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Legionella pneumophila Protease Inactivates Interleukin-2 and Cleaves CD4 on Human T Cells

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The role of the *Legionella pneumophila* protease in the pathogenesis of Legionnaires' disease is unclear. In this study, we assessed the effect of purified protease preparations on human recombinant interleukin-2 (IL-2), the IL-2 receptor, and several additional human T-cell surface proteins to determine whether protease contributes to the virulence of *L. pneumophila* by interfering with human T-cell activation and function. IL-2-induced proliferation of CTLL-2 cells was inhibited by coincubation with protease (10 to 100 U/ml). Protease at concentrations of ≥10 U/ml cleaved human recombinant IL-2 as judged by sodium dodecyl sulfate-polyacrylamide gel electrophoresis of reaction mixtures containing ^{125}I-labeled IL-2 and protease. Protease treatment of activated human T cells did not inhibit binding of a monoclonal antibody directed against the α subunit of the IL-2 receptor and did not interfere with binding of IL-2 to IL-2 receptors on the lymphocytes. Treatment of blood mononuclear cells or activated T cells with protease (50 U/ml) inhibited the binding of a monoclonal antibody directed against CD4. In contrast, protease treatment did not inhibit the binding of antibodies against CD3, CD8, class II major histocompatibility complex, and the transferrin receptor. Heat inactivation (65°C for 20 min) of the protease or treatment with the metal chelator EDTA ablated the inhibitory effect of the protease. The ability of the protease to degrade IL-2 and cleave CD4 on human T cells suggests that protease may contribute to the pathogenesis of Legionnaires' disease by impeding T-cell activation and immune function.

Legionella pneumophila, the causative agent of Legionnaires' disease, is a facultative intracellular pathogen capable of entering and growing within mononuclear phagocytes (12). *L. pneumophila* produces a 38-kDa zinc metalloprotease that possesses both hemolytic and cytotoxic activities (9, 14, 19). Although the protease is not required for the intracellular growth of *L. pneumophila* (24), it has been suggested that the proteolytic and cytotoxic properties of the protease may be involved in the pathogenesis of Legionnaires' disease (14, 18, 19).

The gene encoding the *L. pneumophila* protease has been cloned in *Escherichia coli* (19, 26), and its DNA sequence has been determined (3). The deduced amino acid sequence for the protease gene, along with protease inhibitor studies, indicated that the *L. pneumophila* protease is structurally and functionally similar to *Pseudomonas aeruginosa* elastase (3). Of interest, elastase has been shown to inactivate enzymatically a variety of human cytokines including interleukin-2 (IL-2) (16, 27), degrade surface receptors on human lymphocytes (17), and interfere with normal lymphocyte function in vitro (27).

Horwitz and coworkers (4, 13) have clearly demonstrated that cell-mediated immunity (CMI) plays a vital role in host defense against *L. pneumophila*. The structural and functional similarities between *L. pneumophila* protease and *P. aeruginosa* elastase suggested that the protease may contribute to the pathogenesis of Legionnaires' disease by altering the CMI response mounted against *L. pneumophila*. The negative effect of protease on cellular immunity may be mediated through proteolytic inactivation of IL-2, a cytokine required for activation of T cells, or by degradation of cell surface receptors involved in T-cell activation and function. To test these ideas, we assessed the effect of purified *L. pneumophila* protease on recombinant human IL-2, the IL-2 receptor (IL-2R), and several additional human T-cell surface proteins. The results of these experiments show that *L. pneumophila* protease degrades human IL-2 and selectively cleaves CD4 on human T cells.

