© Med Sci Monit, 2004; 10(3): BR84-89
PMID: 14976460

Received: 2003.04.14
Accepted: 2003.09.24
Published: 2004.03.01

Normalization of peripheral blood cell composition by lactoferrin in cyclophosphamide-treated mice

Authors' Contribution:
A Study Design
B Data Collection
C Statistical Analysis
D Data Interpretation
E Manuscript Preparation
F Literature Search
G Funds Collection

Jolanta Artym[1], Michał Zimecki[1], Marian Kruzel[2]

[1] Department of Experimental Therapy, Institute of Immunology and Experimental Therapy of the Polish Academy of Science, Wrocław, Poland
[2] Department of Integrative Biology and Pharmacology, University of Texas, Health Science Center at Houston, Texas 77030, U.S.A.

Source of support: Department of Experimental Therapy, Institute of Immunology and Experimental Therapy of the Polish Academy of Science, Wrocław, Poland.

Summary

Background: Cyclophosphamide (CP) is used in the treatment of autoimmune disorders and leukemia. The compound induces severe leuko- and neutropenia. Lactoferrin (LF) is a protein which plays a role in the innate immunity. In this study we evaluated the usefulness of LF in reversing CP-induced lympho- and neutropenia in mice.

Material/Methods: CBA mice were treated with CP (350 mg/kg body weight, intraperitoneally) and given LF as a 0.5% addition to drinking water. Alternatively, LF was administered orally (seven doses, 1 mg each) on alternate days following CP injection. Control groups received CP or LF only. Blood samples were taken before treatment and on days 4, 8, 15 and 22 following CP injection to determine leukocytosis and cell types in blood smears.

Results: Mice treated with CP showed severe leukopenia, strong eosinophilia (day 4), and an altered lymphocyte/neutrophil ratio (days 8–22). Treatment of mice with LF for 21 days partially normalized the cell composition in CP-treated mice (increased percentage of lymphocytes and decreased eosinophil content). The content of leukocytes increased upon LF treatment on days 4, 8, 15 and 22 (by 36.8, 39.5, 72 and 70.7%, respectively). More importantly, LF partly normalized the neutrophil and lymphocyte composition on day 22 (neutrophils: 29.2% in control mice, 50.6% in CP-treated, and 39.16% in CP/LF-treated; lymphocytes: 66.18% in control mice, 35% in CP-treated and 48.8% in CP/LF-treated). Administration of LF alone did not change the cell numbers or composition.

Conclusions: LF given orally to CP-immunocompromised mice accelerates reconstitution of lymphopoiesis and myleopoiesis.

key words: mice • leukopenia • cyclophosphamide • lactoferrin • reconstitution

Full-text PDF: http://www.MedSciMonit.com/pub/vol_10/no_3/3621.pdf

Word count: 1716
Tables: 1
Figures: 2
References: 54

Author's address: Michał Zimecki, Department of Experimental Therapy, Institute of Immunology and Experimental Therapy of the Polish Academy of Science, ul. Weigla 12, 53-114 Wrocław, Poland, e-mail: zimecki@immuno.iitd.pan.wroc.pl

BACKGROUND

Cyclophosphamide is an alkylating compound used in the treatment of some autoimmune disorders, such as systemic lupus erythematosus [1], nephritis [2], multiple sclerosis [3], rheumatoid arthritis [4], Wegener's granulomatosis [5] and lymphomas [6]. CP induces profound leukopenia [7] and neutropenia [8], and toxic effects in the bone marrow and liver [9], including stromal cells. The drug preferentially acts on B lymphocytes [10], probably due to their longer life span in comparison with T cells [11]. Consequently, the humoral immune response is more deeply affected [12] than the cellular immune responses, mediated by T cells [13]. A characteristic feature of CP action is increased eosinophilia in mice [14]. The agent also depletes a subpopulation of peritoneal macrophages [15]. The effects of CP on the immune system are dose-dependent, and at lower doses may be stimulatory [16].

Administration of CP to mice infected with various pathogens leads to an impaired immunity [17,18]. Because of the toxic side-effects of CP used in therapy, a wide spectrum of natural and synthetic substances have been tested to accelerate repopulation of the lymphoid organs and reconstitute immune system functions, such as thymic hormones [19], cytokines [20–22], synthetic compounds [23], as well as fungal [24] and bacterial products [25].

Lactoferrin (LF) belongs to the family of proteins involved in iron metabolism and is closely associated with the innate immune system of mammals [26]. The protein exhibits antibacterial [27,28], antifungal [29], antiviral [30] and antitumor [31] activities. Receptors for LF have been found on T [32] and B cells [33], macrophages/monocytes [34], and brush border intestinal cells [35]. In some conditions, the LF molecule can pass the intestinal barrier intact [36]. Also, the LF-derived peptides exhibit immunotropic activities [28,29,31]. LF administered orally stimulates both local and systemic immune responses [37]. LF was found to promote T [38] and B cell maturation and their acquirement of immunocompetence [39], and it also increases neutrophil recruitment [40]. Opinions on the ability of LF to stimulate myelopoiesis are contradictory [41–44]; this could, however, be due to the application of different experimental systems.

