Review Article

Esophageal inflammation in gastroesophageal reflux disease (GERD): role of chemokines

Yuji Naito[1,*], Norimasa Yoshida[2], Toshikazu Yoshikawa[3]

Departments of [1]Medical Proteomics, [2]Molecular Gastroenterology and Hepatology, and [3]Inflammation and Immunology, Graduate School of Medical Science, Kyoto Prefectural University of Medicine, Kyoto, Japan

Chemokines, especially CXC family, play a key role in neutrophil-mediated esophageal inflammatory disease by attracting neutrophils to the site of inflammation. Recent *in vitro* and *in vivo* reports suggest that esophageal squamous epithelial cells can produce these chemokines by the stimulation with gastric acid, bile acids, and pancreatic protease. Transcriptome analysis has confirmed the signaling pathway and several transcriptional factors associated with esophageal inflammation. Detailed studies of the interaction between esophageal epithelium and gastric/duodenal refluxates should make it possible to identify a key therapeutic target molecule that regulates esophageal inflammation.

Rec.8/28/2006, pp428-436

*Correspondence should be addressed to:
Yuji Naito, Medical Proteomics, Kyoto Prefectural University of Medicine, Kawaramachi-Hirokoji, Kamigyo-ku, Kyoto 602-8566, Japan. Phone: +81-75-251-5505, Fax: +81-75-252-3721, e-mail: ynaito@koto.kpu-m.ac.jp

Key words gastroesophageal refelux disease (GERD), chemokines, interluekin-8 (IL-8), transcriptome

Introduction

Gastroesophageal reflux disease (GERD) is a common disease in Western countries and increasingly in Asian countries. Proton-pump inhibitors (PPIs) have been the mainstay of medical therapy for GERD, both in erosive and nonerosive reflux disease. However PPI therapy alone may not result in complete recovery of esophageal mucosal breaks and symptoms such as heartburn[1]. It has been reported that more than 10% of patients show a relapse of esophagitis, even if a PPI is used for maintenance therapy[2,3]. The pathophysiology of GERD involves contact of the esophageal epithelium with gastric/duodenal juice in the refluxate. Recently, several studies have shown that esophageal mucosal immune and inflammatory responses, characterized by specific cytokine and chemokine profiles, may determine the diversity of esophageal phenotypes of GERD. In response to chemical agents such as gastric acid, bile acids, and pancreatic protease, neutrophils are recruited to the site of inflammation and generate reactive oxygen and nitrogen oxide species. Extravascularly migrated neutrophils infiltrate the region around target cells, depending on the concentration of the chemoattractants including interleukin (IL)-8, and take actions advantageous to the body, such as killing bacteria and cancer cells, while they may also injure normal cells and tissue. This paper describes the potential role of chemokine and activated neutrophils for esophageal inflammation in GERD.

Esophageal IL-8 expression in GERD patients

Fitzgerald et al.[4] firstly reported that reflux esophagitis is characterized by an acute inflammatory response with significantly increased levels of the proinflammatory cytokines (IL-1β, IL-8, and interferon-γ) comparing with non-inflamed squamous esophagus. Isomoto et al.[5] have also demonstrated that the presence of intraepithelial neutrophils and eosinophils, which also indicate reflux esophagitis, is associated with high mRNA levels of IL-8 and regulated on activation normal T-cell expressed and presumably secreted (RANTES), respectively, and that the IL-8 levels are significantly decreased after PPI treatment. Their data indicate that chemokine production locally in the esophageal mucosa may be involved in the development and progression of reflux esophagitis, and that gastric acid may play a role in the induction of esophageal inflammation. The importance of gastric acid in the development of esophageal inflammation is also supported by the recent study using an experimental esophagitis model in rats[6].

We have recently investigated the relationship between the IL-8/monocyte chemoattractant protein 1 (MCP-1) mRNA expression and endoscopic grading of reflux esophagitis according to the Los Angeles classification[7]. The expression of IL-8 mRNA determined by the real time PCR correlates the endoscopic severity of GERD (Fig.1) and increases in patients with non-erosive reflux disease (NERD) compared to normal subjects. There is no correlation between the MCP-1 mRNA expression and endoscopic severity, or between severity of subjective symptoms (QUEST score) and endoscopic grading. Kanazawa et al.[8] also reported the higher expression levels of IL-8 mRNA in esophageal mucosa of patients with NERD than those in asymptomatic controls. These results including ours suggest that IL-8 is implicated in the pathogenesis of early stage of GERD, and that mucosal IL-8 level may be a candidate to evaluate the quality of esophageal inflammation.

Proinflammatory cytokines may also be involved in the molecular events that characterize the pathway from inflammation to metaplasia, dysplasia, and adenocarcinoma. O'Riordan et al.[9] examined the expression of IL-8 and IL-1b in patients with esophagitis, metaplasia, dysplasia or adenocarcinoma. They found that in all patients, IL-8 and IL-1β were elevated, whereas the transcription factor NF-κB activation was commonly activated only in the Barrett's epithelium and adenocarcinoma. The association of NF-κB activation with cytokine upregulation may be crucial evident in patients with adenocarcinoma.