MATERIALS AND METHODS

Protease purification. The *L. pneumophila* protease used in all of the assays outlined in this study was purified by the rapid method of Rechnitzer et al. (22). Briefly, *L. pneumophila* Knoxville 1 (serogroup 1) was grown overnight in 1-liter flasks containing 200 ml of albumin-yeast extract broth (12). Incubation was on a rotary shaker (150 rpm) at 37°C. Culture supernatants were obtained by centrifugation at 15,000 × *g* for 10 min at 4°C. Ammonium sulfate was gradually added to pooled supernatants to achieve 60% saturation, and the suspension was gently stirred for 24 h at 4°C. The precipitate was collected by centrifugation (25,000 × *g* for 30 min at 4°C), dissolved in 50 mM Tris-HCl buffer (pH 8.0) containing 0.25 M NaCl, and dialyzed against the same buffer for 24 h. This material was applied to a MonoQ Sepharose column (Pharmacia-LKB, Uppsala, Sweden) equilibrated with 50 mM Tris-HCl buffer, pH 8.0. After the column was washed with 5 column volumes, the protease was eluted from the column with a stepwise NaCl gradient (0.25 to 1.0 M). Fractions were screened for protease activity on casein agar plates as described by Conlan et al. (7), and fractions containing peak protease activity were pooled and concentrated by ultrafiltration with a Centriprep-30 apparatus (Amicon, Beverly, Mass.). Protease activity was determined with hide powder azure substrate (Sigma,

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St. Louis, Mo.) as described previously by Sahney et al. (25), with 1 U arbitrarily defined as the proteolysis resulting in a change of absorbance of 0.001 per hour. Protein concentrations were determined by the bicinchoninic acid assay (Pierce Chemical Co., Rockford, Ill.). Criteria for purity of the final protease preparations were based on a single band on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) visualized by silver staining. Purified protease was then frozen in aliquots at −70°C and thawed as needed.

Two protease preparations were used throughout the course of this investigation. The specific activity of the first preparation was 1,225,000 proteolytic units per mg of protein, whereas that of the second preparation was 1,541 proteolytic units per mg of protein. Although there was a substantial difference in the specific activity of each of the protease preparations, both preparations had identical effects on the biological activity of IL-2 and on surface molecules found on human T cells.

IL-2 bioassay. IL-2-dependent mouse CTLL-2 cells (10) were used to assess the effect of *L. pneumophila* protease on recombinant human IL-2. In these experiments, triplicate cultures of CTLL-2 cells (4×10^3 cells per culture) cultivated in RPMI 1640 (GIBCO, Grand Island, N.Y.) plus 5% heat-inactivated fetal calf serum were incubated with known concentrations of recombinant human IL-2 (obtained from supernatant fluids of P3Y3 cells transfected with human IL-2 cDNA) and various amounts of *L. pneumophila* protease for 22 h at 37°C. CTLL-2 cell cultures incubated with IL-2 alone served as positive controls in these experiments. The cultures were pulsed with [^{3}H]thymidine for the last 4 h of incubation, and the level of thymidine incorporation by CTLL-2 cells was determined as described previously (6). All experiments were repeated at least three times.

SDS-PAGE and autoradiography. Human ^{125}I-IL-2 (35 to 40 μCi/μg; Dupont NEN Products, Boston, Mass.) was incubated with different concentrations of *L. pneumophila* protease (0.01 to 1,000 U/ml) for 1 h at 37°C. The buffer used in these experiments was Hanks balanced salts solution, pH 7.4 (HBSS). The final volume of the reaction mixtures was 100 μl. After incubation, samples were heated for 5 min at 100°C in SDS-PAGE sample buffer, and then a portion of each reaction mixture was electrophoresed through a reducing SDS–15% polyacrylamide gel (16). After electrophoresis, the gel was dried and subjected to autoradiography, using X-OMAT AR film (Kodak, Rochester, N.Y.). In some experiments, a 100-fold excess of unlabeled IL-2 (2 μg/ml) was added to the reaction mixtures containing ^{125}I-IL-2 during incubation with protease. In other experiments, protease and ^{125}I-IL-2 were incubated together in pooled normal human serum (NHS) ranging in concentration from 0.1 to 95% to assess the effect of serum on enzymatic activity of the protease.

