In this work, we investigated the anticancer activity of orally administered recombinant human lactoferrin (rhLF) alone and in combination with chemotherapy in tumor-bearing mice. rhLF inhibited the growth of squamous cell carcinoma (O12) tumors in T cell-immunocompromised nu/nu mice by 80% when administered at 1,000 mg/kg (2.9 g/m²) by oral gavage twice daily for 8 days (p < 0.001). Similar activity was observed in syngeneic, immunocompetent BALB/c mice, where orally administered rhLF (1,000 mg/kg, 2.9 g/m² once daily) halted the growth of mammary adenocarcinoma TUBO. Oral rhLF (200 mg/kg, 0.57 g/m²) was also used alone and in combination with cis-platinum (5 mg/kg) to treat head-and-neck squamous cell carcinoma in a syngeneic murine model. Monotherapy with oral rhLF or cis-platinum caused 61% or 66% tumor growth inhibition over placebo, respectively. Mice receiving both therapies showed 79% growth inhibition, a statistically significant improvement over each drug alone. We then demonstrated that administration of oral rhLF (300 mg/kg, 0.86 g/m²) to tumor-bearing or naive mice resulted in (i) significantly increased production of IL-18 in the intestinal tract, (ii) systemic NK cell activation and (iii) circulating CD8+ T-cell expansion. These data suggest that oral rhLF is an immunomodulatory agent active against cancer as a single agent and in combination chemotherapy, exerting its systemic effect through stimulation of IL-18 and other cytokines in the gut enterocytes. rhLF has been administered orally to 211 people without a single serious drug-related adverse event. Thus, rhLF shows promise as a safe and well-tolerated novel immunomodulatory anticancer agent. Copyright 2004 Wiley-Liss, Inc.

PMID: 15221967 [PubMed - indexed for MEDLINE]
Ingestion of bovine lactoferrin (bLF) has been reported to show anti-infective, anti-cancer, and anti-inflammatory effects. In particular, it has become evident that oral bLF had a beneficial effect on infections of both digestive and nondigestive tract tissue in various animal models. Furthermore, the effects of bLF have been indicated in clinical studies on patients with Helicobacter pylori infection, chronic hepatitis C, tinea pedis, and other diseases. Immunomodulation in the intestine and systemic sites has been suggested to mediate the protective effects of oral bLF against infection. Recently, we demonstrated the beneficial effects of oral bLF in influenza virus infected mice. BLF administration reduced the lung consolidation score and the number of infiltrating leukocytes in bronchoalveolar lavage fluid. We also investigated the effect of oral bLF on the transcription of genes related to immunity in the small intestine of mice using the quantitative RT-PCR method. We found that intake of bLF increased the expression of IL-12p40, IFN-beta, and NOD2. Thus, oral bLF activates the transcription of important immune-related genes in the small intestine, and such transcriptional activation may promote systemic host immunity.

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Combination chemoprevention using tea polyphenols as one of the components has received growing consideration in recent years. The present study was designed to evaluate the antiproliferative and apoptosis inducing effects of bovine lactoferrin (bLF) and black tea polyphenol (Polyphenon-B: P-B) combination on 7,12-dimethylbenz[a]anthracene (DMBA)-induced hamster buccal pouch (HBP) carcinogenesis. Topical application of DMBA for 14 weeks induced buccal pouch tumours that showed aberrant expression of cytokeratins, a marker for epithelial carcinomas. This was associated with increased cell proliferation and evasion of apoptosis as revealed by upregulation of proliferating cell nuclear antigen, NF-kappaB, mutant p53, Bcl-2 and downregulation of Bax, Fas and caspase 3 protein expression. Although dietary administration of bLF and Polyphenon-B alone significantly reduced tumour incidence, combined administration of bLF and Polyphenon-B was more effective in inhibiting HBP carcinogenesis by restoring normal cytokeratin expression, inhibiting cell proliferation and inducing apoptosis. These findings suggest that a "designer item" approach will be useful for human oral cancer prevention strategies.

