**Note**

**Effects of Lactoferrin and Lactoferricin® on the Release of Interleukin 8 from Human Polymorphonuclear Leukocytes**

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A single-letter code is used to indicate the amino acid sequence of the peptides. Basic residues are underlined, and numbers indicate the sequence positions in human and bovine LF.

**Abbreviations:** bLF, bovine lactoferrin; bLCfn, bovine lactoferricin®; hTF, bovine transferrin; EIA, enzyme immunoassay; hLF, human lactoferrin; hLCfn, human lactoferricin®; hTF, human transferrin; PMNs, human polymorphonuclear leukocytes (neutrophils).
of IL-8 in the supernatant was analyzed by a two-site enzyme immunoassay (EIA) system as described previously.172 Human LF and LFcin, as well as bovine LF and LFcin, were effective for inducing IL-8 release from PMNs as shown in Table and Fig. 2. A significant difference between the control and sample groups was observed in the cases of bovine LF and LFcin at a concentration of 100 μg/ml (1.2 μM and 32 μM, respectively), as shown in Table.

LF is homologous to transferrin (TF) in structure, and both proteins have iron-binding properties.181 Therefore, we compared the effect of these proteins, the results being shown in Fig. 2. Neither human nor bovine TF were effective, although human and bovine LF were effective, suggesting that LF interacted with PMNs by a mechanism distinct from that of TF, and that the observed LF-induced stimulation of IL-8 release probably was not mediated by the stimulation of iron transport by LF. The fact that human and bovine LFcin were also effective for inducing IL-8 release, although the effective molar concentration of LFcin was much higher than that of LF, suggests that the region in the LF molecule corresponding to LFcin might have been responsible for this stimulatory effect of LF on the release of IL-8 from PMNs.

During this study, we noticed that bovine LF was more effective for inducing an increase in IL-8 release than was human LF. Similarly, bovine LFcin was more potent than human LFcin. This observation prompts us to suggest that the stimulatory effect of LF and LFcin on IL-8 release was not mediated by LF-receptors, because if PMNs have specific receptors for LF, it would be reasonable to assume that human LF and LFcin would be more effective than bovine LF and LFcin in the case of human PMNs being used. In connection with this, we have found that protamine, mainly consisting of arginine residues (about 70% of the entire amino acid sequence), was effective for inducing an increase in IL-8 release from PMNs; protamine acted as a inducer for IL-8 release below at 11 μg/ml (ca. 2 μM; Fig. 2). We assume that the non-specific binding ability of the cationic molecule was important for LF and LFcin to interact with PMNs and to trigger these biological effects. The effects demonstrated with these basic peptides, as well as those by LF, were obtained under non-cytolytic conditions, as determined by the release of lactate dehydrogenase.191

Up to now, some immunostimulatory effects of LF on PMNs influencing the inflammatory response have been described, including the up-regulation of hydroxy radical production20 and the promotion of adherence to the endothelium,21 although there is still controversy.22 Interestingly, and the up-regulation of these biological responses has been shown to involve IL-8.23,24 In addition, Willem et al. have shown that IL-8 induced LF release from PMNs.25,26 Considering these observations and our findings in this present work, it seems that both LF induced by IL-8 and IL-8 induced by LF acted to up-regulate the functions of PMNs, and that active peptide LFcin may perform the same role as that of LF.

References
Stimulation of IL-8 Release from PMNs by LF and LFcin®

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