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Proteolytic Activation of the Interleukin-1β Precursor by *Candida albicans*

ANNIE BEAUSÉJOUR, DANIEL GRENIER, JEAN-PAUL GOULET, AND NOËLLA DESLAURIERS*

Groupe de Recherche en Écologie Buccale, Faculté de Médecine Dentaire et Faculté des Sciences et de Génie, Université Laval, Québec, Québec, Canada

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Chronic inflammation rather than invasion is characteristic of some forms of superficial candidiasis such as denture stomatitis. We hypothesized that *Candida albicans* may play a critical role in the pathogenesis of inflammatory lesions observed in chronic candidiasis by activating the proinflammatory cytokine interleukin-1β (IL-1β) from epithelial stores of the precursor. The aim of this study was therefore to demonstrate the proteolytic cleavage and activation of the inactive precursor of IL-1β (pro-IL-1β) by *C. albicans*. After incubation of either blastospores or hyphae with the inactive precursor, proteolytic cleavage was monitored by sodium dodecyl sulfate-polyacrylamide gel electrophoresis Western immunoblotting analysis, and the biological activity of the cleavage products was tested in a bioassay. We report here that late-stationary-growth-phase blastospores as well as hyphae of *C. albicans*, but not exponentially growing cells, can efficiently cleave pro-IL-1β to yield fragments of molecular masses compatible with mature biologically active IL-1β (17 to 19 kDa). Assays conducted in the presence of selected proteinase inhibitors suggest that the cleavage of pro-IL-1β involves the participation of one or more aspartyl proteinases. Cleavage products showed a dose-dependent IL-1β-like activity in a thymocyte proliferation bioassay, which was inhibited by anti-IL-1β neutralizing antibodies. The present data thus suggest a role for *C. albicans* proteinases in the activation and maintenance of the inflammatory response at epithelial surfaces.

Candida-associated denture stomatitis is the most common *Candida* infection and is estimated to affect about 50% of complete-denture wearers (4, 6). A characteristic feature of this form of candidiasis is a chronic inflammation of the palatal mucosa in contact with the prosthesis, and a diagnostic criterion is the topographical relationship between the inflamed mucosa and *Candida albicans* growth on the fitting surface of the denture (5). The carriage rate of *Candida* among denture wearers is very high, both in stomatitis patients (93%) and in denture wearers with clinically healthy mucosa (86%), compared to the general population (30 to 40%) (6). The mechanism by which *C. albicans*, a common oral commensal, may occasionally change to a pathogenic form is not clear. One hypothesis is that infective strains of serotype A are more virulent than others, although no mechanisms have been proposed (reviewed in references 4 and 34). Budtz-Jorgensen and Bertram have suggested that both an increase in yeast counts and the unique microbial ecology of denture plaque may contribute to the emergence of the pathogenic potential of *C. albicans* (reviewed in reference 5). We propose here that the proteolytic activity of *C. albicans* may participate in the pathogenesis of the inflammatory lesions observed in *Candida*-associated stomatitis via the enzymatic activation of the proinflammatory cytokine interleukin-1β (IL-1β) from epithelial stores of the precursor.

IL-1 is thought to play a central role in the induction, progression, and maintenance of an inflammatory response (reviewed in reference 17). While inflammation is obviously critical to the mediation of host responses to injury and infection, inappropriate or prolonged inflammatory responses can produce a variety of pathological conditions including inflammatory dermatoses (16, 30). Keratinocytes are the primary source of IL-1 in both normal and diseased epithelia (21, 22, 58). IL-1β is produced in large quantities in keratinocytes and stored in the superficial layers of the epithelium as an inactive, 31- to 33-kDa precursor (pro-IL-1β) (36). While monocytes produce a cysteine proteinase that cleaves pro-IL-1β to produce the 17- to 18-kDa active form (28), keratinocytes do not produce an IL-1β convertase enzyme (ICE) (36). Consequently, IL-1β activity is not observed with normal keratinocytes, despite high precursor content.