Our recent data [45] indicated that oral LF treatment of CP-immunocompromised mice rapidly reconstituted the ability of mice to generate a cellular immune response; in addition, the content of T and B cells was significantly elevated. The purpose of this work was to investigate the effects of oral LF treatment of mice subjected to a sublethal dose of CP on the level of peripheral blood leukocytes and cell-type pattern in the peripheral blood. In this study we found that orally administered LF can accelerate the renewal of peripheral leukocytes and normalize an abnormal blood picture in CP-immunocompromised mice.

MATERIAL AND METHODS

Mice

CBA 8- to 12-week-old mice of both sexes were provided by the Animal Facility of the Institute of Immunology and Experimental Therapy, Wrocław, Poland. The mice were fed a commercial, granulated food and water *ad libitum*. The local ethics committee approved the study.

Treatment of mice with cyclophosphamide and lactoferrin

The mice were fed bovine lactoferrin (Morinaga, Japan) by gavage (1 mg in 0.2 ml water, seven doses on alternate days) or were given LF in drinking water (0.5% final concentration). Cyclophosphamide (ASTA Medica, Frankfurt, Germany) was given intraperitoneally (350 mg/kg).

Determination of leukocyte number and blood cell composition

The mice were bled under general anesthesia using a Pasteur pipette (0.1 ml of blood) from the retro orbital plexus. Leukocytosis was determined by diluting the blood in a hemocytometer and counting the cells in a Burker camera. The smears were subsequently reviewed histologically; the pathologist viewing and interpreting the slides was blinded to the type of experiment and treatment.

Statistics

Each group of mice consisted of 6 animals. All data are expressed as mean values ±standard error (SE). Differences between groups were analyzed by the Student's unpaired t-test. A p value of 0.05 or less was considered significant.

RESULTS

The effect of orally administered LF (pulse treatment) on leukocytes in CP-immunocompromised mice

The mice were given CP intraperitoneally (350 mg/kg) followed by seven doses of LF (1mg each) on alternate days. One day after the last dose of LF, the samples of blood were taken and the white blood cell (WBC) pattern was analyzed in stained blood smears. The results (Table1) show that 2 weeks following administration of CP, the neutrophil/lymphocyte ratio was reversed (the percentage of neutrophils rose from 32 to 70 and that of lymphocytes fell from 62.4 to 21). The treatment of mice with LF partially normalized the neutrophil/lymphocyte ratio (decrease of neutrophils from 70 to 51% and increase of lymphocytes from 21 to 40. 4%). Mice treated with LF alone showed minor and statistically insignificant changes in the neutrophil and lymphocyte content. Other changes caused by LF in the CP-treated mice involved a decrease in the eosinophil content and a significant (from 1.8 to 4.8%) increase in neutrophil precursors (bands).

Table 1. Changes in the circulating blood cell profile following oral LF administration in CP-treated mice. Mice were given CP (350 mg/kg b.w.) i.p. and seven oral doses (1 mg each, by gavage) on alternate days. 24 h after the last dose of LF, the mice were bled and the leukocyte content was determined in a hemocytometer. Determination of neutrophil and lymphocyte content was performed using blood smears stained with Giemsa and May-Grünwald reagents.

% of cells types	Control	CP	CP/LF	LF
Bands	1.8	2.8	4.8	2.5
Segments	32	70	51	24.6
Eosinophils	3.6	6	3.8	2.33
Basophils	0	0.2	0	0
Lymphocytes	62.4	21	40.4	70.5
Monocytes	0	0	0	0

The data are presented as mean values from 5 mice/group (percentage of respective cell types) ± standard error:
Bands: Control/CP (NS), Control /CP LF (P<0.01), CP/CP LF (NS), Control/LF (NS), CP/LF (NS), CP LF/ LF (P<0.02);
Segments: Control/CP (P<0.001), Control /CP LF (P<0.01), CP/CP LF (P<0.02), Control/LF (NS), CP/LF (P<0.001), CP LF/ LF (P<0.01);
Eosinophils: Control/CP (P<0.05), Control /CP LF (NS), CP/CP LF (NS), Control/LF (NS), CP/LF (P<0.01), CP LF/ LF (NS);
Lymphocytes: Control/CP (P<0.001), Control /CP LF (P<0.01), CP/CP LF (P<0.01), Control/LF (NS), CP/LF (P<0.001), CP LF/ LF (P<0.001)

