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and diversity of both activators of the transcription factor and its target genes are already immense and still require additional definition.

CONCLUSIONS

Aggregatebacter actinomycetemcomitans serotype b was able to trigger apoptosis of host cells either by direct or indirect mechanisms. The study has shown that *A. actinomycetemcomitans* serotype b induces apoptosis in epithelial cells and apoptotic mechanisms involved caspase-3, Bax, PARP-1 and NF-KB

RECOMMENDATIONS

An Apoptosis mechanism was involved caspase-3, Bax, PARP-1 and NF-KB in mice gingival epithelium induced crude toxin *Aggregatebacter actinomycetemcomitans* serotype b. Moreover, the finding that tissue injury is associated with elevated NF-KB activation could open up new diagnostic possibilities and therapeutic strategies to prevent tissue destruction in periodontal disease.

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2012 Aggregatibacter actinomycetemcomitans infection enhances apoptosis in vivo through a caspase-3-dependent mechanism in experimental

Apoptosis is thought to play an important role in periodontal disease progression (Gamonal et al., 2001; Vaziri et al., 2004). The results demonstrated that inoculating with *A. actinomycetemcomitans* significantly stimulated apoptosis in the gingival epithelium. Apoptotic cells were detected in gingival epithelium mice by TUNEL staining. It is possible that crude toxin *A. actinomycetemcomitans* through its cytolethal distending toxin (CDT) could stimulate apoptosis (Shvedova et al., 2012). Crude toxin *A. actinomycetemcomitans* serotype b could induce apoptosis through direct and indirect mechanisms (Nalbant and Zadeh, 2002). Crude toxin Aa serotype b increases the apoptosis through Bax, PARP-1, NF-KBand caspase-3 expression in the gingival epithelium. Positive cells were detected by immunohistochemistry in histologic specimens using a specific antibody compared with normal rats after *A. actinomycetemcomitans* inoculation. A commonly used biochemical marker of apoptotic cell death is the induction of Caspase 3. Since Caspase 3 is an "executioner" caspase and is a reliable marker for impending apoptosis (Kang et al., 2012).

The author decided to evaluate the levels of Caspase 3 in gingival epithelium mice infected by *A. actinomycetemcomitans* was detectable at 24 hours, because suggested that the caspase 3 activation was preceded the observable cytotoxic effect of *A. actinomycetemcomitans* on HIGK by 24 hours (Alaoui-El-Azher et al., 2010) Previously, the occurrence of apoptosis in chronic periodontitis has mainly been studied by morphologic criteria as well as by the widely used TUNEL technique that is based on the detection of DNA fragmentation. An increased number of TUNEL-positive inflammatory cells have been reported in periodontitis (Gamonal et al., 2003). This finding is compatible with the study demonstrating that cells with caspase-3 expression are mainly localized in in gingival tissue from patients with periodontitis (Bantel et al., 2005).

This study showing NF-KB activation was considerably high with all three apoptotic markers, even though the cells often revealed no classical apoptotic alterations. There are several possibilities to explain this finding. Poly (ADP-ribose) polymerase-1 (PARP-1) participate in DNA repair as DNA damage activates PARP-1 to catalyze extensive polymerization of ADP-ribose from its substrate NAD to nuclear proteins. When activated by DNA damage, PARP-1 consumes NAD to form branched poly (ADP-ribose) on target proteins. PARP over-activation could allow NAD depletion and consequent necrosis or apoptosis, thus leading to an inflammatory condition in many diseases (Giansanti et al., 2010).

Poly (ADPribose) formation also has effects on gene transcription through interactions with transcription factors (Viscenzo et al, 2010). Post-translational modification of proteins by poly (ADP-ribosyl)ation is involved in the regulation of a number of biological functions. PARP-1 act as a DNA nick sensor and is activated by DNA breaks to cleave NAD(+) into nicotinamide and ADP-ribose to synthesize long branching poly (ADP-ribose) polymers (PAR) covalently attached to nuclear acceptor proteins. Whereas activation of PARP-1 by mild genotoxic stimuli facilitate DNA repair and cell survival, severe DNA damage triggers different pathways of cell death including PARP-mediated cell death through the translocation of apoptosis inducing factor (AIF) from the mitochondria to the nucleus (Wang et al., 2009). PAR and PARP-1 have also been described as having a function in transcriptional regulation through their ability to modify chromatin-associated proteins and as a cofactor of different transcription factors, most notably NF-KB (Quesada et al, 2007).

