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Review

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Effects of lactoferrin on elicitation of the antigen-specific cellular and humoral cutaneous response in mice*

Wpływ laktoferryny na fazę efektorową komórkowej i humoralnej skórnej odpowiedzi immunologicznej u myszy

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Summary

Immune contact dermatitis is an inflammation of the skin resulting from exposure to allergens in the environment. The aim of this study was to compare the actions of lactoferrin (LF), a natural immunomodulator, on the elicitation phases of the cellular and humoral, cutaneous immune responses to oxazolone and toluene diisocyanate (TDI), respectively. LF was given i.v. in a 10 mg/mouse dose, together with the eliciting doses of the antigens. The ear edema and the number of lymphocytes in the draining lymph nodes were measured. In addition, the production of IL-2 in the cultures of lymph node cells and the content of IL-4 in lymph node cells were determined. LF had a profound inhibitory effect on the eliciting phase of the immune response to oxazolone as measured by the ear edema and lymph node cell number. The suppressive effect of LF on the effector phase of the immune response to TDI was moderate. LF had some stimulatory effect on the *ex vivo* content of IL-4 in lymphocytes in the immune response to TDI. On the other hand, it significantly inhibited IL-2 *in vitro* production in the immune response to oxazolone. The data strongly suggest that LF exerted differential actions on the activities of antigen-specific Th1 and Th2 cells involved in respective types of the cutaneous immune responses.

Key words: **contact dermatitis • oxazolone • toluene diisocyanate • lactoferrin • mice**

Streszczenie

Kontaktowe zapalenie skóry to stan zapalny w obrębie skóry rozwijający się po kontakcie z alergenem środowiskowym. Celem tego opracowania było porównanie działania laktoferryny (LF), naturalnego białka o działaniu immunomodulującym, na fazę efektorową skórnej odpowiedzi immunologicznej typu komórkowego i humoralnego, wywołanej odpowiednio przez oksazolon i dwuizocyjanian toluenu (TDI). LF podawano dożylnie w dawce 10 mg/mysz, jednocześnie z dawką wywołującą antygenów. Wielkość reakcji oceniano mierząc obrzęk uszu, liczbę limfocytów w drenujących węzłach chłonnych oraz wytwarzanie IL-2 i IL-4 przez te komórki. Laktoferryna wyraźnie hamowała efektorową fazę odpowiedzi komórkowej na oksazolon, zmniejszając obrzęk uszu i liczbę komórek w węzłach chłonnych. Słabiej działała natomiast na

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efektorową fazę odpowiedzi humoralnej na TDI. W testach *ex vivo* LF zwiększała wytwarzanie IL-4 w komórkach węzłów chłonnych podczas odpowiedzi na TDI, hamowała natomiast wytwarzanie IL-2 w hodowlach limfocytów w odpowiedzi na oksazolon. Uzyskane wyniki wyraźnie sugerują, że białko działa w zróżnicowany sposób na swoiste antygenowo limfocyty Th1 i Th2 zaangażowane w odpowiednie typy skórnych reakcji immunologicznych.

Słowa kluczowe: **kontaktowe zapalenie skóry • oksazolon • dwuizocyjanian toluenu • laktoferryna • myszy**

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Abbreviations: **LF** – lactoferrin; **TDI** – toluene diisocyanate; **PBS** – phosphate buffered saline; **i.v.** – intravenously; **Th1** – T helper type 1 cells; **Th2** – T helper type 2 cells; **MIF** –macrophage migration inhibitory factor; **BG** – background

Introduction

The incidence of allergic contact dermatitis is increasing due to the increasing number of irritants and allergens present in the environment. Non-immune contact reactions to household and industrial chemicals affect the majority of the population [6]. Immune allergic contact dermatitis, affecting susceptible individuals, is a complex skin disorder dependent on several types of immunocompetent cells, where both cellular and humoral components may play a role [12]. Therapeutic approaches to limit symptoms of skin allergy encompass use of topical corticosteroids and calcineurin inhibitors, which are not devoid of side-effects [9]. New experimental, therapeutic strategies propose application of sublingual immunotherapy, intervention in prostanoid metabolism, probiotics, leflunomide, and other treatments (reviewed by [17]). In addition, such new categories of therapeutics as biologics are also considered [19].

Lactoferrin belongs to evolutionarily old proteins, involved in iron metabolism and contained in excretory fluids and secondary granules of neutrophils of mammals [22]. The protein exhibits a broad spectrum of biological activities [1] and is involved in host defense against pathogens [21]. Effects of LF on the immune responses mediated by Th1 and Th2 cells are different in physiological and pathological states [11]. The majority of reports describe stimulation of the cellular immune response by LF where LF acts as an adjuvant [2,18,20,31]. A Th1-mediated response was also achieved in LF-transgenic mice [14]. The stimulation of the immune response by LF may involve maturation of T [33] and B cells [34] and action on antigen-presenting cells and antigen-specific T cells by increasing MHC (major histocompatibility complex) class II antigen expression and relevant cytokine production [18]. The immunostimulatory properties of LF could be best demonstrated in immunocompromised mice [3,4,5]. On the other hand, application of LF in sensitized mice inhibited expression of the cellular immune response [13,32].