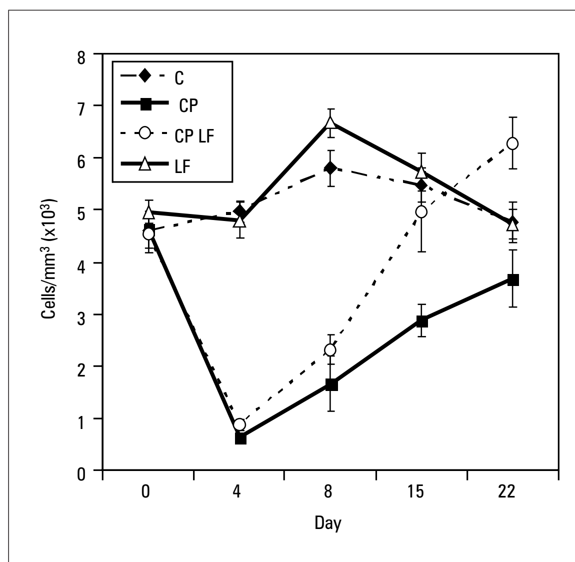

Figure 1. Changes in leukocytosis following LF given in drinking water to CP-treated mice. Mice were given CP (350 mg/kg b. w.) i.p. and LF in drinking water (0.5% solution) for the whole period of the experiment (30 days). The mice were bled under general anesthesia using a Pasteur pipette (0.1 ml of blood) from the retro orbital plexus. Leukocytosis was determined by diluting blood in a hemocytometer and counting the cells in a Burker camera. The data are presented as mean values from 5 mice/group (cell number/mm^3) ±standard error: *Day 0*: Control/CP (NS), Control /CP LF (NS), CP/CP LF (NS), Control/LF (NS), CP/LF (NS), CP LF/ LF (NS); *Day 4*: Control/CP (P<0.001), Control /CP LF (P<0.001), CP/CP LF (NS), Control/LF (NS), CP/LF (P<0.001), CP LF/ LF (P<0.001); *Day 8*: Control/CP (P<0.001), Control /CP LF (P<0.001), CP/CP LF (NS), Control/LF (NS), CP/LF (P<0.001), CP LF/ LF (0.001); *Day 14*: Control/CP (P<0.001), Control /CP LF (NS), CP/CP LF (<0.05), Control/LF (NS), CP/LF (P<0.001), CP LF/ LF (NS); *Day 22*: Control/CP (NS), Control /CP LF (P<0.02), CP/CP LF (P<0.001), Control/LF (NS), CP/LF (NS), CP LF/ LF (P<0.01).

Effect of orally administered LF (continuous treatment) on leukocytes in CP-immunocompromised mice

The mice were given CP intraperitoneally (350 mg/kg) followed by continuous administration of LF in drinking water for the duration of the experiment. One day after completion of the protocol, the blood samples were taken and the WBC pattern was analyzed in stained blood smears. The reconstituting effects of LF on the level of circulating leukocytes are shown in Figure 1. The deep leukopenia caused by CP, observed on day 4, started to normalize in the CP-treated mice, but even on day 22 it did not return to its initial value (79.5% in the controls). Administration of LF accelerated the elevation of the leukocyte content, so that on day 15 the number of leukocytes was 107% vs. 62% of the control values in mice treated only with CP. In fact, the level of leukocytes in the CP/LF-treated mice exceeded the initial, control values by 35.8%.

The changes in the blood cell type composition in the respective groups of mice are presented in Figure 2. CP-treatment caused the characteristic changes in the percentages of lymphocytes (decrease) and neutrophils (increase), thus reversing the lymphocyte/neutrophil ratio. In addition, the drug caused a strong eosinophilia, observed on day 4. Administration of LF led to normalization of the cell composition beginning on day 4, i.e. LF increased the percentage of lymphocytes, decreased the content of neutrophils, and lowered the eosinophilia. Another characteristic feature of LF action was a significant increase in the immature neutrophil (bands) level. The treatment with LF alone did not cause any significant changes in the blood cell composition as in the case of pulse treatment (data not shown). Similar effects were observed in mice given a 0.2% LF solution (not shown).

Discussion

Here we report that oral administration of LF restored the leukocyte levels and normalized the cell pattern in

