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Expression of Cytokines in Bacterial and Viral Infections and Their Biochemical Aspects

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Cytokines are very important in the host defense system, and play a critical role in protection against bacterial and viral infections. Cytokines are also involved in the pathogenesis and development of symptoms in infections. In this article, *Helicobacter pylori* (*H. pylori*) infection as bacterial infection, and influenza virus infection, encephalomyocarditis virus (EMCV) infection, and herpes simplex virus (HSV) infection as viral infection are mentioned. In *H. pylori* infection, various chemokines, especially interleukin (IL)-8, induce inflammatory responses in the gastroduodenal mucosa. Furthermore, IL-6, IL-7, tumor necrosis factor (TNF)-α, and interferon (IFN)-γ are involved in both protection and pathogenesis. In influenza virus infection, IFN-α/β, IFN-γ, and IL-6 play protective roles. In EMCV infection, IL-6 and TNF-α play important roles as a protective and exacerbative factor in acute myocarditis, respectively. Furthermore, in HSV infection, the production of inflammatory cytokines is closely correlated with the pathogenesis of herpetic keratitis, and IFN-γ plays an important role in enhancing viral clearance from the cornea and trigeminal ganglions.

Key words: cytokines, encephalomyocarditis virus, *Helicobacter pylori*, herpes simplex virus, influenza virus.

Cytokines, (glyco)proteins that are secreted from various kinds of cells, are involved in host defense and inflammatory responses. It is supposed that cytokines play an important role in protection against bacterial and viral infections and also in pathogenesis of infectious diseases. The defense mechanism and pathogenesis vary depending on such factors as the kind of pathogens, the stages of infections (acute or chronic), and the site of infections (systemic or topical).

In this article, the author mentions the functions of various kinds of cytokines in bacterial and viral infections, and proposes a hypothesis regarding roles of cytokines in infections.

1. *Helicobacter pylori* infection

The author mentions *Helicobacter pylori* (*H. pylori*) infection as a representative of bacterial infections.

1) *Helicobacter pylori* infection and gastroduodenal diseases. It is well known that *H. pylori* causes various kinds of gastroduodenal diseases including acute and chronic gastritis, duodenal ulcer, gastric ulcer, and gastric cancer (*1–4*). However, the pathogenesis of these diseases caused by *H. pylori* is not well understood. Some immunological responses are involved in the development of inflammation of the stomach (*5*).

It is supposed that several potential virulent factors derived from *H. pylori* are involved in the development of lesions resulting from gastric inflammation. For example, vacuolating cytotoxin, which is an 87-kDa protein encoded by *vacA*, causes vacuolization of gastric epithelial cells (*6–8*). This vacuolating cytotoxin is closely associated with CagA protein, of which the molecular weight is 128 kDa (*9, 10*). However, Tummuru *et al.* (*11*) reported that the mutated *cagA* gene, which can not express CagA protein, did not affect cytotoxin expression. This indicates that *vacA* and *cagA* are independently expressed.

Urease plays an important role in the pathogenesis of *H. pylori* infection by protecting the bacteria from the acid environment of the stomach, promoting colonization, and inducing the production of ammonia. In addition, recent studies have shown that urease is a potent chemoattractant factor for monocytes obtained from peripheral blood mononuclear cells (PBMC) and mucosal macrophages (*12, 13*).

Thus, several virulent factors are involved in the pathogenesis of gastroduodenal diseases.