NF-KB targets a variety of genes associated with apoptosis and early response genes, including p53. NF-KB plays a significant role in deciding a cell's fate; it seems that NF-KB can play both sides of the fence, being pro-apoptotic in some situations and anti-apoptotic in others. How NF-KB effects apoptosis depends on the cell type, the activating stress, and co-factors (Proprawa et al., 2011). Although the mechanism by which NF-KB regulates apoptosis has yet to be fully resolved (Sonis, 2002). The pathway of NF-KB activation by inflammatory cytokines and bacterial products has been intensively studied.

Upon TNF, IL-1, or endotoxin LPS stimulation, NF-KB nuclear translocation can be observed rapidly. In addition, other stimuli, such as hypoxic injury and oxidative stress, have been shown to activate NF-KB (Kim et al., 2010).NF-KB as a critical regulator of apoptosis in the immune, hepatic, epidermal and nervous systems, on the mechanisms through which it operates and on its role in tissue development, homoeostasis and cancer. NF-KB could directly affect apoptosis-controlling genes; second, NF-KB could affect the cell cycle in such a way as to sensitize or desensitize the cell to apoptotic signals; or, third, NF-KB could interact with cellular proteins that themselves effect cell survival (Kucharczak et al., 2003).

Activation of NF-KB contributed to the induction of p53 and Bax led to apoptosis (Ravichandran et al., 2010). The multifunctional aspects of NF-KB help balance and regulate the extent and consequences of inflammatory responses, proliferation, immune function, and apoptosis. It appears that NF-KB has the potential to play a regulatory role in a wide variety of disease processes. The scope

to the manufacturer's instructions. This kit detects double-strand breaks in genomic DNA and identifies most stages of apoptosis. The number of apoptotic cells (TUNEL+) was counted at × 400 magnifications with a microscope. Cells counts were obtained by one examiner and confirmed by a second independent examiner with similar results.

Statistical analysis

Differences between five groups were determined by using multivariate analysis of variance. Significance levels were set at 5%.

RESULTS AND DISCUSSION

Induction of crude toxin *A. actinomycetemcomitans* serotype b at 24 hrs. result apoptosis cell was 8-fold higher than at baseline ($P< 0.05$) . Moreover, for caspase-3, Bax, PARP-one expression was two-fold higher than for the non-infected animals ($P< 0.05$). Also, after inoculation crude toxin Aa serotype b had NF-KB level that were three-fold higher than non infected animals ($P< 0.05$). Induction of crude toxin *A. actinomycetemcomitans* serotype b showed a significant increase in factors apoptosis ($P< 0.05$).

Table 1. Number of cell expressing apoptosis, caspase-3, Bax, PARP-1, andNF-KBin the cell of epitel gingiva exposed in toxin bacteria *A. actinomycetemcomitans*

	Sample size	Mean ± SD Control	Mean ± SD Toxin Aa serotype b	p value
Apoptosis	15	5.17 ± 0.27	45.17 ± 0.68	p < 0.05
Caspase-3	15	19.76 ± 0.67	46.35 ± 0.95	
Bax	15	29.97 ± 0.69	63.17 ± 0.71	p < 0.05
PARP-1	15	25.21 ± 1.72	48.9± 1.01	
NF-Kb	15	20.21± 0.15	60.23 ± 0.78	p < 0.05

Crude toxin Aa serotype b increases the apoptosis expression in the gingival epithelium of rats. Bax, PARP-1, NF-KB and caspase-3 positive cells were detected by immunohistochemistry in histologic specimens using a specific antibody. For more obviously result in this study can be shown in Figure 2.