Since information on the effects of LF on the eliciting phase of the cellular immune response is scanty [13,32], and regarding the humoral immune response is not available, the aim of this study was to compare the actions of LF on the eliciting phase of both types of the immune response. For this purpose we selected models of cutaneous immune response to oxazolone [23] and toluene diisocyanate [27] since the first compound selectively develops the delayed type hypersensitivity and the second one the humoral immune response. The advantage of using the cutaneous responses to these antigens is that the same parameters, such as ear edema and cell numbers in the draining lymph nodes, could be measured and compared in parallel at elicitation of both cellular and humoral immune responses. A high, intravenous dose of LF was chosen, shown previously to induce high endogenous corticosterone levels [30], thus potentially capable of inhibiting the immune response [32].

Materials and Methods

Mice

BALB/c female mice, 8–10 weeks old, delivered by the Institute of Laboratory Medicine, Łódź, Poland, were used for the study. The mice were fed commercial food and water *ad libitum*. The local ethics committee approved the study.

Reagents

Oxazolone (4-ethoxymethylene-2-phenyl-2-oxazolin-5-one), toluene diisocyanate (TDI), acetone, trypan blue, and Triton X-100 were from Sigma-Aldrich. Low endotoxin bovine lactoferrin (0.16 E.U./mg, <25% iron saturated) was obtained from Morinaga Milk Industry Co., Japan. FCS (fetal calf serum) was from Gibco, rIL-2 from R&D Systems, ELISA kit for determination of IL-4 activity from eBioscience, ELISA kit for measurement of serum corticosterone from Assay Designers, USA. LF was dissolved in

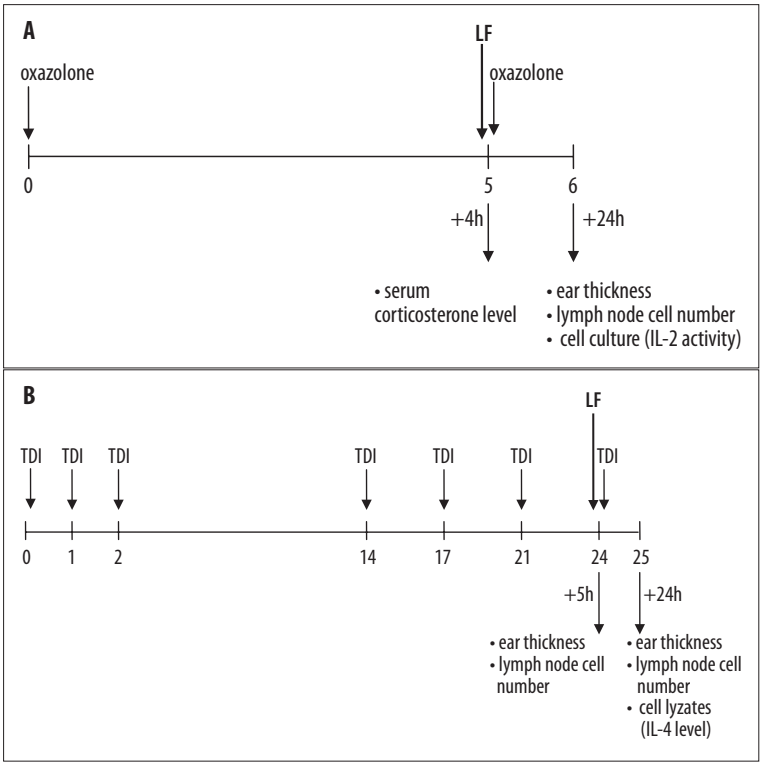

Fig. 1. Experimental design for the performance of the cellular (oxazolone) (**A**) and humoral (toluene diisocyanate) (**B**) immune response and application of LF

phosphate-buffered saline (PBS) and filtered through 0.2 μm Millipore filters before injection in mice.

Contact sensitivity to oxazolone

The test was performed according to Noonan and Halliday [23] with some modifications. Mice were shaved on the abdomen (2×2 cm area) and after 24 h 100 μl of 0.5% oxazolone in acetone was applied. The contact sensitivity reaction was elicited 5 days later by application of 25 μl of 1% oxazolone in acetone on both sides of the ears. The ear edema was measured 24 h later using a spring caliper (Mitutoyo, Japan). For the experiments 5 mice were used per group and the reaction was elicited on both ears (10 determinations per group). The results are presented as the ear thickness, expressed in millimeters. Mice treated only with the eliciting dose of antigen served as a background (BG) group. Mice challenged with 0.5% oxazolone on the abdomen and 1% oxazolone on both sides of the ears are referred to below as the control group (full blown, not manipulated inflammation). 24 h after the eliciting dose of oxazolone, also the draining lymph nodes were isolated and the total number of lymphocytes and the activity of IL-2 in cultures of lymph node cells were determined.