2) *cagA* and inflammation of gastroduodenal tissue. As described above, the role of the *cagA* gene in the pathogenesis of gastroduodenal diseases has not yet been elucidated. Therefore, we examined the relationship between the *cagA* gene and the degree of inflammation in gastroduodenal mucosa (*14*). One hundred sixty patients (85 men and 75 women; age range, 19–85 years; mean age, 54.5 years) participated in the study. Endoscopic findings in the patients were as follows: normal mucosa, 15 patients; gastric ulcer, 40 patients; duodenal ulcer, 25 patients; gastric cancer, 30 patients; and chronic gastritis without ulcer, 25 patients. Table I shows that 112 of 160 patients (70%) were positive for *H. pylori* by culture or histological staining. Nine patients were positive for *H. pylori* only by histological staining. In the 103 patients with a positive culture for *H. pylori*, a test for *cagA* gene was positive in 89

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(86.4%). The positive rate of the *cagA* gene was significantly higher in patients with duodenal ulcer or patients with gastric cancer, although there were no significant differences between patients with duodenal ulcer and those with gastric ulcer.

In terms of the histological severity of the biopsy specimens, specimens from patients with normal mucosa without *H. pylori* infection had no infiltration of mononuclear cells (MNC) or polymorphonuclear cells (PMN). Specimens infected with *H. pylori* had significantly more severe infiltration of PMN and MNC than those without *H. pylori* both in the antrum and corpus (Table II).

Furthermore, specimens infected with *cagA*-positive strains had significantly more severe infiltration of PMN than those infected with *cagA*-negative strains both in the antrum and corpus. *cagA*-positive specimens also had significantly more severe infiltration of MNC than *cagA*-negative specimens in the antrum, but not in the corpus.

3) Expression of cytokine mRNA and *cagA* gene. We examined the expression level of various kinds of cytokines in the biopsy specimens. In general, gastric mucosal inflammation caused by *H. pylori* is more severe in the antrum than that in the corpus (*15, 16*). Therefore, we compared the expression of cytokine mRNA in the specimens of the antrum with those of the corpus (*14, 17*).

The results showed that interleukin (IL)-1β and IL-7 mRNA were found in the specimens from normal mucosa without infection with *H. pylori*, whereas IL-6, IL-8, IL-10, and tumor necrosis factor (TNF)-α mRNA were not found. The levels of expression of IL-6 and IL-10 mRNA were significantly higher in specimens from noncancerous tissue of gastric cancer than those from chronic gastritis without ulcer in the corpus. The level of IL-6 mRNA was significantly higher in specimens from cancerous tissues than in those from chronic gastritis without ulcer and gastric ulcer or duodenal ulcer. The level of IL-10 mRNA expression was also significantly higher in specimens from cancerous tissues than in those from gastric ulcer or duodenal ulcer in the antrum and chronic gastritis without ulcer in the corpus.

Next, we examined the relationship between the expression of cytokine mRNA and the histological findings in *H. pylori*–positive patients. The levels of expression of IL-7, IL-8, and TNF-α mRNA were significantly higher in specimens with moderate to severe infiltration of MNC and PMN than in those with normal to mild infiltration in the corpus. Furthermore, the levels of IL-8 and TNF-α mRNA expression were significantly higher in specimens with moderate to severe infiltration of MNC and PMN than in those with normal to mild infiltration in the antrum.

The levels of expression of IL-6, IL-7, IL-8, IL-10, and TNF-α mRNA were significantly higher in *H. pylori*–positive than in *H. pylori*–negative specimens both in the antrum and corpus (Table III). The level of IL-8 mRNA expression was also significantly higher in *cagA*-positive than in *cagA*-negative specimens both in the antrum and corpus. On the other hand, the levels of expression of IL-6, IL-7, IL-10, and TNF-α mRNA were not significantly higher in *cagA*-positive than in *cagA*-negative specimens.

Thus, it was concluded that *cagA*-positive strains induce the expression of IL-8 mRNA and that IL-8 may play an important role in the pathogenesis of gastroduodenal diseases associated with *H. pylori* infection.

4) Induction of various cytokines and *cagA* gene.