Although ICE is exquisitely specific in cleaving pro-IL-1β into a biologically active 17- to 18-kDa fragment, it is not the only proteinase that can activate pro-IL-1β. Serine proteinases such as neutrophil elastase and cathepsin G, mast cell chymase, and granzyme A can all generate active fragments from pro-IL-1β (2, 24, 37). It has been postulated that this property may contribute to the pathology of rheumatoid arthritis and other forms of acute or chronic inflammation (16). The possibility that microbial enzymes display such convertase-like activity was tested with *Streptococcus pyogenes*, and it was found that exotoxin B, a cysteine proteinase, possessed ICE-like activity (27). The ability of mucosal pathogens to cleave and activate epithelial stores of pro-IL-1β has not yet been investigated. Generating proinflammatory cytokines by enzymatic cleavage may represent a novel pathogenic mechanism for proteolytic microbes, especially if the inflammatory reaction is inefficient in clearing the microorganisms. We therefore hypothesized that, given the strategic storage of pro-IL-1β in superficial epithelial cells of squamous epithelia (15, 21), membrane-bound and secreted proteinases may act as virulence factors in chronic candidiases of the skin and mucosae by triggering a damaging inflammation cascade. The aim of this study was to test this hypothesis by demonstrating the proteolytic cleavage and activation of pro-IL-1β by clinical isolates of *C. albicans.*

* Corresponding author. Mailing address: Groupe de Recherche en Écologie Buccale, Université Laval, Québec, Québec, Canada G1K 7P4. Phone: (418) 656-2067. Fax: (418) 656-2861. E-mail: Noella.Deslauriers@greb.ulaval.ca.

MATERIALS AND METHODS

***C. albicans* strains and culture conditions.** *C. albicans* isolates 2 through 6 from *Candida*-associated stomatitis patients were cultured in modified Sabouraud medium (20 g of glucose and 10 g of tryptose per liter) in shaking flasks (100 rpm) at 25°C. Blastospores were harvested at various times during growth (between 8 h [exponential growth phase] and 120 h [stationary growth phase]), washed three times in sterile RPM1-F12 (Gibco BRL, Burlington, Ontario, Canada), pH 6, and resuspended at 1.5×10^9 cells/ml in RPMI-F12 culture medium unless otherwise indicated. In selected experiments, age-matched culture supernatants of the blastospores were also collected for analysis. Mycelium formation was induced by incubation of washed, stationary-growth-phase (96-h) blastospores in Iscove's modified Dulbecco medium (Sigma Chemical Co., St. Louis, Mo.) for 4 h at 37°C as previously described (14). Blastospores, mycelial cells, and culture supernatants prepared as described above were used to determine their potential to degrade pro-IL-1β.

Fluorometric assay for ICE-like activity. The fluorometric assay for the determination of ICE-like activity was adapted from that of Moncla et al. (38). The fluorogenic substrate acetyl-Tyr-Val-Ala-Asp-7-amido-4-methylcoumarin was obtained from Peptides International (Louisville, Ky.). A 10 mM stock solution was prepared in dimethyl formamide and kept at −80°C. Prior to the assay, the substrate was diluted to 50 μM in 0.1 M Tris HCl (pH 8.0), and 30-μl drops were applied to a cellulose paper strip (6 by 66 mm; Whatman Inc., Fairfield, N.J.). After drying at room temperature, *C. albicans* culture supernatants (20 μl), or 3×10^8 blastospores in 20 μl, were applied to the spots. Hydrolysis was monitored by UV illumination (366 nm) after a 2-h incubation at 37°C in a humidified atmosphere. *Treponema denticola* ATCC 35405 cells were used as a positive control (1).