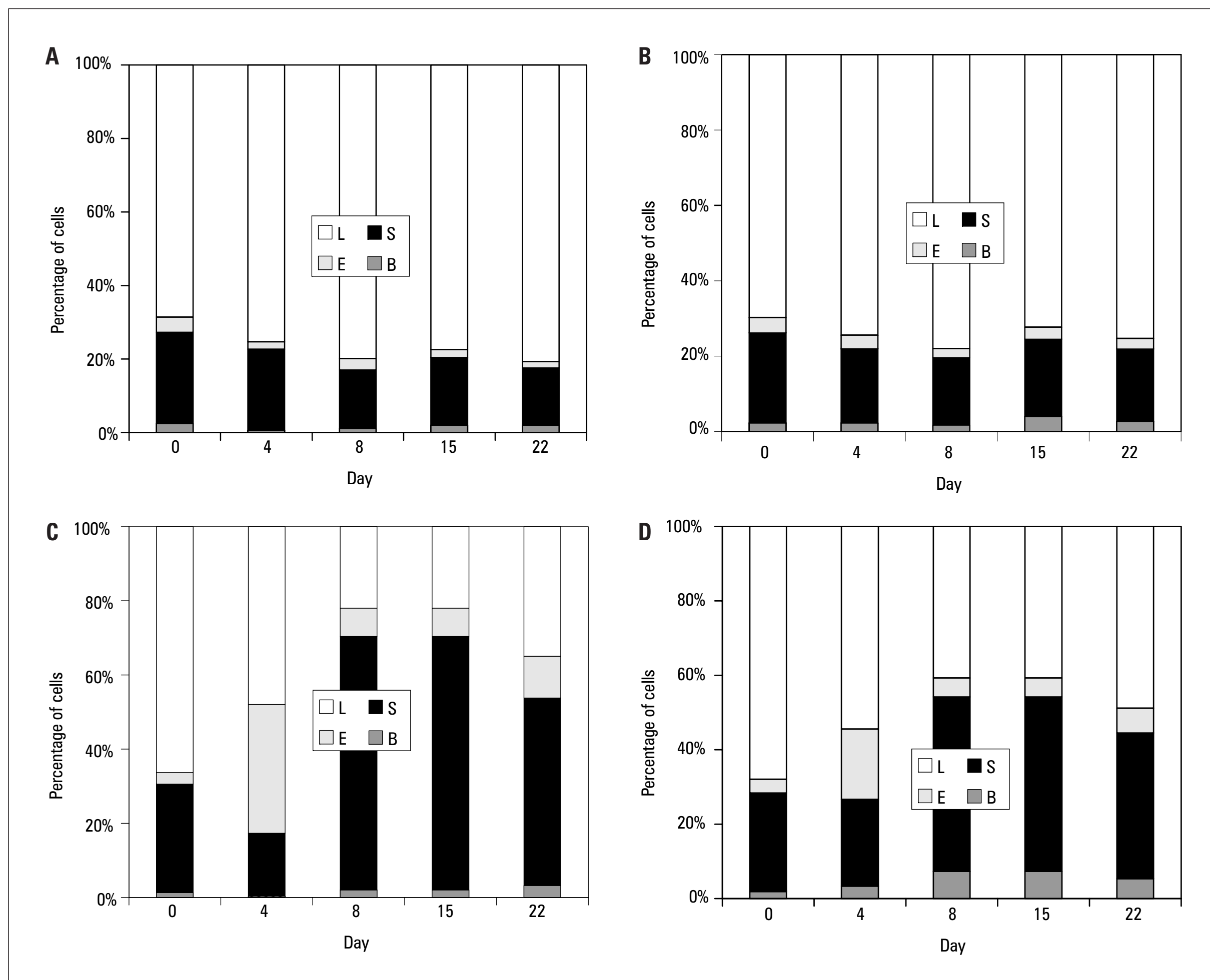

Figure 2. Effect of LF given in drinking water to CP-treated mice on blood cell type composition in the peripheral blood. Mice were treated with CP and LF as described in Figure 1. The mice were bled under general anesthesia using a Pasteur pipette (0.1 ml of blood) from the retro orbital plexus and determination of cell content was performed using blood smears stained with Giemsa and May-Grünwald reagents. (**A**) Untreated mice; (**B**) LF-treated mice; (**C**) CP-treated mice; (**D**) CP/LF-treated mice. The data are presented as mean values from 5 mice/group (percentage of respective cell types) ±standard error: *Day 0*: *Bands*: Control/CP (P<0.05), Control /CP LF (NS), CP/CP LF (NS), Control/LF (NS), CP/LF (P<0.05), CP LF/ LF (NS); *Segments*: Control/CP (NS), Control /CP LF (NS), CP/CP LF (NS), Control/LF (NS), CP/LF (NS), CP LF/ LF (NS); *Eosinophils*: Control/CP (NS), Control /CP LF (NS), CP/CP LF (NS), Control/LF (NS), CP/LF (NS), CP LF/ LF (NS); *Lymphocytes*: Control/CP (NS), Control /CP LF (NS), CP/CP LF (NS), Control/LF (NS), CP/LF (NS), CP LF/ LF (NS). *Day 4*: *Bands*: Control/CP (NS), Control /CP LF (P<0.001), CP/CP LF (P<0.001), Control/LF (P<0.01), CP/LF (P<0.01), CP LF/ LF (NS); *Segments*: Control/CP (P < 0. 05), Control /CP LF (NS), CP/CP LF (P<0.01), Control/LF (NS), CP/LF (NS), CP LF/ LF (NS); *Eosinophils*: Control/CP (P<0.001), Control /CP LF (P<0.001), CP/CP LF (P<0.001), Control/LF (P<0.01), CP/LF (P<0.001), CP LF/ LF (P<0.001); *Lymphocytes*: Control/CP (P<0.001), Control /CP LF (P<0.001), CP/CP LF (<0.02), Control/LF (NS), CP/LF (P<0.001), CP LF/ LF (P<0.001). *Day 8*: *Bands*: Control/CP (NS), Control /CP LF (P<0.001), CP/CP LF (P<0.001), Control/LF (NS), CP/LF (NS), CP LF/ LF (P<0.001); *Segments*: Control/CP (P<0.001), Control /CP LF (P<0.001), CP/CP LF (P<0.001), Control/LF (NS), CP/LF (P<0.001), CP LF/ LF (P<0.001); *Eosinophils*: Control/CP (P<0.01), Control /CP LF (P<0.05), CP/CP LF (P<0.05), Control/LF (NS), CP/LF (P<0. 001), CP LF/ LF (P<0. 001); *Lymphocytes*: Control/CP (P<0.001), Control /CP LF (P<0.001), CP/CP LF (<0.001), Control/LF (NS), CP/LF (P<0.001), CP LF/ LF (P<0.001). *Day 14*: *Bands*: Control/CP (NS), Control /CP LF (P<0.001), CP/CP LF (P<0.001), Control/LF (P<0.02), CP/LF (P<0.01), CP LF/ LF (P<0.01); *Segments*: Control/CP (P<0.001), Control /CP LF (P<0.001), CP/CP LF (P<0.01), Control/LF (NS), CP/LF (P<0.001), CP LF/ LF (P<0.001); *Eosinophils*: Control/CP (P<0.001), Control /CP LF (P<0.01), CP/CP LF (P<0.01), Control/LF (NS), CP/LF (P<0.001), CP LF/ LF (P<0.02); *Lymphocytes*: Control/CP (P<0.001), Control /CP LF (P<0.001), CP/CP LF (<0.02), Control/LF (NS), CP/LF (P<0.001), CP LF/ LF (P<0.001). *Day 22*: *Bands*: Control/CP (NS), Control /CP LF (P<0.01), CP/CP LF (P<0.05), Control/LF (NS), CP/LF (NS), CP LF/ LF (P<0.001); *Segments*: Control/CP (P<0.001), Control /CP LF (P<0.001), CP/CP LF (P<0.02), Control/LF (NS), CP/LF (P<0.001), CP LF/ LF (P<0.001); *Eosinophils*: Control/CP (P<0.001), Control /CP LF (P<0.001), CP/CP LF (P<0.001), Control/LF (P<0.05), CP/LF (P<0.001), CP LF/ LF (P<0.001); *Lymphocytes*: Control/CP (P<0.001), Control /CP LF (P<0.001), CP/CP LF (<0.02), Control/LF (P<0.05), CP/LF (P<0.001), CP LF/ LF (P<0.001).