TABLE I. **Expression of *cagA* gene and endoscopic findings.**

		Hp+			*Hp*−	*Hp*+/total (%)	*cagA*+/(*cagA*+ and *cagA*−) (%)
		cagA+	*cagA*−	Not tested[a]			
Chronic gastritis	(*n*=50)	24	6	3	17	66.0	80.0
Gastric ulcer	(*n*=40)	23	4	4	9	77.5	85.2
Duodenal ulcer	(*n*=25)	25	0	0	0	100.0	100[b]
Gastric cancer	(*n*=30)	17	4	2	7	76.7	81.0
Normal mucosa	(*n*=15)	0	0	0	15	0.0	—
Total	(*n*=160)	89	14	9	48	70.0	86.4

Hp+, *H. pylori* positive; *Hp*−, *H. pylori* negative; *cagA*+, *cagA* gene positive; *cagA*−, *cagA* gene negative. [a]The presence of *cagA* gene was not tested because *H. pylori* was positive only by histological staining. [b]The positive rate of *cagA* gene was significantly higher in patients with duodenal ulcer than patients with chronic gastritis and patients with gastric cancer ($p < 0.05$). [Reproduced from Yamaoka, Y. *et al.* (1996) *Gastroenterology* **110**, 1744–1752 (*14*).]

TABLE II. ***H. pylori* infection and histological severity of gastritis.**

Location	Grade	MNC infiltration			PMN infiltration		
		Hp+		*Hp*−	*Hp*+		*Hp*−
		cagA+	*cagA*−		*cagA*+	*cagA*−	
Antrum	Normal	0	0	15	13	7	45
	Mild	7	5	21	9	4	3
	Moderate	32	7	12	29	2	0
	Severe	50	2	0	38	1	0
Corpus	Normal	0	0	15	15	6	47
	Mild	7	1	27	20	3	1
	Moderate	44	9	6	37	5	0
	Severe	38	4	0	17	0	0

NOTE. MNC infiltration: *Hp*+ *vs.* *Hp*−, $p < 0.0001$ (antrum) and $p < 0.0001$ (corpus); *cagA*+ *vs.* *cagA*−, $p < 0.001$ (autrum) and NS (corpus). PMN infiltration: *Hp*+ *vs.* *Hp*−, $p < 0.0001$ (antrum) and $p < 0.0001$ (corpus); *cagA*+ *vs.* *cagA*−, $p < 0.001$ (autrum) and NS (corpus) by Mann-Whitney U test. *Hp*+, *H. pylori* positive; *Hp*−, *H. pylori* negative; *cagA*+, *cagA* gene positive; *cagA*−, *cagA* gene negative. [Reproduced from Yamaoka, Y. *et al.* (1996) *Gastroenterology* **110**, 1744–1752 (*14*).]

TABLE III. **Expression of cytokine mRNA and *H. pylori* infection.**

	No. studied	IL-1β (%)	IL-6 (%)	IL-7 (%)	IL-8 (%)	IL-10 (%)	TNF-α (%)
Antrum							
Hp+	112	108 (96.4)	34 (30.4)	65 (58.0)	84 (75.0)	17 (15.2)	51 (45.5)
cagA+	89	87 (97.8)	29 (32.6)	58 (65.2)	72 (80.9)	16 (18.0)	44 (49.4)
cagA−	14	12 (85.7)	4 (28.6)	6 (42.9)	6 (42.9)	0 (0.0)	5 (35.7)
Hp−	48	44 (91.7)	1 (2.1)	12 (25.0)	6 (12.5)	0 (0.0)	6 (12.5)
Hp+ *vs.* *Hp*−		NS	$p < 0.0001$	$p < 0.0005$	$p < 0.0001$	$p < 0.005$	$p < 0.0001$
cagA+ *vs.* *cagA*−		NS	NS	NS	$p < 0.005$	NS	NS
Corpus							
Hp+	112	107 (95.5)	50 (44.6)	59 (52.7)	93 (83.0)	16 (14.3)	57 (50.9)
cagA+	89	87 (97.8)	39 (43.8)	49 (55.1)	81 (91.0)	14 (15.7)	44 (49.4)
cagA−	14	13 (92.9)	5 (35.7)	6 (42.9)	6 (42.9)	1 (7.1)	7 (50.0)
Hp−	48	44 (91.7)	0 (0.0)	4 (8.3)	3 (6.3)	0 (0.0)	4 (8.3)
Hp+ *vs.* *Hp*−		NS	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	$p < 0.005$	$p < 0.0001$
cagA+ *vs.* *cagA*−		NS	NS	NS	$p < 0.0005$	NS	NS