SDS-PAGE Western immunoblotting analysis of pro-IL-1β cleavage. Recombinant pro-IL-1β (9 ng) (33 kDa; Cistron Biotechnology, Pine Brook, N.J.) and 1.9×10^7 *Candida* cells in RPMI-F12 medium were incubated in 24-μl volumes at 37°C for 8 h unless otherwise indicated. When culture supernatants of *C. albicans* were tested, a 12-μl aliquot was mixed with an equal volume of RPMI-F12 medium containing 24 ng of pro-IL-1β. Following the incubation, sodium dodecyl sulfate (SDS) sample buffer was added, and the mixture was boiled for 5 min. Polyacrylamide gel electrophoresis (PAGE) was performed with a minigel system (Bio-Rad Laboratories, Mississauga, Ontario, Canada). Samples (32 μl) were loaded on a 12% polyacrylamide gel, and the electrophoresis was carried out at 100 V for 90 min (31). Proteins were electrophoretically transferred to a nitrocellulose membrane (Gibco BRL) for 60 min at 100 V (54). The membrane was blocked in 20 mM Tris–500 mM NaCl buffer (Tris-buffered saline) containing 18% glucose, 10% glycerol, 0.6% Tween 20, and 2.5% bovine serum albumin (blocking buffer) and stained by a three-layer immunoenzymatic technique. Membranes were sequentially incubated with anti-IL-1β goat polyclonal antibodies (R & D Systems, Minneapolis, Minn.) diluted to 2 μg/ml in Tris-buffered saline–0.5% bovine serum albumin, biotin-labeled donkey anti-goat immunoglobulin G (Jackson ImmunoResearch Laboratories Inc., West Grove, Pa.) diluted to 1.2 μg/ml in blocking buffer, and streptavidin-alkaline phosphatase (Cedarlane Laboratories Ltd., Hornby, Ontario, Canada) diluted 1/1,000 in blocking buffer. Membranes were developed in a solution containing nitroblue tetrazolium chloride (1.65 mg) and 5-bromo-4-chloro-3-indolylphosphate *p*-toluidine salt (0.8 mg) in 5 ml of 100 mM Tris-HCl (pH 9.5) containing 100 mM NaCl and 50 mM $MgCl_2$. Positive controls, recombinant pro-IL-1β, and recombinant mature IL-1β (Cistron Biotechnology) were included in each gel. Pancreatic α-chymotrypsin (12 μg/ml; Sigma) was also used as a positive control for the generation of a 18- to 19-kDa fragment from recombinant pro-IL-1β, as already reported (2, 36). Negative controls included omitting each reagent in turn.

Effect of heat treatment and proteinase inhibitors on pro-IL-1β cleavage. A preliminary characterization of the enzymatic activity involved heating suspensions of *C. albicans* for 30 min at temperatures ranging from 56 to 100°C prior to adding the pro-IL-1β substrate. In other assays, the following proteinase inhibitors, at a final concentration of 5 mM, were added to 1.9×10^7 *Candida* cells in 0.1 M citrate buffer (pH 6.0) 15 min before adding the pro-IL-1β: EDTA (Sigma) and 1,10-phenanthroline (Sigma) for metalloproteinases, iodoacetamide (Sigma) for cysteine proteinases, 4-(2-aminoethyl)benzensulfonyl fluoride (AEBSF; ICN Biochemical Inc., Aurora, Ohio) for serine proteinases, *N*α-*p*-tosyl-L-lysyl chloromethyl ketone (TLCK; Sigma) for serine and cysteine proteinases, and pepstatin A (Sigma) for aspartyl proteinases.

Effect of pH on pro-IL-1β cleavage. The enzymatic cleavage of pro-IL-1β by *Candida* cells was tested by using 0.1 M buffers at various pHs: citrate buffer at pH 4.0, 5.0, and 6.0; phosphate buffer at pH 7.0 and 8.0; Tris HCl buffer at pH 9.0; and carbonate buffer at pH 10.0. The mixtures were incubated at 37°C for 8 h, and postincubation pHs were verified with a microelectrode. Controls included omitting *C. albicans* cells to detect spontaneous hydrolysis of pro-IL-1β.

Bioassay for determining IL-1β activity. The assay mixtures analyzed by SDS-PAGE Western immunoblotting were also analyzed, after filtration, by using a murine thymocyte proliferation assay adapted from that of Gery and Waksman (20). The bioassay measured the comitogenic effect of mature IL-1β, or biologically active fragments derived from pro-IL-1β, on concanavalin A (ConA; Flow Laboratories, Mississauga, Ontario, Canada) submitogenic stimulation of thymocyte proliferation. Thymocyte cultures were grown in RPM1-F12 supplemented with 25 mM HEPES, gentamicin (50 μg/ml), penicillin (100 U/ml), streptomycin (100 μg/ml), 5% decomplemented fetal calf serum (FetalClone; HyClone Laboratories Inc., Logan, Utah), 50 μM β-mercaptoethanol, and 3 mM glutamine. ConA was added at 0.75 or 2.0 μg/ml, as indicated, to 1.5×10^6 thymocytes/well in flat-bottom Nunclon 96-well culture plates (Nunc InterMed, Roskilde, Denmark). Standard curves were determined by simultaneously adding ConA and graded concentrations (0 and 1,000 pg/ml) of recombinant mature IL-1β. To titrate the biological activity of the *Candida* supernatants incubated (8 h in RPMI-F12 medium, pH 6.0) in the presence of 24 ng of pro-IL-1β, standard curves were constructed in the presence of control *Candida* supernatants (i.e., supernatants of *Candida* incubation in RPMI-F12 medium without pro-IL-1β.) Such standard curves were required because of an intrinsic inhibitory activity of *Candida* soluble products in supernatants (see below). An MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] colorimetric assay adapted from that of Mosmann (42) was used to measure cell proliferation after a 72-h incubation at 37°C (13). Specificity was confirmed with anti-human IL-1β neutralizing antibodies used at 1 μg/well. The means ± standard deviations of data obtained from duplicate cultures and from three similar experiments were calculated.