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Lactoferrin, an evolutionarily old protein of the transferrin family, is among the proteins constituting the system of innate immunity; its action, however, also extends to the regulation of acquired immunity and other immunological phenomena. The actions of LF, confirmed in numerous in vitro and in vivo models, include participation in iron homeostasis, immunoregulatory properties, anti-inflammatory, anti-tumor, and analgesic actions, regulation of bone metabolism, participation in embryonic development, reproductive functions, and others. LF plays an important role in the normal development of a newborn. The anti-tumor properties of LF were discovered about a decade ago and have been confirmed in many laboratory, preclinical, and clinical studies. The immunomodulatory properties of LF play a major role in its anti-tumor actions. Such actions of LF appeared particularly effective in cancer patients with impaired immunity. The growth of tumors is facilitated by low expressions of MHC and co-stimulatory antigens on tumor cells and the induction of suppressor cells and other inhibitory products by tumors. Enhancement of an anti-tumor immunological response may, therefore, restrict tumor growth. Studies showed that LF elevates the number and increases the activity of T and B lymphocytes and NK cells, stimulates the release of a number of cytokines (IL-1, -6, -8, -18, IFN-gamma, TNF alpha), increases phagocytic activity and cytotoxicity of monocytes/macrophages, accelerates the maturation of T and B cells, and elevates the expression of several types of cellular receptors, such as CD4, zeta chain of the CD3 complex, LFA-1, CD11, ICAM-1, and selectin P. Apart from its immunomodulatory properties, LF exhibits direct anti-tumor actions, such as lytic, pro-apoptotic, anti-proliferative, anti-angiogenic, anti-oxidant activity and the chelation of iron ions. LF also possesses chemo-preventive properties, regulates the activity of phase I and II enzymes participating in the activation and detoxification of carcinogens, and regulates the composition of the intestinal microflora. In this way it prevents the generation of tumors and their development at early stages of carcinogenesis.

PMID: 16885906 [PubMed - indexed for MEDLINE]
Antimicrobial peptides have been shown to exert cytotoxic activity towards cancer cells through their ability to interact with negatively charged cell membranes. In this study the cytotoxic effect of the antimicrobial peptide, LfcinB was tested in a panel of human neuroblastoma cell lines. LfcinB displayed a selective cytotoxic activity against both MYCN-amplified and non-MYCN-amplified cell lines. Non-transformed fibroblasts were not substantially affected by LfcinB. Treatment of neuroblastoma cells with LfcinB induced rapid destabilization of the cytoplasmic membrane and formation of membrane blebs. Depolarization of the mitochondria membranes and irreversible changes in the mitochondria morphology was also evident. Immuno- and fluorescence-labeled LfcinB revealed that the peptide co-localized with mitochondria. Furthermore, treatment of neuroblastoma cells with LfcinB induced cleavage of caspase-6, -7 and -9 followed by cell death. However, neither addition of the pan-caspase inhibitor, zVAD-fmk, or specific caspase inhibitors could reverse the cytotoxic effect induced by LfcinB. Treatment of established SH-SY-5Y neuroblastoma xenografts with repeated injections of LfcinB resulted in significant tumor growth inhibition. These results revealed a selective destabilizing effect of LfcinB on two important targets in the neuroblastoma cells, the cytoplasmic- and the mitochondria membrane. Copyright (c) 2006 Wiley-Liss, Inc.

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Experimental evidence from previous studies supports the conclusion that orally administered lactoferrin (LF) restores the immune response in mice treated with a sublethal dose of cyclophosphamide (CP). The aim of this study was to elucidate potential benefit of LF in mice undergoing chemotherapy with busulfan (BU) and CP, followed by intravenous (i.v.) injection of bone marrow cells. CBA mice were treated orally with busulfan (4 mg/kg) for 4 consecutive days, followed by two daily doses of CP delivered intraperitoneally (i.p.) at a dose of 100 mg/kg and reconstituted next day with i.v. injection of 10(7) syngeneic bone marrow cells. One group of these mice was given LF in drinking water (0.5% solution). After treatment, mice were immunized with ovalbumin (OVA) to subsequently measure delayed type hypersensitivity responsiveness and with sheep red blood cells to determine humoral immunity by evaluation of splenic antibody-forming cells. As expected, both humoral and cellular immune responses of mice that were treated with these chemotherapeutic agents was markedly impaired. Here we report that this impairment was remarkably attenuated by oral administration of LF. Humoral immunity fell to levels that were 66-88% lower than that of untreated animals. Humoral immunity of LF-treated animals was equivalent to that of untreated mice within 1 month. Cellular immune responses were inhibited by chemotherapy treatment to a lesser degree, reaching levels that were approximately 50% lower than those of untreated animals. Again, LF mitigated this decrease, resulting in responses that were only slightly lower than those observed in untreated animals. Furthermore, when mice were given a
lethal dose of BU (4 x 25 mg daily doses, i.p.) followed by a bone marrow transplant, LF caused enhanced lympho-, erythro-, and myelopoiesis in the bone marrow and appearance of transforming splenic lymphoblasts, similar to effects caused by administration of recombinant human granulocyte-macrophage colony-stimulating factor (GM-CSF). In summary, our study suggests that LF may be a useful agent to accelerate restoration of immune responsiveness induced by chemotherapy in bone marrow transplant recipients.