B – bands; S – segments; E – eosinophils; L – lymphocytes.

the peripheral blood of CP-immunocompromised mice. To our knowledge this is the first demonstration of the reconstituting effect of LF on leukocytes in immunocompromised mice. The described activity of LF is in agreement with our previous reports demonstrating that LF can promote maturation of double-negative thymocytes from normal mice and B cells from neonatal mice [39]. We and others also reported that LF can accelerate neutrophil recruitment in humans and animals [40,44]. Results presented in this work also showed that in the conditions of deep neutropenia caused by CP, LF induced the appearance of neutrophil precursors, as demonstrated by the increase in band forms. Our unpublished data also revealed that treatment of mice with LF induced a strong mobilization/recruitment of myelocytes and band forms in bone marrow. Such a finding is in contradiction with other studies showing no or negative effects of LF on myelopoiesis [41,42]; others, however, have found a positive influence of LF on myelopoiesis [43,44]. Eosinophilia, induced by CP administration, is a known phenomenon [14] and it also occurred in our system. LF was able to significantly down-regulate the transient, rapid rise in the percentage of eosinophils. Taken together, oral LF administration to CP-treated mice led to significant correction of the abnormal blood cell pattern and diminution of leukopenia.

Analysis of blood smears revealed that LF accelerated not only myelopoiesis (the rise in immature neutrophil forms) but also the appearance of young lymphocyte forms (not shown). In our recent studies [45] we demonstrated, by use of the panning technique, that the content of $CD3^+$, $CD4^+$ and Ig^+ cells in the spleens of CP-treated mice was significantly elevated by treatment with LF. A rise in the level of circulating $CD4^+$, $CD8^+$ and asialo GMI^+ cells was also demonstrated in rats given LF in drinking water [46].

Although LF may directly interact with immature T and B cells [38,39] and T-cell lines [47], it is most probable that the reconstituting action of LF on lympho- and neutropenia is indirect and involves T- and B-tropic cytokines elicited by the interaction of LF with cells which escaped the cytotoxic action of CP (e.g. endothelial and stromal cells or macrophages). LF was shown to release IL-6, TNFα and IL-10 when given per os or i.v. to mice [48]. Recently we showed (manuscript submitted) that i.v. injection of LF released IL-1 into the circulation. LF also induces considerable amounts of TNFα and IL-6 in human PBMC cultures [49]. It is possible that the remaining macrophage subpopulation, resistant to CP action [15], may be a potent source of IL-6, a B- and T-tropic cytokine [50]. Some authors also provided evidence for induction of colony-stimulating factors by LF [43].