RESULTS

Cleavage of pro-IL-1β by *C. albicans*. Aliquots from a *C. albicans* culture were recovered at various times (up to 120 h) to test the capacity of blastospores and culture supernatants to hydrolyze a fluorogenic substrate that closely matches the amino-terminal sequence of the cleavage site of monocyte ICE. No hydrolysis was detected with *C. albicans*, whereas *T. denticola* ATCC 35405, which was used as a positive control (1), caused an intense, fluorescent reaction.

The ability of blastospores and culture supernatants to cleave the natural pro-IL-1β substrate was then analyzed by SDS-PAGE Western immunoblotting. Weak activity was detected with exponentially growing cells (<18 h), whereas more efficient cleavage was observed with stationary-growth-phase (≥72-h) blastospores (Fig. 1A). The major digestion product derived from pro-IL-1β had an apparent molecular mass of approximately 21 kDa (band c). Additional bands with molecular masses in the 17- to 19-kDa range were also detected (bands a and b). Fragments with molecular masses greater than 21 kDa were also produced but were not studied further because their sizes were not compatible with mature, biologically active IL-1β (17 to 19 kDa) (2, 24, 37). In addition, mycelial cells recovered after a 4-h induction at 37°C showed a pattern of cleavage similar to that of the 96-h blastospores from which they were generated (data not shown). When blastospores culture supernatants (cultured for the same time periods) were tested for hydrolytic activity against pro-IL-1β, efficient cleavage also occurred, generating fragments a, b, and c (Fig. 1B). However, it took 120 h for culture supernatants to produce the major 21-kDa band, which is generated by 72-h stationary-growth-phase blastospores (Fig. 1B, lane 6, versus Fig. 1A, lane 4).

The fact that a significant decrease in the 33-kDa recombinant pro-IL-1β band with the production of few digestion products was observed with exponential-growth-phase blastospores suggests that pro-IL-1β binds to the cell surface. This was confirmed by analyzing whole blastospores by SDS-PAGE Western immunoblotting following an 8-h incubation with pro-IL-1β and two washes in phosphate-buffered saline. About half of the pro-IL-1β was recovered, apparently unaffected, from the washed blastospores (data not shown).

Four other *C. albicans* isolates obtained from four different patients with denture stomatitis were grown to the stationary phase and tested for the ability to cleave pro-IL-1β at pH 6. These isolates were all found to generate a fragment profile compatible with mature, biologically active IL-1β (17 to 19 kDa) (2, 24, 37), although there was some heterogeneity in the molecular masses and intensities of the various fragments (Fig.

