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Research Article

This paper describes a suppressor T cell factor which protects mice against intraabdominal abscesses caused by Bacteroides fragilis. This soluble cell-free factor (ITF) is derived from splenic T cells from mice immunized with capsular polysaccharide (CP) of B. fragilis. Mice receiving ITF are protected from developing abscesses caused by B. fragilis to the same degree as animals receiving intact immune splenic T cells. The factor appears to be small in molecular size as protective activity is dialyzable through a 12,000-mol wt exclusion dialysis membrane and is present in fractions intermediate between the bed and void volumes of a P2 Biogel column. The protective effect of ITF is antigen-specific to B. fragilis alone. Mice given a complex inoculum of B. fragilis, enterococcus, and another anaerobe develop abscesses even after receiving column-purified ITF. The activity of ITF also is eliminated after adsorption with B. fragilis CP coupled to sheep erythrocytes but not with an unrelated CP coupled to sheep erythrocytes. ITF, therefore, appears to have a binding site for B. fragilis CP. ITF is heat-labile and loses efficacy after protease digestion, suggesting that the active material is a protein. These studies define a suppressor cell factor with activity in a model system resembling human disease and offer promise for increased understanding of the diversity of cell-mediated immune systems.

A Soluble Suppressor T Cell Factor Protects Against Experimental Intraabdominal Abscesses

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Abstract

This paper describes a suppressor T cell factor which protects mice against intraabdominal abscesses caused by *Bacteroides fragilis*. This soluble cell-free factor (ITF) is derived from splenic T cells from mice immunized with capsular polysaccharide (CP) of *B. fragilis*. Mice receiving ITF are protected from developing abscesses caused by *B. fragilis* to the same degree as animals receiving intact immune splenic T cells. The factor appears to be small in molecular size as protective activity is dialyzable through a 12,000-mol wt exclusion dialysis membrane and is present in fractions intermediate between the bed and void volumes of a P2 Biogel column. The protective effect of ITF is antigen-specific to *B. fragilis* alone. Mice given a complex inoculum of *B. fragilis*, enterococcus, and another anaerobe develop abscesses even after receiving column-purified ITF. The activity of ITF also is eliminated after adsorption with *B. fragilis* CP coupled to sheep erythrocytes but not with an unrelated CP coupled to sheep erythrocytes. ITF, therefore, appears to have a binding site for *B. fragilis* CP. ITF is heat-labile and loses efficacy after protease digestion, suggesting that the active material is a protein. These studies define a suppressor cell factor with activity in a model system resembling human disease and offer promise for increased understanding of the diversity of cell-mediated immune systems.

Introduction

Bacteroides fragilis, an extracellular gram-negative anaerobe accounting for the majority of positive anaerobic blood cultures, is a common isolate from intraabdominal abscesses, particularly abscesses arising from a colonic source (1). Experimental work, first in a rat and later in a mouse model, has established that T cells, rather than antibody, are responsible for immunity to intraabdominal abscesses induced by the organism (2, 3). These T cells were characterized further as suppressor cells by phenotype (3).

This paper presents evidence that in addition to immune T cells, a cell-free immune T cell factor (ITF)[1] prepared from an immune splenic T cell population protects experimental animals from developing intraabdominal abscesses caused by *B. fragilis*. Preliminary purification of this factor reveals that the active material is low in molecular weight, can bind to *B. fragilis* capsular polysaccharide (CP), and induces antigen-specific immunity to abscesses. The material is heat-labile and loses efficacy after protease, but not nuclease, digestion.

Methods

Bacterial strains. B. fragilis (no. 23745; American Type Culture Collection, Rockville, MD) and *Bacteroides distasonis* (no. 8503; American Type Culture Collection) were employed from the stock collection of the Channing Laboratory, Boston, MA. *Fusobacterium varium* (TVDL 37) was obtained from the stock culture collection of the Tufts University Veterinary Diagnostic Laboratory, Jamaica Plains, MA. *Streptococcus faecalis* (enterococcus) 2988T was a urinary clinical isolate from the Bacteriology Laboratory, Beth Israel Hospital, Boston, MA. Anaerobes were incubated in prereduced peptone yeast glucose medium (Scott Laboratories, Fiskeville, RI) within an anaerobic isolator (Lab-Line Instruments, Melrose Park, IL) for 24–48 h and frozen in 5-ml aliquots in gas tight vials at −70°C. The enterococcus was grown in Todd Hewitt broth (Difco Laboratories, Detroit, MI) incubated for 24 h at 37°C and frozen in the same manner.

