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Human Lactoferrin Activates Transcription of IL-1β Gene in Mammalian Cells

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Lactoferrin (Lf) has been suggested to play roles in primary defense against microbial infection and other cellular processes including immunomodulation. Lf is known to bind to DNA and implicated to activate transcription. In the present study, we demonstrated that Lf stimulated transcription of IL-1β gene, one of natural genes containing putative Lf binding site (LBS) in the 5′-flanking sequences. K562 cells treated with a combination of Lf and PMA showed a synergistic induction in the level of IL-1β mRNA over treatment with PMA alone. Synergistic stimulation of IL-1β expression by Lf and PMA was also confirmed by IL-1β/Luc reporter gene assays. Analysis of Lf domains revealed that the transcriptional domain of Lf is located within the N-terminal 90 amino acids, termed NIa and that the C-terminal half lobe lacked the transactivating activity. The NIa, the N-terminal half lobe as well as intact Lf stimulated transcription of IL-1β gene in the transfected K562 cells along with PMA, while the C-terminal half lobe did not. Our results suggest that Lf may play some roles in transcription of IL-1β gene and may also regulate transcription of other natural genes containing LBS.

Key Words: **cytokine; DNA binding domain; IL-1β; lactoferrin; transactivation.**

Lactoferrin (Lf) is a non-heme iron-binding glycoprotein which is predominantly found in human milk and in other body fluids secreted from glandular epithelium cells of salivary glands, biliary tract and lacrimal gland. It is a major component of the secondary granules of polymorphonuclear leukocytes (1) and is released from neutrophils during inflammatory responses (1, 2). Lf is known to play roles in primary defense against microbial infections probably due to its ability to bind to ferric ions which are essential nutrients for bacterial growth (3, 4). Lf was shown to exert a number of immunological responses such as regulation of myelopoiesis (5), activation of NK cells (6), inhibition of granulopoiesis (7) and stimulation of lymphokine-activated killer cell activity (8). A number of investigators reported that Lf affected production and release of cytokines such as TNF-α, IL-1β, IL-8, NO, and GM-CSF (9–14).

Regulation of these processes by Lf is not understood clearly, although some of these functions are thought to be independent of its ability to bind ferric ions. Lf, after binding at the cell surface, was internalized and appeared in nucleus, where it was bound to DNA (15). Specific lactoferrin binding sites (LBS) have been identified from random human sequence and one of specific LBS, GGCACTTGC, was demonstrated to activate transcription of a reporter gene containing the sequence (16).

The amino acid sequence of Lf, like all transferrins, demonstrates a striking 2-fold internal homology, with about 40% amino acid identity between the N- and C-terminal halves (17). The three-dimensional structure of human lactoferrin has revealed that it is subdivided into two lobes, N- and C-lobes, each associated with one metal-binding site as other transferrin family (18, 19). The N- and C-terminal halves appear to have the same folding based on the similar supersecondary structure (18).

Lf is an extremely polyfunctional protein and binds to a number of different molecules. The N-terminal domain is responsible for binding of heparin, bacterial lipopolysaccharide, human lysozyme and DNA (20, 21). In the present study, we showed that Lf plays roles in transcription of IL-1β gene containing five putative Lf binding sites in the 5′-flanking sequences. Analysis of IL-1β mRNA levels by RT-PCR showed that Lf and

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phorbol myristate acetate (PMA) synergistically stimulated IL-1β gene expression. We demonstrated that the transcriptional domain of Lf was located within the N-terminal 90 amino acid region and that the C-terminal half lobe lacked the transactivating activity.