Hp+, *H. pylori* positive; *Hp*−, *H. pylori* negative; *cagA*+, *cagA* gene positive; *cagA*−, *cagA* gene negative. [Reproduced from Yamaoka, Y. *et al.* (1996) *Gastroenterology* **110**, 1744–1752 (*14*).]

Next, we examined the relationship between the *cagA* gene and various kinds of cytokine proteins using the enzyme-linked immunosorbent assay (ELISA) method (*18*). Results showed that mucosal levels of IL-1β, IL-6, IL-8, and TNF-α were significantly higher in *H. pylori*–positive than in *H. pylori*–negative patients. Furthermore, the mucosal levels of IL-1β and IL-8 were higher in specimens infected with *cagA*-positive strains than in those infected with *cagA*-negative strains. In *H. pylori*–positive patients, the mucosal level of IL-8 was closely correlated with that of IL-1β and the mucosal level of IL-6 was closely correlated with that of TNF-α.

These findings suggest that the ability to induce cytokines differs among the strains; *cagA*-positive strains induce various kinds of cytokines and may cause severe inflammation, whereas *cagA*-negative strains induce IL-8 and IL-1β only weakly and may cause only mild inflammation. However, as most patients infected with the *cagA*-positive strains have gastritis, these strains might not be equivalent to ulcerogenic strains.

5) Chemokines and *H. pylori* infection. It is clarified from the results in the above studies that various cytokines were involved in the pathogenesis of gastroduodenal diseases. In particular, IL-8 was supposed to play an important role in the development of inflammation of gastric mucosa. Therefore, we examined the expression of chemokines in the gastric mucosa in *H. pylori* infection (*19*).

Chemokines are a kind of cytokines showing chemotactic activity for leukocytes. There are over 20 members of the chemokine superfamily at present, and all of them are involved directly or indirectly in inflammatory responses. Chemokines are classified into two major families on the basis of the arrangement of the first two of four conserved cysteine residues. The C-X-C chemokines consist of those members whose first two cysteines (C) are separated by one amino acid (X), while in the C-C chemokines, the first two cysteines are adjacent to one another.

In general, C-X-C chemokines mainly show chemotactic activities for neutrophils but not monocytes, whereas C-C chemokines show chemotactic activities for monocytes and lymphocytes, but have little effect on neutrophils (*20*). The former group includes IL-8 and GROα, and the latter group includes RANTES, MCAF, MIP-1α, and MIP-1β.

We examined chemokine expression and production patterns in gastric mucosa by RT-PCR and ELISA methods using gastric biopsy specimens (*19*). Furthermore, we compared the expression of chemokines in the antrum with that in the corpus in the patients with and without *H. pylori* infection.

The results showed that *H. pylori* infection increased the rates of expression of mRNA for IL-8, GROα, RANTES, and MIP-1α. The levels of these chemokines were correlated with cellular infiltration. *cagA* gene–positive *H. pylori* infection increased the expression of mRNA for IL-8 and GROα.

Thus, it is concluded from these results that *H. pylori* infection increased the expression of C-X-C chemokines (IL-8 and GROα), but not the production of C-C chemokines.

6) Urease and *H. pylori* infection. Thus, various kinds of cytokines are induced by infection with *H. pylori*, and *cagA* gene products are involved in the production of cytokines. However, products derived from *H. pylori* other than CagA protein may be related to the production of cytokines.