Isolation and cultivation of human T cells. Peripheral blood mononuclear cells (BMNC) were isolated from heparinized blood by gradient centrifugation, using Accuspin System-Histopaque-1077 (Sigma). BMNC were washed three times with HBSS and suspended in RPMI 1640 supplemented with 10% fetal calf serum and 5 μg of phytohemagglutinin (PHA) per ml (Sigma). Approximately 2×10^6 mononuclear cells were added to each well of several 24-well plates (Falcon, Lincoln Park, N.J.), and the plates were incubated for 48 to 72 h at 37°C in 5% CO_2–95% air. The PHA-stimulated cells were harvested from the 24-well plates, washed several times in HBSS, adjusted to a cell concentration of 10^5 cells per ml, and suspended in RPMI 1640 supplemented with 10% fetal calf serum and recombinant human IL-2 (200 U/ml). These PHA-activated, IL-2-expanded T blasts were cultured in 150-mm^3 tissue culture flasks (Corning Glass Works, Corning, N.Y.) at 37°C for 3 days in 5% CO_2–95% air prior to use in protease susceptibility assays.

IL-2 binding assay. The ^{125}I-IL-2 binding assay was performed as described previously with slight modifications (11). Briefly, IL-2-expanded PHA-activated BMNC were incubated for 1 min in low-pH buffer (10 mM citrate, 140 mM NaCl; pH 3.0) to remove all cell-associated IL-2. The treated cells (10^6 per tube) were suspended in binding buffer and incubated with serial dilutions of ^{125}I-IL-2 for 1 h at 37°C in a total reaction volume of 100 μl. Binding buffer consisted of RPMI 1640 medium containing 5 mg of bovine serum albumin (Sigma) per ml and 0.1% sodium azide to prevent internalization of bound IL-2. After incubation, cell suspensions were layered over 150 μl of a mixture of 20% olive oil and 80% *n*-butylphthalate (Sigma) in 250-μl microcentrifuge tubes and centrifuged for 15 s at maximum speed in an Eppendorf microcentrifuge (Brinkmann Instruments, Inc., Westbury, N.Y.). After centrifugation, the tips of the microcentrifuge tubes were cut off, and the amount of radioactivity associated with cell pellets and corresponding supernatants was measured in a gamma counter (Pharmacia, Gaithersburg, Md.). Nonspecific binding of ^{125}I-labeled IL-2 to T cells was determined by incubating cells in the presence of a 100-fold excess of unlabeled IL-2. Binding data were subjected to Scatchard plot analysis as described previously (11).

T-cell surface protein analysis by flow cytometry. BMNC or IL-2-treated, PHA-activated T blasts were harvested by centrifugation, washed two times with HBSS, adjusted to a concentration of 5.0×10^6 cell per ml in HBSS, and incubated with various concentrations of protease for 1 h at 37°C with occasional agitation. T cells incubated without added protease served as positive controls in these experiments. After incubation, T cells were washed two times, directly or indirectly stained with appropriate antibodies, and subjected to immunofluorescence analysis as described previously (6). Negative controls were cells incubated with second antibody alone, i.e., fluorescein isothiocyanate-conjugated goat anti-mouse immunoglobulin. Analysis was performed on a FACSCAN (Becton Dickinson, San Jose, Calif.). For each sample, 5×10^5 cells were analyzed. Dead cells were excluded from fluorescence-activated cell sorter (FACS) analysis by using a combination of forward-angle and 90°C light scatter. The following antibodies (kindly provided by Eckhard Podack, University of Miami) were used in these experiments: OKT3, anti-CD3; 2.06, anti-class II major histocompatibility complex (MHC); L5.1, anti-transferrin receptor and anti-Tac, anti-IL-2Rα. Fluorescein isothiocyanate-conjugated CD4 antibodies and phycoerythrin-conjugated CD8 antibodies were obtained from Coulter Immunology, Hialeah, Fla.