MATERIALS AND METHODS

Cell culture and transfection. Monkey kidney COS-1 and Hela cells were cultured in DMEM containing 10% fetal bovine serum, penicillin (100 U/ml) and streptomycin (100 μg/ml). Human promonocytic U937 and human myelogenous leukemia K562 cells were cultured in RPMI 1640 (Gibco BRL) containing 10% fetal bovine serum, 2 mM glutamate, penicillin (100 U/ml) and streptomycin (100 μg/ml). For COS-1 cells, transient transfections were carried out by Lipofectamine method as described by manufacturer (Gibco BRL). U937 and K562 cells were transfected by electroporation (100 μF capacitance, 400 V and 400 Ω wave controller resistance) with use of Gene Pulsar (Bio-Rad). pCH110 (Amersham) was introduced in all experiments in order to correct for transfection efficiency.

Plasmid construction. A reporter gene, L4E1b/CAT, was constructed by replacing the GAL4 binding sites of pG5E1b/CAT with a 50 bp DNA fragment containing four tandem repeats of lactoferrin binding site (GGCACTTGC). IL-1β/luciferase reporter plasmid was constructed by ligation of PCR-amplified IL-1β upstream DNA fragment (-3753 to $+550$) (22) to pGL-basic plasmid (Promega). The plasmids expressing different domains of lactoferrin were constructed by inserting PCR-amplified lactoferrin cDNA fragments into pRcCMV plasmid (Invitrogen), and the resulting plasmids were named as pLf-T, pLf-C, pLf-N, pLf-NIa, pLf-NIb, and pLf-NII. The positions of amino acid residues of Lf and its fragments are as follows: T (1–692), C (342–692), N (1–345), NIa (1–90), NIb (252–320), and NII (91–251). GAL4/Lf fusion plasmids were constructed by inserting PCR-amplified lactoferrin cDNA fragments into pSG424, and the resulting plasmids were designated as pSG-T, pSG-N, and pSG-C. pG5E1b/CAT was used as a reporter gene for assaying transcriptional activities of GAL4 fusion plasmids.

RT-PCR. Lf (Iron-saturated form, Sigma) was added to K562 cells to final concentration of 10 μg/ml at the cell density of 1×10^6 cells. Immunodepleted Lf was prepared by using rabbit anti-human Lf (Sigma) and was added to K562 cells. After 24 h of incubation, PMA (Sigma) was added at the concentration of 10 ng/ml and incubated for another 24 h. To test the effects of Lf and its fragments on transcription of IL1-β gene, K562 cells were transfected with pLf-T, pLf-C, pLf-N, and pLf-NIa as described above and the transfected cells were treated or not treated with PMA after 24 h of transfection at the concentration of 10 ng/ml. Cells were harvested to measure IL-1β mRNA levels by RT-PCR. Total RNA was extracted from the samples using High pure RNA isolation kit (Roche), and 1 μg of total RNA was reverse transcribed and amplified using specific primers for IL-1β and β-actin. RT-PCR was carried out by using Titan One tube RT-PCR kit (Roche). β-actin mRNA levels were used as internal controls.

CAT and Luciferase reporter gene assays. Transfected cells were treated with Lf and/or PMA 2 h after transfection as described above and harvested, or were harvested 48 h after transfection without any treatment for enzyme assays. The CAT assay was performed as described previously (23) and the luciferase assay by the method using enhanced luciferase assay kit (Promega). The reporter enzyme activities were normalized on the basis of β-galactosidase activities. All experiments were repeated at least five times.

In vitro transcription/translation. Lf and its fragments were *in vitro* translated with the TNT T7 quick coupled transcription/translation system (Promega). *In vitro* transcription/translation was carried out with 1 μg of pLf-T, pLf-N, or pLf-C plasmid DNA in 50 μl of reaction mixture supplemented with 50 μCi of [^{35}S]methionine (Perkin–Elmer Life Sciences) and appropriate amounts of TNT T7 Quick Master Mix (Promega) for 90 min at 30°C. The reaction products were electrophoretically separated on 15% SDS–polyacrylamide gel, which was dried and analyzed by autoradiography (24).

