQLRVLLEAGELTAYGDL was used as a control. Selectin t-Butoxy carbonyl (t-BOC) peptides were synthesized on a peptide synthesizer (430; Applied Biosystems, Inc., Foster City, CA) using standard t-BOC/bicyclohexyl-carbodiimide/dichloromethane coupling chemistry. Syntheses were performed at the 0.5-mmol scale using standard t-BOC amino acids, with the following side chain protecting groups: Arg (touensulfonyl), Asp (Obenzyl), Cys (4-methylbenzyl), Glu (Obenzyl), His (dinitrophenyl), Lys (2-chlorobenzoxycarbonyl), Ser (benzyl), Thr (benzyl), and Tyr (2-bromobenzoxycarbonyl). All histidine, glutamine, arginine, and asparagine residues were double coupled. The relevant COOH-terminal amino acid–coupled 4-(carboxamidonethyl)benzyl ester linked polystyrene was used as the starting resin. The final resin was dried and subjected to hydrofluoric acid cleavage and deprotection. The cleaved, dried peptide was purified to >90% purity by HPLC using a 300-Å pore size, 1 × 25-cm reverse-phase column (C4; Vydac, Hesperia, CA) with binary gradients of 0.05% TFA/acetonitrile and 0.1% TFA/water, and lyophilized. Amino acid compositions were verified on an amino acid composition system (420A/130A/920A; Applied Biosystems, Inc.).

Adherence of Neutrophils to Selectin-coated Surfaces. Terasaki tissue culture plates were coated with 10 µg/ml E-selectin (courtesy of R. Lobb, Biogen, Cambridge, MA [23]) or P-selectin (courtesy of R. McEver, University of Oklahoma, Oklahoma City, OK [20, 28]), then blocked with albumin (5 mg/ml), washed, and incubated for 10 min at 37°C with 10 µg/ml of purified PT or B oligomer, recombinant purified S2, S3, or S4, S2 analogues containing site-specific mutations, or 1 mg/ml synthetic peptides. LPS (10 µg/ml) was tested as a control for residual endotoxin in inclusion body preparations of recombinant subunits. Human neutrophils were isolated according to the manufacturer’s specifications using neutrophil isolation medium (Cardinal Associates, Santa Fe, NM). Neutrophils were then cultured overnight in human serum and the adherent cells were counted in a hemocytometer. Neutrophils were then centrifuged and washed 3 times with PBS and reincubated on the tissue culture plates.

Figure 1. Sequence comparison of the NH2-terminal regions of subunits S2 and S3 of PT and the selectins. Sequences were aligned according to the algorithm of Needleman and Wunsch (34) using the GENA-LIGN program, resulting in an alignment score of 68. Boxed residues are those conserved among the proteins and residues in capital letters indicate identities with the consensus sequence of the C-type lectins (CRD) as defined by Drickamer (35). Shaded regions are predicted to be α helical (MICROGENIE program).
trophils (10⁶/ml) were added without washing the plate and allowed to adhere for 30 min at 37°C. The plate was then inverted for 10 min and rinsed by brief submersion in buffered saline. The number of neutrophils adherent to the surfaces in a 40x microscope field was quantitated and expressed as a percentage of adherence in buffer taken as 100%. Repetition of the assay with the substitution of albumin for the selectin used in coating the surfaces revealed no effect on leukocyte adherence by any PT component tested, indicating the effects observed with the selectins were specific.

Uregulation of the Integrin CD11b/CD18 on Neutrophils. Neutrophils (5 µl of 10⁶ cells/ml) were allowed to spread for 30 min at 37°C in Terasaki tissue culture wells. Adherent neutrophils were exposed to 5 µl of PT subunit (50 µg/ml), synthetic peptides (0.5 mM), LPS (10 µg/ml), or fNLLP (5 × 10⁻⁴ M) for 30 min at 37°C. After aspiration of the supernatant fluid, 5 µl of C3bi-coated erythrocytes (10⁵ cells/ml; C3bi is a specific ligand for the integrin CD11b/CD18 [32]) were added for 30 min at 37°C. Uncoated, IgM- or IgG-coated erythrocytes were used as controls. After washing, the number of erythrocytes bound per 100 leukocytes (adherence index) was quantitated visually under an inverted microscope. Intoxication of the leukocytes by PT did not affect the assay results during the 30-min incubation period tested since induction of enhanced adherence of C3bi-coated erythrocytes by fNLLP was equivalent in the presence or absence of PT (basal value of 296 increased to 1,628 or 1,265 particles/100 leukocytes, respectively).