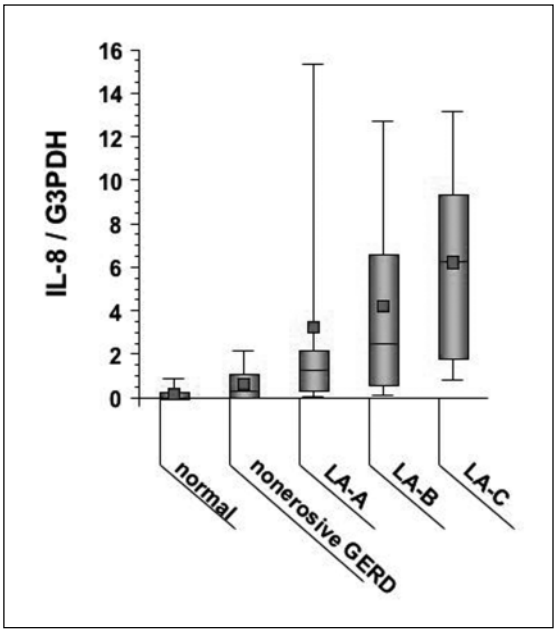

Fig.1 Relationship between IL-8 mRNA expression and the endoscopic grade of gastroesophageal reflux disease (GERD)

Samples were taken from non-erosive mucosa in normal control and non-erosive GERD and mucosal breaks in patients with esophagitis. Expression of IL-8 mRNA was quantified by real-time PCR and was corrected for that of G3PDH mRNA. Endoscopic features were assessed according to the Los Angels (LA) classification according to the criteria of severity.
Published with permission[7].

Chemokine expression in experimental esophagitis

We evaluated CINC-1 mRNA levels in the rat esophagus because rat CINC-1 is a counterpart of human GRO, a member of the IL-8 family, and is well known as a potent chemotactic factor for rat neutrouphils[10,11]. Chemotactic activity of rat CINC-1 was previously demonstrated in the *in vivo* setting by Suzuki et al.[12]. Yamaguchi et al.[13] have shown time-dependent changes in inflammatory markers such as cytokine expression, myeloperoxidase (MPO) activity, and lipid peroxides in esophageal mucosa exposed to gastroduodenal contents in rats. Specifically, the expression of tumor necrosis factor-α (TNF-α) and CINC-1 mRNA were recognized in the early phase at 3 and 6 h after induction of esophagitis, prior to significance increases in the lesion index, wet weight, and lipid peroxides (Fig.2). Katada et al.[14] recently assesses the protective effect of rebamipide against

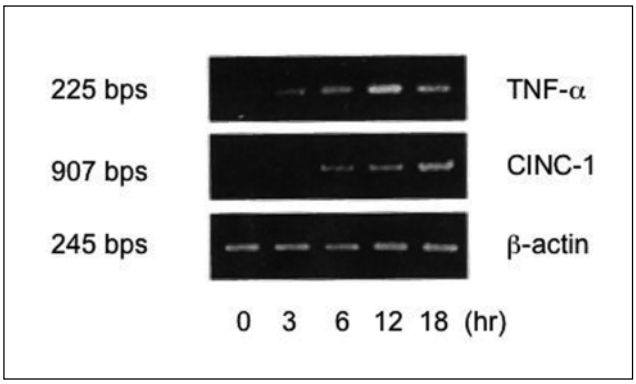

Fig.2 The expression of TNF-α and CINC-1 mRNA in esophageal tissue at 0, 3, 6, 12, and 18 h after the induction of reflux esophagitis in rats
Published with permission[13].

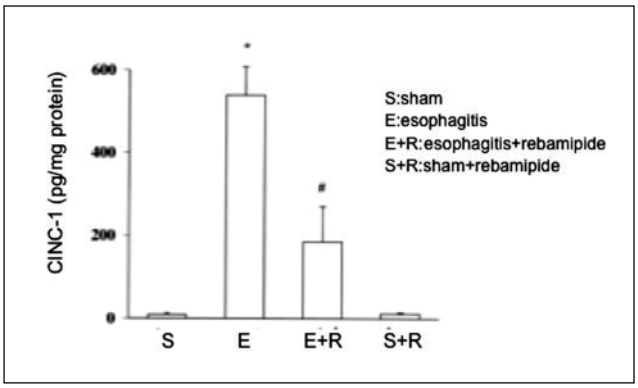

Fig.3 Effect of rebamipide on CINC-1 protein in esophageal mucosa at 12 hr after the induction of reflux esophagitis in rats
$p < 0.01$ when compared to the sham group, $^{\#}p < 0.05$ when compared to the esophagitis group.
Published with permission[14].

acute reflux esophagitis in rats. Rebamipide inhibited the increase in mucosal CINC-1 protein as well as reduced the expression of CINC-1 mRNA in the early phase, 6 hr after the induction (Fig.3). It has been also reported that rebamipide can directly inhibit IL-8 production from cultured gastric epithelial cells. These data suggest that the neutrophil-related inflammatory changes and the earlier increase in cytokine expression may be involved in the pathogenesis of reflux esophagitis.