RESULTS

Effect of protease on IL-2-induced lymphocyte proliferation. Theander et al. (27) showed that *P. aeruginosa* elastase can interfere with IL-2-induced lymphocyte proliferation. To determine whether *L. pneumophila* protease exerted a similar effect on IL-2-driven cell proliferation, IL-2-dependent mouse CTLL-2 cells were coincubated with protease and recombinant human IL-2 and cultured for 24 h. The results from dose-response experiments showed that the protease, ranging in concentration from 10 to 100 U/ml, inhibited

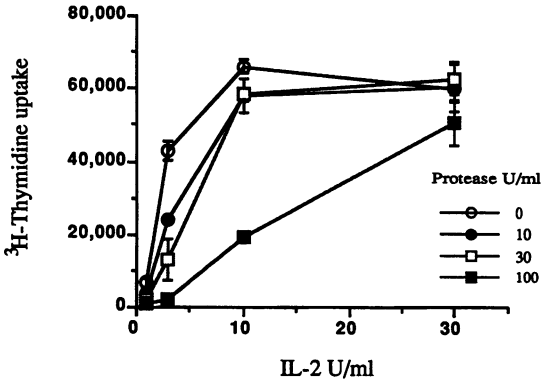

FIG. 1. Effect of protease on IL-2-induced CTLL-2 cell proliferation. CTLL-2 cells were coincubated with various concentrations of IL-2 and protease for 18 h at 37°C. After incubation, the cultures were pulsed with [^{3}H]thymidine for 4 h, and the level of thymidine incorporation by CTLL-2 cells was determined (5).

IL-2-induced proliferation of CTLL-2 cells (Fig. 1). Maximal inhibition was achieved with protease at a concentration of 100 U/ml. However, protease-induced inhibition decreased as the concentration of IL-2 increased (Fig. 1). Vital staining showed that protease treatment did not have a toxic effect on CTLL-2 cells (15). These results indicated that the protease was able to interfere with the biological activity of IL-2. The protease may have inhibited IL-2 bioactivity by proteolytic inactivation of IL-2 or by cleavage of IL-2R found on the CTLL-2 cell surface.

Proteolytic cleavage of IL-2 by protease. To determine whether IL-2 was susceptible to proteolytic cleavage by the protease, human ^{125}I-IL-2 (20 ng/ml) was incubated with increasing concentrations of protease for 1 h at 37°C and analyzed by SDS-PAGE and then by autoradiography. The results of a representative experiment are shown in Fig. 2. IL-2 treated with protease at concentrations of ≥10 U/ml showed altered migration on the gels compared with untreated samples. Moreover, at very high protease concentrations, e.g., 1,000 U/ml, IL-2 was almost completely degraded (Fig. 2). Similar results were obtained when a 100-fold excess of unlabeled IL-2 was added to reaction mixtures that contained ^{125}I-IL-2 and protease (15). This finding indicated that degradation of radiolabeled IL-2 in previous experiments did not result from extremely high protease-to-substrate ratios in the reaction mixtures. The results of these

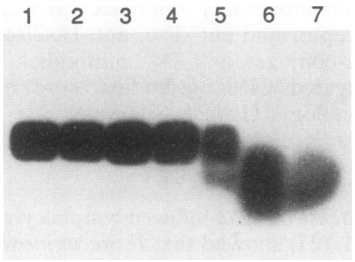

FIG. 2. Effect of protease on human IL-2. Recombinant human ^{125}I-IL-2 (20 ng/ml) was incubated with various concentrations of protease for 1 h at 37°C. After incubation, samples were heated at 100°C for 5 min and electrophoresed through a reducing SDS–15% polyacrylamide gel. The gel was dried and subjected to autoradiography. Lanes: 1, no protease; 2, 0.01 U of protease per ml; 3, 0.1 U/ml; 4, 1 U/ml; 5, 10 U/ml; 6, 100 U/ml; 7, 1,000 U/ml.