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Lactoferrin, a member of the transferrin family, is iron-binding and a strongly cationic 76 kDa glycoprotein. In breast milk it is secreted in high concentrations from glandular epithelia and is also present in other exocrine fluids including saliva. In the present study, we examined the biological mechanisms of apoptosis induced by pepsin-digested-lactoferrin peptide (Lfnp) in the human oral squamous cell carcinoma cell line SAS. We found that treatment with Lfnp induced cell death with apoptotic nuclear changes, preceded by the cleavage of caspase-3 and poly (ADP-ribose) polymerase (PARP) in the apoptotic cells. Treatment with Lfnp induced phosphorylation of extracellular signal-regulated kinase (ERK1/2), a member of the MAP kinase family, at early stages of apoptosis. Another MAP kinase, c-Jun N-terminal kinase/stress-activated protein kinase (JNK/SAPK), was also phosphorylated by treatment with Lfnp. Pretreatment of SAS cells with SP600125, a JNK/SAPK inhibitor, diminished Lfnp-induced apoptosis, as assessed by determining released lactate dehydrogenase activity. On the other hand, the MEK1 inhibitors PD98059 or U0126 showed no effect on repression of cell death, but rather an increase. These results suggest that JNK/SAPK activation may play an important role in Lfnp-induced apoptotic cell death of human oral squamous cell carcinoma cells.

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Bovine lactoferricin (LfcinB) is a cationic, amphipathic peptide that is cytotoxic for human and rodent cancer cells. However, the mechanism by which LfcinB causes the death of cancer cells is not well understood. Here, we show that in vitro treatment with LfcinB rapidly induced apoptosis in several different human leukemia and carcinoma cell lines as determined by DNA fragmentation assays and phosphatidylserine headgroup inversion detected by Annexin V binding to the surface of cancer cells. Importantly, LfcinB treatment did not adversely affect the viability of untransformed human lymphocytes, fibroblasts, or endothelial cells. Studies with different LfcinB-derived peptide fragments revealed that the cytotoxic activity of LfcinB resided within the amino acid sequence FKCRRWQWRM. Treatment of Jurkat T leukemia cells with LfcinB resulted in the production of reactive oxygen species followed by caspase-2-induced dissipation of mitochondrial transmembrane potential and subsequent activation of caspase-9 and caspase-3. Selective inhibitors of caspase-2 (Z-VDVAD-FMK), caspase-9 (Z-LEHD-FMK), and caspase-3 (Z-DEVD-FMK) protected both leukemia and carcinoma cells from LfcinB-induced apoptosis. Conversely, a caspase-8 inhibitor (Z-IETD-FMK) had no effect, which argued against a role for caspase-8 and was consistent with the finding that death receptors were not involved in LfcinB-induced apoptosis. Furthermore, Jurkat T leukemia cells that overexpressed Bcl-2 were less sensitive to LfcinB-induced apoptosis, which was characterized by mitochondrial swelling and the release of cytochrome c from mitochondria into the cytosolic compartment. We conclude that LfcinB kills cancer cells by triggering the mitochondrial pathway of apoptosis at least in part through the generation of reactive oxygen species.

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Lactoferrin, a naturally occurring glycoprotein found in breast milk, has previously been shown to have antimicrobial properties and recently has been demonstrated to inhibit malignant tumor growth, presumably through immunomodulation. We hypothesized that intratumoral injection of human and murine recombinant lactoferrin would decrease the growth of malignant tumors in vivo. Using an orthotopic murine model for both squamous cell carcinoma and fibrosarcoma of the floor of the mouth, we administered lactoferrin directly into the tumors using variable dosing strategies. Additionally, we performed in vitro experiments to assess whether the effects of lactoferrin are due to direct cytotoxicity. Our results revealed growth inhibition of 50% (p=0.03) and 54% (p=0.01) as compared with controls for both human and murine tumor cells in immunodeficient and immunocompetent mice, respectively. There was a more dramatic effect in immunocompetent models which may identify immunomodulation as an important mechanism of action for lactoferrin. Support for immunomodulation as a possible mechanism was the lack of any difference between controls and the experimental groups in vitro. Lactoferrin proved effective in reducing malignant tumor
growth in a murine model. These properties offer hope for its use as a primary or adjuvant chemotherapeutic agent. Further investigation focused on mechanism and delivery is needed. Copyright 2003 S. Karger AG, Basel