RESULTS AND DISCUSSION

Lf and PMA synergistically stimulate expression of IL-1β. Lf was shown to activate transcription of a CAT reporter constructs containing four tandem Lf consensus binding sites, 5′-GGCACTTGC-3′ (16). To test whether Lf activates transcription of any natural genes in cells, we searched human genes which contain the Lf consensus binding site and found that human IL-1β gene, among other candidate genes, contained five putative LBS in the 5′-flanking sequences. The effect of Lf on transcription of IL-1β gene was analyzed by measuring IL-1β mRNA levels, which was shown in Fig. 1A. K562 cells were stimulated to accumulate IL-1β mRNA with PMA (Fig. 1A) but showed little if any response to LPS treatment (data not shown) as observed in U937 cells previously (25). K562 cells treated with Lf produced only a slight increase in IL-1β mRNA level, which was detected only after longer cycles of RT-PCR. However, K562 cells stimulated with a combination of Lf and PMA showed a synergistic induction in the level of IL-1β mRNA over treatment with PMA alone. Synergistic stimulation of IL-1β expression by Lf and PMA was also confirmed by Luciferase reporter assays in the transfected K562 cells treated with a combination of Lf and PMA or treated either alone (Fig. 1B). In contrast, previous treatment of Lf with anti-human Lf abrogated the synergistic stimulation of IL-1β gene, indicating that the effect was attributed to Lf (Figs. 1A and 1B).

Our results appear to be correlated with the previous observation that Lf activated macrophages to secrete inflammatory cytokines such as TNF-α, IL-8 and NO (13). It is known that IL-1β release by macrophages not only mediates tissue damage but also activates a cascade of cytokines that include TNF-α (13). Thus, it is supposed that Lf may play roles in production of IL-1β and also other inflammatory cytokines.

However, Lf inhibits the production of GM-colony stimulating factor (GM-CSF) from monocyte-induced fibroblast (9). Lf also inhibits the release of IL-1β, IL-2 and TNF-α from mixed lymphocyte cultures (10). Therefore, the effects of Lf on cytokines production are unclear and may depend on types of cells and conditions of cell growth. Indeed, the inhibitory effect on production of GM-CSF by Lf was only observed when Lf was added before adherence of the monocyte for culture but not observed at the time of adherence or after adherence (9).