Immune response to toluene diisocyanate (TDI)

The test was performed according to Yamamoto [27] with minor modifications. Mice were shaved on the abdomen and after 24 h 50 μl of 3% TDI in acetone was applied for 3 consecutive days. After 14 days the reaction was elicited by application of 25 μl of 0.3% TDI on both sides of the ears. The procedure was repeated four times every 3 days. 5 h and 24 h after the last challenge with TDI, the ear thickness was measured using a spring caliper. For the experiments 5 mice were used per group. The reaction was elicited on both ears (10 determinations per group). The results are presented as the ear thickness, expressed in millimeters. Mice treated only with the eliciting dose of antigen served as a background (BG) group. Mice sensitized 3% TDI on the abdomen and challenged with 0.3% TDI on both sides of the ears are referred to below as the control group (full blown, not manipulated inflammation). 5 h and 24 h after the eliciting dose of TDI, the draining lymph nodes were also isolated and the total number of lymphocytes was determined. 24 h after the eliciting dose of TDI, the cells from the lymph nodes was prepared and the activity of IL-4 was determined.

Administration of lactoferrin

Lactoferrin (LF) was administered intravenously into the retroorbital plexus, in a dose of 10 mg/mouse, 1 h before the eliciting dose of oxazolone or 1 h before the fourth eliciting dose of TDI. These mice are referred to in the figures and figure legends as the "LF" group. For detailed protocols of the experiments, see the schemes (Figure 1AB).

Determination of lymph node cell numbers

Superficial parotid, mandibular and accessory mandibular lymph nodes were isolated, homogenized by pressing against a stainless screen into PBS, washed and re-suspended in PBS containing 0.2% trypan blue. The total cell numbers were determined using a light microscope and Bürker's hemocytometer. The results are presented as the mean number of lymphocytes from 10 determinations (5 mice) ±SE.

Determination of serum corticosterone levels

10 mg of LF was administered i.v. 1 h before the eliciting dose of antigen (oxazolone). Four hours later, the mice were bled and the sera separated to determine corticosterone

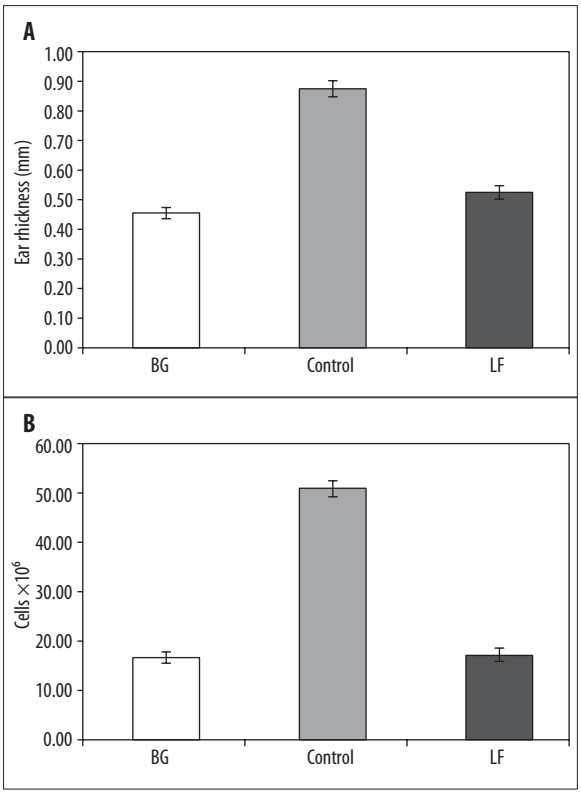

Fig. 2. Effects of LF on the elicitation phase of the contact sensitivity to oxazolone: the ear thickness (**A**) and the total number of cells in the draining lymph nodes (**B**). 10 mg of LF was administered *i.v.* 1 h before elicitation of the response to oxazolone. (**A**) 24 h after the challenge with the eliciting dose of oxazolone the thickness of both auricles was measured. The results are presented as the mean of the ear thickness, expressed in millimeters, from 10 determinations (5 mice) ±SE. Statistics: BG *vs* Control P=0.0001; Control *vs* LF P=0.0001 (ANOVA); (**B**) 24 h after the challenge with the eliciting dose of oxazolone the draining lymph nodes from each side were isolated and the number of cells in the draining lymph nodes was measured. The results are presented as the mean number of lymphocytes from 10 determinations (5 mice) ±SE. Statistics: BG *vs* Control P=0.0001; Control *vs* LF P=0.0001 (ANOVA of Kruskal-Wallis)

content. The levels of corticosterone were determined using immunoenzymatic assay according to the manufacturer's instructions. The results are shown in nanograms/ml of corticosterone as mean values from 5 mice ±SE.