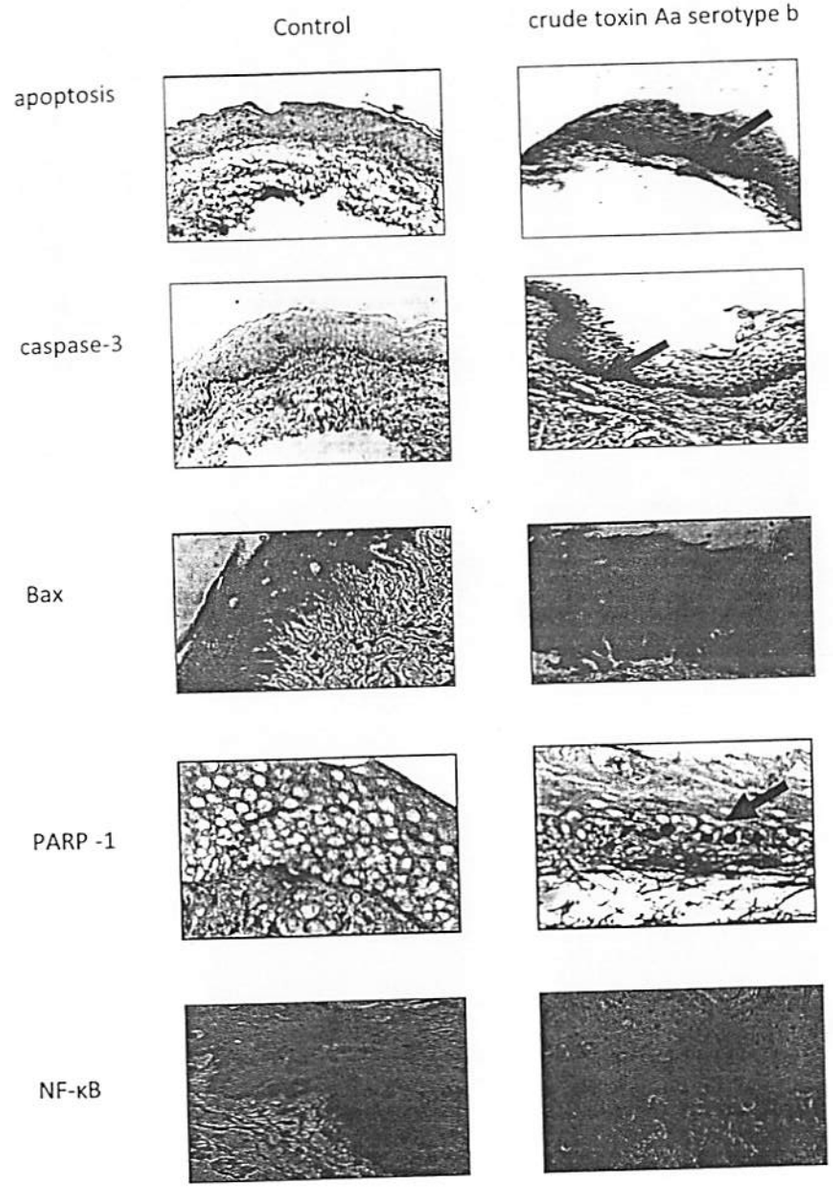

Figure 2. Crude toxin Aa serotype b increases the apoptosis, caspase-3, Bax, PARP-1, and NF-KB expression in the gingival epithelium of rats.

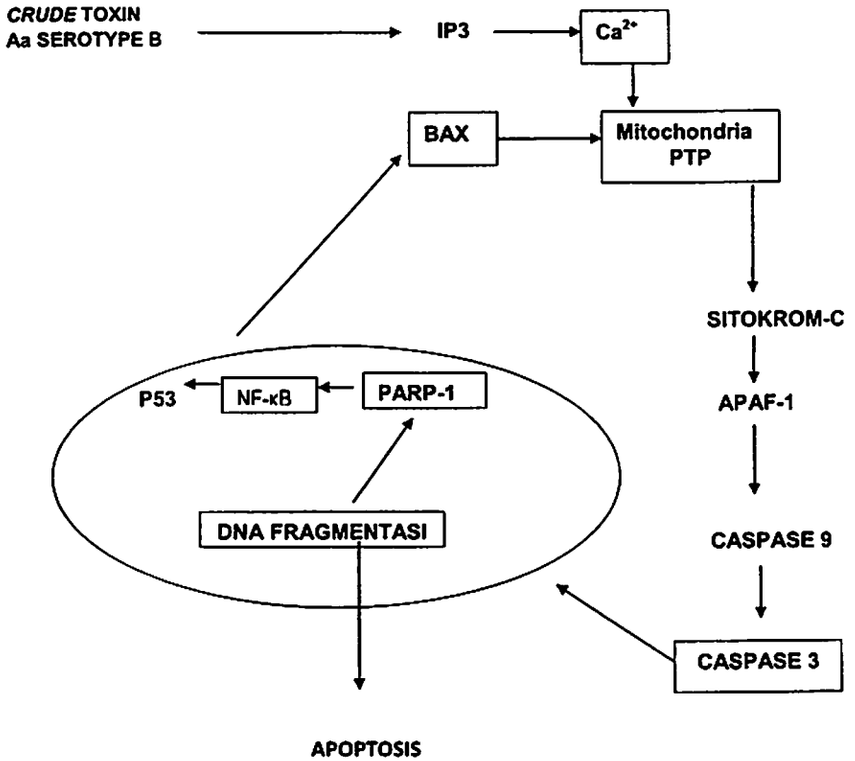

Figure 1. Theoretical framework of the research

OBJECTIVES OF THE STUDY

The study determined whether the pathogenic mechanisms of *A. actinomycetemcomitans* serotype b -induced gingival epithelium based on apoptosis mechanism is associated with caspase-3, Bax, PARP-1, and NF-KB expression.

