Quantitation of human lactoferrin as an inflammation marker by an enzyme-linked immunosorbent assay (ELISA)

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Quantifizierung von Human Lactoferrin als Entzündungsparameter mittels Enzymimmunoassay vom Sandwich-Typ (ELISA)

Human lactoferrin (Lac) is an iron-binding glycoprotein of $M_R = 76{,}000$ and is found especially in milk and many other body fluids, such as tears, saliva, nasal and bronchial secretions, urine, seminal fluid, and in pancreatic fluid. Although the biological significance of Lac is still under discussion there is evidence for its antibacterial activity in milk and blood. Other biological roles of Lac include regulatory functions during intestinal iron absorption, in myelopoiesis, in neutrophil aggregation and adherence and in inflammation [1]. Lac appears to be synthesized mainly by polymorphonuclear leukocytes (PMN). It is localized intracellularly in the secondary or "specific" granules of these cells [2]. The cellular content of Lac is around 3–5 µg/10^6 cells. Normally Lac occurs in plasma only in trace amounts. During inflammation it is released from PMN with other lysosomal proteins [3, 4]. Measurement of Lac may be useful because studies suggest that Lac-concentrations in blood might reflect the activation of the total blood granulocyte pool [5]. Here we describe an enzyme-linked immunosorbent assay (ELISA) for human lactoferrin.

Materials and methods

Lactoferrin was isolated from human colostrum by copper chelate chromatography, ion-exchange chromatography, and gel filtration. The purity of the preparations was assessed by SDS- and immuno-electrophoresis. Antibodies against purified Lac were raised in sheep. Trace amounts of antibodies against human serum albumin were eliminated by absorption with normal human serum. IgG was isolated from monospecific antiserum by precipitation with n-octanoic acid and further purified on DEAE-cellulose. IgG was conjugated with calf intestinal alkaline phosphatase (Boehringer Mannheim) by condensation with glutardialdehyde [6]. Polystyrene tubes were coated with IgG by incubation of the tubes with a solution containing 20 µg IgG/ml in 0.01 mol/l NH_4HCO_3 at room temperature over night, washed and lyophilized. For the assay serum, EDTA-plasma or citrated plasma samples were routinely diluted 1/50 in phosphate buffer-saline, pH 7.4. 500 µl of the diluted samples were incubated in the IgG-coated tubes for 2 h at 25°C. A standard curve was derived over the range of 1 ng/ml to 10 ng/ml of human Lac as well as a blank value. Lac-standards for RID (Behring, Marburg) were used as external controls and diluted 1/10^6 before use. After removing the samples by aspiration and three washes with saline-polyoxyethylene(20)-sorbitan monolaurate the tubes were reincubated another 2 h at 25°C with 500 µl of conjugate in saline-Tris/HCl-buffer, pH 8.0. After aspiration of the conjugate the tubes were washed 3 times. 500 µl of 10 mmol/l 4-nitrophenylphosphate in 1 mol/l diethanolamine/HCl-buffer, pH 9.8, 0.5 mmol/l $MgCl_2$ were added and the tubes incubated at 25°C for 1 h. The enzymic reaction was stopped with 500 µl of 2 mol/l NaOH and the absorbance was read at 405 nm. Clinical studies were performed with citrated plasma samples.

Results and discussion

Linear calibration curves were obtained in the range of 0 to 10 ng Lac/ml. Correlation coefficients were higher or equal to 0.995. The Lac-concentration of samples could be calculated by linear regression. The detection limit, defined as mean absorbance of a blank value plus three times the standard deviation was about 0.25 ng Lac/ml. Within-run imprecision (coefficient of variation, $n = 10$) was in the range of 1.1 to 6.6% with the standards and 2.3 to 5.3% with samples (serum, EDTA-plasma, citrated plasma) in the range of 65 to 250 ng Lac/ml. Between-run imprecision ($n = 10$) was from 2.8 to 7.9% with the standards and 3.7 to 10.4% with the samples. Within-run imprecision ($n = 4$) for one indoor control plasma and two external Lac-standards (see Materials and methods) ranged from 1.4 to 7.3%, the between-run imprecision ($n = 10$) was 12.5%, 10.3%, and 8.0% for these samples. The normal range of Lac-concentrations in serum, EDTA-plasma or citrated plasma of apparently healthy persons were (mean ± SD) found to be 202.4 ± 40.8 ng/ml ($n = 10$), 115.4 ± 35.4 ng/ml ($n = 35$), and 136.6 ± 51.4 ng/ml ($n = 37$), respectively. The higher values for serum samples could be interpreted as release of Lac during blood clotting. Similar differences between serum and plasma were observed by others with RIA [7]. In clinical cases Lac-concentrations found exceeded the normal ranges by a factor of 5 to 10. Remarkably, preliminary data of patients suffering from multiple trauma and/or severe infections showed a concomitant release of Lac from the specific granules and elastase from the azurophilic ones. The latter enzyme has been taken as a main indicator for the participation of polymorphonuclear proteins in inflammatory reactions [8]. The results suggest that in contrast to in-vitro stimulation of PMNs [9] both types of granules has been equally involved in phagocytosis and extracellular liberation of the lysosomal proteins in these underlying kinds of inflammation. Measured Lac-concentrations exceeded that of the PMN-elastase-α_1-proteinase inhibitor complex.

On the other hand, measurement of PMN granule constituents in plasma samples drawn in very short intervals (5–10 min) before and after open heart surgery indicate that Lac is released more readily and to a greater degree than elastase in the first 2–3 h following surgery. The clinical meaning of the different in-vivo discharge of specific granules compared to azurophilic ones is currently under investigation.

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