Hamaguchi et al.[6] have reported that gastric acid might directly induce chronic inflammation associated with increased expression of several kinds of cytokines. They have established a rat chronic acid reflux esophagitis model, and expression of both C-X-C chemokines such as MIP-2 and CINC-2α, and C-C chemokines such as MCP-1 and MIP-1α mRNA have been found to increase significantly in esophageal mucosa. They have shown that treatment with rabeprazole, a PPI, almost completely inhibits its development of chronic acid-reflux esophagitis and significantly decreases expression of CINC-2α compared with control, indicating that gastric acid may play a role in the induction of esophageal cytokine production. However, the possibility that PPIs may directly affect the function of esophageal mucosal cells still remains.

IL-8 production by esophageal epithelial cells

Judging from the immunohistochemical staining[4,7], there is evidence that esophageal epithelial cells produce IL-8 protein. In order to confirm the epithelial cells as a potential source of cytokines, we have determined whether cultured human esophageal epithelial cells (HEEC) produce IL-8 and have identified molecular mechanism involved in IL-8 production[15]. Stimulation of HEEC with cholic acid or taurochenodeoxycholic acid resulted in IL-8 production via p38 mitogen-activated protein kinase (MAPK) phosphorylation-dependent pathway. The investigation of luciferase activity for the promotor lesion of *IL-8* gene also demonstrated that insertion of a vector with a mutation at the binding sites for NF-κB suppressed post-stimulation activity by 100%, indicating that NF-κB is an essential transcription factor, and that NF-IL6 and AP-1, with 30-70% suppression, are also involved[15]. These data indicates the main transcription factors involved in the expression of IL-8 are NF-κB and, in part, NF-IL6 and AP-1.

Role of pancreatic protease in esophageal inflammation

Reflux of the duodenal contents with gastric acid has been reported to contribute to the development of esophageal mucosal damage and Barrett's esophagus[16,17]. Trypsin, one of the proteases in the pancreatic juice, has in particular been reported to play an important role in the development of esophageal mucosal injury[18]. Previous studies have demonstrated that trypsin has a synergistic effect on the development of mucosal injury with bile acid, though trypsin alone does not induce esophageal mucosal injury[19], and that trypsin, in an alkaline environment, causes severe hemorrhagic erosive esophagitis, though bile salts under acidic and alkaline conditions do not induce morphologic

injury[20]. In recent studies it has been shown that trypsin induces the expression of proinflammatory cytokines on epithelial cells through the activation of specific receptors, protease-activated receptors (PARs)[21]. Although the expression of PARs in esophageal mucosa has not been clarified, human esophageal cells can produce IL-8 and cyclooxygenase-2-dependent prostaglandin E_2 by stimulation with trypsin or synthetic PAR-2 ligands[22,23]. These data suggest that trypsin might play a crucial role in esophageal injury or inflammation induced by reflux of the duodenal contents, including pancreatic proteases.

Recently, we created a rat chronic gastroduodenal reflux esophagitis model with esophagogastroduodenal anastomosis and investigated the role of pancreatic trypsin based on determinations of esophageal ulceration, infiltration of inflammatory cells, mucosal hyperplasia, and gene expression of inflammatory mediators[24]. A side-to-side esophagogastroduodenostomy was created to induce mixed gastroduodenal reflux. The most striking finding of our study was that camostat mesilate (CMM), a serine protease inhibitor, attenuated esophageal mucosal injury and cellular proliferation induced by gastroduodenal reflux. The significant role of pancreatic juices in the pathogenesis of reflux esophagitis has previously been demonstrated. Mud et al.[18] have reported that the presence of active trypsin in the esophagus contributes to the development of reflux esophagitis. They clearly showed that both bile acid and gastric juices alone do not induce esophageal mucosal damage, while combined with trypsin reflux they both induce severe esophagitis. Johnson et al.[20] have also reported that in a rabbit model, HCl at physiologic pH values does not break the mucosal barrier or cause esophagitis, and that bile salts at physiologic concentrations under both acid and alkaline conditions break the mucosal barrier but fail to cause morphologic injury. Instead, proteolytic enzymes such as pepsin in an acid environment and trypsin in an alkaline environment cause severe hemorrhagic erosive esophagitis. In our study, chronic treatment with CMM significantly inhibited both ulceration and hyperplasia scores 8 weeks after surgery, and significantly reduced the increase in trypsin activity in esophageal lumen 2 weeks after surgery. These results provide the evidence that pancreatic trypsin is involved in the development of chronic esophagitis induced by gastroduodenal reflux.

The significant role of pancreatic trypsin in esophageal inflammation should be clarified by the discovery that esophageal epithelial cells can produce inflammatory cytokines via a PAR-2 activated pathway. Four PARs have been cloned, and PAR-2 is activated most sensitively by trypsin[25]. Recent studies have revealed that PAR-2 plays an important role in inflammatory