Adherence of Neutrophils to Cultured Endothelial Cells. Human umbilical vein endothelial cells (first passage; Clonetics, San Diego, CA) were subcultured at confluence into Terasaki tissue culture wells. After 24 h, confluent monolayers were used for adherence assays (26). Monolayers were stimulated with 10 ng/ml TNF-α (Boehringer Mannheim Biochemicals, Indianapolis, IN) at 37°C for 4 h to induce expression of E-selectin. Human neutrophils (10⁵/ml) were labeled with fluorescein (26), and preincubated for 15 min at 37°C with peptides (0.5 mM in HAP [PBS containing 0.5 mg/ml human serum albumin, 3 mM glucose, and 0.3 U/ml aprotinin]) or HAP as a control buffer. For some experiments, selectin- or integrin-mediated adherence was differentiated by also preincubating the neutrophils with 50 µg/ml of the antibody IB4 against the CD18 integrins (33). Neutrophils were allowed to adhere to the monolayer for 15 min at 37°C and unbound cells were removed by submersion of the plate in saline buffer. The number of adherent neutrophils was counted in a 40x microscope field and expressed as a percentage of adherence in control wells with neutrophils treated with HAP buffer alone.

Results

Similarity of Sequence of Subunits S2 and S3 and the Selectins. The amino acid sequence of the NH2-terminal regions of the lectin domains of the selectins and the carbohydrate recognition domains of S2 and S3 were compared. Significant sequence similarity was observed between PT subunit residues 19–52 and selectin residues 15–46 (Fig. 1). A strong prediction of α-helical structure for >10 sequential residues was found for residues 28–40 of E-selectin, 27–43 of L-selectin, and 33–42 of S3; shorter α-helical regions were predicted for P-selectin 29–35 and S2 35–46 (Fig. 1). Based on these similarities, purified subunits S2 and S3, analogues of the subunits containing site-specific mutations, and peptides corresponding to the region in Fig. 1, were made and tested in assays of selectin function in vitro and in vivo.

The Ability of Purified Subunits and Subunit Analogues to Block Leukocyte Adherence to Selectins and Upregulate Integrins. In view of the apparent structural similarities of the NH2-terminal regions of S2 and S3 to E-selectin and P-selectin, we tested PT, B oligomer, and individual PT subunits for functional selectin-like activity in vitro. Neutrophils adhere avidly to substrates coated with E-selectin and P-selectin; PT and B oligomer were highly effective competitive inhibitors of this interaction, competing in a dose-dependent manner (Fig. 2). The maximum inhibition of leukocyte adherence to both selectins by the B oligomer was ≈75% and was achieved at concentrations of 2 µM (150 µg/ml). Substantial inhibition of adherence (>25%) was still evident at concentrations of 130 nM (10 µg/ml).

To examine the specificity of this inhibition of adherence, the effects of B oligomer were compared with isolated subunits and subunit chimeras (Fig. 3). Inhibition of adherence to P-selectin equivalent to that seen with 130 nM of the native B oligomer was achieved by 30 nM (10 µg/ml) of partially purified S2 (27 ± 3%); S3 was consistently less effective (2 ± 9%). Conversely, inhibition of neutrophil adherence to E-selectin equivalent to that seen with 130 nM of the native B oligomer was achieved by 30 nM (10 µg/ml) of partially purified S3 (36 ± 5%); S2 was consistently less effective (9 ± 7%). These effects were dependent on the amino acid sequence of the lectin domain of the subunits since chimeric subunits having interchanged carbohydrate recognition do-
Glutamic acid, tyrosine, arginine, and glycine (Fig. 4), the residues known to change the carbohydrate specificity of S2 from nonsialylated to the sialylated ligands preferred by S3.