Determination of IL-2 activity in the cultures of lymph node cells

Superficial parotid, mandibular and accessory mandibular lymph nodes were isolated 24 h after the eliciting dose of antigen (oxazolone). The organs were homogenized by pressing against a stainless screen into culture medium (RPMI 1640, supplemented with 10% fetal calf serum, L-glutamine, sodium pyruvate, 2-mercaptoethanol and antibiotics) at a density of 5×10^6 cells/ml/well in a 48-well culture plate. After 48 h incubation in a cell culture incubator, the supernatants were harvested. IL-2 activity was determined using the indicator cell line CTLL-2 [26]. The cell proliferation was determined using the MTT colorimetric method [15]. The results are presented as mean optical density (OD) values from 5 mice ±SE.

Determination of *ex vivo* IL-4 content in the lymph node cells

Superficial parotid, mandibular and accessory mandibular lymph nodes were isolated 24 h after the fourth eliciting dose of antigen (TDI). Lymph nodes were homogenized by pressing the organs against a steel mesh in fresh RPMI 1640 solution. Cells were counted and re-suspended in RPMI 1640 (4×10^6 cells/ml). 250 µl (1×10^6 cells) of samples was taken and 50 µl of Triton X-100 was added to lyze the cells. The samples were centrifuged (10000×g, 4°C), the pellets were discarded and the supernatants were frozen at –20°C. The activity of IL-4 in the supernatants was determined using IL-4 immunoenzymatic assay according to the manufacturer's instructions. The results are shown in picograms/ml of IL-4 as mean values from 5 mice ±SE.

Statistical analysis

The results of one representative experiment from three independent experiments are presented. The results are presented as mean values ± standard error (SE). Brown-Forsyth's test was used to determine the homogeneity of variance between groups. When the variance was homogeneous, analysis of variance (one-way ANOVA) was applied, followed by *post-hoc* comparisons with Tukey's test to estimate the significance of the differences between groups. Nonparametric data were evaluated with Kruskal-Wallis' analysis of variance, as indicated in the text. Significance was determined at $P<0.05$. Statistical analysis was performed using STATISTICA 7 for Windows.

Results

Effects of lactoferrin on contact sensitivity to oxazolone

The experiments were performed as shown in Figure 1A (scheme). The administration of LF before elicitation of the response had a significant, inhibitory effect on the contact sensitivity to oxazolone as measured by the ear edema (40.0% inhibition *versus* control) (Figure 2A) and the lymphocyte count in the draining lymph nodes (67.6% inhibition *versus* control) (Figure 2B).

The measurement of IL-2 activity in cultures of lymph node cells, derived from mice 24 h following administration of the eliciting dose of oxazolone, showed that LF significantly lowered (27.2% inhibition) IL-2 production in these cells (Figure 3).

Effect of lactoferrin on serum corticosterone level in mice sensitized to oxazolone

In sensitized mice given the eliciting dose of antigen (oxazolone), the serum corticosterone levels rose four-fold on the next day (Figure 4). The pretreatment with LF did not change the corticosterone levels.

Effects of lactoferrin on the humoral immune response to toluene diisocyanate (TDI)

The experiments were conducted as presented in Figure 1B. As shown in Figure 5A, lactoferrin, administered 1 h before

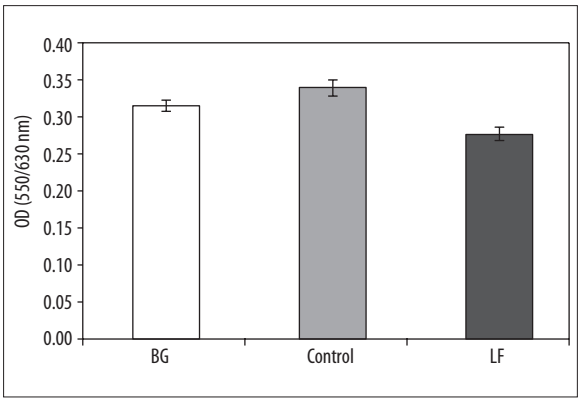

Fig. 3. Effect of LF administration on *in vitro* IL-2 production by draining lymph node cell cultures. Mice were treated with LF before the eliciting dose of antigen (oxazolone). 24 h later the draining lymph nodes were isolated, and cell suspensions were prepared and cultured for 48 h. IL-2 activity was determined by a bioassay using the indicator cell line CTLL-2. The results are presented as mean optical density (OD) values from 5 mice ±SE. Statistics: BG *vs* Control NS (P=0.2732); Control *vs* LF P=0.0001 (ANOVA)