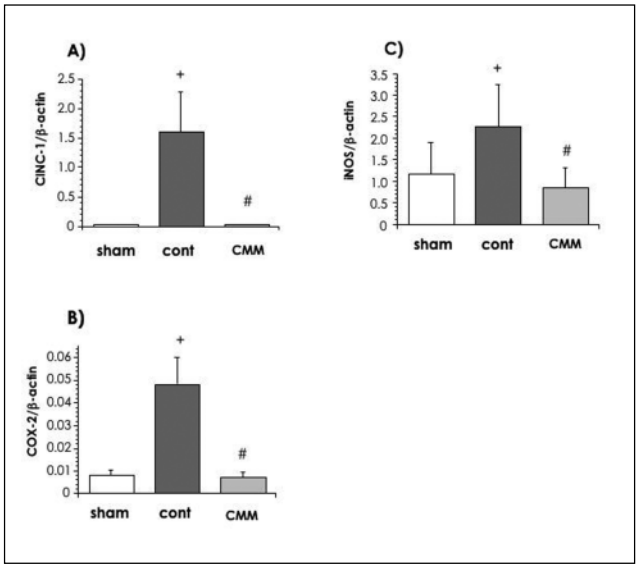

Fig.4 mRNA expression of CINC-1 (A), COX-2 (B), and iNOS (C) in the esophageal mucosa 8 weeks after the esophagogastroduodenal anastomosis

mRNA expression was determined by real-time PCR. Relative expression was calculated as the density of the product divided by β-actin. n = 5, $^{+}p < 0.05$ versus sham group and $^{\#}p < 0.05$ versus control group.
Published with permission[24].

processes in the colon[26] and pancreas[27,28]. It has also been reported that trypsin induces the expression of proinflammatory cytokines such as IL-6 and IL-8 on respiratory epithelial cells through a PAR-2 activated pathway[21]. More recently, our preliminary study has demonstrated that human esophageal epithelial cells can produce IL-8 via the activation of PAR-2 by trypsin or a synthetic PAR-2 ligand[22]. We confirmed the mRNA expression of *PAR-1* and *PAR-2* in rat esophageal epithelium by RT-PCR. However, further studies will be necessary to clarify the role of the trypsin-PAR-2 pathway in the pathogenesis of the inflammatory response in reflux esophagitis.

To further analyze the effect of CMM on the esophageal inflammation, we assessed mRNA expression for *CINC-1*, cyclooxygenase-2 (*COX-2*), and inducible nitric oxide synthase (*iNOS*), which are reported to play a role in acute and chronic inflammation. As shown in Fig.4, mRNA expression for these genes was significantly increased by real-time PCR in the control group 2 weeks after the esophago-gastroduodenal anastomosis compared with the sham-operated group. These increases were significantly decreased in the CMM group. The trypsin

activity in the esophageal lumen was significantly increased 2 weeks after surgery compared with the sham-operated rats. The treatment with CMM also significantly inhibited the increase in trypsin activity in the esophageal lumen.

Transcriptome analysis for esophageal cells

Some studies on reflux esophagitis have been based on the analysis of the expression of a single molecule, or of a relatively limited number of these molecules in esophageal mucosa. Recently, DNA microarray techniques have become available that have enabled the characterization of the mRNA expression pattern of large number of genes simultaneously[29]. We have identified specific gene expression profiles of the esophageal epithelial cells in the experimental esophagitis, which is created EGD anastomosis in rats as a model of combined-type chronic reflux esophagitis[24,30]. We used the GeneChip of rat toxicology U34 array (Affymetrix), which contained 1,031 probes selected from the UniGene database. Among the 1,031 probes on this array, there are 368 probes (36%) that shows a more than 1.5-fold difference in expression between the sham-operated and esophagitis groups 2 weeks after the GDE operation; 185 probes (17.9%) are up-regulated and 183 probes (17.7%) are down-regulated. Genes that are up-regulated at least 1.5-fold were involved in cellular stress responses (heat shock protein 60, 70, Dna-J), apoptosis-related genes (caspase 2, 3, 6, bcl-2 asscociated death agonist), transcriptional factors (nuclear factor κB), DNA damage-associated genes (mismatch repair protein), and enzymes (cytochrome c oxidase, glutathione s-transferase).

To further refine the list of esophagitis-affected genes, we next investigated which of these genes are known to interact biologically. To this end we carried out pathway analysis on the above 368 up- and down-regulated genes using an Ingenuity Pathway Analysis tool (IPA). Of these genes, 207 mapped to genetic networks, as defined by the IPA tool. Three caronical networks associated with inflammation were found to be significant in that they had more of the identified genes present than would be expected by chance (Table 1): IL-6 signaling, p38 MAPK signaling, and IL-2 signaling. We recently demonstrated that the expression of IL-6 mRNA is increased in patients with reflux esophagitis, especially in endoscopic negative reflux disease (not published data). In addition, the role of IL-6 in esophagitis was reported by Cheng et al.[31]. They have demonstrated that acid-induced platelet activating factor (PAF) formation induces the production of IL-6 in esophageal mucosa. As IL-6 signaling molecules were most significantly affected in this model of esophagitis, further studies will be necessary to clarify the role of this signaling in the pathogenesis of GERD.