Under certain conditions, when neutrophils engage selectins on the surface of activated endothelial cells, integrins of the CD11/CD18 family are rapidly upregulated on the neutrophil surface. Subunits S2 and S3 (50 µg/ml, 150 nM) were as effective as fNLLP, PT, and native B oligomer at enhancing the binding of a CD11b/CD18 ligand (C3bi-coated erythrocytes) to neutrophils (>300% of control; Fig. 5); subunit S4 was ineffective. LPS at a concentration representative of the level of contamination of the subunit preparations was also ineffective, as expected in assays performed in the absence of serum. These effects were independent of the A subunit of the toxin as indicated by the equivalent response to PT and B oligomer. Furthermore, the upregulation occurred within 30 min of exposure to the PT subcomponents as opposed to the time frame of hours for the ADP-ribosylation effects of the holotoxin. This result indicates that concentrations of the subunits that inhibit leukocyte attachment to selectins also cause rapid and significant upregulation of the CD11b/CD18 integrin.

The Ability of S2 and S3 Peptides to Block Leukocyte Adherence to Selectins or Endothelial Cells vs. Upregulate Integrins In Vitro. Various peptides derived from residues 24–45 of the native selectins or from the corresponding region 28–53 of S2 and S3 were synthesized and tested in vitro for two selectin functions: the ability to inhibit neutrophil adherence to E-selectin and upregulate the leukocyte integrin CD11b/CD18. Peptides corresponding to the COOH-terminal half of the lectin domains of Fig. 1 were uniformly less effective at blocking leukocyte adhesion but retained strong activity for upregulation of the CD11b/CD18 integrin (Table 1). Thus, peptides corresponding to the NH2-terminal half of the re-
Table 1. Ability of Peptides Derived from Selectins or PT Subunits to Block Neutrophil Adherence to E-Selectin-coated Surfaces and to Upregulate the Integrin CD11b/CD18

| Peptide*          | Leukocyte binding† | EC3bi binding§ |
|-------------------|--------------------|----------------|
| E-selectin        |                    |                |
| 32KEIEYLNSILSYS⁴⁵ | −2 ± 5             | +243           |
| 27VAIQKEIEYLNSILSYS⁴⁵ | −35 ± 11         | +71            |
| 24THLVAIQKEIEYL³⁸ | > −50              | +73            |
| L-selectin        |                    |                |
| 37VAIQNKAEIYEKTLPS⁴⁵ | −26 ± 5           | +163           |
| 34TLVAIQNKAEIYEYL³⁸ | > −50             | +104           |
| P-selectin        |                    |                |
| 21VAIQNKNEIDYNKVLPPYS⁴⁵ | −9 ± 6            | +113           |
| S2                |                    |                |
| 32GDLQELRHVTRGWS⁵³ | −7 ± 7            | +232           |
| 28VAELRGSDLQELRHVTRGWS⁵³ | +27 ± 5         | +48            |
| 32RALTVAILRGSDLQEL⁴⁵ | > −50            | +104           |
| S3                |                    |                |
| 32AELQTYLRQITPGWS⁵³ | −9 ± 8            | +201           |
| 32RALTVAILRGNAELQTYL⁴⁵ | > −50           | +122           |

* Peptides are offset to highlight regions of overlap.
† Leukocytes were added to wells coated with E-selectin in the presence of 0.5 mM of peptide. Values are the percent of inhibition of attachment as compared with control wells receiving buffer alone and are mean ± SD of triplicate experiments.
§ Erythrocytes coated with C3bi (EC3bi) were incubated with leukocytes pretreated with 0.5 mM of peptide. Values are the percent of increase in attachment as compared with control wells pretreated with buffer alone and are representative of triplicate experiments.

Figure 6. PT peptides inhibit adherence of neutrophils to endothelial cells. Fluoresceinated neutrophils were incubated with peptides (0.5 mM) in HAP buffer; peptides are designated by the numbers of the first and last residues as in Fig. 1. Control cells were treated with HAP buffer alone. Without washing, the neutrophils were allowed to adhere to confluent monolayers of human endothelial cells. After washing, the number of adherent neutrophils was counted per 40× microscope field and expressed as a percentage of adherence in the control HAP wells (mean of 63 ± 9 neutrophils/field). Values represent mean ± SD of triplicate experiments with triplicate wells.
strong predictions of extensive $\alpha$-helical structure in the regions of the biologically active peptides; shorter regions were found in P-selectin and S2. Taken together, the similarities of primary sequence, tertiary structure, and general functional requirements of the prokaryotic and eukaryotic proteins suggested that these proteins might have similar physiological effects.