MATERIALS AND METHODS

This is an experimental research which was conducted using test only group design. As this is an experiment with the purpose for getting the evidence, the study uses animal for the object of the experiment (Robert, L.W, 1998). This experimental research uses male rat (*Mus musculus* strain Swiss Webster balb c).

The analysis unit was gingival epithelial cell of the bucco-anterior rat mandible, physically fit, aged at 2.5 mo., weighing 150 to 250 g. All animal procedures were approved by the Institutional Animal Care and Use, Committee Airlangga University. After a subsequent period of one day, the rats were divided into two groups of approximately 30 rats.

A. actinomycetemcomitans serotype b (ATCC 43718) was incubated in *A. actinomycetemcomitans* growth medium with 35 mg of rifampin (Sigma-Aldrich, St. Louis, MO) for two days. Crude toxin *A. actinomycetemcomitans* serotype b was inoculated in gingival epithelium. First treatment (treatment group): 15 rats were applied topically with crude toxin *A. actinomycetemcomitans* serotype b (ATCC 43718) at 100µg/ml at the bucco-anterior mandibular gingiva using Hamilton syringe (Reno, Nevada USA). Second treatment (control group): 15 rats were given sterile distillated water topically at the bucco-anterior mandibular gingiva with aid disposable oral sponge swab (Rynell Inc, USA). Rats were euthanized 24 hrs. after the inoculation period was completed.

Histomorphometric analysis of caspase-3, Bax, PARP-1NF-KB:

Bucco anterior mandibullar gingiva was fixed in 10% neutral buffered formalin and routinely processed. Thick sections 4 µm were made from the paraffin blocks and mounted on three aminopropyltriethoxysilane coated slides. The anterior region was examined in each specimen and was established by being sectioned to a level where the gingival epithelium was visible. All data were analyzed by a blinded examiner who did not know the group to which an animal belonged.

The identification of these cells was confirmed by an experienced examiner. To evaluate the number of cells expressing caspase-3, Bax, PARP-1, NFkB sections were stained by immunohistochemistry with an antibody against caspase-3(*Santa CruzBiotechnology, Sc- 7148),* Bax(*LAB VISION,Clone 2C8),* PARP-1 (*TREVIGEN, Clone A6.4.12*), NF-KB p65 (*Biovision, 3012-100).* Sections were examined at × 400 magnification. The number of positive cells was evaluated cell counts were obtained by one examiner and confirmed by a second independent examiner with similar results.

Detection of apoptotic cells

Apoptotic cells were detected by an *in situ* TUNEL (terminal deoxynucleotidyltransferase-mediated dUTP-biotin nick end labeling) assay (DeadEnd fluorometric TUNEL system kit; Promega, Madison, WI) according

and NF-KB was up to 3-fold higher than controlled mice ($P < 0.05$). Besides, Aa- serotype-b induced periodontal destruction in mice gingival epithelium significantly through acaspase-3- dependent mechanism.

KEYWORDS

Science and Clinical Laboratory, aggregatebacter, actinomycetemcomitans Serotype b, apoptosis, Caspase-3, gingival epithelium, experimental design, Indonesia

INTRODUCTION

Researchers thought Apoptosis can contribute to periodontal disease progression especially aggressive periodontitis. It suggests that apoptosis of gingival epithelium cells should contribute to the loss of attachment in junctional epithelium. In this case, loss of gingival epithelium and fibroblast has shown the largest cellular change that occurs with periodontal disease progression (Chung et al., 2007). Infection by *A. actinomycetemcomitans* serotype b has shown that it induces apoptosis gingival epithelium *in vitro*(Kato et al, 2000). However, it relatively little is known about how *Aggregatebacter actinomycetemcomitans* serotype b induces apoptosis *in vivo* and affects periodontal tissue destruction in aggressive periodontitis.