Mice. C57BL6 male mice were purchased from The Jackson Laboratory (Bar Harbor, ME). All animals were screened on arrival by serology for active murine virus infection and were housed in cages containing no more than 10 animals. They were given Purina mouse chow (Ralston Purina Co., St. Louis, MO) and water ad libitum, and were from 6 to 10 wk of age at the time of experiments.

Mouse immunization. CP antigen of *B. fragilis* 23745 was isolated and purified by methods previously described (4). Mice were immunized with 10 μg of antigen in 0.1 ml phosphate-buffered saline (PBS) subcutaneously three times a week for 3 wk and were utilized in experiments during the fourth week after immunization was initiated.

Spleen cell preparations. Spleens from immunized or nonimmunized mice were removed by blunt dissection, gently teased apart, and ground over a wire mesh screen. Cell suspensions in balanced salt solution (BSS) with 5% fetal calf serum (FCS) (M. A. Bioproducts, Walkersville, MD) were counted by trypan blue dye exclusion after filtering through glass wool.

Nylon wool column processing. Nylon wool columns were utilized to obtain T cell-enriched spleen cell suspensions. Methods employed here have been described previously (2).

Factor preparation. Lysates of T cell-enriched fractions from both immunized and nonimmunized mice were prepared by serial freeze-thawing known concentrations of cells. Suspensions containing 2.5 × 10^7 cells/ml were shell-frozen with dry ice and alcohol, and then, thawed for several minutes in a boiling water bath, without warming the solution, sequentially four times. Debris was filtered out by passage over glass wool, and the volume returned to starting volume with BSS and 5% FCS. 1-ml aliquots were stored at −80°C. Microscopic examination of the solution revealed no intact cells.

Adsorption of ITF. CP of either *B. fragilis* or type III group B *Streptococcus,* extracted and purified as previously described (4, 5), was coupled to sheep erythrocytes (SRBC) with chromium chloride by the method of Baker et al. (6). Purified CPs were added to 10%

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1. *Abbreviations used in this paper:* CP, capsular polysaccharide; GBS, group B *Streptococcus;* ITF, immune T cell factor; NITF, nonimmune T cell factor; RI, refractive index; SRBC, sheep erythrocytes.

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solutions of SRBC in the presence of 1% chromium chloride. After a 5-min incubation at room temperature, cells were washed in saline. Coupling was confirmed by specific hemagglutination of sensitized erythrocytes in microtiter plates by rabbit antisera raised to purified CP. ITF, prepared as above, was incubated with SRBC coupled to either CP or with SRBC alone at 4°C for 30 min. SRBC concentrations were 5×10^7 and 2.5×10^8 cells for 2.5×10^7 cell equivalents of ITF. After 30-min incubation, SRBC were removed by centrifugation.

Dialysis of T cell factor. 1 ml of the lysate prepared from T cells from immunized mice (ITF) was placed into a 12,000-mol wt exclusion dialysis membrane (Arthur H. Thomas Co., Philadelphia, PA) and dialyzed at 4°C against 10 ml of 5 mM ammonium acetate buffer, pH 7.1. Dialysis proceeded for 24 h with two changes of the dialysate. The contents of the dialysis bag were removed and frozen immediately at −80°C. The dialysate was pooled (total volume, 30 ml) and frozen at −80°C.

Column chromatographic purification of T cell lysate. 1 cm^3 of ITF containing the equivalent of 5×10^7 cells/ml was fractionated on an S-200, 0.9 × 50-cm column (Pharmacia, Uppsala, Sweden). Buffer was PBS, pH 7.2, and the column was run at 4°C at a speed of 8 cm^3/h. Fractions were read at 280, 260, and 210 nm in a spectrophotometer (Perkin-Elmer, Norwalk, CT) and pooled fraction peaks tested for protective activity in mice. 3 ml of active peaks were, in turn, loaded on a P2 Biogel (Bio-Rad Laboratories, Richmond, CA) column, 1.6 × 90-cm (Amicon Corp., Danvers, MA) and run at 4°C in 5 mM ammonium acetate buffer, pH 7.1, at a speed of 8 cm^3/h. Refractive index (RI) was monitored (Waters Associates, Milford, MA), fractions pooled, and testing again performed in mice.