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BACKGROUND: Bovine lactoferrin (LFB) and its pepsin-generated peptide lactoferricin (LfcinB) possess antitumor activities. The mechanism underlying the antitumor activities of LfcinB in vivo has not been elucidated. In this study the antitumor activities exerted by LFB, LfcinB and murine lactoferricin (LfcinM) on murine tumor cell lines and experimental tumors were investigated. MATEIALS AND METHODS: The protein and peptides were tested against Meth A fibrosarcoma, B16F10 melanoma and C26 colon carcinoma cells in vitro and their derived tumors in vivo, exploring the mechanisms of antitumor activity by way of histological and scanning electron microscopical studies. RESULTS: LfcinB exerted significant cytotoxic activity against the three tumor cell lines and experimental tumors were investigated. MATERIALS AND METHODS: The protein and peptides were tested against Meth A fibrosarcoma, B16F10 melanoma and C26 colon carcinoma cells in vitro and their derived tumors in vivo, exploring the mechanisms of antitumor activity by way of histological and scanning electron microscopical studies. RESULTS: LfcinB exerted significant cytotoxic activity against the three tumor cell lines in vitro and significantly reduced the size of solid Meth A tumors. Scanning electron micrographs revealed tumor cell membrane disruption and eventually cell lysis, while extensive hemorrhagic necrosis was apparent in tumor sections one day after LfcinB treatment. No species-specific antitumor effect of LfcinM was observed. CONCLUSION: Our study demonstrated that LfcinB elicits an antitumor effect mediated through a direct mechanism of action not observed with LFB or LfcinM.

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In experimental studies, bovine lactoferrin (bLF) has been found to significantly inhibit colon, esophagus, lung, and bladder carcinogenesis in rats when administered orally in the post-initiation stage. Furthermore, concomitant administration with carcinogens resulted in inhibition of colon carcinogenesis, possibly by suppression of phase I
enzymes, such as cytochrome P450 1A2 (CYP1A2), which is preferentially induced by carcinogenic heterocyclic amines. Enhancement of the activities of their phase II counterparts, such as glutathione S-transferase might have also played a critical role in post-initiation suppression in a study of tongue carcinogenesis. Anti-metastatic effects were moreover detected when bLF was given intragastrically to mice bearing highly metastatic colon carcinoma 26 cells (Co 26Lu), with apparent enhancing influence on local and systemic immunity. Marked increase in the number of cytotoxic T and NK cells in the mucosal layer of the small intestine and peripheral blood cells was thus found, this in turn enhancing the production of Interleukin 18 (IL-18) and caspase-1 in the epithelial cells of the small intestine, with possible consequent induction of interferon (IFN)-gamma positive cells. Furthermore, bLF has been found to exert anti-hepatitis C virus (HCV) activity in a preliminary clinical trial in patients with chronic active hepatitis due to this virus, a main causative factor in hepatocellular carcinoma development in Japanese. More extensive clinical trials are now underway in the National Cancer Center Hospital and other institutes to further explore the preventive potential against colon carcinogenesis.

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Ceruloplasmin and laprote based on natural protein antioxidants were used in the treatment and prevention of postoperative complications in 174 patients after extensive interventions for cancer. Development of pyoseptic complications during the postoperative period is associated with activation of lipid peroxidation, decreased functional activity of the antioxidant component of detoxication, suppressed T-cellular immunity, and development of polyorgan failure in the presence of endogenous toxemia. Systemic treatment with laprote (50 pts) and ceruloplasmin (33 pts) after surgical cleansing and draining of purulent focus stimulated activation of antioxidant defense, decreased the intensity of oxidative processes, normalized the lymphocytic component of immunity, and promoted resolution of polyorgan, primarily hepatic failure. Local therapy with laprote (60 pts) promoted rapid regression of local pyoinflammatory processes. Intraoperative blood loss complicated by hemorrhagic shock had a deep impact on the oxidative/antioxidant system, which correlated with the severity of hypoxia. Addition of ceruloplasmin, a potent antioxidant, to therapy of these patients (n = 31) optimized the course of recovery by stimulating the resolution of posthypoxic polyorgan failure, recovery of the oxidant/antioxidant balance, and decreasing the incidence of postoperative pyoinflammatory complications.

