CLEAVAGE OF THE HUMAN C5A RECEPTOR BY PROTEINASES DERIVED FROM PORPHYROMONAS GINGIVALIS

Cleavage of Leukocyte C5a Receptor

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ABSTRACT/SUMMARY

The anaerobic bacteria P. gingivalis has been implicated as a primary causative agent in adult periodontitis. Several proteinases are produced by this bacteria and it is suggested that they contribute to virulence and to local tissue injury resulting from infection by P. gingivalis. Collagenases and cysteine proteinases (i.e., the gingipains)¹ have been characterized as the predominant vesicular enzymes produced by this bacterium. It has been shown that an arginine-specific cysteine proteinase from P. gingivalis, called gingipain-1 or Arg-gingipain, can selectively cleave complement components C3 and C5. In the case of C5, cleavage by Arg-gingipain results in the generation of C5a, a potent chemotactic factor for PMNs. Since these bacterial proteinases are capable of generating pro-inflammatory factors at sites of infection, we examined the possibility that gingipains or other proteinases from

*Abbreviations: P. gingivalis, Porphyromonas gingivalis; Arg-gingipain (gingipain-1), a cysteinyl proteinase from P. gingivalis that is specific for Arg-X bonds; Lys-gingipain (gingipain-2), a cysteinyl proteinase from P. gingivalis that is specific for Lys-X bonds; PMA, Phorbol-myristate-acetate; PMN, polymorphonuclear leukocytes; C3 and C5, blood complement components; C3a and C5a, bioactive fragments derived from C3 and C5, respectively, and MOPS, 3-(N-Morpholino)propanesulfonic acid; AP, adult periodontitis.
this bacterium might attack or destroy cell surface proteins, such as receptor molecules. Using an affinity-purified rabbit antibody raised against residues 9-29 of the C5a receptor (i.e., C5aR; CD88), the signal transmitting element for the pro-inflammatory mediator C5a, we demonstrated that the mixture of proteinases in P. gingivalis vesicles cleaves the C5a receptor on human neutrophils. This vesicular proteinase activity did not require cysteine activation which indicates that proteinases other than the gingipains may be responsible for cleavage of the C5aR molecule. In addition, the purified Lys-gingipain, but not Arg-gingipain, also cleaved C5aR on the human neutrophils. The N-terminal region of C5aR (residues 9-29, PDYGHYDDKDTLDLNTPVDKT) was readily cleaved by chymotrypsin, but not by trypsin, despite the presence of potential trypsin (i.e., lysyl-X) cleavage sites. The specific sites of C5aR 9-29 peptide cleavage were determined by mass spectrometry for both chymotrypsin and Lys-gingipain. These studies suggest that the proteolytic activity in the bacterial vesicles that is responsible for cleaving C5aR is primarily a non-tryptic proteinase, distinct from either Arg- or Lys-gingipain. Consequently, there appear to be additional proteinase(s) in the vesicles that attacks the cell surface molecule C5aR which are not the same (i.e., Arg- and Lys-gingipain) as were shown to generate pro-inflammatory activity from complement components C3 and C5. Evidence that the proteinases which attack the inflammatory precursor molecules (i.e., C3 and C5) exhibit different specificities than those that attack receptors to these bioactive complement products makes a particularly interesting story of how this bacteria avoids major host defense mechanisms.

It is well known that generation of pro-inflammatory factors such as C3a and C5a at extra-vascular sites can promote edema, leukocyte recruitment and cellular activation responses that could lead to the release of toxic oxygen products and to phagocytosis of the bacteria. Destruction of receptors to these cellular activating factors generated by bacterial proteinases may eliminate the ability of these (i.e., complement-derived) and other mediators to carry out their anti-bacterial actions and thereby limit the host’s defense mechanisms in responses to the infecting bacteria. The concept of anti-bacterial responses (i.e., oxygen radical generation and phagocytosis) being effectively eliminated at the injury site, by bacterial proteinases acting at the cellular receptor level, has not been studied in detail. In this case, the situation is particularly unusual because, once the bacterial gingipains generate potent plasma-derived inflammatory factors that can enhance edema and deliver essential nutrients to the bacteria, other bacterial proteinases may destroy their cellular receptors. These receptors transmit the signal activation mechanisms in the infiltrating cells that elicit bacterial killing. It is this series of events which might explain the ability of these anaerobes to persist and flourish in gingival tissue.

INTRODUCTION

Severe adult periodontitis is characterized by an acute inflammatory response with excessive granulocyte infiltration into the gingival tissue which become a chronic disease typified by extensive tissue degradation and eventual bone erosion and loss of teeth (White and Mayrand, 1981). Adult periodontitis (AP) affects 36% of Americans over the age of 19 and severity of the disease increases significantly with age (Mayrand and Holt, 1988). About half of all Americans have lost their teeth to AP by the age of 65 and approximately 10-20% of AP patients are refractory to present day treatment including antibiotics. In third world countries, where poor nutrition is a major contributing factor, AP is even more pervasive and tooth loss is the norm for adults over 30 years of age. It is agreed that gram negative anaerobes are mainly responsible for AP and that the bacterium Porphyromonas gingivalis is a major pathogen in this form of oral disease. Gingival crevicular fluid accumulates in periodontitis