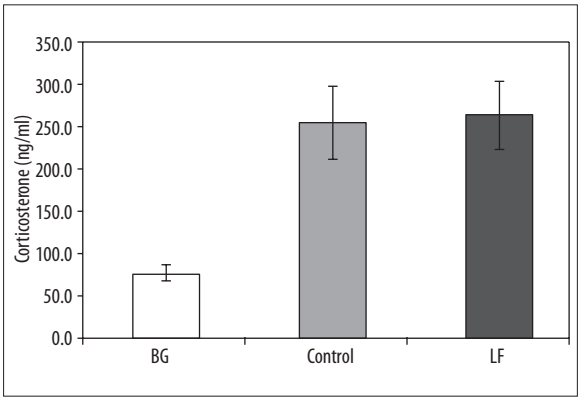

Fig. 4. Effect of LF administration on serum corticosterone levels. Mice were treated with 10 mg of LF before the eliciting dose of antigen (oxazolone). Four hours later the mice were bled and the sera separated to determine the corticosterone levels. The results are shown in nanograms/ml of corticosterone as mean values from 5 mice ±SE. Statistics: BG *vs* Control NS (P=0.0568); Control *vs* LF NS (P=0.9593) (ANOVA)

the fourth eliciting dose of antigen, had a small effect (3.9% inhibition) on the humoral immune response to TDI, measured as the ear thickness 5 h after the eliciting dose of TDI. In addition, LF had no effect on the cutaneous response to TDI when the numbers of cells in the draining lymph nodes were measured 5 h after the last dose of antigen (Figure 5B).

In addition, we did not register any effect of LF on 5 h measurement of ear edema when the protein was divided into four 2.5 mg doses and applied before eliciting doses of antigen (data not shown).

The action of LF on the TDI response measured 24 h following the last administration of antigen was moderately inhibitory when the ear edema was measured (7.5% inhibition *versus* control) (Figure 6A). On the other hand, the action of LF was more inhibitory when lymph node cell numbers were determined (30.2% inhibition) (Figure 6B).

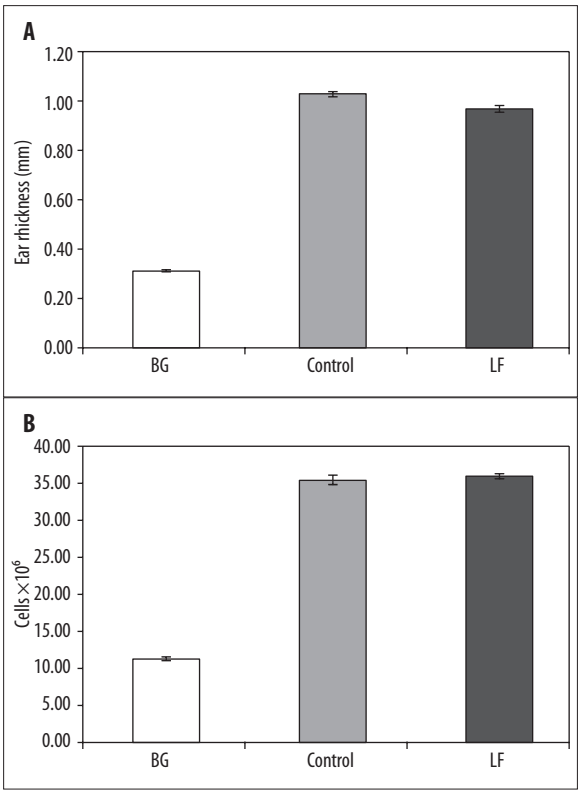

Fig. 5. Effect of LF on the elicitation phase of the humoral immune response to TDI (measurements after 5 h): the ear thickness (**A**) and the total number of cells in the draining lymph nodes (**B**). 10 mg of LF was administered 1 h before the last (fourth) eliciting dose of antigen. (**A**) 5 h after the last eliciting dose of antigen, the ear edema of both auricles was measured using a spring caliper. The results are presented as the mean of the ear thickness, expressed in millimeters, from 10 determinations (5 mice) ±SE. Statistics: BG *vs* Control P=0.0001; Control *vs* LF NS (P=1.0000) (ANOVA of Kruskal-Wallis); (**B**) 5 h after the last eliciting dose of antigen, the draining lymph nodes for each ear were isolated and the total cell numbers in the organs were determined. The results are presented as the mean value from 10 determinations (5 mice) ±SE. Statistics: BG *vs* Control P=0.0001; Control *vs* LF NS (P=1.0000) (ANOVA of Kruskal-Wallis)

The measurement of IL-4 concentration in lymph node cells derived from mice at 24 h after administration of the last dose of TDI, and lyzed with Triton X-100, showed that LF, given with the last eliciting dose of TDI, increased that parameter (Figure 7).

Discussion

The application of the cutaneous models of the cellular and humoral immune responses, where the manifestation of the response upon application of the eliciting dose of antigen can be analyzed using the same parameters, enabled us to compare in parallel the effects of LF on the effector phases of the respective types of the immune response. In both cases the inflammatory antigen-specific responses are mediated by antigen-specific T helper cells [8,16,24,25], although in the humoral immune response the antibodies also play a role in the early phase of the inflammatory reaction [8].