PMID: 11757307 [PubMed - indexed for MEDLINE]
In order to determine the effects of the multifunctional iron-binding glycoprotein, lactoferrin (LF), and related compounds on tumor growth and metastasis, bovine LF (bLF), and bLF hydrolysate and lactoferricin (bLFcin), active products generated by acid-pepsin hydrolysis were administered orally to BALB/c mice bearing subcutaneous (s.c.) implants of the highly metastatic colon carcinoma 26 (Co 26Lu). bLF and the bLF hydrolysate demonstrated significant inhibition of lung metastatic colony formation from s.c. implanted tumors without appreciable effects on tumor growth. bLFcin displayed a tendency for inhibition of lung metastasis. On the other hand, bLF did not exert marked anti-metastatic activity in athymic nude mice bearing Co 26Lu, though bLF had a tendency to inhibit the lung metastatic colony formation associated with anti-asialoGM1 antibody (Ab) treatment. AsialoGM1+ and CD8+ cells in white blood cells were increased after treatment with bLF. In vitro, the viability of Co 26Lu-F55 cells was markedly decreased when co-cultured with white blood cells from mice administrated bLF p.o., but recovered on treatment with anti-asialoGM1 Ab or anti-CD8 mAb and complement. The results suggest bLF and related compounds might find application as tools in the control of metastasis and that asialoGM1+ and CD8+ cells in the blood are important for their inhibitory effects.

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measured using MTT colorimetric method. The spontaneous and LPS-induced activity of TNF-alpha and IL-6 were measured with bioassays using indicator cell lines WEHI-164.13 and 7TD1, respectively. We demonstrated that LF inhibited the proliferative response, both spontaneous and LPS-induced, in all groups of patients. Lactoferrin alone was a good inducer of IL-6 and TNF-alpha production by monoclear cells in vitro. Addition of LF to the cultures of LPS-activated mononuclear cells stimulated IL-6 production, most markedly in the group of septic survivor patients (mean 1479, 1452, 1728, 1980 pg/ml on day 1, 2, 3 and 6 respectively). Lactoferrin also upregulated TNF-alpha production. That effect was very significant in the septic survivor patients (mean 7407, 6739, 7498 and 8509 pg/ml on day 1, 2, 3 and 5 respectively) and less pronounced in the group of trauma patients. We conclude that lactoferrin exhibited regulatory actions on the altered reactivity of PBMC from patients with sepsis and multiple injury. Lactoferrin is a good inducer of IL-6 and TNF-alpha production. However, in most cases of septic nonsurvivors LF could not abolish low reactivity of cells with regard to cytokine production.

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The effect of saliva on the adhesion of Candida albicans to epithelial cells was examined in vitro by using saliva from healthy controls and patients with oral squamous cell carcinoma. The adhesion of C. albicans to established epithelial tumor cells was reduced by 40% by salivary treatment of the C. albicans or epithelial cells. The inhibitory activity of saliva was almost completely abolished by anti-secretory immunoglobulin A antibody, concanavalin A, and mannose. Compared with saliva from healthy individuals, that from patients who had received chemoradiotherapy for oral carcinoma showed reduced suppression of C. albicans adhesion, which accompanied decreased salivary secretory immunoglobulin A and lactoferrin concentrations. A greater number of C. albicans cells adhered to buccal cells obtained from patients who had received chemoradiotherapy than to those from healthy individuals. Treatment of either epithelial cells or C. albicans with anticancer drugs induced an increase in adherence of epithelial cells and yeast cells. In contrast, concanavalin A- and mannose-pretreated C. albicans exhibited reduced adhesion to epithelial cells. No further decrease of C. albicans adhesion was observed when both epithelial cells and yeast phase C. albicans were treated with mannose. In conclusion, the inhibition of C. albicans adhesion by saliva depends largely on mannose residues on salivary glycoproteins and mannose is one of the binding ligands on both C. albicans and epithelial cells. In addition, anticancer therapy may induce oral C. albicans overgrowth by decreasing salivation and the concentrations of glycoproteins in saliva inhibiting C. albicans adhesion and by increasing the adhesive properties of both C. albicans and oral epithelial cells.

PMID: 7714204 [PubMed - indexed for MEDLINE]