We examined by RT-PCR and ELISA methods whether *H. pylori* urease stimulates the gastric epithelial cells to induce cytokines (*21*). First, by using peripheral blood mononuclear cells (PBMC) and a gastric cancer cell line (MKN-45 cells), we confirmed the ability of purified *H. pylori* urease to induce the expression of IL-1β, IL-6, IL-8, IL-10, IL-12, IL-18, and TNF-α mRNA. However, interferon (IFN)-γ mRNA was detected neither before nor after the stimulation of the purified urease. The production of IL-6 and TNF-α, but not IL-8, increased significantly in a dose-dependent manner after the addition of urease to PBMC. In particular, a large amount of TNF-α was detected in the supernatants.

On the other hand, the expression of IL-6 and TNF-α mRNA was induced in response to exposure to *H. pylori* urease in MKN-45 cells. IL-1β, IL-8, IL-12, and IL-18 mRNA were constitutively detected both before and after the stimulation of the purified urease. IL-10 and IFN-γ mRNA were detected neither before nor after the stimulation.

In MKN-45 cells, the production of IL-6, IL-8, and TNF-α was significantly induced by the addition of 10 μg of urease per ml. However, *H. pylori* UreB proteins stimulated the production of IL-6 and TNF-α, but not IL-8, significantly in a dose-dependent manner in PBMC. The production of IL-8 and TNF-α, but not IL-6, was also significantly induced by

the addition of UreB protein to MKN-45 cells.

Furthermore, human gastric epithelial cells were incubated with purified *H. pylori* urease for 3 h, and the expressions of mRNA were determined by RT-PCR method. The stimulation with the purified urease induced the expressions of IL-6 and TNF-α mRNA, and there was no expression of mRNA in the unstimulated cells. IL-1β, IL-8, IL-10, and IL-18 mRNA were expressed constitutively both before and after the stimulation with the purified urease. IL-12 and IFN-γ mRNA expressions, on the other hand, were detected neither before nor after the stimulation.

These results suggest that the human gastric epithelial cells produce inflammatory cytokines by stimulation with *H. pylori* urease, which indicates that the epithelial cells were involved in the mucosal inflammation that accompanied *H. pylori* infection.

7) IFN-γ and *H. pylori* infection. IFN-γ and IL-1β mRNA were detected in the almost all biopsy specimens, whereas IL-2, IL-3, IL-4, IL-5, IL-9, IFN-β, and TNF-α mRNA were not detected in any specimens and IL-6 mRNA was detected in only a few specimens. IFN-γ is thought to be important in immune responses because it induces the expression of the class II major histocompatibility complex of antigen-presenting cells and activates macrophages and natural killer cells.

We first examined the colonizing abilities of eight *H. pylori* strains with a short-term infection test in order to select *H. pylori* strains that could colonize the mouse stomach (*22*). Only three strains (ATCC 43504, CPY2052, and HPK127) colonized the stomach of C57BL/6 wild-type mice, although all of the strains except for ATCC 51110 could colonize the stomach of IFN-$\gamma^{-/-}$mice. The number of *H. pylori* organisms colonizing the stomach in wild-type mice was lower than that in IFN-$\gamma^{-/-}$mice. These findings suggested that IFN-γ may play a protective role in *H. pylori* infection, although the degree of its protective ability was estimated to be low.