Heat-stability testing of column-purified ITF. 1-ml aliquots of active fractions from the P2 Biogel column were incubated for 30 min at 37 and 56°C and for 2 h at room temperature and 4°C. After incubation, these fractions were frozen again at −80°C and then injected into mice to test for protection against abscesses.

Enzyme digestion of ITF. 1-ml aliquots of ITF were incubated for 2.5 h at room temperature with: (*a*) no enzyme, (*b*) 1 mg pronase B (Calbiochem-Behring Corp., San Diego, CA), (*c*) deoxyribonuclease and ribonuclease (Worthington Biochemicals, Freehold, NJ) in 0.1- and 0.5-mg quantities, respectively, or (*d*) 1 mg trypsin (Worthington Biochemicals). After 2 h, 1 mg of trypsin soybean inhibitor (Sigma Chemical Co., St. Louis, MO) was added to the tube containing trypsin, and the tube was incubated for the additional 30 min. Treated ITF then was placed in 12,000-mol wt exclusion dialysis membranes and dialyzed for 24 h against 10 cm^3 of 5 mM ammonium acetate, pH 7.1, as above at 4°C with two changes of the dialysate. Dialysates were injected into mice to test for protective effect.

Transfer of spleen cells or factor or column fractions into mice. 2.5×10^6 cells or 0.1–0.2 cm^3 of factor or column fractions were injected into mice by the intracardiac route. After 24 h, animals were challenged intraperitoneally with *B. fragilis* or complex inocula containing *B. fragilis* at a concentration of 1×10^6 organisms, enterococcus, and *B. distasonis* or *Fusobacterium varium,* each at a concentration of 1×10^7 organisms. Organisms were mixed 50:50 vol/vol with sterile cecal contents from meat-fed rats as adjuvant. Animals were sacrificed 6 d later and examined for abscesses. Animals with one or more gross abscesses containing polymorphonuclear leukocytes (PMN) by gram stain were scored as positive for abscesses. For the experiments with complex inocula, abscess contents were cultured and gram-negative rods of differing colonial morphologies, usually 5–10 colonies/plate, were subcultured and identified using standard anaerobic identification procedures.

Statistics. Fisher's Exact Test and Comparison of Proportions were utilized for statistical analyses.

Results

Identification of a protective ITF. Splenic cells from mice immunized with CP and naive mice were passed over nylon wool columns to eliminate B cells and macrophages, and varying numbers of T cells were lysed by sequential freezing and thawing. The cell-free lysates, ITF, and nonimmune T cell lysate (NITF) were transferred intracardiacally to naive mice. 24 h later, animals were challenged with *B. fragilis* and sterile cecal contents, and 6 d later, examined for abscesses. Mice with one or more abscesses on exploration and examination of the abscess contents for PMN were recorded as positive. Animals listed as negative for abscesses contained no intraperitoneal collections. Results appear in Table I.

ITF at concentrations of $2.5–25 \times 10^6$ cell equivalents prevented the development of abscesses following challenge with viable *B. fragilis* to the same degree as 2.5×10^6 intact immune T cells. Neither 2.5×10^6 nonimmune T cells or 25×10^6 T cell equivalents of NITF provided any protection. Even a dose of 0.25×10^6 cell equivalents of ITF protected five of eight animals from developing abscesses, a significant degree of protection ($P < 0.01$) compared with NITF. Crude factor prepared by lysing immune T cells, a process which left no cells intact by microscopic examination, was as active as intact cells in preventing abscess formation in mice. Protective ITF was prepared from mouse splenic T cells at least 46 d following completion of the immunization protocol.