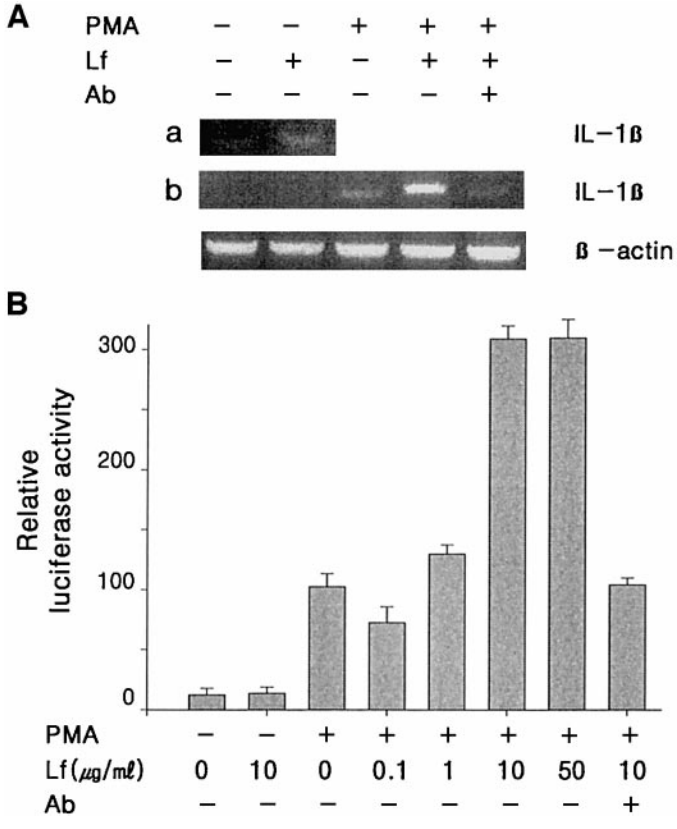

FIG. 1. Synergistic stimulation of IL-1β gene by Lf and PMA. (A) Increased IL-1β mRNA levels in K562 cells treated with Lf and PMA measured by RT-PCR. A, 45 cycles of PCR; B, 25 cycles of PCR. β-Actin mRNA levels were measured in parallel by RT-PCR as internal control (25 cycles of PCR). (B) Increased expression of IL-1β/luciferase reporter gene in transfected K562 cells by PMA and Lf. K562 cells were transfected with IL-1β/luciferase reporter plasmid and pCH110. After 2 h of transfection, Lf was added to the transfected cells and incubated for 24 h. PMA was then added at final concentration of 10 ng/ml and incubated for 24 h. Cells were harvested and assayed for luciferase activity.

The transcriptional domain is located in the N-terminal region of Lf. To identify the transcriptional domains of Lf, COS-1 cells were cotransfected with the reporter construct pL4E1b/CAT containing four tandem repeats of Lf consensus binding site and a plasmid expressing Lf or its fragments, and tested for their abilities to direct transcription of the reporter gene. The COS-1 cells transfected with plasmid expressing intact Lf exhibited positive CAT activity (Fig. 2A) as previously observed in K562 human myelogenous leukemia cells (16). Transcriptional activation was also obtained with the construct expressing the N-terminal half lobe or the N-terminal 90 amino acid region, which was compared to little transcriptional activity of the C-terminal half lobe (Fig. 2A). The result shows that transcriptional domain is localized in the N-terminal 90 amino acid region.

Cotransfection of cells with IL-1β/Luc reporter plasmid and Lf-expressing plasmid resulted in expression of the reporter gene as in the case of the minimal promoter containing the LBS (Fig. 2B), confirming that Lf was active for transcription of IL-1β gene. The NIa portion of Lf was shown to activate transcription of IL-1β as well as intact Lf (Fig. 2B). As shown in Fig. 2, the activities of each Lf domain directing IL-1β transcription were almost same as those on the minimal promoter containing four Lf binding sites. We also confirmed the transcriptional activities of Lf domains in K562 and U937 cells and obtained very similar patterns as in COS-1 cells (data not shown), suggesting that cell-specific proteins may not be required for activating transcription of a gene containing LBS by Lf.