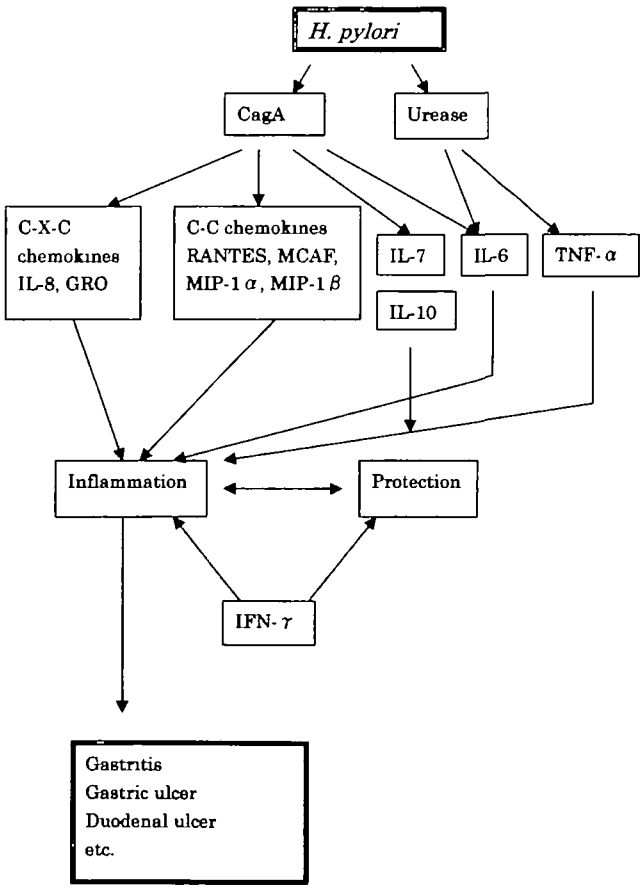

Fig. 1. **Scheme of the development of gastroduodenal diseases by *Helicobacter pylori*.**

In contrast, in a long-term infection test done to examine the contribution of IFN-γ to gastric inflammation, CPY-2052-infected wild type mice developed a severe infiltration of MNC in the lamina propria and erosion in the gastric epithelium 15 months after infection, whereas CPY2052-infected IFN-$\gamma^{-/-}$mice showed no inflammatory symptoms.

This result clearly demonstrated that IFN-γ plays an important role in the induction of gastric inflammation caused by *H. pylori* infection.

From the results obtained in our studies, the following scheme may be proposed (Fig. 1). Namely, CagA and urease derived from *H. pylori* induce the production of cytokines, and the cytokines produced cause inflammatory responses and/or protect against infection. Finally, gastroduodenal mucosal lesions characterized by infiltration of MNC and PMN develop *via* actions of various cytokines.

2. Viral infection

Various kinds of cytokines also play an important role in many viral infections. In this section, the author describes roles of cytokines in influenza virus infection, encephalomyocarditis virus (EMCV) infection, and herpes simplex virus (HSV) infection.

1) Influenza virus infection. In influenza virus infection, cytokines appear in the acute phase, namely 1 to 3 days after infection. IFN plays an especially important role in the protection against influenza virus infection. There are three types of IFN: first, IFN-α, which is produced in the leukocytes infected with viruses; second, IFN-β, which is produced in fibroblasts infected with viruses or treated with synthetic double-stranded RNA; third, IFN-γ, which is produced in sensitized lymphocytes stimulated with antigens. Recently, it has been clarified that IFN-γ is produced in type 1 helper T cells (Th1).

In order to investigate roles in the protection from influenza virus infection, we administered anti–IFN-α/β antibody to mice infected with the influenza virus. The results showed that all the mice treated with the antibody died within 7 days post-infection, while the mice in the control groups survived. Virus titers in the lungs of the mice that were not given the antibody peaked on day 3 and then decreased again. Also, IFN was detectable both in lung homogenates and serum. In mice given the antibody, no interferon was detectable and virus yields in the lung increased until death. These results suggest that IFN-α/β produced in the respiratory tract plays an important role in the early stages of influenza virus infection (*23*).

Next, we examined the role of IFN-γ in the protection against influenza virus infection. Anti–IFN-γ antibody was administered to mice infected with the influenza virus. Sixty percent of the mice receiving the anti–IFN-γ antibody died, while the mice that did not receive it survived. Thus, IFN-γ also plays an important role in the protection against influenza virus infection.