Aggressive Periodontitis is characterized by severe and rapid destruction of the supporting apparatus of the teeth, which may lead to tooth loss early in life. Gingival epithelial cell is exquisitely sensitive to the toxin *Aggregatebacter actinomycetemcomitans* serotype b so that may lead to disruption of the epithelial protective barrier, facilitating invasion and perturbation of the underlying connective tissue (Nishimura et al., 2003). The cytolethal distending toxin (Cdt), expressed by the periodontal pathogen *Aggregatebacter actinomycetemcomitans,* inhibits the proliferation of cultured epithelial cells by arresting the cell cycle (Ohara et al., 2011). The gingival epithelium is an early line of defense against microbial assault. When damaged, bacteria collectively gain entry into underlying connective tissue where microbial products can affect infiltrating inflammatory cells, leading to the destruction of the attachment apparatus.

In connection with the above evidence, the data from in vitro cell cultures and a few reports of patient biopsies had suggested that apoptosis might play a role in periodontitis-associated gingival tissue damage (Gamonal et al., 2001). However,

the relative contribution of apoptosis and the functional role of caspase-3, Bax, PARP-1 and NF-KB in periodontal tissue damage remained largely unknown in vivo. In this study, the researcher demonstrates by rat model has been developed to study the pathogenic mechanisms of *A. actinomycetemcomitans* serotype b -induced gingival epithelium destruction through caspase-3,Bax, PARP-1 and NF-KB. This model is characterized by infecting the animals with a crude toxin of *A. actinomycetemcomitans* serotype b that adheres to the gingival epithelium. For more obviously framed, the theoretical basis in this study can be shown in Figure 1.

FRAMEWORK

Apoptosis is a controlled and regulated process and it plays an active role in cell death or cell suicide. Apoptosis and cell proliferation are in balance in healthy organisms, but an imbalance station in diseases preventing them from undergoing apoptosis. In the condition above, the sensitivity of cells on apoptosis depends on the balance of pro- and anti-apoptotic bcl-2 proteins. Bcl-2 and bcl-XL are anti-apoptotic, but Bad, Bax and Bid are pro-apoptotic proteins. The interaction between pro- and anti-apoptosis proteins leads to the formation of permeability transition pores (PTP) in the mitochondrial membranes. The mitochondria contain pro-apoptosis proteins (cytochrome C) and released through these pores leading to the formation of the apoptosome and activation of the Caspase cascade. Once cytochrome C released into the cytosol, apoptotic peptidase activating factor-1 (APAF-1) leaded to the recruitment of procaspase 9 into apoptosome consisting of cytochrome c, apoptotic protease activating factor-1(Apaf-1), and procaspase-9.. The apoptosome can then recruit procaspase-3, which is cleaved and activated by the active caspase-9, and can release it to mediate apoptosis (Zimmermann et al, 2001). PARP-1 and nuclear factor kappa B (NF-KB) have both been suggested to play a crucial role in inflammatory disorders. PARP-1 can act as a co activator of NF-KB (Hassa & Hottiger, 2002). Activation of NF-KB contributed to the induction of p53 and led to apoptosis (Sonis, 2002). For more obviously framed, the theoretical basis in this study can be shown in Figure 1.

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Factors Enhancing Apoptosis *In Vivo* through a Caspase-3, Bax, PARP-1 and NFkB Expression in Mice Gingival Epithelium

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ABSTRACT

Aggregatebacter actinomycetemcomitans serotype b in periodontal pockets indicates future periodontal disease progression. *A. actinomycetemcomitans* serotype-b has virulence factors, such as leukotoxin and cytolethal distending toxin (CDT) which may induce rapid tissue destruction by promoting apoptosis of a number of host cell types. This study tested the hypothesis that periodontal destruction is induced by crude toxin *A. actinomycetemcomitans* (Aa) serotype b based on apoptosis mechanism associated with caspase-3, Bax, PARP-1, and NF-KB expression. Thirty adult mice Swiss Webster strain were randomly divided into two groups (toxin and control). Gingival epitheliums were inoculated with crude toxin Aa serotype b and euthanized at base and 24 hours after inoculation. Apoptotic cells in gingival epithelium were measured by a TUNEL (terminal deoxynucleotidyltransferase-mediated dUTP-biotin nick end labeling) assay. The induction of caspase-3, Bax, PARP-1 and NF-KB was evaluated by immunohistochemistry. After Aa serotype b toxin was induced, the apoptotic cell in gingival epithelium was 8-fold higher than controlled mice ($P < 0.05$). The results show that the induction of caspase-3, Bax, PARP-1 was 2-fold higher

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