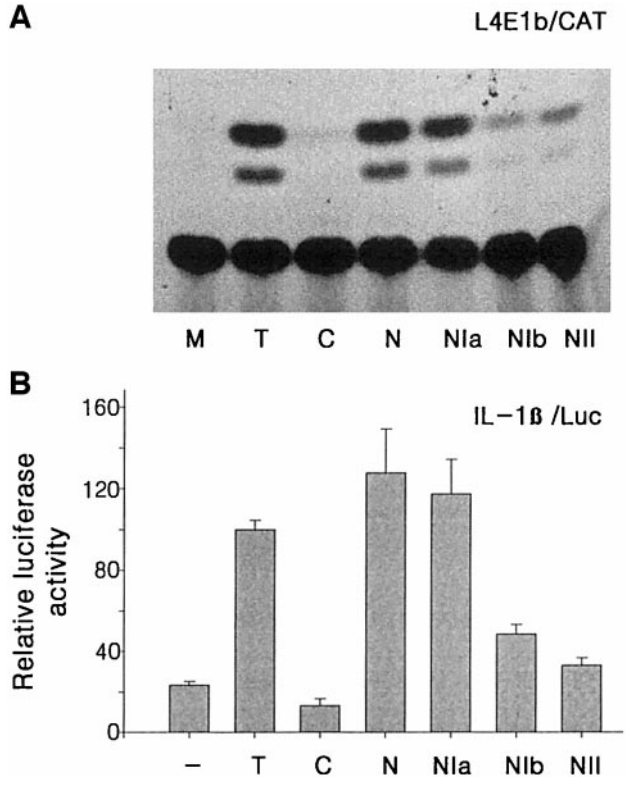

FIG. 2. Activation of reporter gene by Lf and its N-terminal fragments. (A) Transcriptional activation was determined by measuring CAT activity from reporter construct containing four tandem repeats of LBS (L4E1b/CAT) in COS-1 cells cotransfected with a plasmid expressing Lf or its fragment. (B) IL-1β/luciferase reporter construct containing five putative LBS in the 5′-flanking sequence was used for measurement of transcriptional activation in K-562 cells cotransfected with a plasmid expressing Lf and its derivatives. Lf and its different fragments expressed in transfected cells are represented as follows: T, intact Lf (1–692); C, C-lobe (342–692); N, N-lobe (1–345); Nia (1–90); Nib (252–350); NII (91–251). The numbers in parentheses indicate the positions of amino acid residues of Lf and its fragments.

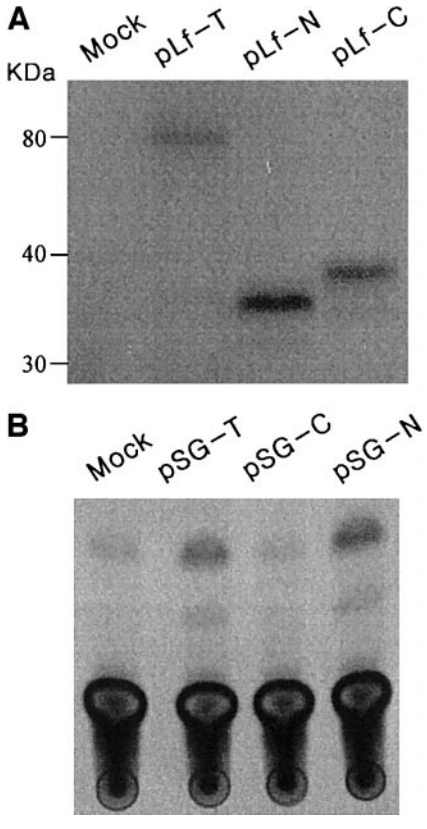

FIG. 3. *In vitro* transcription/translation of Lf and its fragments and analysis of transactivation of Lf and its fragments by GAL4/Lf fusion proteins. (A) *In vitro* transcription/translation products of mock (pRcCMV), pLf-T, pLf-N, and pLf-C. The positions of amino acid residues are indicated in the legend to Fig. 2. (B) GAL4 DNA binding domain was fused with Lf, N-lobe, and C-lobe and transfected into COS-1 cells along with pG5E1b/CAT reporter plasmid and pCH110 as an internal control plasmid. GAL4/C-lobe fusion protein fails to activate the reporter gene.

The C-terminal half lobe lacks transactivation domain. The amino acid sequence of Lf, like all transferrins, demonstrates a striking 2-fold internal homology, with about 40% amino acid identity between the N- and C-terminal halves (17). Nevertheless, the N-lobe of Lf activated transcription of L4E1b/CAT and IL-1β/Luc reporter genes, while the C-lobe of Lf did not (Figs. 2A and 2B). The C-terminal half lobe was expressed equally well as Lf and the N-terminal half lobe (Fig. 3A). It has been previously reported that Lf possesses two DNA binding sites with different affinities and that the high-affinity binding site was located in the N-terminal portion of Lf and the second binding site was located in the larger Lf fragment comprising C-terminal region (21). Analysis of transcriptional activation domains by GAL4/Lf fusion proteins in COS-1 cells revealed that Lf fragments containing the N-terminal 90 aa region contained the transactivating activity but the C-terminal half lobe did not (Fig. 3B). Thus, failure of the C-terminal half lobe to activate transcription of the reporter gene was probably due to very weak activation domain or even absence of it in the C-terminal domain. We attempted to locate the DNA binding domain and activation domain within the N-terminal 90 amino acid region by expressing Lf fragments from residue 1 to 52 and from residue 47 to 90 but failed to separate the DNA binding and activation activities clearly probably due to conformational change or instability of each fragment.