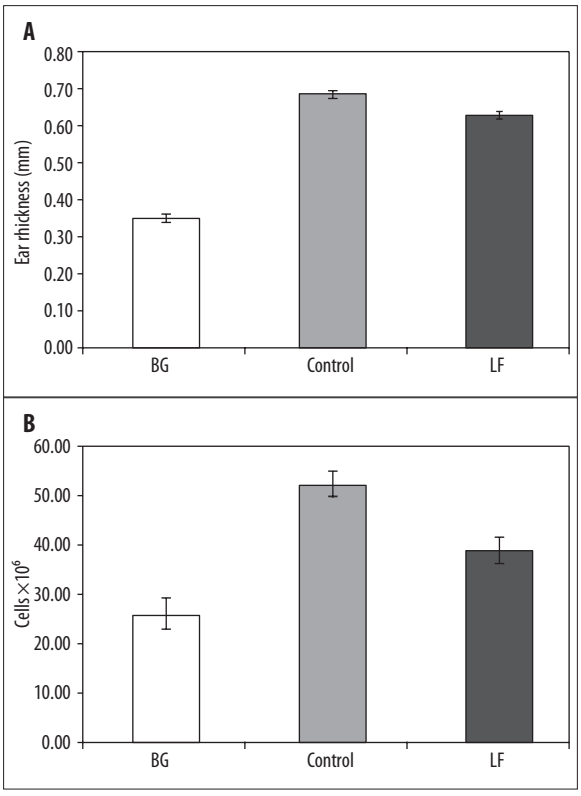

Fig. 6. Effect of LF on the elicitation phase of the humoral immune response to TDI (measurements after 24 h): the ear thickness (**A**) and the total number of cells in the draining lymph nodes (**B**). 10 mg of LF was administered 1 h before the last (fourth) eliciting dose of antigen. (**A**) 24 h after the last eliciting dose of antigen, the ear edema of both auricles was measured using a spring caliper. The results are presented as the mean of the ear thickness, expressed in millimeters, from 10 determinations (5 mice) ±SE. Statistics: BG *vs* Control P=0.0001; Control *vs* LF P=0.0088 (ANOVA); (**B**) 24 h after the last eliciting dose of antigen, the draining lymph nodes were isolated and the total numbers of cells in the organs were determined. The results are presented as the mean value from 10 determinations (5 mice) ±SE. Statistics: BG *vs* Control P=0.0034; Control *vs* LF NS (P=0.0720) (ANOVA)

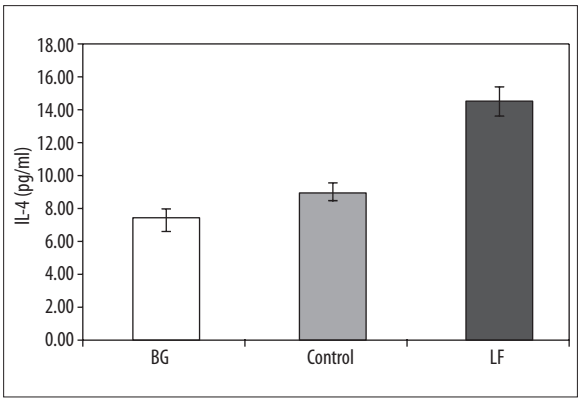

Fig. 7. Effect of LF on *ex vivo* IL-4 production by draining lymph node cell cultures. Mice were treated with LF before the last eliciting dose of antigen (TDI). 24 h later the draining lymph nodes were isolated and cell suspension prepared. The levels of IL-4 were determined by ELISA. The results are shown in picograms/ml of IL-4 as mean values from 5 mice ±SE. Statistics: BG *vs* Control NS (P=0.8681); Control *vs* LF P=0.0426 (ANOVA)

The significant inhibition of the effector phase of the cellular immune response to oxazolone by LF (Figure 2AB) could be explained by the ability of LF to induce endogenous release of corticosteroids [30], which are known to preferentially inhibit Th1 type-mediated immune responses [10]. That phenomenon was also found in our studies on the effect of immobilization stress [29] and in the immune response to an intracellular pathogen [28]. However, such a mechanism of suppression is unlikely or may have only marginal significance since corticosterone levels in mice subjected to the eliciting dose of oxazolone and injected with LF had only slightly elevated corticosterone serum concentration (Figure 4). Rather, a second mechanism of the differential action of LF on the effector phases of the cellular and humoral immune responses was relevant, since LF was found to suppress proliferation and cytokine production of antigen-specific Th1 but not Th2 cell lines in vitro, and this phenomenon was associated with a decrease of IL-2 receptors but not IL-4 receptors on these cells [35]. Therefore, it seems plausible that in the presented models LF suppressed the cellular immune response by decreasing recruitment and activity of Th1 cells. Such an assumption was supported by the decreased IL-2 production by the lymph node cells derived from mice treated with LF before elicitation of the response to oxazolone (Figure 3). Consequently, that could result in significant inhibition of Th1 cell mobilization to the lymph nodes adjacent to the application site of the eliciting dose of antigen.