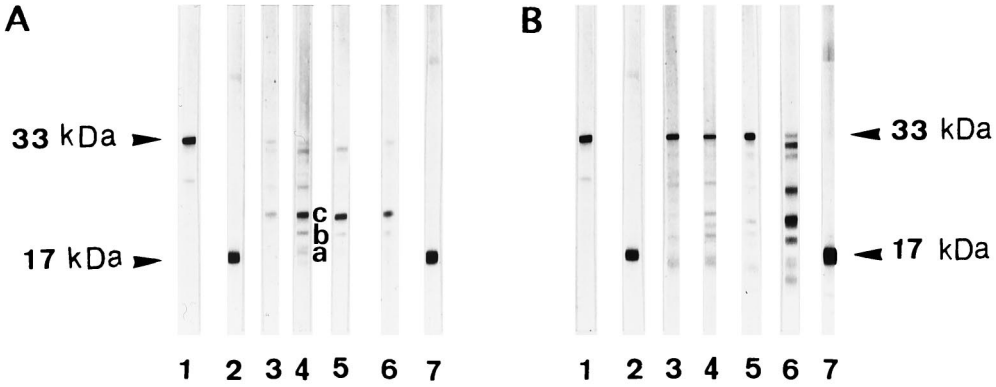

FIG. 1. SDS-PAGE Western immunoblotting analysis of the cleavage of pro-IL-1β by *C. albicans* as a function of growth phase. Recombinant pro-IL-1β (24 ng) was incubated with cells or supernatants at 37°C for 8 h in RPMI-F12 medium (pH 6.0). (A) *C. albicans* (isolate 2) blastospores in exponential (8 h; lane 3) and late stationary (72, 96, and 120 h; lanes 4 through 5, 6) growth phases. Purified recombinant pro-IL-1β (33 kDa; lane 1) and mature IL-1β (17 kDa; lanes 2 and 7) are shown for reference. (B) Culture supernatants corresponding to the *C. albicans* blastospores used for panel A. Supernatants were harvested during the exponential (8 h; lane 3) and late stationary (72, 96, and 120 h; lanes 4 through 6) growth phases. Purified recombinant pro-IL-1β (33 kDa; lane 1) and mature IL-1β (17 kDa; lanes 2 and 7) are shown for reference. Bands a, b, and c are approximately 17, 18, and 21 kDa, respectively.

2). Clinical isolate 2 was further investigated for its capacity to cleave pro-IL-1β and generate biologically active fragments.

The patterns shown in Fig. 1 were obtained with an 8-h incubation at pH 6.0 in RPM1-F12 medium. We used SDS-PAGE Western immunoblotting to determine whether changing the reaction conditions would modify pro-IL-1β cleavage by stationary-growth-phase blastospores. The data obtained are summarized in Table 1. Pro-IL-1β cleavage by *C. albicans* blastospores did not occur at pH 4.0. At pH 5.0, two pro-IL-1β cleavage fragments, one of approximately 21 kDa (band c) and a second of approximately 19 kDa (band b), were generated. Reactions at pH 6.0 appeared to be optimal for the generation of these two fragments as well as a 17-kDa fragment (band a). In general, raising the pH from 6.0 to 7.0 produced a more heterogeneous pattern of digestion and decreased the yield of low-molecular-mass (17- to 19-kDa) fragments. Virtually no digestion occurred at pH 8.0. Control assays at different pHs, without *Candida* cells or culture supernatants, indicated that no significant spontaneous degradation of pro-IL-1β occurs under these conditions (data not shown). Measurements at the end of the incubation period revealed that pHs remained constant throughout the enzymatic assay.

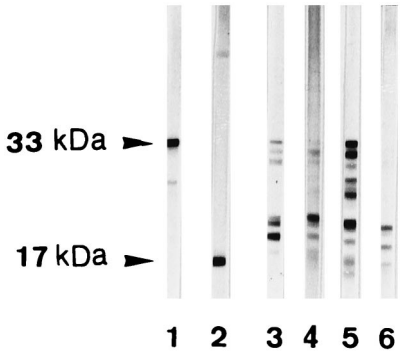

FIG. 2. SDS-PAGE Western immunoblotting analysis of the cleavage of pro-IL-1β by different clinical isolates of *C. albicans*. Blastospores from *C. albicans* isolates 3 through 6 (lanes 3 through 6) from four different patients with denture stomatitis were harvested in the stationary growth phase (96 h) and incubated with pro-IL-1β for 8 h at pH 6.0 in RPMI-F12. Purified recombinant pro-IL-1β (33 kDa) and mature IL-1β (17 kDa) (lanes 1 and 2) are shown for reference.

Pro-IL-1β cleavage by *C. albicans* cells was assayed after various incubation times (Table 1). Cleavage was observed after a 3-h incubation at pH 6.0 but was much more evident after 8 h. Following an 18-h incubation, the pattern became sketchy because the high-molecular-mass (≥21-kDa) fragments were further degraded or unstable under these conditions. Moreover, the substrate (pro-IL-1β) became undetectable in the assay medium when the enzymatic reaction was allowed to continue for 18 h.

We used the optimal conditions (pH 6.0, 8-h incubation) to evaluate the effect of class-specific proteinase inhibitors on the cleavage of pro-IL-1β by *C. albicans* (Table 1). Pepstatin A, an

TABLE 1. Generation of pro-IL-1β cleavage fragments by *C. albicans* blastospores under various assay conditions

Assay conditions	Pro-IL-1β fragments[a]		
	a	b	c
Controls			
Recombinant mature IL-1β	+	−	−
α-Chymotrypsin cleavage	−	+	−
Incubation period (h) at pH 6.0			
3	−	+	+
8	+	+	+
18	+	+	−
Reactional pH for an 8-h assay			
4	−	−	−
5	−	+	+
6	+	+	+
7	−	−	+
8	−	−	−
Proteinase inhibitor at pH 6 for an 8-h assay			
EDTA	+	+	+
AEBSF	+	+	+
TLCK	+	+	+
1,10-Phenanthroline	+	+	+
Iodoacetamide	+	+	+
Pepstatin A	−	−	−