Then, we detected IL-6 activity in the bronchoalveolar fluid from the mice infected with the influenza virus, and we confirmed that IL-6 was produced in the mast cells from the infected mice. Furthermore, we were unable to detect IL-6 in the bronchoalveolar fluid from the mast cell–deficient mice infected with the influenza virus. When anti–IL-6 antibody was administered to the mice infected with the influenza virus, the mortality of the mice treated with the antibody was significantly higher than that of the untreated control mice or the normal serum-treated mice (*24*). Thus, IL-6 also plays an important role in the protection against influenza virus infection.

2) Cytokines and viral myocarditis. It is possible that cytokines play a role in the myocardial injury produced by viral myocarditis. It was reported that IL-1α, IL-1β, TNF-α, and granulocyte-colony stimulating factor (G-CSF) were elevated in the serum of patients with acute viral myocarditis (*25*). Although IFN, TNF and IL-6 reportedly possess antiviral properties, their effects on viral myocarditis are unclear.

To investigate the role of cytokines in viral myocarditis induced by the encephalomyocarditis virus (EMCV) in mice, we determined cytokine mRNA levels expressed in the heart after EMCV infection by the RT-PCR method. Four-week-old C57BL/6 mice were inoculated intraperitoneally with EMCV. The results showed that the expressions of IL-1β, IL-6, IL-10, TNF-α, IFN-α, IFN-β, and IFN-γ mRNA were enhanced in the heart during the acute stage 2 to 3 days after EMCV infection.

In addition, we examined cytokine levels in heart tissue and plasma by the ELISA and bioassay methods. IL-6, TNF-α, and IFN concentrations were elevated in the blood and heart of infected mice compared with uninfected mice.

Finally, we examined the effects of the anti-cytokine antibody *in vivo*. Treatment with anti–IL-6 or anti–IFN-γ antibody resulted in significantly reduced survival and increased myocardial damage in mice with viral myocarditis. In contrast, the anti–TNF-α antibody improved survival rate and lesions of myocardial lesions. These results suggest that IL-6 and TNF-α play important roles as a protective and exacerbative factor in acute myocarditis, respectively.

3) Cytokines and herpetic keratitis. It is well known that cytokines, especially IFN, show protective activity in herpes simplex virus (HSV) infections. We investigated the role of cytokines in the pathogenesis of acute herpetic keratitis (HK). The kinetics of cytokines expression in the corneas and the trigeminal ganglia (TG) of C57BL/6Cr (B6) mice was examined after HSV type 1 (HSV-1) infection and the influence of the targeted disruption of the IFN-γ gene on the clinical course of HK and/or viral clearance was also observed (*26*).

After corneal infection with HSV-1 Amakata strain, all corneas developed typical dendritic keratitis. Quantitative analysis using the ELISA method revealed that the expressions of IL-1α, IL-5, IL-6, and IFN-γ in corneas and TGs were significantly elevated immediately after infection, peaked between days 2 and 7 post-infection, and then diminished. One exception was IFN-γ, whose expression significantly persisted in the TGs until day 30 post-infection.

An additional experiment using IFN-$\gamma^{-/-}$ mice revealed that there was no significant difference in the peak level of viral replication in corneas and TGs between IFN-$\gamma^{-/-}$ mice and B6 mice, although IFN-$\gamma^{-/-}$ mice showed a significant delay of virus clearance in both the corneas and TGs ($p < 0.005$) and a higher mortality rate than that of B6 mice after HSV-1 infection ($p < 0.01$). It is suggested from these results that the production of inflammatory cytokines is closely correlated with the pathogenesis of HK, and that IFN-γ plays an important role in enhancing viral clearance from the cornea and TG.

Thus, in general, IFN-α and β play protective role in viral infections and IFN-γ, TNF-α and IL-6 show both protective function and induction of inflammatory responses.

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