TABLE 1. Effect of *L. pneumophila* protease on expression of T-cell surface proteins[a]

Surface protein	No. of expt	% Positive	% Decrease in median fluorescence intensity
CD3	2	99.1 ± 0.4	8.0 ± 5.0
CD4	6	48.2 ± 13.7	77.0 ± 22.1
CD8	8	43.2 ± 15.4	3.1 ± 5.3
IL-2Rα	3	87.9 ± 5.0	1.0 ± 1.4
Transferrin receptor	2	36.4 ± 4.7	0
MHC class II	2	60.8 ± 6.4	0

[a] Untreated or protease-treated (50 U/ml), IL-2-expanded, PHA-activated T cells were stained with fluorescent MAbs to the indicated cell surface protein and subjected to FACS analysis. Percent positive represents those cells detected in FACS channels to the right of cells incubated with the indirect control antibody (fluorescein isothiocyanate-conjugated goat anti-mouse immunoglobulin) alone. For these control stained cells, <2% of the cells were fluorescence positive in any experiment. Percent decrease in median fluorescence intensity represents the relative decrease in fluorescence intensity for those cells that stained positive with the MAb to the indicated surface protein. Data are presented as the means ± standard deviations.

experiments indicated that recombinant human IL-2 was susceptible to proteolytic cleavage by the protease. Of interest, we found that digestion of radiolabeled IL-2 by protease was inhibited in ≥1% NHS (15).

Effect of protease on IL-2 receptors. To determine whether protease affected IL-2 receptors, human T blasts were treated with protease (50 and 100 U/ml) for 1 h at 37°C and then subjected to FACS analysis, using IL-2Rα antibodies. The results of these experiments showed that protease treatment did not alter the binding of anti-Tac (IL-2Rα) antibodies to activated T cells (Table 1), which suggested that this receptor subunit was not digested by the protease. Since functional high-affinity IL-2 receptors contain two additional subunits, β and γ, we also assessed the effect of protease on the binding of ^{125}I-labeled IL-2 to PHA-activated T cells. When these binding data were subjected to Scatchard plot analysis (Fig. 3), both untreated and protease-treated T-cell blasts yielded curvilinear graphs, indicative of both high- and low-affinity IL-2 receptors. Linear regression analysis of the portion of these curves that

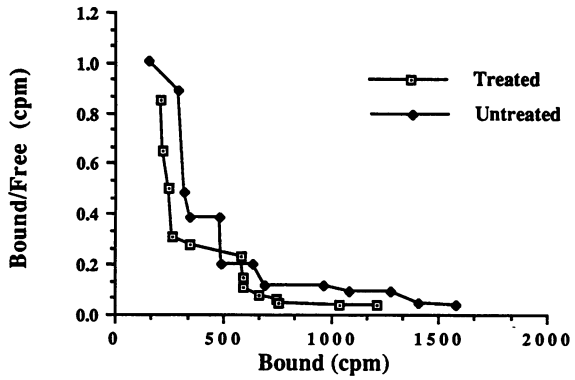

FIG. 3. Effect of protease on expression of high-affinity IL-2 receptors. Untreated or protease-treated (50 U/ml), IL-2-expanded, PHA-activated T blasts were incubated with serial dilutions of ^{125}I-labeled human IL-2 for 1 h. The resulting binding data were analyzed by Scatchard plots. The linear portion of the curve between approximately 200 and 800 cpm represents high-affinity IL-2 receptors.