Lactoferrin has been recently tried in preclinical and clinical studies and showed benefit in the treatment of some types of cancer and hepatitis C infection [reviewed in 51]. The lower cost and lack of side-effects of LF, in comparison with interferon alpha [52], may make it an attractive alternative for the treatment of hepatitis C. In our own clinical trial we demonstrated the positive effect of preoperational, oral LF treatment of patients on their immune status following surgery [53]. Interestingly, oral treatment of multiple sclerosis patients with LF normalized selected immune parameters and improved their health conditions (to be published). Similar properties were exhibited by a substance also derived from colostrum, a proline rich polypeptide, which improved the clinical status of Alzheimer's disease patients [54]. In conclusion, we suggest that the clinical application of LF may also include the reversal of undesirable, chemotherapy-induced leukopenia in neoplastic and autoimmune diseases.

CONCLUSIONS

1. Oral administration of LF to CBA mice given a sublethal dose of cyclophosphamide results in an accelerated reconstitution of circulating lymphocytes and neutrophils and leads to a normalization of the white blood cell pattern.

2. These results suggest that LF given orally may be effective in the reconstitution of chemotherapy--impaired lympho- and myleopoiesis in humans.

REFERENCES:

1. Lacki JK, Mackiewicz SH, Leszczynski P, Muller W: The effect of intravenous cyclophosphamide pulse on peripheral blood lymphocytes in lupus erythematosus patients. Rheumatol Int, 1997; 17: 55-60
2. Mok CC, Ho CT, Chan KW et al: Outcome and prognostic indicators of diffuse proliferative lupus glomerulonephritis treated with sequential oral cyclophosphamide and azathioprine. Arthritis Rheum, 2002; 46: 1003-13
3. Weiner HL, Cohen JA: Treatment of multiple sclerosis with cyclophosphamide: critical review of clinical and immunologic effects. Mult Scler, 2002; 8: 142-54
4. Leandro MJ, Edwards JC, Cambridge G: Clinical outcome in 22 patients with rheumatoid arthritis treated with B lymphocyte depletion. Comment in: Ann Rheum Dis, 2002; 6: 863-66, Ann Rheum Dis, 2002; 61: 883-88
5. Dabrowski A, Droszcz W: Wegener's granulomatosis – clinical analysis of 18 patients group. Med Sci Monit, 2000; 6: 151-57
6. Asou N, Suzushima H, Hishimura S et al: Long-term remission in an elderly patient with mantle cell leukemia treated with low-dose cyclophosphamide. Am J Haematol, 2000; 63: 35-37
7. Shalit I, Kletter Y, Halperin D et al: Immunomodulatory effects of moxifloxacin in comparison to ciprofloxacin and G-CSF in a murine model of cyclophosphamide-induced leukopenia. Eur J Haematol, 2000; 66: 287-96
8. Vogels MT, Hermsen CC, Huys HL et al: Roles of tumor necrosis factor alpha, granulocyte-macrophage colony-stimulating factor, platelet-activating factor, and arachidonic acid metabolites in interleukin-1-induced resistance to infection in neutropenic mice. Infect Immun, 1994; 62: 2065-70
9. Anton E: Ultrastructural changes of stromal cells of bone marrow and liver after cyclophosphamide treatment in mice. Tissue Cell, , 1997; 29: 1-9
10. Hemendinger RA, Bloom SE: Selective mitomycin C and cyclophosphamide induction of apoptosis in differentiating B lymphocytes compared to T lymphocytes *in vivo*. Immunopharmacology, 1996; 35: 71-82
11. Fulcher DA, Basten A: B cell life span: a review. Immunol Cell Biol, 1997; 75: 446-55
12. Masnaya NV, Ratner GM: Peculiarities of primary humoral immune response in mice with cytostatic disease. Bull Exp Biol Med, 2000; 129: 370-73
13. Turk JL: Studies on the mechanism of action of methotrexate and cyclophosphamide on contact sensitivity in the guinea pig. Int Arch Allergy, 1964; 24: 191-200

