行政院國家科學委員會專題研究計畫　期中進度報告

胃幽門螺旋桿菌引起 TRAIL 所誘發之細胞凋亡訊息傳導的調節機轉(1/2)

計畫類別： 整合型計畫
計畫編號： NSC91-2320-B-002-193-
執行期間： 91 年 08 月 01 日至 92 年 07 月 31 日
執行單位： 國立臺灣大學醫學院免疫學研究所

計畫主持人： 許秉寧

計畫參與人員： 吳怡瑩, 吳詠萱, 許秉寧

報告類型： 精簡報告

處理方式： 本計畫可公開查詢

中　華　民　國 92 年 5 月 29 日

行政院國家科學委員會專題研究計劃成果報告

中文：幽門螺旋桿菌引起 TRAIL 所誘發之細胞凋亡訊息傳導的調節機轉

英文：Mechanisms of Helicobacter induced modulation of TRAIL death signal transduction

計劃類別： □個別計劃 ■主題計劃

計劃編號： NSC 91——2320B——002——193

執行期間： 91 年 8 月 1 日至 92 年 7 月 31 日

個別型計劃： 計劃主持人：許秉寧

處理方式： □可立即對外提供參考

(請打✓) ■一年後可對外提供參考

□兩年後可對外提供參考

(必要時，本會得展延發表時限)

執行單位：台大醫學院

中華民國 92 年 5 月 25 日

Abstract

Key words: *Helicobacter pylori*; TRAIL; apoptosis; human gastric epithelial cells; mitochondria..

Helicobacter pylori (*H. pylori*) causes a common chronic infection of humans that causes gastritis and peptic ulcer diseases. The enhanced gastric epithelial cell apoptosis observed during infection with *H. pylori* has been suggested to be of significance in the pathogenesis of gastritis. In addition to direct triggering cell apoptosis by *H. pylori* cytotoxins, in our preliminary study, we demonstrated that *H. pylori* could modulate intracellular death signal transduction regulatory mechanisms and confer sensitivity to TNF-related apoptosis inducing ligand (TRAIL) -mediated apoptosis in gastric epithelial cells. TRAIL, a new member of TNF superfamily molecule, induces apoptosis in transformed cell lines of diverse origin but not in most primary cells, indicating that the apoptosis induced by TRAIL is under regulation. The gastric epithelial cell lines, AGS and KATO III, which were resistant to TRAIL-induced cell death in vitro; however, when both AGS and KATO III cells exposed to *H. pylori*, they exhibited significant apoptosis to TRAIL. The effect of induction of TRAIL apoptosis by *H. pylori* is only observed when it is in the presence of live *H. pylori* but not heat-killed bacteria. Moreover, the *H. pylori* induced enhancement of TRAIL mediated apoptosis in gastric epithelial cells could be specifically blocked by Caspase-3 and Caspase-8 inhibitors, indicating that the alteration of TRAIL sensitivity by *H. pylori* is via modulation of intracellular TRAIL death signal transduction regulation. Thus, in addition to direct trigger apoptosis in gastric epithelial cells, *H. pylori* can induce sensitivity to TRAIL-mediated apoptosis

by altering intracellular signal transduction regulation in gastric epithelial cells. In this project, we attempt to further investigate the possible molecular mechanisms of TRAIL induced death signals modulated by Helicobacter as well as the pathogenesis of gastric mucosa damage induced by *H. pylori* infection in an *in vitro* co-culture system. We were able to demonstrated that the induction of TRAIL sensitivity by *H. pylori* is independent of expression of *H. pylori* virulent factors Vac A and Cag A; and is dependent on viable bacteria and direct contact with cells. *H. pylori* induced sensitivity to TRAIL-mediated apoptosis in gastric epithelial cells is dependent on activation of caspase-8 downstream pathway to convey the death signal to mitochondria**,** leading to activation of mitochondrial pathway and breaking the apoptosis resistance. This study will elucidate the mechanism of apoptosis signal transduction modulation after interaction with *H. pylori*.

Introduction

H. pylori, which infects about 50% of the world's population, is associated with duodenal ulcer and peptic ulcer diseases. Recent studies have shown there is increased apoptosis of gastric epithelial cells during *H. pylori* infection (1-4). The enhanced gastric epithelial cell apoptosis observed during infection with *H. pylori* has been suggested to be of significance in the etiology of gastritis, peptic ulcers, and neoplasia. There are a number of mechanisms that may be involved, including the direct cytotoxic effects of the bacteria, as well as inflammatory responses elicited by the infection (4-7). Recent studies have suggested that T helper type 1 (Th1) cells are selectively increased during infection (8-11). Th1 cytokines, such as gamma interferon (IFN-γ) and tumor necrosis factor alpha (TNF-α), can increase the release

of proinflammatory cytokines, augmenting apoptosis induced by *H. pylori* (7). *H. pylori* infection could also induce gastric mucosa damage by increasing expression of Fas in gastric epithelial cells, leading to gastric epithelial cell apoptosis through Fas/FasL interaction with infiltrating T cells (6, 12). These findings suggest a role for immune-mediated apoptosis of gastric epithelial cells during *H. pylori* infection.

