Interferon- γ and Tumor Necrosis Factor- α Levels in Sera and Whey of Cattle with Naturally Occurring Coliform Mastitis

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ABSTRACT. Concentrations of interferon- γ (IFN- γ) and tumor necrosis factor- α (TNF- α) were determined in serum and whey samples from cattle with naturally occurring coliform mastitis for two weeks after onset using bovine IFN- γ and TNF- α -specific ELISA. In serum and whey samples from healthy cows, IFN- γ was almost undetectable and TNF- α was detected at low levels. At the onset of illness, IFN- γ in sera and whey and TNF- α in whey from the mastitic cows were significantly higher than their respective values in healthy cows. Concentrations of IFN- γ and TNF- α in whey from mastitic cattle decreased significantly as the cows recovered.

KEY WORDS: bovine mastitis, interferon- γ , tumor necrosis factor- α .

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Mastitis is a major cause of economic loss in the dairy industry. Although many efforts have been made to prevent this disease, control of mastitis is still a major problem for herd management. Dairy cattle must continually undergo pregnancy, parturition and lactation. These stressful conditions, which lower host immunological states, may be a reason for the difficulty in solving this problem.

During the past decade, many studies on mouse and human cytokines have revealed that many kinds of cytokine are responsible for inflammatory disorders, activation of immune cells and clearance of pathogens. Therefore, determination of cytokine levels during the course of mastitis is thought to be useful for elucidating the pathogenesis of mastitis. Although there have been several studies on cytokines in experimental and naturally occurring mastitis of cattle [10, 11, 13, 14, 16, 17, 19, 20], it is very difficult to review the kinetics and to clarify the significance of cytokine production in the course of mastitis from these studies due to factors such as limited kinds of cytokines studied, various assay methods, and different observation periods.

Neutrophils play an important role in defence against mastitis [17]. Infiltration of neutrophils into inflammatory sites is enhanced by interleukin (IL)-1 [2]. The cells are activated by interferon (IFN)- γ [9, 18] and tumor necrosis factor (TNF)- α [1, 14]. Phagocytosis by neutrophils is also enhanced through acute phase proteins induced by IL-6 [8]. Therefore, we determined the concentrations of these cytokines and IL-1 receptor antagonist (ra), which antagonizes IL-1 activity, in sera and whey of cattle with naturally occurring mastitis from onset to cure using bovine cytokine-specific ELISA. The results for IL-1 β , IL-1ra and IL-6 have already been reported [6, 23]. In this paper, the results for IFN- γ and TNF- α are presented.

Twenty-eight mastitic and 27 healthy Holstein dairy cattle, reared in the Toh-yo district of Ehime Prefecture, Japan, were examined. At the onset of illness (day 0), the mastitic cattle showed clinical signs such as redness, hardness, swelling and pain in the udder, or the presence of clots in milk. Four cows showed anastasia and were euthanatized 2 to 7 days later due to poor prognosis. Fresh milk samples taken from the abnormal quarters of all the mastitic cattle on day 0 were examined bacteriologically. Causative bacteria isolated were *Escherichia coli* from 11 cows, *Klebsiella pneumoniae* from 16 cows and *Proteus* sp. from 1 cow. The mastitic cattle were treated with antibiotics to which the causative agent was susceptible, and all of the cows, except for the euthanatized ones, were judged to have recovered by day 14 after the onset of mastitis on the basis of disappearance of the above-mentioned clinical signs. Healthy cows were judged to be normal on the basis of their being clinically healthy, negative for pathogens in milk, and having a low individual-quarter milk somatic cell count (less than 1×10^5 cells/ml). Days after parturition of the mastitic and healthy cows varied from within a week to 36 weeks.

Serum samples were collected from the cows with acute clinical mastitis at the onset of illness (day 0) and on days 3, 7 and 14 after onset. The serum samples were stored at -30°C until use. Milk samples from the abnormal quarters of those cows were collected at the same time as the serum samples. The milk samples were centrifuged (2,500 g for 20 min at 4°C), and the cell-free aqueous whey fractions were collected as described by Goto *et al.* [5]. The whey samples were then frozen at -30°C until use. Sera and whey samples from normal cows were also obtained and frozen at -30°C as described above.

The concentrations of bovine IFN- γ and TNF- α in the serum and whey samples were determined by sandwich ELISA using affinity-purified IgGs specific for the bovine cytokines as described previously [7]. The detection limits

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of the assays were 0.2 ng/ml for IFN- γ and 0.5 ng/ml for TNF- α . The concentrations of these cytokines are presented as means (ng/ml) $\pm$ standard errors. The statistical significance of difference in cytokine concentrations between sample groups was analyzed using the Mann-Whitney U test.

IFN- γ and TNF- α concentrations of the healthy and mastitic cattle are shown in Fig. 1. In the healthy cattle, IFN- γ was almost undetectable and TNF- α was detected at low levels (less than 10 ng/ml) in serum and whey samples. In mastitic cattle, IFN- γ in sera and whey and TNF- α in whey on day 0 showed significantly higher concentrations than their respective concentrations in the healthy cattle. The concentrations of IFN- γ and TNF- α in whey reduced significantly as the cows recovered. On the other hand, IFN- γ in serum showed no significant changes. There were no significant differences between either cytokine concentrations in cows with *E. coli* mastitis or those with *K. pneumoniae* mastitis (data not shown).