In the NIa region of human Lf, there are two basic clusters, Arg^{2}-Arg^{3}-Arg^{4}-Arg^{5} and Arg^{28}-Lys^{29}-Val^{30}-Arg^{31}. Previous reports showed that each consecutive arginine residue of the first basic cluster from residue 2 to 5 contributed to the interaction of Lf with ligands such as heparin, lipid A, human lysozyme and DNA (20). It was postulated that binding of Lf to ligands was predominantly mediated through the second basic cluster. The loop region from residue 28 to 34 amino acids was also essential for the high affinity binding of LPS (26). The region of human Lf which binds specifically to receptors on mitogen-stimulated lymphocytes is also located within the identified transcriptional domain (27).

The N-terminal 90 aa region is responsible for the synergistic stimulation of IL-1β gene expression. All of Lf and its fragments containing the N-terminal 90 aa region were able to stimulate transcription of human IL-1β gene synergistically with PMA but the C-terminal half lobe was not (Fig. 4). The IL-1β mRNA level in K562 cells transfected with pLf-C was similar to that of mock-transfected cells when treated with PMA. However, the amounts of IL-1β mRNA were increased to higher levels in the transfected K562 cells expressing the NIa, the N-terminal half lobe, or the intact Lf than those in the mock transfected cells or in the cells expressing the C-terminal half lobe when induced with PMA (Fig. 4). Our results indicated that the N-terminal half lobe and NIa, the N-terminal 90 aa

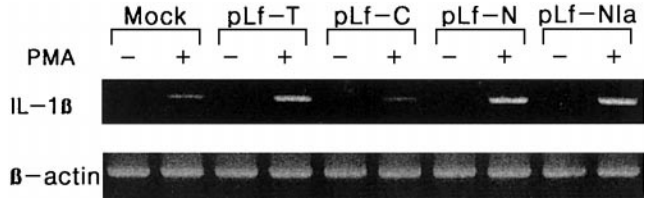

FIG. 4. Synergistic stimulation of IL-1β gene in the transfected K562 cells expressing Lf or its fragment containing the N-terminal 90 aa region. Upon treatment of the transfected K562 cells with PMA (10 ng/ml), the amounts of IL-1β mRNA were increased to higher levels in the cells transfected with pLf-T, pLf-N, and pLf-NIa than those in the cells transfected with pLf-C or in mock-transfected cells. IL-1β mRNA levels were measured by RT-PCR (25 cycles) and β-actin mRNA levels were measured in parallel as internal control (25 cycles).

region also synergistically stimulated transcription of IL-1β gene as Lf.

In human IL-1β gene, the five putative lactoferrin binding sites are located between −3202 and −3193 (GGCACTTGC), between −3137 and −3129 (GGAACTTGC), between −2384 and −2376 (GTCACGTGC), between −1357 and −1348 (GGCACTGTGC), and between −1052 and −1043 (GGAACTTGC). The LBS located between −3202 and −3193 is perfect match to the reported LBS but other sites are not. Gel mobility shift assay revealed that Lf bound to all five sites (data not shown). We do not know presently which sites are important for synergistic activation of IL-1β by Lf and PMA. A PMA-responsive enhancer is located between position −2982 and −2795 upstream of the transcription start site (28). A LPS-responsive region from −3757 to −2729 was essential for maximal LPS induction of IL-1β gene (22). In addition, NFIL-6 sites both proximal and distal to the cap site are also important for LPS-induced activation of the human IL-1β gene (22, 29). We are presently analyzing the putative Lf bindings sites to identify Lf binding sites which are important for induction of IL-1β in conjunction with other responsive elements.