Table 1 Caronical pathways significantly affected in the esophageal mucosa

		Genes	
Pathway	Significance	Up-regulated	Down-regulated
IL-6 Signaling	2.18×10^{-6}	COL1A1, IL1A, JUN, MAP2K2, MAPK14, NFKB1, STAT3, TNF	CYP19A1, FOS, HSPB1, MAPK9, NRAS, TNFRSF1B
p38 MAPK Signaling	7.57×10^{-3}	IL1A, MAPK14, TGFB3, TNF	HSPB1, MAPK12, MYC, TNFRSF1B
IL-2 Signaling	1.06×10^{-2}	IL2, IL2RB, JUN, MAP2K2	FOS, NRAS

COL1A1	collagen, type I, alpha 1
CYP19A1	cytochrome P450, family 19, subfamily A, polypeptide 1
FOS	v-fos FBJ murine osteosarcoma viral oncogene homolog
HSPB1	heat shock 27kDa protein 1
IL1A	interleukin 1, alpha
IL2	interleukin 2
IL2RB	interleukin 2 receptor, beta
JUN	v-jun sarcoma virus 17 oncogene homolog (avian)
MAP2K2	mitogen-activated protein kinase kinase 2
MAPK9	mitogen-activated protein kinase 9
MAPK12	mitogen-activated protein kinase 12
MAPK14	mitogen-activated protein kinase 14
MYC	v-myc myelocytomatosis viral oncogene homolog (avian)
NFKB1	nuclear factor of kappa light polypeptide gene enhancer in B-cells 1 (p105)
NRAS	neuroblastoma RAS viral (v-ras) oncogene homolog
STAT3	signal transducer and activator of transcription 3
TGFB3	transforming growth factor, beta 3
TNF	tumor necrosis factor (TNF superfamily, member 2)
TNFRSF1B	tumor necrosis factor receptor superfamily, member 1B

In order to focus on inflammation, the expression of genes to be studied is narrowed down to 40 probes, which are selected using a keyword "*cytokine*" by a soft of NetAffx™ Analysis Center (http://www.affymetrix.com/jp/analysis/index.affx). The expression of these probes at each time point after the GDE operation is shown to be within the limits of 6.0-fold up-regulation (red) or down-regulation (blue) compared to that in the sham-operated group (yellow) using a GeneSpring software. Fig.5 shows that the probes associated with "cytokine" were divided into several clusters on the basis of time-kinetics. In group A (Fig.5), many genes, including the early-response genes (IL-1 and cyclooxygenase), were up-regulated predominantly during the experiment for 8 weeks. In particular, the expression of many probe sets for interleukin (IL-1α, IL-1β, IL-1 receptor) was markedly up-regulated, and their expression continued or was even further enhanced during the period of esophagitis. In group

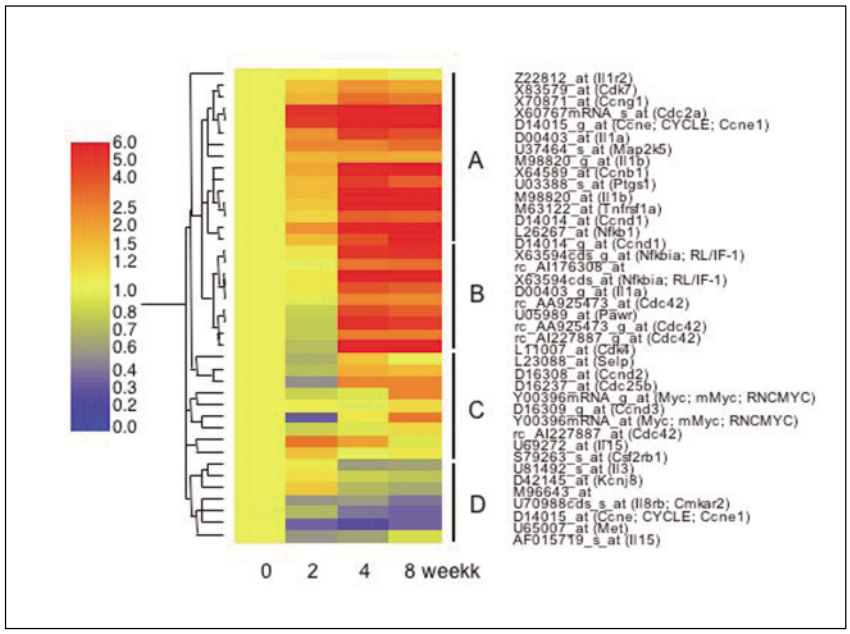

Fig.5 Expression clusters of cytokine-related genes in esophageal mucosa after esophagogastroduodenostomy in rats

In the hierarchical-clustering analysis, a fold-change ratio was calculated using the sham-operated rats and esopahgitis samples. Red indicates up-regulated genes, and blue indicates down-regulated genes. Yellow indicates the same expression level as the normoxia sample. Published with permission[30]