14. Mu HH, Penny R, Sewell WA: Interleukin-5 is necessary for eosinophilia induced by cyclophosphamide in immunized mice. Immunology, 1993; 79: 452-58
15. Marcinkiewicz J, Bryniarski K, Ptak W: Cyclophosphamide uncovers two separate macrophage subpopulations with opposite immunogenic potential and different patterns of monokine production. Cytokine, 1994; 6: 472-77
16. Miyauchi A, Hiramine C, Tanaka S, Hojo K: Differential effects of a single dose of cyclophosphamide on T cell subsets of the thymus and spleen in mice: flow cytofluorometry analysis. Tohoku J Exp Med, 1990; 162: 147-67
17. Wang E, Simard M, Ouellet N et al: Pathogenesis of pneumococcal pneumonia in cyclophosphamide-induced leukopenia in mice. Infect Immun, 2002; 70: 4226-28
18. Correa EB, Cunha JM, Bunn-Moreno MM, Madeira ED: Cyclophosphamide affects the dynamics of granuloma formation in experimental visceral leishmaniasis. Parasitol Res, 1992; 78: 154-60
19. Ohmori H, Kamo M, Yamakoshi K et al: Restoration of immunocyte functions by thymosin alpha1 in cyclophosphamide-induced immunodeficient mice. Immunopharmacol Immunotoxicol, 2001; 23: 75-82
20. Fibbe WE, van der Meer JW, Falkenburg JH et al: A single low dose of human recombinant interleukin 1 accelerates the recovery of neutrophils in mice with cyclophosphamide-induced neutropenia. Exp Hematol, 1989; 17: 805-8
21. Santosuosso M, Divangahi M, Zganiacz A, Xing Z: Reduced tissue macrophage population in the lung by anticancer agent cyclophosphamide: restoration by local granulocyte macrophage colony-stimulating factor gene transfer. Blood, 2002; 99: 1246-52
22. Morrissey PJ, Conlon P, Braddy S et al: Administration of IL-7 to mice with cyclophosphamide-induced lymphopenia accelerates lymphocyte repopulation. J Immunol, 1991; 146: 1547-52
23. Kalechman Y, Sotnik-Barkai I, Albeck M, Sredni B: The effect of AS101 on the reconstitution of T-cell reactivity following irradiation or cyclophosphamide treatment. Exp Hematol, 1992; 20: 1302-8
24. Wang GL, Lin ZB: The immunomodulatory effect of lentinan. Yao Xue Xue Bao, 1996; 31: 86-90
25. Zaidi SI, Singh KP, Raisuddin S et al: Protein A induced abrogation of cyclophosphamide toxicity is associated with concomitant potentiation of immune function of host. Immunopharmacol Immunotoxicol, 1990; 12: 479-512
26. Lonnerdal B, Iyer S: Lactoferrin: molecular structure and biological function. Annu Rev Nutr, 1995; 15: 93-110
27. Ellison RT III, Giehl TJ, LaForce FM: Damage of the outer membrane of enteric gram-negative bacteria by lactoferrin and transferrin. Infect Immun, 1988; 56: 2774-81
28. Tomita M, Bellamy W, Takase M et al: Potent antibacterial peptides generated by pepsin digestion of bovine lactoferrin. J Dairy Sci, 1991; 74: 4137-42
29. Wakabayashi H, Abe S, Okutomi T et al: Cooperative anti-Candida effects of lactoferrin or its peptides in combination with azole antifungal agents. Microbiol Immunol, 1996; 40: 821-25
30. Sato R, Inanami O, Tanaka Y et al: Oral administration of bovine lactoferrin for treatment of intractable stomatitis in feline immunodeficiency virus (FIV)-positive and FIV-negative cats. Am J Vet Res, 1996; 57: 1443-46
31. Yoo YC, Watanabe S, Watanabe R et al: Bovine lactoferrin and lactoferricin, a peptide derived from bovine lactoferrin, inhibit tumor metastasis in mice. Jpn J Cancer Res, 1997; 88: 184-90
32. Mazurier J, Legrand D, Hu WL et al: Expression of human lactotransferrin receptors in phytohemagglutinin-stimulated human peripheral blood lymphocytes. Isolation of the receptors by antiligand-affinity chromatography. Eur J Biochem, 1989; 179: 481-87
33. Hashizume S, Kuroda K, Murakami H: Identification of lactoferrin as an essential growth factor for human lymphocytic cell lines in serum-free medium. Biochim Biophys Acta, 1983; 763: 377-82
34. Birgens HS, Hansen NE, Karle H, Kristensen LO: Receptor binding of lactoferrin by human monocytes. Br J Haematol, 1983; 54: 383-91
35. Hu WL, Mazurier J, Montreuil J, Spik G: Isolation and partial characterization of a lactotransferrin receptor from mouse intestinal brush border. Biochemistry, 1990; 29: 535-41
36. Teraguchi S, Ozawa K, Yasuda S et al: The bacteriostatic effects of orally administered bovine lactoferrin on intestinal Enterobacteriaceae of SPF mice fed bovine milk. Biosci Biochim, 1994; 58: 482-87
37. Debbabi H, Dubarry M, Rautureau M, Tome D: Bovine lactoferrin induces both mucosal and systemic immune response in mice. J Dairy Res, 1998; 65: 283-93
38. Zimecki M, Mazurier J, Machnicki M et al: Immunostimulatory activity of lactotransferrin and maturation of CD4- CD8- murine thymocytes. Immunol Lett, 1991; 30: 119-23
39. Zimecki M, Mazurier J, Spik G, Kapp JA: Human lactoferrin induces phenotypic and functional changes in murine splenic B cells. Immunology, 1995; 86: 122-27
40. Zimecki M, Spiegel K, Wlaszczyk A et al: Lactoferrin increases the output of neutrophil precursors and attenuates the spontaneous production of TNF-alpha and IL-6 by peripheral blood cells. Arch Immunol Ther Exp (Warsz), 1999; 47: 113-18
41. Bagby GC Jr, McCall E, Layman DL: Regulation of colony-stimulating activity production. Interactions of fibroblasts, mononuclear phagocytes, and lactoferrin. J Clin Invest, 1983; 71: 340-44
42. Rado TA, Bollekens J, St Laurent G et al: Lactoferrin biosynthesis during granulocytopoiesis. Blood, 1984; 64: 1103-9
43. Rich IN, Sawatzki G: Lactoferrin, the signal for colony stimulating factor production? Negative-feedback regulation versus supply-and-demand regulation of myelopoiesis. Blood Cells, 1989; 15: 400-6
44. Rich IN: The macrophage as a production site for hematopoietic regulator molecules: sensing and responding to normal and pathophysiological signals. Anticancer Res, 1988; 8: 1015-40
45. Artym J, Zimecki M, Kruzel ML: Reconstiuton of the cellular immune response by lactoferrin in cyclophosphamide-treated mice is correlated with renewal of T-cell compartment. Immunobiology, 2003; 207: 187-205
46. Tsuda H, Sekine K, Takasuka N et al: Prevention of colon carcinogenesis and carcinoma metastasis by orally administered bovine lactoferrin in animals. Biofactors, 2000; 12: 83-88
47. Zimecki M, Mazurier J, Spik G, Kapp JA: Lactoferrin inhibits proliferative response and cytokine production of TH1 but not TH2 cell lines. Arch Immunol Ther Exp (Warsz), 1996; 44: 51-56
48. Zimecki M, Kruzel M: Protective effect of lactoferrin against LPS-induced endotoxemia is mediated by IL-10. Proceedings of the 5th World Congress on Trauma, Shock, Inflammation and Sepsis, 2000 Feb 29 – Mar 4; Munich, Germany. Monduzzi Editore; 2000
49. Wlaszczyk A, Zimecki M, Adamik B et al: Immunological status of patients subjected to cardiac surgery: effect of lactoferrin on proliferation and production of interleukin 6 and tumor necrosis factor alpha by peripheral blood mononuclear cells *in vitro*. Arch Immunol Ther Exp (Warsz), 1997; 45: 201-12
50. Hilbert DM, Cancro MP, Scherle et al: T cell derived IL-6 is differentially required for antigen-specific antibody secretion by primary and secondary B cells. J Immunol, 1989; 143: 4019-24
51. Tsuda H, Sekine K, Fujita K, Ligo M: Cancer prevention by bovine lactoferrin and underlying mechanisms – a review of experimental and clinical studies. Biochem Cell Biol, 2002; 80: 131-36
52. Czerwionka-Szaflarska M, Chrobot A, Szaflarska-Szczepanik A: Studies of the effectiveness of interferon alpha treatment for chronic hepatitis C in children. Med Sci Monit, 2000; 6: 964-70
53. Zimecki M, Wlaszczyk A, Wojciechowski R et al: Lactoferrin regulates the immune responses in post-surgical patients. Arch Immunol Ther Exp (Warsz), 2001; 49: 325-33
54. Leszek J, Inglot AD, Janusz M, Byczkiewicz F et al: Colostrinin proline-rich polypeptide complex from ovine colostrum – a long-term study of its efficacy in Alzheimer's disease. Med Sci Monit, 2002; 8: PI93-96