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REFERENCES

1. Masson, P. L., Heremans, J. F., and Schonne, E. (1969) Lactoferrin, an iron-binding protein in neutrophilic leukocytes. *J. Exp. Med.* **130,** 643–658.
2. Slater, K., and Fletcher, J. (1987) Lactoferrin derived from neutrophiles inhibits the mixed lymphocyte reaction. *Blood* **69,** 1328–1333.
3. Arnold, R. R., Brewer, M., and Gauthier, J. J. (1980) Bactericidal activity of human lactoferrin: Sensitivity of a variety of macroorganisms. *Infect. Immun.* **28,** 893–899.
4. Brines, R. D., and Brock, J. H. (1983) The effect of trypsin and chymotrypsin on the *in vitro* antimicrobial and iron-binding properties of lactoferrin in human milk and bovine colostrums. Unusual resistance of human lactoferrin to proteolytic digestion. *Biochim. Biophys. Acta* **759,** 655–672.
5. Rado, T. A., Wei, X., and Benz, E. J. (1987) Isolation of lactoferrin cDNA from a human myeloid library and expression of mRNA during normal and leukemic myelopoiesis. *Blood* **70,** 989–993.
6. Nishiya, K., and Horwitz, P. A. (1982) Contrasting effects of lactoferrin on human lymphocyte and monocyte natural killer cell activity and antibody-dependent cell-mediated cytotoxicity. *J. Immunol.* **129,** 2519–2523.
7. Bagby, G. C., McCall, E., and Layman, D. L. (1983) Regulation of colony-stimulating activity production: Interaction of fibroblasts, mononuclear phagocytes, and lactoferrin. *J. Clin. Invest.* **71,** 340–344.
8. Shau, H., Kim, A., and Golub, S. H. (1992) Modulation of natural killer and lymphokine-activated killer cell cytotoxicity by lactoferrin. *J. Leukocyte Biol.* **51,** 343–349.
9. Zucali, J. R., Broxmeyer, H. E., Levy, D., and Morse, C. (1989) Lactoferrin decreases monocyte-induced fibroblast production of myeloid colony-stimulating activity by suppressing monocyte release of interleukin-1. *Blood* **74,** 1531–1536.
10. Crouch, S. P. M., Slater, K. J., and Fletcher, J. (1992) Regulation of cytokine release from mononuclear cells by the iron-binding protein lactoferrin. *Blood* **80,** 235–240.
11. Penco, S., Pastorino, S., Bianchi-Scarra. G., and Garre, C. (1995) Lactoferrin down-modulates the activity of the granulocyte macrophage colony-stimulating factor promoter in Interleukin-1β-stimulated cells. *J. Biol. Chem.* **270,** 12263–12268.
12. Shinoda, I., Takase, M., Fukuwatari, Y., Shimamura, S., Koller, M., and Konig, W. (1996) Effects of lactoferrin and lactoferricin on the release of interleukin 8 from human polymorphonuclear leukocytes. *Biosci. Biotechnol. Biochem.* **60,** 521–523.
13. Sorimachi, K., Akimoto, K., Hattori, Y., Ieiri, T., and Niwa, A. (1997) Activation of macrophages by lactoferrin: Secretion of TNF-alpha, IL-8 and NO. *Biochem. Mol. Biol. Int.* **43,** 79–87.
14. Choe, Y., and Lee, S. (1999) Effect of lactoferrin on the production of tumor necrosis factor-α and nitric oxide. *J. Cell. Biochem.* **76,** 30–36.
15. Garre, C., Bianchi-Scarra, G., Sirito, M., Musso, M., and Ravazzolo, R. (1992) Lactoferrin binding sites and nuclear localization in K562(S) cells. *J. Cell. Physiol.* **153,** 477–482.
16. He, J., and Furmanski, P. (1995) Sequence specificity and transcriptional activation in the binding of lactoferrin to DNA. *Nature* **373,** 721–724.
17. Metz-Boutigue, M. H., Jolles, J., Mazurier, J., Schoentgen, F., Legrand, D., Spik, G., Montreuil, J., and Jolles, P. (1984) Human lactotransferrin: Amino acid sequence and structural comparisons with other transferrins. *Eur. J. Biochem.* **145,** 659–676.
18. Anderson, B. F., Baker, H. M., Dodson, E. J., Norris, G. E., Rumball, S. V., Waters, J. M., and Baker, E. N. (1987) Structure of human lactoferrin at 3.2Å resolution. *Proc. Natl. Acad. Sci. USA* **84,** 1769–1773.
19. Anderson, B. F., Baker, H. M., Norris, G. E., Rice, D. W., and Baker, E. N. (1989) Structure of human lactoferrin: Crystallographic structure-analysis and refinement at 2.8Å resolution *J. Mol. Biol.* **209,** 711–734.
20. Van Berkel, P. H. C., Geerts, M. E. J., van Veen, H. A., Mericskay, M., de Boer, H. A., and Nujens, J. H. (1997) N-terminal stretch Arg2, Arg3, Arg4 and Arg5 of human lactoferrin is essential for binding to heparin, bacterial lipopolysaccharide, human lysozyme and DNA. *Biochem. J.* **328,** 145–151.
21. Kanyshkova, T. G., Semenov, D. V., Buneva, V. N., and Nevinsky, G. A. (1999) Human milk lactoferrin binds two DNA molecules with different affinities. *FEBS Lett.* **451,** 235–237.
22. Shirakawa, F., Saito, K., Bonagura, C. A., Galson, E. L., Fenton, M. J., Webb, A. C., and Auron, P. E. (1993) The human prointerleukin 1 beta gene requires DNA sequences both proximal and distal to the transcription start site for tissue-specific induction. *Mol. Cell. Biol.* **13,** 1332–1344.
23. Gorman, C. M., Moffat, L. F., and Howard, B. H. (1982) Recombinant genomes which express chloramphenicol acetyltransferase in mammalian cells. *Mol. Cell. Biol.* **2,** 1044–1051.
24. Lee, B.-C., Kim, H., Hwang, Y.-I., and Lee, S.-C. (2001) The *in vitro* translocation of *Escherichia coli* ribose-binding protein via various targeting routes. *J. Biochem. Mol. Biol.* **34,** 118–122.
25. Knudsen, P. J., Dinarello, C. A., and Strom, T. B. (1986) Pros-