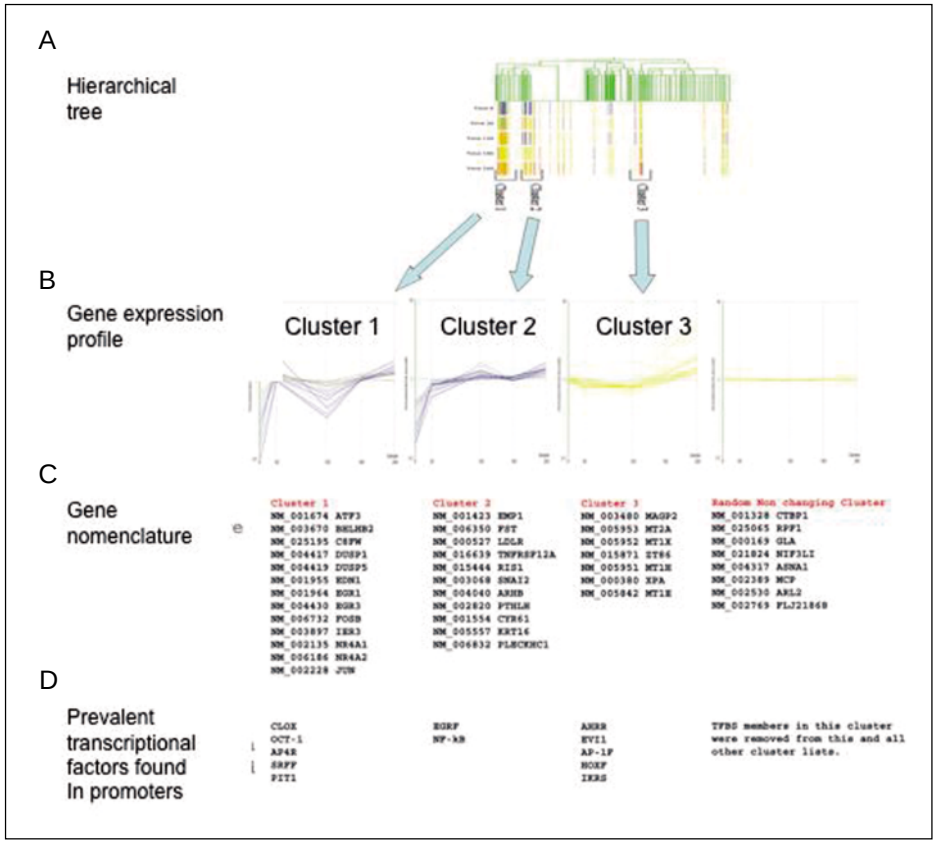

Fig.6 Co-regulatory analysis of the low pH-induced transcriptome of SKGT4 oesophageal cells using the promoters of gene sets defined by hierarchical clustering

(A) Hierarchical clustering map of the genes in the ordered list. Gene-expression profiles have separated into distinct clusters. Blue represents low-expression levels, which move through a range of colours to red for high level of expression. Three gene-expression clusters have been marked for further bioinformatic analysis. (B) Expression profiles of each of the three clusters demonstrating the similarities of the genes within the individual clusters in comparison to a random selection of non-changing profiles. (C) Gene nomenclature of those genes chosen for promoter analysis from each cluster inclusive of the control, non-changing gene set. (D) Statistically significant TFBSs found within the majority of gene promoters within each individual cluster after comparison with the TFBS sets of the other clusters and the control non-changing gene set. Published with permission[32].

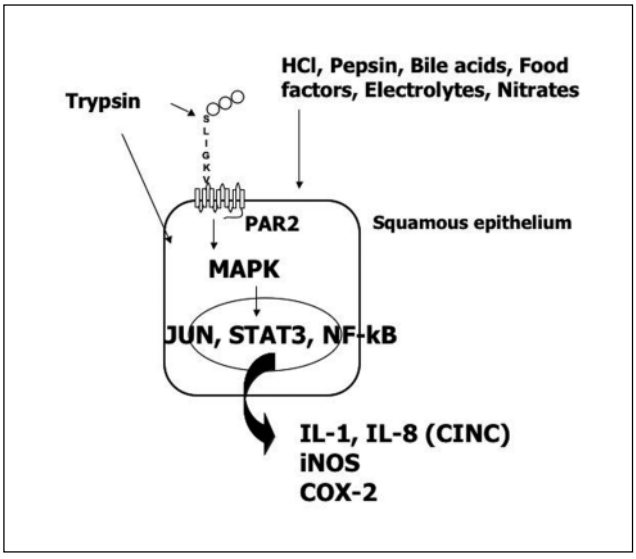

Fig.7 Esophageal epithelium can produce several products associated with inflammation in response to gastric/duodenal refluxate

B, nuclear factor-κB and cell cycle-related genes {cell division cycle 42 homolog (Cdc42) and cyclin-dependent kinase 4 (Cdk4)} were up-regulated at 4 and 8 weeks after the operation. These increases in cell cycle-related genes may result in hyperplasia of the epidermis and basal cells of the esophagus, which are features of esophagitis described in previous reports (5, 10, 11). The gene expression of group C showed irregular changes during the experiment. Group D included genes such as IL-3 and Met that were down-regulated at least two points compared to the sham.

Recently, Duggan et al.[32] have assessed regulation of gene expression in response to low pH in esophageal cells by a transcriptomic and bioinformatics approach. Their initial experiments demonstrate maximum induction of *EGR1* gene expression at pH 6.5. Subsequent DNA array experimentation revealed significant induction of gene expression such functional categories as DNA damage response (*EGR1-4, ATF3*) and cell-cycle control (*GADD34, GADD45, p57*). In addition, comparative promotor transcription factor binding site analysis (MatInspector, http://www.genomatix.de/) identified transcription factors that may co-ordinately regulate gene expression clusters, Cluster 1: Oct-1, AP4R; Cluster 2: NF-κB, EGRF; Cluster 3: IKRS, AP-1F (Fig.6). This methods employed in their study can add to the tools available to analyze the vast amounts of data generated from transcriptomic experiments and assist in identifying sequential bilological events. Especially, there is increasing evidence for the involvement of *EGR1* in the regulation of immune response both in immune cells and epithelial cells. Katada et al.[33] have found that *EGR1* mRNA expression resulted in a response at early stage of hypoxia and their expression continued or was enhanced during the reoxygenation using a DNA microarray technique. These data suggest that this molecule *EGR1* may be a candidate for a target of a novel therapeutic strategy for hypoxia-reoxygenation or inflammation.