Antigen specificity of ITF. To evaluate the antigen specificity of ITF activity, ITF was adsorbed with SRBC coupled with either *B. fragilis* CP or an unrelated CP. CP of *B. fragilis* or type III group B *Streptococcus* (GBS) were bound to SRBC using chromium chloride. ITF was incubated with unbound SRBC or SRBC coupled to *B. fragilis* or GBS CP at a ratio of 2 and 10 SRBC per cell equivalent of ITF. After incubation at 4°C for 30 min, SRBC were removed by centrifugation and 25×10^6 cell equivalents of ITF in 0.2 cm^3 transferred intracardiacally to naive mice. Mice were challenged as usual with *B. fragilis* 24 h later. Table II lists the results.

Mice receiving unabsorbed ITF and ITF absorbed with SRBC alone or SRBC coupled to GBS CP were protected against abscesses caused by *B. fragilis.* Animals receiving ITF absorbed with SRBC coupled to *B. fragilis* CP developed abscesses as did control animals receiving NITF. Therefore, absorption of ITF with SRBC coupled to *B. fragilis,* but not to the unrelated CP, eliminated the protective effect of the factor. These results indicate that ITF derived from immune

Table I. Comparative Ability of Splenic T Cells and T Cell Lysates to Protect Mice Against Abscesses

Cells or factor transferred	No. of cells or equivalents	No. of mice with abscesses/total	Percent protection
Nonimmune T cells	2.5×10^6	8/8	0
Immune T cells	2.5×10^6	1/8‡	87.5
NITF	25×10^6*	10/10	0
ITF	25×10^6*	1/8§	87.5
ITF	2.5×10^6*	1/8§	87.5
ITF	0.25×10^6*	3/8‖	62.5

* Factor was prepared by freezing and thawing a known concentration of spleen cells, filtering, and injecting a 0.2 cm^3 volume of factor at the cell-equivalent dose listed.
‡ $P < 0.001$ compared with nonimmune cells by the Fisher's exact test.
§ $P < 0.001$ compared with NITF by the Fisher's exact test.
‖ $P < 0.01$ compared with NITF by the Fisher's exact test.

Table II. Antigen Specificity of Protective Activity of T Cell Factor

Factor transferred	Prior adsorption of factor	No. of mice with abscesses/total	Percent protection
NITF	—	9/10	10
ITF	—	0/10‡	100
ITF	SRBC	2/20‡	90
ITF	*B. fragilis* CP coupled to SRBC	18/20§	10
ITF	Group B streptococcal CP coupled to SRBC	0/20‡	100

* Factor prepared from 2.5×10^7 immune T cells was adsorbed with SRBC or CPs-coupled by chromium chloride to SRBC for 10 min at 4°C. The ratio of SRBC to immune T cell-equivalents was 2:1 and 10:1 in groups of 10 mice each. Data were pooled.
‡ $P < 0.001$ compared with NITF by Fisher's exact test.
§ $P = 0.5$ compared with NITF by Fisher's exact test.

splenic T cells is capable of specific binding to the *B. fragilis* CP.

Sizing and partial purification of ITF. To assess an approximate molecular size of the component which confers protection, ITF was dialyzed in a 12,000-mol wt exclusion dialysis membrane for 24 h at 4°C against 5 mM ammonium acetate, pH 7.1. ITF, dialysis bag contents, and dialysate were tested in mice to determine protective capacity. Results appear in Table III.

Mice receiving nondialyzed ITF were protected from developing abscesses caused by *B. fragilis*. Animals not receiving ITF all developed abscesses. The active component of the lysate appeared to be smaller than 12,000 mol wt since the dialysis bag contents lost protective capacity, while the dialysate was protective despite a 30-fold dilution from the initial volume of 1 ml.

The T cell lysate was purified partially by molecular sieve chromatography. ITF was loaded initially on an S-200 column and pooled fractions tested for protective activity in mice. In multiple runs, protection was conferred by fractions near and at the bed volume of the column (data not shown). A protective peak which eluted at 26 ml from the S-200 column (where 12 ml represented void volume and 43 ml bed volume) was placed on a P2 Biogel column. 2-ml fractions from the P2 Biogel column were pooled as marked on the elution profile recorded by RI monitoring (Fig. 1). The P2 Biogel