[a] Approximate molecular masses of fragments as in Fig. 1: a, ≈17 kDa; b, ≈19 kDa; c, ≈21 kDa.

inhibitor of aspartyl proteinases, was the only inhibitor tested that prevented the cleavage of pro-IL-1β into low-molecular-mass (17- to 21-kDa) fragments. Cleavage was also completely abolished by prior heating of *C. albicans* for 30 min at 70 or 100°C (data not shown).

Production of biologically active fragments from pro-IL-1β by *C. albicans*. Supernatants from *C. albicans* blastospores incubated with and without pro-IL-1β in RPM1-F12 medium (pH 6.0, 8 h) were tested in the IL-1β bioassay. Since the bioassay is based on costimulation by ConA and mature IL-1β (or eventually biologically active fragments derived from pro-IL-1β), it was expected that neither ConA (at suboptimal mitogenic concentrations) nor mature recombinant IL-1β alone would produce significant proliferation. Assaying the costimulatory activity of pro-IL-1β cleavage products on ConA-stimulated thymocytes was, however, hampered by the inhibitory activity of soluble material released from *Candida* blastospores during the 8-h incubation in RPMI-F12 medium on ConA stimulation. Control supernatants of *Candida* blastospores incubated in RPMI-F12 medium without pro-IL-1β inhibit in a dose-dependent manner the proliferative response to ConA and to ConA plus 1,000 pg of mature recombinant IL-1β per ml (data not shown). This inhibition starts weakening when the control supernatant is diluted 1/100 and is completely lifted at a dilution of 1/1,000. However, such high dilutions precluded the detection of IL-1β-like activity in similarly diluted assay supernatants (*Candida* cells incubated with pro-IL-1β).

We hypothesized that the inhibition could be due to ConA sequestration by soluble *Candida* mannan or mannoproteins (7) and that ConA signalization would be somewhat limited when the proliferative assay was carried out in the presence of these soluble *Candida* products. We thus increased the ConA concentration to 2.0 μg/ml to overcome this inhibition. Figure 3 shows that under these conditions, the thymocytes proliferated following a normal dose-response curve with graded concentrations of recombinant mature IL-1β notwithstanding the presence of control *Candida* supernatant (*Candida* cells incubated in RPMI-F12 medium without pro-IL-1β) diluted 1/10. These conditions thus enabled us to assess the biological activity of assay supernatants (supernatants of *Candida* cells incubated with pro-IL-1β). At a 1/10 dilution, assay supernatants containing pro-IL-1β cleavage products can elicit a proliferative response corresponding to the costimulatory activity of 20 to 30 pg of mature recombinant IL-1β per ml. This IL-1β-like costimulatory activity was neutralized in the presence of anti-IL-1β antibodies, and the proliferative response decreased to the level of ConA stimulation alone, indicating an almost complete loss of comitogenic activity.