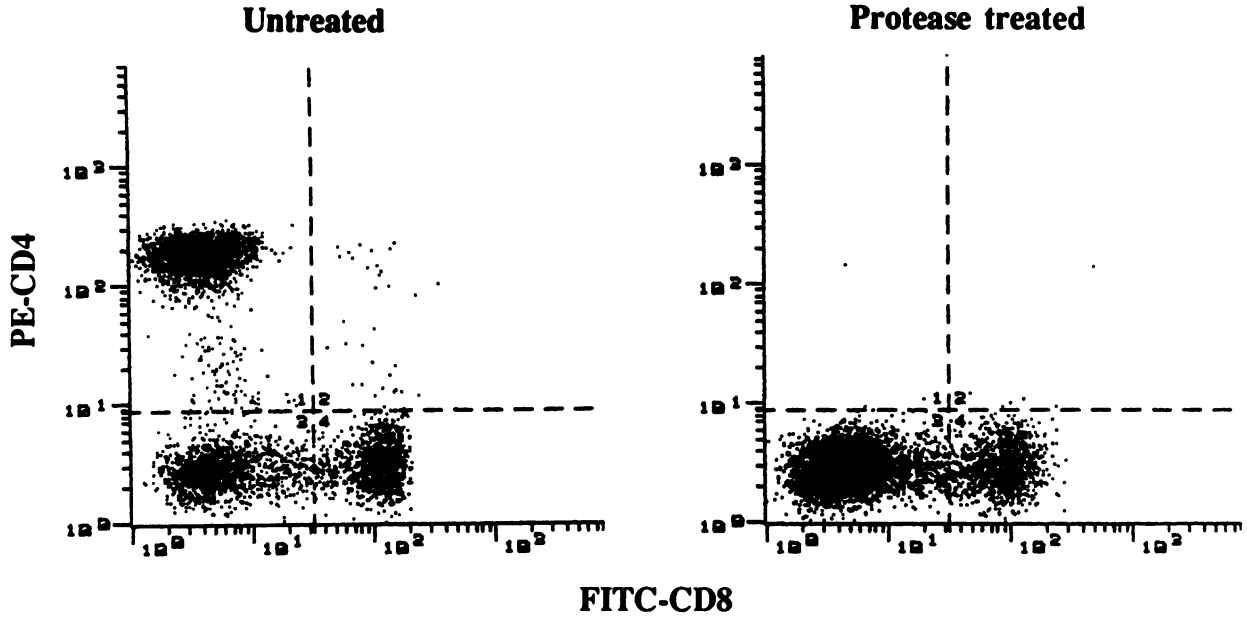

FIG. 4. Effect of protease on expression of CD4 and CD8. Untreated or protease-treated (50 U/ml) fresh BMNC were stained with the indicated MAbs and subjected to two-color FACS analysis. PE, phycoerythrin; FITC, fluorescein isothiocyanate.

corresponded to high-affinity IL-2 receptors indicated that there were ca. 785 high-affinity sites on the untreated cells compared with ca. 700 high-affinity sites on the protease-treated cells. These results indicated that the protease did not have an appreciable effect on high-affinity binding of IL-2 to activated T cells. Taken together, the results of FACS analysis and IL-2 binding experiments indicated that the protease did not have a substantial proteolytic effect on any subunit of the human IL-2 receptor. Therefore, it is likely that inhibition of IL-2-induced proliferation of CTLL-2 cells by protease resulted from proteolytic cleavage of IL-2 rather than from an effect of protease on the IL-2 receptor.

Protease cleaves the CD4 receptor on human T cells. A previous report (17) demonstrated that *P. aeruginosa* elastase can degrade the CD4 receptor on human lymphocytes. To determine whether protease also cleaves CD4, BMNC were treated with different amounts of protease and analyzed by FACS analysis, using a CD4 monoclonal antibody (MAb). Protease heated at 65°C for 20 min (which abolishes enzymatic activity but does not completely denature protein structure [17]) was used as a control in these experiments. Treatment of BMNC with 50 U of protease per ml for 1 h at 37°C reduced binding of the CD4 MAb by >90% in each of five separate experiments as assessed by FACS analysis. In the histogram shown, 49% of the BMNC expressed CD4, but after protease treatment <1% of the cells were CD4 positive (Fig. 4, upper left quadrant). These protease-treated CD4 cells were now detected as unstained cells (Fig. 4, lower left quadrant). This effect was specific in that surface expression of CD8 was unaffected, as 21% of the cells were CD8 positive before and after protease treatment (Fig. 4, lower right quadrant). Treatment of BMNC with 50 U of heat-inactivated protease per ml did not alter the binding of the CD4 antibody. Moreover, we found that the effect of the protease on CD4 was abrogated in the presence of 50 mM EDTA (15). It is important to note that previous studies showed that the protease is a metalloenzyme that requires Zn^{2+} for enzymatic activity (9, 17). Of interest, as little as 10 U of protease per ml was sufficient to reduce binding of the CD4 MAb to BMNC by 50%. Time course experiments revealed that removal of CD4 from BMNC was detectable after 15 min of incubation with protease and was maximal after 1 h (15).