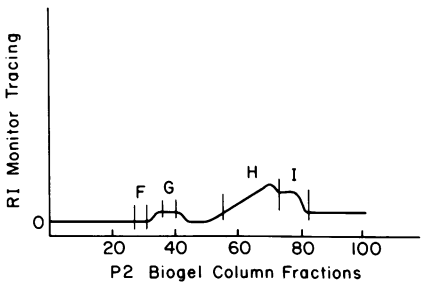

Figure 1. Elution profile of ITF purified on a P2 Biogel column. The elution profile of a representative P2 Biogel column fractionation of partially purified ITF was recorded by monitoring RI. Column speed was 8 cm^3/h at 4°C with 5 mM ammonium acetate buffer, pH 7.1. Two cm^3 fractions were pooled as marked.

column has an exclusion size of 1,800 mol wt for proteins. Doses of 0.2 cm^3 of these pooled fractions were transferred to mice which were challenged and examined for abscesses. Table IV presents the results.

Mice given 0.2 ml of peaks G and H were protected from abscess formation, while bed volume peak I and void volume peak F did not protect. All mice developed abscesses in the control group which did not receive any factor in transfer. These results are representative of eight experiments testing P2 column fractions in mice, and the elution profile was representative of other P2 column runs fractionating protective peaks from the S-200 column. On the P2 Biogel column, protective activity resided in column fractions intermediate between the void volume and bed volume. In subsequent experiments, pooled fractions from several column runs were utilized after testing for ability to protect mice against abscesses. Fractions capable of protecting >80% of mice in a test group were used.

Antigen specificity of the protective action of column fractions. To assess whether the protective capacity of these fractions was specific for *B. fragilis* or induced more general immunity, a complex inoculum was given to mice. Anaerobes other than *B. fragilis* require a facultative aerobic organism to form abscesses in the animal model (7). Enterococcus was the aerobe employed, while *B. distasonis* served as the other anaerobe. The challenge inoculum was designed to provide two means of inducing abscesses, either *B. fragilis* alone or the combination of an anaerobe with an aerobic organism. Results of the experiment utilizing *B. distasonis* are listed in Table V. Mice were not protected from abscess formation in either group in this experiment. Mice receiving active column

Table III. Protective Capacity of ITF and Dialyzed Lysate

Factor transferred	No. of mice with abscesses/total	Percent protection
—	8/8	0
ITF	1/8	87.5*
Dialysis bag contents	6/8	25
Dialysate	0/8	100*

* $P < 0.001$ comparing the two groups to dialysis bag contents by the Fisher's exact test.

Table IV. Testing P2 Biogel Column Fractions for Protection Against Abscesses in Mice

Group	P2 Biogel fractions transferred into mice	No. of mice with abscesses/total
E	None	5/5
F	Fractions 27–30	3/5
G	Fractions 36–40	1/5
H	Fractions 55–72	1/5
I	Fractions 73–82	4/4

Table V. Antigen Specificity of Protection Mediated by P2 Biogel Column Fractions

Fractions transferred	Challenge organism	No. of mice with abscesses/total	Abscess cultures
Fractions 55–72‖	*B. fragilis**	10/11	
	B. distasonis‡		*B. distasonis*
	Enterococcus§		Enterococcus
—	*B. fragilis*	12/13	*B. fragilis*
	B. distasonis		*B. distasonis*
	Enterococcus		Enterococcus

* *B. fragilis* ATCC 23745.
‡ *B. distasonis* ATCC 8503.
§ Enterococcus 2988T (clinical isolate).
‖ Mice received column fractions 55–72 (group H in Table IV) which previously had been found to protect >80% of mice.

fractions developed abscesses in 10 of 11 animals, but no *B. fragilis* was cultured from the abscess contents. 12 of 13 mice in the control group not receiving factor developed abscesses, and all of these abscesses still contained the starting inoculum.

Another experiment similarly designed was performed substituting *F. varium* for *B. distasonis* as the anaerobic organism. The conditions were identical otherwise and the same active column fractions were transferred. Results of this experiment were similar. 12 of 12 animals receiving column fractions developed abscesses compared with 12 of 13 animals not receiving fractions. Those mice receiving column fractions, however, did not have *B. fragilis* cultured from the abscesses. Protection, then, was antigen-specific to *B. fragilis* alone without cross protection even to another Bacteroides species.