Conclusion

These results, including our data, suggest that the inhibition or regulation of proinflammatory cytokine pathway may be an important target for future therapeutic strategies. Clinically, detailed studies of the interaction between esophageal epithelium and gastric/duodenal refluxate should make it possible to identify a key therapeutic target molecule that regulates esophageal inflammation (Fig.7).

References

1) Chiba N: Proton pump inhibitors in acute healing and maintenance of erosive or worse esophagitis: a systematic overview. Can J Gastroenterol, 11 Suppl B: 66B-73B, 1997.
2) Robinson M, Lanza F, Avner D, Haber M: Effective maintenance treatment of reflux esophagitis with low-dose lansoprazole. A randomized, double-blind, placebo-controlled trial. Ann Intern Med, 124: 859-867, 1996.
3) Escourrou J, Deprez P, Saggioro A, Geldof H, Fischer R, Maier C: Maintenance therapy with pantoprazole 20 mg prevents relapse of reflux oesophagitis. Aliment Pharmacol Ther, 13: 1481-1491, 1999.
4) Fitzgerald RC, Onwuegbusi BA, Bajaj-Elliott M, Saeed IT, Burnham WR, Farthing MJ: Diversity in the oesophageal phenotypic response to gastro-oesophageal reflux: immunological determinants. Gut, 50: 451-459, 2002.
5) Isomoto H, Wang A, Mizuta Y, Akazawa Y, Ohba K, Omagari K, Miyazaki M, Murase K, Hayashi T, Inoue K, Murata I, Kohno S: Elevated levels of chemokines in esophageal mucosa of patients with reflux esophagitis. Am J Gastroenterol, 98: 551-556, 2003.
6) Hamaguchi M, Fujiwara Y, Takashima T, Hayakawa T, Sasaki E, Shiba M, Watanabe T, Tominaga K, Oshitani N, Matsumoto T, Higuchi K, Arakawa T: Increased expression of cytokines and adhesion molecules in rat chronic esophagitis. Digestion, 68: 189-197, 2003.
7) Yoshida N, Uchiyama K, Kuroda M, Sakuma K, Kokura S, Ichikawa H, Naito Y, Takemura T, Yoshikawa T, Okanoue

T: Interleukin-8 expression in the esophageal mucosa of patients with gastroesophageal reflux disease. Scand J Gastroenterol, 39: 816-822, 2004.