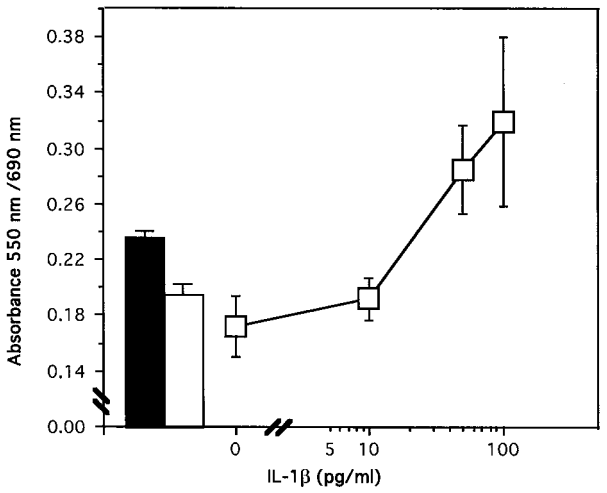

FIG. 3. IL-1β-like costimulatory activity from proteolytic cleavage of pro-IL-1β by *C. albicans*. A dose-response curve of mature recombinant IL-1β was generated by using the bioassay described in Materials and Methods. To titrate the biological activity of the supernatants of *Candida* incubated in the presence of 24 ng of pro-IL-1β, this standard curve was constructed in the presence of control supernatants (i.e., supernatants of *C. albicans* incubated in RPMI-F12 medium for 8 h at 37°C without pro-IL-1β). These adapted standard curves were required because of the inhibitory effect of *Candida* supernatants on this bioassay. The ConA concentration was increased to 2.0 μg/ml to overcome this inhibitory activity. Control supernatants were diluted (1/10) and added simultaneously with graded concentrations of recombinant mature IL-1β and ConA. The closed bar shows the proliferative response when the assay is conducted in the presence of pro-IL-1β cleavage products (assay supernatants) instead of mature recombinant IL-1β. At this dilution (1/10), *Candida* supernatants containing cleavage products generated from pro-IL-1β generate a proliferative response corresponding to the costimulatory activity of 20 to 30 pg of mature IL-1β per ml. This biological activity was neutralized by antibodies directed against mature IL-1β (open bar). Means ± standard deviations from three experiments are shown.

DISCUSSION

C. albicans commonly colonizes various stratified squamous epithelia, including those of the oral cavity, the pharynx, the eosophagus, the vagina, and the epidermis. Because it possesses several attributes that are critical for colonizing these sites, it is considered not only a successful commensal but also an opportunistic pathogen since the expression of *C. albicans* virulence is often signalled by a breakdown in surveillance by innate and acquired defense mechanisms, for example, in AIDS. However, defense mechanisms may also participate in the pathogenesis of superficial candidiases in situations where *C. albicans* is allowed to sustain a chronic inflammatory reaction. The inflammatory lesions observed in mucosal candidiases are thought to be triggered by *Candida* aggressins (44, 45), and although several authors (9, 35) report that a number of *C. albicans* constituents can act as inflammatory aggressins in vivo, no mechanism has been proposed to explain the transition from asymptomatic carriage to proinflammatory potential. *Candida* proteinases are suspected to act as virulence factors, possibly by facilitating fungal access and adherence to epithelial cells, invasion of host tissues, and interference with host defense mechanisms (reviewed in references 18, 35, and 49). We report here that *C. albicans* proteinase(s) can cleave pro-IL-1β to produce biologically active fragments. This is the first time to our knowledge that *Candida* proteinases have been proposed as proinflammatory agents at mucosal surfaces.

C. albicans proteinases can apparently cause limited proteolysis of the Hageman factor, leading to activation of the kallikrein-kinin system, which in turn generates bradykinin and causes increased vascular permeability (26). The release of inflammatory mediators through cell wall-induced complement activation via the alternative cascade or direct production of a neutrophil chemotactic attractant by the fungus may also lead to inflammatory reactions (10, 11, 29). However, neither complement nor Hageman factor is available at the skin surface or in the upper layers of stratified mucosal epithelia (53). Therefore, direct activation of pro-IL-1β reserves would be an alternate but powerful inflammatory mechanism. One may argue that inflammation would benefit the host rather than the fungus. However, in chronic inflammatory diseases such as denture stomatitis, the inflammatory reaction not only is inefficient but accounts for much of the etiopathology.

The extracellular proteolytic activity of *C. albicans* has been extensively studied and results mainly from secreted aspartyl proteinases (Sap) whose activity is restricted to the acidic pH

range (reviewed in reference 11). Sap isoenzymes are not specific to a single substrate and can break down a number of host proteins found in the oral cavity, including salivary proteins, immunoglobulin A, mucin, and epithelial keratin (8, 25, 43, 48, 50). Sap isoenzymes are now known to be the products of a family of genes that are expressed and regulated differentially (33, 39, 49, 51, 56). The expression of the *SAP1* and *SAP3* genes is regulated during phenotypic switching from the white to the opaque form of strain WO-1 (41). Sap2 is the main enzyme secreted in vitro by the yeast form of many strains of *C. albicans*, including white and opaque cells of strain WO-1 (23, 56, 57). The expression of the *SAP4*, *SAP5*, and *SAP6* genes has been detected at neutral pH during serum-induced yeast-to-hypha transition, whereas the expression of the *SAP7* gene has not yet been detected under any in vitro conditions (23, 56). Among the products of the eight *SAP* genes identified so far, only the products of genes *SAP1* to -3 and a putative Sap8 protein have been isolated and characterized (40, 55). While it is becoming clear that the pattern of Sap production is environmentally regulated in *C. albicans*, at least in vitro, the attribution of a particular proteolytic pattern to commensal versus pathogenic strains of *C. albicans* remains speculative. Moreover, as the entire substrate specificity or spectrum of *C. albicans* proteinases has not yet been defined, a more complete characterization of proteolytic enzymes as putative virulence factors is clearly needed to clarify these issues.