Selectins on activated endothelia rapidly and reversibly capture surface carbohydrates of leukocytes in the process of leukocyte rolling at sites of inflammation (19). This selectin-carbohydrate interaction also rapidly triggers the upregulation of a second leukocyte adhesion molecule, the CD11b/CD18 integrin (26). The B oligomer of PT (independent of the ADP-ribosyltransferase activity of the A subunit) is also known to bind to leukocyte carbohydrates and rapidly induces signal transduction leading to such effects as mitogenicity (10) and enhancement of bacterial uptake by CD11b/CD18 (7). The shared features of a rapid effect targeted to leukocyte carbohydrates and the CD11b/CD18 integrin are consistent with the hypothesis that the selectins and PT lectins may use similar structural motifs to achieve common effects on leukocyte function.

The B oligomer, individual subunits S2 and S3, and peptides from the S2 and S3 lectin domains strongly inhibited leukocyte adherence to selectin-coated surfaces. B oligomer was equally effective against the P- and E-selectin with 50% inhibition at $\approx 0.5 \mu M$. The individual S2 subunit demonstrated greater activity against P-selectin and the S3 subunit showed greater activity against the E-selectin. This preference was a property, at least in part, of the region 19–53 of these proteins since chimeric subunits containing swap mutations caused a parallel shift in the preferred selectin targeted by the subunit and individual site mutations at residues 43, 44, 45, 50, and 51, which caused S2 to switch carbohydrate affinity to that of S3, also improved the ability of S2 to inhibit leukocyte adherence to E-selectin. This is further supported by the ability of peptides derived from the 19–53 lectin domain of the subunits to block adherence of leukocytes to selectin-coated surfaces. Strong inhibition of adherence was evident for peptides derived from the more NH$_2$-terminal half of this region while the second selectin function of upregulation of the integrin CD11b/CD18 was a property of all peptides throughout the region, indicating the possibility of dissociating the structural requirements for these two physiologic responses. These findings are consistent with the ability of the P-selectin peptide 19–43, which overlaps with the NH$_2$-terminal portion of the homologous domain studied here, to strongly inhibit leukocyte adherence to P-selectin–coated surfaces (90% inhibition at 0.5 mM vs. S3 28–45 $>50\%$ inhibition at 0.5 mM) (29).

When neutrophils engage selectins on the surface of activated endothelia, integrins are rapidly upregulated on the neutrophil surface in order to effect transmigration of the neutrophil across the endothelial barrier (26). PT subunits not only acted as competitive analogues of selectins in leukocyte adherence assays, but also strongly upregulated the CD11b/CD18 integrin on the neutrophil surface. CD11b/CD18 ac-

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**Figure 7.** PT peptides upregulate the leukocyte integrin CD11b/CD18. Neutrophils adherent to plastic surfaces were incubated with peptides (0.5 mM); peptides are designated by the numbers of the first and last residues as in Fig. 1. The cells were washed and overlaid with erythrocytes coated with C3bi (EC3bi), a specific ligand for CD11b/CD18. After washing away unbound EC3bi, the number of EC3bi per 100 leukocytes (adherence index) was determined under an inverted microscope. Values represent the mean ± SD of triplicate experiments with triplicate wells.

200% of control values (Fig. 7). In contrast, three NH$_2$-terminal peptides (S2 and S3 28–45 and E-selectin 24–38) upregulated the integrin significantly less (<50% of resting control levels). Thus, the same S2 and S3 peptides found to be most effective at blocking leukocyte adherence to endothelial cells shared the lowest ability to upregulate the integrin.