Partial characterization of column-purified ITF. Column-purified ITF was evaluated for heat stability by incubating previously active P2 Biogel column fractions at different temperatures and injecting the fractions into mice to assess protective efficacy against abscesses. A control sample was incubated at 4°C since activity had been preserved at this temperature during the column chromatography processing. Table VI indicates results.

Animals receiving no factor in transfer developed abscesses while those receiving fractions incubated for 2 h at 4°C were all protected. Processing fractions for 2 h at room temperature preserved protective capacity, but heating to 37 or 56°C for 30 min eliminated the protective effect. The extreme heat lability of column-purified ITF indicates that the active component was not antibody.

Table VI. Lability of Partially Purified ITF

Fractions transferred	Treatment of fractions	No. of mice with abscesses/total
—*	—	6/6
+‡	4°C for 2 h	0/5§
+	Room temperature for 2 h	1/5‖
+	37°C for 30 min	5/5
+	56°C for 30 min	5/5

* Mice received no fractions in transfer.
‡ Mice received fractions like group H in Table IV which previously had protected >80% of mice.
§ $P < 0.01$ compared with animals receiving no fractions by the Fisher's exact test.
‖ $P < 0.05$ compared with animals receiving no fractions by the Fisher's exact test.

To determine whether the active constituent of ITF was a protein or nucleic acid, factor was subjected to enzyme digestion with either pronase or trypsin, or with the nucleases, DNase and RNase. Based on the previous experiment, enzyme digestions were performed at room temperature for 2.5 h. After 2 h, trypsin soybean inhibitor was added to the trypsin tube to neutralize enzyme activity. To avoid manipulating ITF excessively but to remove the enzymes before injecting mice, ITF was dialyzed as before and 0.2 cm^3 of dialysate was transferred to mice. The enzymes were retained in the dialysis bags. Control ITF was processed in an identical fashion without enzyme and also was dialyzed. Results appear in Table VII.

Mice not receiving factor all formed abscesses, while dialysate of ITF without enzyme treatment was protective. DNase/RNase treatment did not alter protection. Both pronase and trypsin digestion, however, eliminated the protective effect of ITF against abscesses caused by *B. fragilis* ($P < 0.001$ compared with ITF). These results suggest that ITF is a protein or, alternatively, that activity depends on a protein cofactor.

Discussion

Despite conventional teaching that host defense to extracellular bacteria involves humoral factors usually in conjunction with phagocytic cells, we have found that immunity to experimental intraabdominal abscesses caused by the "extracellular" pathogen *B. fragilis* is T cell-dependent. The T cells have been defined further as suppressor by phenotype (3). In this report we demonstrate that a cell-free factor derived from splenic T cells from mice immunized with *B. fragilis* CP protects animals against abscesses caused by this organism as effectively as intact T cells. This cell-free lysate (ITF) appears to be small in size since protective activity is dialyzable through a 12,000-mol wt dialysis membrane, and after column chromatography, protection is mediated by fractions intermediate between the void volume and bed volume of a P2 Biogel column, possibly suggesting a size < 1,800 D. 1,800 D represents the exclusion size for proteins on the P2 Biogel column. The broad protective

Table VII. Protective Efficacy of Enzyme Digested and Dialyzed ITF

Factor transferred	Enzyme digestion	No. of mice with abscesses/total	Percent protection
—	—	10/10	0
ITF*	—	1/15	93
ITF	Pronase‡	11/14¶	21.4
ITF	DNase/RNase§	2/14	85.7
ITF	Trypsin‖	8/8¶	0

* ITF was processed under identical conditions as enzyme-digested factor but without enzyme. Factor was incubated at room temperature for 2.5 h and then dialyzed against 5 mM ammonium acetate, pH 7.1, at 4°C for 24 h with two changes of the dialysate. 30 cm^3 of dialysate was pooled and 0.2 cm^3 injected into mice.
‡ 1 cm^3 of ITF was incubated with 1 mg pronase for 2.5 h at room temperature and dialyzed as above. Mice were injected with 0.2 cm^3 dialysate.
§ 1 cm^3 of ITF was incubated with 0.1 mg DNase and 0.5 mg RNase under the same conditions as for pronase digestion.
‖ 1 cm^3 of ITF was incubated with 1 mg trypsin for 2 h at room temperature. 1 mg trypsin soybean inhibitor was added and the mixture incubated for 30 min at room temperature. Processing then was performed as for pronase digestion.
¶ $P < 0.001$ compared with ITF by the Fisher's exact test.

range on the elution profile may be secondary to hydrophobic interactions with the Biogel column which might falsely minimize the molecular size of ITF.