8) Kanazawa Y, Isomoto H, Wen CY, Wang AP, Saenko VA, Ohtsuru A, Takeshima F, Omagari K, Mizuta Y, Murata I, Yamashita S, Kohno S: Impact of endoscopically minimal involvement on IL-8 mRNA expression in esophageal mucosa of patients with non-erosive reflux disease. World J Gastroenterol, 9: 2801-2804, 2003.
9) O'Riordan JM, Abdel-latif MM, Ravi N, McNamara D, Byrne PJ, McDonald GS, Keeling PW, Kelleher D, Reynolds JV: Proinflammatory cytokine and nuclear factor kappa-B expression along the inflammation-metaplasia-dysplasia-adenocarcinoma sequence in the esophagus. Am J Gastroenterol, 100: 1257-1264, 2005.
10) Watanabe K, Iida M, Takaishi K, Suzuki T, Hamada Y, Iizuka Y, Tsurufuji S: Chemoattractants for neutrophils in lipopolysaccharide-induced inflammatory exudate from rats are not interleukin-8 counterparts but gro-gene-product/melanoma-growth-stimulating-activity-related factors. Eur J Biochem, 214: 267-270, 1993.
11) Handa O, Naito Y, Yoshikawa T: Rat cytokine-induced neutrophil chemoattractant-1 (CINC-1) in inflammation. J Clin Biochem Nutr, 38: 51-58, 2006.
12) Suzuki H, Suematsu M, Miura S, Liu YY, Watanabe K, Miyasaka M, Tsurufuji S, Tsuchiya M: Rat CINC/gro: a novel mediator for locomotive and secretagogue activation of neutrophils in vivo. J Leukoc Biol, 55: 652-657, 1994.
13) Yamaguchi T, Yoshida N, Tomatsuri N, Takayama R, Katada K, Takagi T, Ichikawa H, Naito Y, Okanoue T, Yoshikawa T: Cytokine-induced neutrophil accumulation in the pathogenesis of acute reflux esophagitis in rats. Int J Mol Med, 16: 71-77, 2005.
14) Katada K, Yoshida N, Isozaki Y, Tomatsuri N, Ichikawa H, Naito Y, Okanoue T, Yoshikawa T: Prevention by rebamipide of acute reflux esophagitis in rats. Dig Dis Sci, 50 Suppl 1: S97-S103, 2005.
15) Yoshida N, Imamoto E, Uchiyama K, Kuroda M, Naito Y, Mukaida N, Kawabe A, Shimada Y, Yoshikawa T, Okanoue T: Molecular mechanisms involved in interleukin-8 production by normal esophageal epithelial cells. Aliment Pharmacol Ther, symp ser 2: 219-226, 2006.
16) Kauer WK, Peters JH, DeMeester TR, Ireland AP, Bremner CG, Hagen JA: Mixed reflux of gastric and duodenal juices is more harmful to the esophagus than gastric juice alone. The need for surgical therapy re-emphasized. Ann Surg, 222: 525-531; discussion 531-523, 1995.
17) Gillen P, Keeling P, Byrne PJ, Healy M, O'Moore RR, Hennessy TP: Implication of duodenogastric reflux in the pathogenesis of Barrett's oesophagus. Br J Surg, 75: 540-543, 1988.
18) Mud HJ, Kranendonk SE, Obertop H, Van Houten H, Westbroek DL: Active trypsin and reflux oesophagitis: an experimental study in rats. Br J Surg, 69: 269-272, 1982.
19) Salo JA, Kivilaakso E: Contribution of trypsin and cholate to the pathogenesis of experimental alkaline reflux esophagitis. Scand J Gastroenterol, 19: 875-881, 1984.
20) Johnson LF, Harmon JW: Experimental esophagitis in a rabbit model. Clinical relevance. J Clin Gastroenterol, 8 Suppl 1: 26-44, 1986.
21) Asokananthan N, Graham PT, Fink J, Knight DA, Bakker AJ, McWilliam AS, Thompson PJ, Stewart GA: Activation of protease-activated receptor (PAR)-1, PAR-2, and PAR-4 stimulates IL-6, IL-8, and prostaglandin E2 release from human respiratory epithelial cells. J Immunol, 168: 3577-3585, 2002.
22) Yoshida N, Katada K, Kuroda M, Shimozawa M, Uchiyama K, Naito Y, Okanoue T, Yoshikawa T, Soma T, Shimada Y: Trypsin activates human esophageal epithelial cells to produce inflammatory cytokines through protease-activated receptor 2. Gastroenterology, 126: A-501, 2004.
23) Kawabe A, Shimada Y, Soma T, Maeda M, Itami A, Kaganoi J, Kiyono T, Imamura M: Production of prostaglandin E2 via bile acid is enhanced by trypsin and acid in normal human esophageal epithelial cells. Life Sci, 75: 21-34, 2004.
24) Naito Y, Uchiyama K, Kuroda M, Takagi A, Kokura S, Yoshida N, Ichikawa H, Yoshikawa T: Role of pancreatic trypsin in chronic esophagitis induced by gastroduodenal reflux in rats. J Gastroenterol, 41: 198-208, 2006.
25) Bohm SK, Khitin LM, Grady EF, Aponte G, Payan DG, Bunnett NW: Mechanisms of desensitization and resensitization of proteinase-activated receptor-2. J Biol Chem, 271: 22003-22016, 1996.
26) Cenac N, Coelho AM, Nguyen C, Compton S, Andrade-Gordon P, MacNaughton WK, Wallace JL, Hollenberg MD, Bunnett NW, Garcia-Villar R, Bueno L, Vergnolle N: Induction of intestinal inflammation in mouse by activation of proteinase-activated receptor-2. Am J Pathol, 161: 1903-1915, 2002.
27) Nguyen TD, Moody MW, Steinhoff M, Okolo C, Koh DS, Bunnett NW: Trypsin activates pancreatic duct epithelial cell ion channels through proteinase-activated receptor-2. J

Clin Invest, 103: 261-269, 1999.
28) Olejar T, Matej R, Zadinova M, Pouckova P: Expression of proteinase-activated receptor 2 during taurocholate-induced acute pancreatic lesion development in Wistar rats. Int J Gastrointest Cancer, 30: 113-121, 2001.
29) Naito Y, Mizushima K, Yoshikawa T: Global analysis of gene expression in gastric ischemia-reperfusion: a future therapeutic direction for mucosal protective drugs. Dig Dis Sci, 50 Suppl 1: S45-55, 2005.
30) Naito Y, Kuroda M, Uchiyama K, Mizushima K, Akagiri S, Takagi T, Handa O, Kokura S, Yoshida N, Ichikawa H, Yoshikawa T: Inflammatory response of esophageal epithelium in combined-type esophagitis in rats: a transcriptome analysis. Int J Mol Med, in press, 2006.
31) Cheng L, Cao W, Fiocchi C, Behar J, Biancani P, Harnett KM, Corrado G, Zicari A, Cavaliere M, Rea P, Pacchiarotti C, Cerroni F, Pontieri G, Cardi E: HCl-Induced Inflammatory Mediators in Cat Esophageal Mucosa and Inflammatory Mediators in Esophageal Circular Muscle in an In Vitro Model of Esophagitis Increased release of interleukin-6 by oesophageal mucosa in children with reflux oesophagitis. Am J Physiol Gastrointest Liver Physiol, 11: 839-843, 2006.
32) Duggan SP, Gallagher WM, Fox EJ, Abdel-Latif MM, Reynolds JV, Kelleher D: Low pH induces co-ordinate regulation of gene expression in oesophageal cells. Carcinogenesis, 27: 319-327, 2006.
33) Katada K, Naito Y, Mizushima K, Takagi T, Handa O, Kokura S, Ichikawa H, Yoshida N, Matsui H, Yoshikawa T: Gene expression profiles on hypoxia and reoxygenation in rat gastric epithelial cells: a high-density DNA microarray analysis. Digestion, 73: 89-100, 2006.