To assess the effect of protease on other T-cell surface proteins, IL-2-expanded T blasts were treated with protease as described above and subjected to FACS analysis, using appropriate MAbs (Table 1). Protease treatment did not alter the binding of MAbs directed against CD3, CD8, class II MHC, and transferrin receptor. As expected, protease treatment of activated T cells also reduced binding of the CD4 MAb. However, the effect of protease on CD4 found on activated cells was, in some instances, less dramatic than that observed with BMNC. The reason for this difference is unknown at the present time. Nevertheless, the results of these experiments showed that protease selectively degraded CD4 found on human T lymphocytes.

DISCUSSION

Several investigators (7, 8, 14, 18) have implicated the protease as a possible virulence determinant in the pathogenesis of Legionnaires' disease. The similarities between the protease and *P. aeruginosa* elastase (3) suggested that the protease may contribute to the virulence of *L. pneumophila* by impeding the development of CMI, either by inactivating cytokines or by degrading T-cell surface proteins that participate in T-cell activation and immune function. In the present study, we showed that the protease selectively cleaves CD4 on human T cells and inactivates IL-2, a cytokine required for T-cell proliferation.

The ability of protease to inactivate IL-2 is consistent with the observations of Theander et al. (27), who reported that elastase also interferes with the bioactivity of IL-2. The similarity between our results and those reported by Theander et al. was not unexpected since protease and elastase show 100% homology at amino acid residues that interact

with and mediate proteolysis of substrate peptides (3). Recently, Parmely et al. (16) showed that elastase can also inactivate several other cytokines, including human gamma interferon and tumor necrosis factor alpha. Therefore, it is reasonable to assume that these cytokines may also be susceptible to cleavage by *L. pneumophila* protease. Indeed, results from preliminary experiments indicate that human tumor necrosis factor alpha is also cleaved by the protease (15). Moreover, we have determined recently that the protease interferes with IL-4-induced proliferation of CTLL-2 cells (15). Experiments are under way to assess the effect of the protease on other cytokines that may be involved in the immune response to *L. pneumophila*.

We found that cleavage of radiolabeled IL-2 by protease could be inhibited in 1% pooled NHS. This may have resulted from protease inhibitors or from excess competing substrates present in NHS. Of interest, Conlan et al. (8) showed that incubation of *L. pneumophila* protease with purified preparations of α_2-macroglobulin or α-1-antitrypsin inhibitor, the two major protease inhibitors found in human serum (1), did not inhibit the enzymatic activity of the protease. Therefore, it is unlikely that either of these protease inhibitors was responsible for the inhibitory effect of NHS on the enzymatic activity of the protease described in the present study.

The antiprotease activity of NHS does not preclude a role for the protease early in the pathogenesis of Legionnaires' disease since normal lung secretions usually contain only small amounts of most serum components (1). Moreover, it is unlikely that there would be sufficient levels of protease inhibitors or competing substrates to completely inactivate the protease during the inflammatory response induced by *L. pneumophila* because of the copious amount of protease likely to be produced by actively growing legionellae in infected mononuclear phagocytes. In support of this notion, Horwitz and coworkers have determined that the protease is the most abundant protein released by *L. pneumophila* into growth medium and that protease is produced within legionella-infected mononuclear phagocytes (4, 5).