**Discussion**

A number of parallels can be drawn between the structure/function analyses of the prokaryotic lectins S2 and S3 of PT and the eukaryotic selectins. Among the carbohydrates recognized by PT subunits S2 and S3 are the sialylated Lewis x determinant and its nonsialylated relative, the Lewis a determinant (14). This affinity pattern is reminiscent of that of the selectin pair, E-selectin and P-selectin. Carbohydrate recognition appears to be a property of the NH$_2$ termini of subunits S2 and S3 as evidenced by the ability to alter the specificity of carbohydrate recognized by the subunits upon mutational alteration of residues 28–52 (6) and the ability of a peptide from S2 9–23 to directly bind to simple sialylated saccharides (17). Similarly, mutational alteration of NH$_2$-terminal residues 7, 8, and 9 or the 43–48 loop of E-selectin either abolished or enhanced carbohydrate binding ability of the protein (27). Conservation of lysine at positions 111 and 113 of E-selectin and lysine 105 of S3 are critical for lectin function (6, 18, 27). Upon more detailed comparison of the amino acid sequences of the NH$_2$-terminal region of the prokaryotic and eukaryotic proteins, significant similarity was apparent between all three selectins and the two lectin subunits of PT. Over a span of 25 residues (19–53) of the PT subunits and 22 or 23 residues (15–45 or 15–46) of the selectins, nine positions of identity and two positions of conservative substitution were found. E-Selectin, L-selectin, and S3 shared...
tivity was stimulated approximately eightfold above control values, an increase comparable to the well-known activator fNLLP. Upregulation of the integrin function was seen for the B oligomer, the individual subunits (especially S3), and for the peptides from the subunit lectin domains; on a molar basis the purified subunits were fourfold more effective than the B oligomer. Most PT peptides were as effective as native selectin peptides in this function, with the notable exceptions of S2 and S3 28-45 and E-selectin 24-38. These latter three peptides showed less ability to affect integrin function than their respective more COOH-terminal peptides. This was particularly evident for the S3 peptide, which showed virtually no upregulation of the integrin.

The ability of the B oligomer of PT to function in a selectin-like manner, particularly the upregulation of CD11b/CD18, is of potential importance to the biology of pertussis infection. Pretreatment of macrophages with cell-free B oligomer enhances bacterial binding to the CD11b/CD18 integrin by the adhesin FHA, resulting in greater uptake and survival of bacteria intracellularly (7). The upregulation of the CD11b/CD18 integrin shown here by the B oligomer, subunits S2 and S3, and the subunit peptide is consistent with and provides a mechanism for this observed activation of the integrin by pertussis toxin released from bacterial cells. Thus subversion of the selectin- and integrin-based systems provides bacterial entry into a second environmental niche in the lung, which prolongs the length of colonization (5) and is compatible with the 6-wk course of infection, which extends 4 wk beyond the documented time of bacterial adherence to its primary niche on ciliated respiratory epithelial cells. It is not likely that these transient selectin-like effects contribute to the substantial abnormalities in leukocyte trafficking characteristic of whooping cough since the intoxication of leukocytes by ADP-ribosylation by the A subunit of PT is known to be the overwhelming cause of the characteristic circulating leukocytosis in this infection.

The process of leukocyte adherence to endothelial cells involves at least two sequential steps, the selectin-mediated reversible association, which allows leukocytes to roll along an endothelial surface, and the second CD18 integrin-mediated step, which promotes stable association of the leukocyte with the endothelium before transmigration. The ability of selectins to upregulate integrin function presumably contributes to the proper sequential activation of these steps. However, in the design of an antiinflammatory agent it would be desirable to dissociate the two steps such that selectin-mediated rolling could be competitively inhibited without triggering leukocyte adhesion by integrins. The dissociation of the activities of blocking leukocyte adherence to selectins and upregulation of the CD11b/CD18 integrin in the studies with the PT and selectin peptides suggested that these peptides might effectively block leukocyte adherence to endothelial cell monolayers, an in vitro system in which both selectin- and integrin-mediated mechanisms can be demonstrated to contribute to adherence. The S2 and S3 28-45 peptides, which upregulated the integrin the least while strongly blocking adherence of neutrophils to selectin-coated surfaces, were highly effective inhibitors of leukocyte adherence to activated endothelia. Based on the in vitro studies presented here, the peptides predicted to have the greatest net antiinflammatory effect are S2 and S3 28-45, the prokaryotic variants of the native selectin sequences. The identification of prokaryotic members of the selectin family expands the range of possibilities for development of selectin analogues with potential therapeutic value.

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