Index Copernicus

Global Scientific Information Systems for Scientists by Scientists

TM
INDEX
COPERNICUS
INTERNATIONAL

www.IndexCopernicus.com

- EVALUATION & BENCHMARKING
- PROFILED INFORMATION
- NETWORKING & COOPERATION
- VIRTUAL RESEARCH GROUPS
- GRANTS
- PATENTS
- CLINICAL TRIALS
- JOBS
- STRATEGIC & FINANCIAL DECISIONS

Index Copernicus integrates

IC Scientists

Effective search tool for collaborators worldwide. Provides easy global networking for scientists. C.V.'s and dossiers on selected scientists available. Increase your professional visibility.

IC Virtual Research Groups [VRG]

Web-based complete research environment which enables researchers to work on one project from distant locations. VRG provides:

- customizable and individually self-tailored electronic research protocols and data capture tools,
- statistical analysis and report creation tools,
- profiled information on literature, publications, grants and patents related to the research project,
- administration tools.

IC Journal Master List

Scientific literature database, including abstracts, full text, and journal ranking. Instructions for authors available from selected journals.

IC Patents

Provides information on patent registration process, patent offices and other legal issues. Provides links to companies that may want to license or purchase a patent.

IC Grant Awareness

Need grant assistance? Step-by-step information on how to apply for a grant. Provides a list of grant institutions and their requirements.

IC Conferences

Effective search tool for worldwide medical conferences and local meetings.

IC Lab & Clinical Trial Register

Provides list of on-going laboratory or clinical trials, including research summaries and calls for co-investigators.