In mucosal infections, proteolytic enzymes with mucinolytic and keratinolytic activities may enable proinflammatory enzymes to gain access to stratum corneum stores of pro-IL-1β and thus participate in the cleavage and activation of this precursor. Our study of this activity suggests that it is not due to a typical convertase-like enzyme because the classic fluorogenic ICE substrate is not hydrolyzed by *Candida* and because pro-IL-1β is cleaved into multiple fragments rather than a predominant, low-molecular-mass (17- to 18-kDa) fragment as is the case for monocyte ICE (28). We do not know whether the high-molecular-mass (25- to 30-kDa) fragments are intermediates in pro-IL-1β processing to smaller fragments with biological activity (17 to 19 kDa) or whether the cleavage pattern results from a number of concurrently acting proteinases. However, the pepstatin sensitivity of pro-IL-1β cleavage and the increase in enzymatic activity during the stationary growth phase that follows the decrease in the pH of the culture medium suggest a similarity between Saps and the pro-IL-1β-cleaving enzyme(s) (55). In addition, data presented here do not exclude that a pro-IL-1β-cleaving enzyme(s) may be associated with the *Candida* cell surface, a feature already demonstrated for Saps on *C. albicans* cells that adhere to human, nonkeratinized, buccal epithelia (3). The fact that Saps function optimally at pH 4 whereas pro-IL-1β cleavage occurs mainly at pH 6 under our conditions suggests that a putative neutral proteinase (32) may also be at work, possibly with Saps, to cleave the precursor.

Generally, proteolytic processing of pro-IL-1β by ICE or other convertase-like enzymes generates 17- to 19-kDa fragments resistant to further proteolytic degradation. In our study, we observed that the 17- to 19-kDa fragments are also stable end products of *C. albicans* proteolytic activity. In fact, after 18 h of incubation, the 17- to 19-kDa fragments increase in concentration and are the only fragments detectable. The five *C. albicans* isolates studied demonstrated an ability to generate 17- to 19-kDa fragments from pro-IL-1β, although each strain showed a particular cleavage pattern. This finding may be related to different panels of proteinases or to the amount of enzyme released, which may vary by 2 orders of magnitude (12).

As the expression of proteinases by *C. albicans* has been reported for human skin mycoses and the initial stages of mucosal candidiases (3, 46, 47), we suggest a role for pro-IL-1β cleavage and activation by *Candida* proteinases in the pathogenesis of these inflammatory lesions. The mobilization of keratinocyte pro-IL-1β into biologically active cleavage products may have immediate effects on contiguous keratinocytes, fibroblasts, and endothelial cells. As a proinflammatory cytokine, IL-1β initiates and perpetuates the deleterious inflammation cascade by mediating neutrophil emigration and releasing secondary lipid-derived mediators and chemotactic cytokines, which in turn participate in the expression of vascular endothelial adhesion molecules and lead to an amplified recruitment of inflammatory cells (reviewed in references 19 and 52). Such an immunopathologic sequence would persist only in situations where host defense mechanisms are deficient or inefficient.

The transition of *C. albicans* from an innocuous commensal to an opportunistic pathogen thus likely depends on cumulative adaptive attributes that may be controlled by rapid phenotypic variability, which in turn is regulated by microenvironmental stimuli. According to the current, integrated models of composite virulence phenotypes proposed by Cutler (11) and Odds (45), the *C. albicans* virulence profile would vary depending on the site and stage of infection and the nature of the host response. Adherence to and invasion of epithelia, together with the ability to evade local host defenses, may be selected in mucosal microniches, and there is evidence that this selection may be linked to the secretion (and the secretion rate) of proteinases (11, 45). We proposed here that *C. albicans* proteinases may contribute to the inflammatory nature of mucosal candidiasis by the activation of an epithelial, proinflammatory cytokine. That epithelial pro-IL-1β may be mobilized for in situ activation by *Candida* enzymes is presently being tested in assays human keratinocytes cultured as stratified dermal equivalents. A precise characterization of the pro-IL-1β-cleaving enzymes and of their regulation and activity at mucosal surfaces will further clarify how *C. albicans* may occasionally convert to a harmful pathogen and how Saps may act as pathogenic determinants in infections of the stratified epithelia.

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