The inhibitory effect of LF on the humoral immune response to TDI was differential, i.e. in the early phase of the reaction (measurements after 5 h) a small, non-significant suppression of the measured ear edema was observed (3.9% inhibition) (Figure 5A) with no effect on the number of lymphocytes in the draining lymph nodes (Figure 5B). In the second, delayed phase of the allergic reaction to TDI (measurements after 24 h), small inhibition of the ear edema (7.5%) and moderate inhibition of lymph node cell numbers (30.2%) were observed (Figure 6AB). In fact, the cutaneous, antibody-dependent inflammatory response to fluorescein isothiocyanate (FITC) was described as biphasic: the early phase is IgE-mediated and the delayed component is mediated by Th2-type cells [8]. Although the delayed phase of the humoral cutaneous reaction (measurements after 24 h) was slightly inhibited (7.5%), the activity of Th2-type cells, as expressed by IL-4 production, was even increased by LF (Figure 7). These findings were consistent with our previous report indicating lack of suppressive action of LF on an antigen-specific Th2 cell line [35]. It seems, therefore, that the decrease in the cell number of the adjacent lymph nodes could be due rather to inhibition of cell-to-cell communication and/or cell migration by LF. In fact, lactoferrin was shown to regulate LFA-1 on lymphocytes [36] and to suppress production of MIF [32], a cytokine crucial for the elicitation phase of a Th2-type allergic response [7].

In summary, we demonstrated that lactoferrin differentially affected the eliciting phases of the humoral and cellular immune responses in the cutaneous mouse models, and that preferential inhibition of the cellular immune response could be due to directional suppression of Th1 cells.

References

[1] Actor J.K., Hwang S.A., Kruzel M.L.: Lactoferrin as a natural immune modulator. Curr. Pharm. Des., 2009; 15: 1956–1973

[2] Actor J.K., Hwang S.A., Olsen M., Zimecki M., Hunter R.Jr., Kruzel M.L.: Lactoferrin immunomodulation of DTH response in mice. Int. Immunopharmacol., 2002; 2: 475–486

[3] Artym J., Zimecki M., Kruzel M.L.: Effect of lactoferrin on the methotrexate-induced suppression of the cellular and humoral immune response in mice. Anticancer Res., 2004; 24: 3831–3836

[4] Artym J., Zimecki M., Kruzel M.L.: Reconstitution of the cellular immune response by lactoferrin in cyclophosphamide-treated mice is correlated with renewal of T cell compartment. Immunobiology, 2003; 207: 197–205

[5] Artym J., Zimecki M., Paprocka M., Kruzel M.L.: Orally administered lactoferrin restores humoral immune response in immunocompromised mice. Immunol. Lett., 2003; 89: 9–15

[6] Basketter D.A., Wilhelm K.P.: Studies on non-immune immediate contact reactions in an unselected population. Contact Dermatitis, 1996; 35: 237–240

[7] Das R., Moss J.E., Robinson E., Roberts S., Levy R., Mizue Y., Leng L., McDonald C., Tigelaar R.E., Herrick C.A., Bucala R.: Role of macrophage migration inhibitory factor in the Th2 immune response to epicutaneous sensitization. J. Clin. Immunol., 2011; 31: 666–680

[8] Dearman R.J., Kimber I.: Role of CD4+ T helper 2-type cells in cutaneous inflammatory responses induced by fluorescein isothiocyanate. Immunology, 2000; 101: 442–451

[9] Draelos Z.D.: Use of topical corticosteroids and topical calcineurin inhibitors for the treatment of atopic dermatitis in thin and sensitive skin areas. Curr. Med. Res. Opin., 2008; 24: 985–994

[10] Elenkov I.J.: Glucocorticoids and the Th1/Th2 balance. Ann. N Y Acad. Sci., 2004; 1024: 138–146

[11] Fischer R., Debbabi H., Dubarry M., Boyaka P., Tomé D.: Regulation of physiological and pathological Th1 and Th2 responses by lactoferrin. Biochem. Cell Biol., 2006; 84: 303–311

[12] Gober M.D., Gaspari A.A.: Allergic contact dermatitis. Curr. Dir. Autoimmun., 2008; 10: 1–26

[13] Griffiths C.E., Cumberbatch M., Tucker S.C., Dearman R.J., Andrew S., Headon D.R., Kimber I.: Exogenous topical lactoferrin inhibits allergen-induced Langerhans cell migration and cutaneous inflammation in humans. Br. J. Dermatol., 2001; 144: 715–725

[14] Guillén C., McInnes I.B., Vaughan D.M., Kommajosyula S., Van Berkel P.H., Leung B.P., Aguila A., Brock J.H.: Enhanced Th1 response to Staphylococcus aureus infection in human lactoferrin-transgenic mice. J. Immunol., 2002; 168: 3950–3957

[15] Hansen M.B., Nielsen S.E., Berg K.: Re-examination and further development of a precise and rapid dye method for measuring cell growth/cell kill. J. Immunol. Methods, 1989; 119: 203–210