Despite the apparent small size, the protection afforded against abscesses was found to be specific for *B. fragilis*. A complex inoculum of *B. fragilis*, enterococcus, and another Bacteroides species provoked abscess formation whether or not animals received protective column-purified ITF. Mice receiving column fractions in transfer, however, did not have *B. fragilis* cultured from the abscesses. In addition, absorption of ITF with *B. fragilis* CP coupled to SRBC eliminated the protective effect of ITF. In contrast, absorption with SRBC alone or SRBC coupled to an unrelated polysaccharide (from GBS) did not alter protection against abscesses. Besides confirming the antigen-specific activity of ITF, this latter experiment demonstrates that ITF contains a moiety which binds to the CP antigen.

Characterization of this factor is hampered by the lability of its activity. Column-purified ITF is not heat-stable and, in fact, loses its protective effect upon heating to 37°C for 30 min. The apparent size of the material and its heat-lability both indicate that the active constituent of ITF is not antibody-like. Enzyme digestion by both the nonspecific protease, pronase, and by trypsin removed the ability of ITF to protect against abscesses. Digestion with DNase/RNase did not affect protection. ITF appears then to be a protein or at least to depend on a protein cofactor in the preparation.

Recent literature is replete with the definition and partial characterization of a number of cellular factors from both helper and suppressor T cell populations (8–10). Beginning with dialyzable activity from lymph node cell populations containing transfer factor, investigators have defined both antigen-specific and nonspecific regulatory phenomena mediated by cellular factors (8). Though like transfer factor, ITF appears to be low in molecular weight and labile, its activity seems to be exclusively antigen-specific and trypsin-sensitive.

The cells of origin of ITF are suppressor by phenotype since they are Ly-$1^-2^+3^+$, activity is not H-2 restricted, and they bear the IJ surface marker (3; Finberg, R. W., D. L. Kasper, D. F. Zaleznik, S. Spriggs, A. B. Onderdonk, and M. E. Shapiro, manuscript submitted for publication). In other systems examining suppressor cell factors, an inhibitory function has been defined directly. In the case of transfer factor, leukocyte migration inhibition and suppression of footpad reactivity to antigen stimulation in mice have been demonstrated (8, 11). Antigen-specific suppressor factors for a variety of haptens and synthetic polymers have been shown to suppress in vitro spleen cell responses (9, 12). Our system provides a biological model closely resembling human disease, which differentiates this model from others. We, however, have not defined a discrete suppressive activity of ITF despite the suppressor-like nature of the originating T cells. Others have described antigen-specific suppressor factors which trigger other cells which, in turn, release nonspecific inhibitory factors (13, 14). Injection of cryptococcal antigen into mice stimulates a suppressor T cell lymph node population (14). A soluble factor derived from these lymph node cells, in turn, stimulates a splenic suppressor T cell population which has been found to suppress the delayed type hypersensitivity response to the inciting antigen (14). Similar T cell circuits have been defined in response to inert haptens (12). In our experimental abscess system, we have evidence that ITF stimulates a second suppressor-like T cell (Finberg, R. W., D. L. Kasper, D. F. Zaleznik, S. Spriggs, A. B. Onderdonk, and M. E. Shapiro, manuscript submitted for publication).

Although a final effector mechanism has not been established to understand T cell-mediated immunity to experimental intraabdominal abscesses, we have documented that phenotypic suppressor murine splenic T cells (3) in animals immunized with *B. fragilis* CP produce a dialyzable factor (ITF) which appears to be low in molecular weight. This factor, which is heat-labile and likely a protein, protects naive mice against abscesses caused by *B. fragilis* as efficiently as intact T cells. ITF promotes antigen-specific immunity to abscesses and, in fact, binds directly to CP antigen. Further definition of this unique biologic host defense system may advance understanding of the diversity of cell-mediated immune processes.

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