The protease selectively decreased the expression of CD4 on BMNC and activated T cells (Fig. 3 and Table 1). This result was likely due to enzymatic cleavage of the CD4 receptor by protease, since heat-inactivated protease, which is devoid of enzymatic function but structurally intact, did not modulate the CD4 receptor on T lymphocytes. Furthermore, the ability of the protease to cleave CD4 was abrogated in the presence of the metal chelator EDTA. As mentioned previously, protease requires Zn^{2+} for enzymatic activity. Protease treatment did not alter binding of MAbs directed against the CD3, CD8, IL-2Rα, class II MHC, and transferrin receptor on T cells. This suggests that the protease had no effect on any of these surface molecules. It is important to note, however, that only one MAb directed against each of the T-cell proteins was used in our FACS analysis. Therefore, we cannot rule out the possibility that protease treatment may alter binding of other MAbs that recognize different epitopes contained within each of the surface proteins tested in the present study. Nevertheless, our results are in agreement with the findings of Pedersen et al. (17), who reported that elastase, like protease, selectively cleaves CD4 but has no effect on the expression of CD3, CD8, or class II MHC on human T lymphocytes. Of interest, Sahney (23) showed recently by FACS analysis and ligand binding assays that protease can degrade FcRII, FcRIII, and CR1 found on human polymorphonuclear leukocytes. In contrast, CR3 was unaffected by protease treatment. Our results, along with those of Sahney, suggest that the protease can degrade certain cell surface proteins found on neutrophils and lymphocytes that are likely to participate in host defense against *L. pneumophila* infection.

Although the protease is not required for the intracellular multiplication of *L. pneumophila* (26), a role for protease in the pathogenesis of Legionnaires' disease has been proposed for several reasons. First, Conlan et al. (7) showed that introduction of partially purified protease preparations into the lungs of guinea pigs caused lesions morphologically indistinguishable from those observed in the lungs of Legionnaires' disease victims. In more recent studies, Keen and Hoffman (14) and Quinn et al. (18) showed that the protease has both hemolytic and cytotoxic activities. Second, protease is produced in vivo during experimental and natural Legionnaires' disease (18, 28). Finally, Blander and Horwitz (4) showed that vaccination of guinea pigs with purified protease induced a vigorous CMI response and protected immunized animals against lethal aerosol challenge with *L. pneumophila* Philadelphia-1. The results from these studies suggested that the protease may contribute to the virulence of *L. pneumophila*. However, Blander et al. (5) recently found no difference in the ability of strain Philadelphia-1 and an isogenic protease-deficient mutant to cause disease after aerosol challenge in the guinea pig model of Legionnaires' disease. On the basis of these results, the authors concluded that the protease was not a virulence determinant for *L. pneumophila*. It is important to note, however, that results obtained in animal models may not mirror the situation in humans. Therefore, while the protease may not be required for the full expression of virulence by *L. pneumophila* in the guinea pig model, it may still play a role in the pathogenesis of Legionnaires' disease in humans. In human legionellosis, the protease may act as an accessory virulence determinant augmenting virulence by either enhancing other virulence factors or interfering with normal host cell functions, which would be likely to contribute to the pathogenesis of disease. In support of the latter idea, the protease was found recently to interfere with human neutrophil and monocyte function in vitro (21, 23) and to abrogate natural killer cell activity (20).

CMI plays an integral role in host defense against *L. pneumophila*. Activated monocytes and alveolar macrophages have been reported to inhibit the intracellular multiplication of *L. pneumophila* (12). It is generally accepted that macrophage activation occurs following the production of lymphokines by immune T cells (2). The roles of IL-2 and CD4 in T-cell activation and generation of an immune response have been clearly established (2). Our results, which show that protease can degrade IL-2 and cleave CD4 on human T cells, suggest that protease may also act as a virulence factor during human legionellosis by interfering with T-cell activation and immune function. Whether the *L. pneumophila* protease cleaves CD4 and inactivates IL-2 and other cytokines in vivo remains to be determined. Nonetheless, the results of our study suggest a potential role for the protease in the pathogenesis of human Legionnaires' disease.

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