Although, to our knowledge, there are no reports on IFN- γ concentration in bovine mastitis, Taylor *et al.* [21] reported enhanced expression of IFN- γ mRNA in milk cells of cows with naturally occurring mastitis. The observations of the present study support this finding at the protein level. Increased TNF- α levels in whey [11, 13, 14, 16] and presence of the relatively low (less than 10 ng/ml) concentrations of TNF- α in sera [10, 20] from experimental and natural cases of coliform or endotoxin-induced mastitis have been reported. These findings also coincide with the present observations.

The cytokine levels in the cattle that recovered and those that were euthanatized are shown in Table 1. There were no significant differences in serum and whey IFN- γ concentration between these two groups of cattle, indicating that IFN- γ is considered not to be an exacerbating factor of mastitis. Although TNF- α concentrations in sera were not different between the groups, those in whey from the euthanatized cattle on day 0 and day 3 were significantly higher than the values of the recovered cattle on the corresponding days. In

Fig. 1. IFN- γ and TNF- α concentrations in serum and whey samples from healthy and coliform mastitic cattle. Columns and the lines on columns indicate means and standard errors, respectively. Samples were taken from 27 healthy cows and from 28 mastitic cows on day 0, 26 mastitic cows on day 3, 23 mastitic cows on day 7, and 21 mastitic cows on day 14. Statistically significant differences are shown by * and ** (compared to the value in healthy cows) and by # and ## (compared to the value in whey) for $p < 0.05$ and $p < 0.01$, respectively and by ° (compared to the value on day 0) at $p < 0.01$.

our previous reports, we demonstrated significantly higher concentrations of IL-6 and significantly lower IL-1ra/IL-1 β ratios in the sera of euthanatized cows compared to those of the cattle which recovered [6, 23]. These observations may indicate a failure in suppression of IL-1-induced IL-6 production [12] by IL-1ra. Therefore, the systemic level of IL-6 may be critical in determining the prognosis of mastitic cattle. Taking this into consideration, the absence of difference in the systemic level of TNF- α between the euthanatized and recovered cows suggests that TNF- α may not be

Table 1. Cytokine concentrations (ng/ml) in sera and whey of recovered or euthanatized mastitic cattle

Cytokine	Sample	n	Recovered				Euthanatized		
			Day 0	Day 3	Day 7	Day 14	Day 0	Day 3	Day 7
			24	22	22	21	4	4	1
IFN- γ	Serum	mean	50.7	51.9	48.4	39.8	48.8	30.8	121.9
		se	14.7	15.3	13.2	9.7	26.8	16.0	
	Whey	mean	25.0	17.9 [#]	7.6 ^{##}	6.7 ^{##}	26.2	19.7	3.8
		se	5.1	5.1	1.5	1.5	11.4	8.2	
TNF- α	Serum	mean	6.1	5.7	4.7	4.7	9.8	9.1	11.1
		se	2.1	2.1	1.3	1.4	4.6	4.5	
	Whey	mean	130.5	113.1	62.4 ^{##}	31.2 ^{##}	426.5*	719.1**	1.1
		se	76.6	109.4	61.2	30.0	332.1	377.5	

n: number of cows tested, se: standard error.

* and ** significantly different from the value of the recovered cattle on corresponding day at $p < 0.05$ and $p < 0.01$, respectively.

[#] and ^{##} significantly different from the value of the day 0 at $p < 0.05$ and $p < 0.01$, respectively.

directly associated with poor prognosis of coliform mastitis, although locally produced TNF- α may cause tissue damage and induce clinical signs. Sordillo and Peel reported that increased TNF- α concentrations in sera and milk were closely associated with death during acute *E. coli* mastitis [20]. Although the reason for the discrepancy between their results and ours is not clear, one possible explanation may be a difference in the mastitic cattle examined, which were treated with antibiotics in our study, but not in theirs.

Since neutrophils play a key role in defense against mastitis [17], cytokines that activate neutrophils may contribute to the recovery from mastitis. IFN- γ functions as an activating factor of neutrophils [4, 19]. Exogenously administered IFN- γ confers protection against some bacterial infections, including bovine mastitis [9, 18]. TNF- α induces bovine neutrophil activation [1, 15]. The present results showed significant reduction of IFN- γ and TNF- α levels in whey in connection with recovery of cows from mastitis. In our previous report, IL-6 levels in whey also showed significant reduction in connection with recovery. IL-6 contributes to recruitment and activation of neutrophils in order to eradicate pathogens, through acute-phase proteins induced by IL-6 [8]. Therefore, these cytokines may play roles in the recovery from mastitis, probably in collaboration with the antibiotics used. This issue should be examined in a future study.

IFN- γ in sera did not change during the recovery process. To clarify the significance of this issue, the role of antagonistic cytokine against IFN- γ such as IL-10 [3], and IFN- γ inducing cytokines such as IL-12 and IL-18 [22] should be determined in the future.

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