TRAIL (also called Apo2L), a novel TNF superfamily member with strong homology to FasL, is capable of inducing apoptosis in a variety of transformed cell lines *in vitro* (13, 14) , but usually not in normal primary cells. It was shown recently that T cells can kill target cells via TRAIL/ TRAIL receptor interaction (15-20), suggesting that TRAIL might serve as a cytotoxic effector molecule in activated T cells *in vivo*. In addition to its role in inducing apoptosis by binding to death receptors, TRAIL itself can stimulate T cell after T cell receptor (TCR) engagement and augment IFN-γ secretion (21). These findings led us to hypothesize that TRAIL/TRAIL receptor interaction is involved in the interaction between infiltrating T cells and gastric epithelium during *H. pylori* gastritis. Therefore we set out to investigate the role of TRAIL mediated apoptosis in gastric epithelial cells during *H. pylori* infection. Here we report that human gastric epithelial cells sensitized to *H. pylori* confer susceptibility to TRAIL-mediated apoptosis. Our results indicate that *H. pylori* modulates sensitivity to TRAIL-mediated apoptosis in gastric epithelial cells by altering regulation of TRAIL death signal transduction, resulting in gastric mucosa damage during inflammation.

Results and Discussion

TRAIL was shown to induce apoptosis in a number of different tumor cell types,

but usually not in normal primary cells. Recent studies indicated that TRAIL-induced apoptosis occurs through a caspase signaling cascade, and that resistance to TRAIL is controlled by intracellular regulators of apoptosis. To examine a role for TRAIL-induced apoptosis in gastric epithelial cells, recombinant TRAIL proteins were used to induce apoptosis in human gastric epithelial cell lines, AGS and KATO III in the presence or absence of *H. pylori*. The results revealed that both AGS and KATO III cells were resistant to TRAIL-mediated apoptosis despite the expression of TRAIL death receptors in these cells. However, these cells became sensitive to TRAIL-induced cell death in the presence of a subtoxic level (0.05 μg/ml) of actinomycin D, an inhibitor of transcription. The results suggest that apoptosis induced by TRAIL was controlled by short-lived intracellular regulators in these cells. We further studied TRAIL-induced apoptosis in gastric epithelial cell lines after interaction with *H. pylori*. In the absence of TRAIL, *H. pylori* induced apoptosis in 20-30% of AGS and KATO III cells, indicating that *H. pylori* directly triggers apoptosis. However, significantly more apoptosis was induced after adding TRAIL, and TRAIL sensitivity induction was similar in effect to treatment with Actinomycin D. The enhancement of TRAIL sensitivity by *H. pylori* increased with bacterial counts in a dose-dependent manner.

To further delineate the intracellular signal transduction pathway altered by *H. pylori* that results in induction of TRAIL-sensitivity, we investigated the activation of caspases pathways after TRAIL engagement, subsequent to *H. pylori* interaction. During TRAIL engagement, both caspase-3 and caspase-8 were activated in AGS cells, resulting in cell apoptosis after exposure to *H. pylori*. In the absence of *H. pylori*, TRAIL engagement induced activation of caspase-8 but not caspase-3 in AGS cell. Furthermore, the ability to induce TRAIL sensitivity in AGS cells by *H. pylori* was significantly suppressed by either caspase-8 inhibitor, Z-IETD-fmk, or caspase-3

inhibitor, Z-VAD-fmk. These results indicated that *H. pylori* induced TRAIL sensitivity in gastric epithelial cell lines by activation of a downstream caspase cascade. In so doing, the pathogen alters the intracellular regulation of resistance to death receptor induced apoptosis. These observations indicate that *H. pylori* induces TRAIL-mediated apoptosis in gastric epithelial cell lines through a pathway involving the sequential induction of apical caspase-8 activity, caspase -cascade and effector caspase-3 activity. The alteration of signal transduction through TRAIL death receptor seems to be regulated at the level of caspase-8 downstream pathways, possibly involving mitochondrial pathway and its downstream caspase activation. For detecting the caspase processing events distal to caspase-8 activation, we investigated the activation of mitochondria after exposure to *H. pylori* in AGS cells. Our results demonstrated that TRAIL engagement induced breakdown of transmembrane potential of mitochondria after interaction with *H. pylori* but not in the absence of *H. pylori,* consistence with the difference in caspase-3 activation, indicating that activation of mitochondria downstream pathway was required in TRAIL-mediated apoptosis in AGS cells induced by *H. pylori*.

Self-estimation

We are satisfied with the progress we have obtained in recent one year. We will keep following the data we obtained, and the results will submit for publication in the near future.

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