taglandins posttranscriptionally inhibit monocyte expression of interleukin 1 activity by increasing intracellular cyclic adenosine monophosphate. *J. Immunol.* **137,** 3189–3194.

26. Elass-Rochard, E., Roseanu, A., Legrand, D., Trif, M., Salmon, V., Motas, C., Montreuil, J., and Spik, G. (1995) Lactoferrin–lipopolysaccharide interaction: Involvement of the 28–34 loop region of human lactoferrin in the high-affinity binding to *Escherichia coli* 055B5 lipopolysaccharide. *Biochem. J.* **312,** 839–845.
27. Rochard, E., Legrand, D., Mazurier, J., Montreuil, J., and Spik, G. (1989) The N-terminal domain I of human lactotransferrin binds specifically to phytohemagglutinin-stimulated peripheral blood human lymphocyte receptors. *FEBS Lett.* **255,** 201–204.
28. Bensi, G., Raugei, G., Bounamassa, D., Rossini, M., and Melli, M. (1990) An inducible enhancer controls the expression of the human interleukin 1βa gene. *Cell Growth Differ.* **1,** 491–497.
29. Zhang, Y., and Rom, W. M. (1993) Regulation of the interleukin-1β gene by mycobacterial components and lipopolysaccharide is mediated by two nuclear factor-IL6 motifs. *Mol. Cell. Biol.* **13,** 3831–3837.