[16] Herrick C.A., Xu L., McKenzie A.N., Tigelaar R.E., Bottomly K.: IL-13 is necessary, not simply sufficient, for epicutaneously induced Th2 responses to soluble protein antigen. J. Immunol., 2003; 170: 2488–2495

[17] Hsu C.J., Wang L.F.: Emerging treatment of atopic dermatitis. Clin. Rev. Allergy Immunol., 2007; 33: 199–203

[18] Hwang S.A., Kruzel M.L., Actor J.K.: Influence of bovine lactoferrin on expression of presentation molecules on BCG-infected bone marrow derived macrophages. Biochimie, 2009; 91: 76–85

[19] Katoh N.: Future perspectives in the treatment of atopic dermatitis. J. Dermatol., 2009; 36: 367–376

[20] Kocięba M., Zimecki M., Kruzel M., Actor J.: The adjuvant activity of lactoferrin in the generation of DTH to ovalbumin can be inhibited by bovine serum albumin bearing alpha-D-mannopyranosyl residues. Cell. Mol. Biol. Lett., 2002; 7: 1131–1136

[21] Legrand D., Mazurier J.: A critical review of the roles of host lactoferrin in immunity. Biometals, 2010; 23: 365–376

[22] Legrand D., Pierce A., Elass E., Carpentier M., Mariller C., Mazurier J.: Lactoferrin structure and functions. Adv. Exp. Med. Biol., 2008; 606: 163–194

[23] Noonan F.P., Halliday W.J.: Studies of contact hypersensitivity and tolerance *in vivo* and *in vitro*. I. Basic characteristics of the reactions and confirmation of an immune response in tolerant mice. Int. Arch. Allergy Appl. Immunol., 1978; 56: 523–532

[24] Scheper R.J., van Dinther-Janssen A.C., Polak L.: Specific accumulation of hapten-reactive T cells in contact sensitivity reaction sites. J. Immunol., 1985; 134: 1333–1336

[25] van Dinther-Janssen A.C., van Maarsseveen A.C., de Groot J., Scheper R.J.: Comparative migration of T- and B-lymphocyte subpopulations into skin inflammatory sites. Immunology, 1983; 48: 519–527

[26] VanBuskirk A.M., Adams P.W., Orosz C.G.: Nonradioactive alternative to clinical mixed lymphocyte reaction. Hum. Immunol., 1995; 43: 38–44

[27] Yamamoto S., Sugahara S., Ikeda K., Shimizu Y.: Pharmacological profile of a novel phosphodiesterase 7A and -4 dual inhibitor, YM-393059, on acute and chronic inflammation models. Eur. J. Pharmacol., 2006; 550: 166–172

[28] Zhang D., Kishihara K., Wang B., Mizobe K., Kubo C., Nomoto K.: Restraint stress-induced immunosuppression by inhibiting leukocyte migration and Th1 cytokine expression during the intraperitoneal infection of *Listeria monocytogenes*. J. Neuroimmunol., 1998; 92: 139–151

[29] Zimecki M., Artym J., Chodaczek G., Kocięba M., Kruzel M.: Effects of lactoferrin on the immune response modified by the immobilization stress. Pharmacol. Rep., 2005; 57: 811–817

[30] Zimecki M., Artym J., Kocięba M.: Endogenous steroids are responsible for lactoferrin-induced myelopoiesis in mice. Pharmacol. Rep., 2009; 61: 705–710

[31] Zimecki M., Kruzel M.L.: Systemic or local co-administration of lactoferrin with sensitizing dose of antigen enhances delayed type hypersensitivity in mice. Immunol. Lett., 2000; 74: 183–188

[32] Zimecki M., Machnicki M.: Lactoferrin inhibits the effector phase of the delayed type hypersensitivity to sheep erythrocytes and inflammatory reactions to *M. bovis* (BCG). Arch. Immunol. Ther. Exp., 1994; 42: 171–177

[33] Zimecki M., Mazurier J., Machnicki M., Wieczorek Z., Montreuil J., Spik G.: Immunostimulatory activity of lactotransferrin and maturation of CD4-CD8- murine thymocytes. Immunol. Lett., 1991; 30: 119–123

[34] Zimecki M., Mazurier J., Spik G., Kapp J.A.: Human lactoferrin induces phenotypic and functional changes in murine splenic B cells. Immunology, 1995; 86: 122–127

[35] Zimecki M., Mazurier J., Spik G., Kapp J.A.: Lactoferrin inhibits proliferative response and cytokine production of TH1 but not TH2 cell lines. Arch. Immunol. Ther. Exp., 1996; 44: 51–56

[36] Zimecki M., Międzybrodzki R., Mazurier J., Spik G.: Regulatory effects of lactoferrin and lipopolysaccharide on LFA-1 expression on human peripheral blood mononuclear cells. Arch. Immunol. Ther. Exp., 1999; 47: 257–264

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