

Endogenous antibodies to bovine lactoperoxidase in children and adolescents

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Sera from 146 children and adolescents (70 girls and 76 boys, median age 6 years; range 8 months - 18 years) were analysed for the presence of antibodies to bovine lactoperoxidase (LPO) using a solid-phase immunosorbent radioassay. LPO antibodies were present in all but one serum. The antibody titres varied from 1/5 to > 1/5000 (final dilutions). Most of the individuals had antibody titres between 1/50 to 1/5000. Because of the frequent occurrence of LPO antibodies in human beings, the presence of LPO antibodies in the serum must be regarded as a physiological phenomenon.

Key words: endogenous autoantibodies; immunosorbent radioassay; lactoperoxidase.

Accepted for publication 22 January 1987

CLINICAL ASPECTS

Endogenous antibodies to bovine lactoperoxidase (LPO) were demonstrated in human beings from the age of 8 months throughout life and therefore must be regarded as a physiological phenomenon. Absence of LPO antibodies was observed in less than 1 % of the children. From the present work no conclusions can be drawn about the clinical significance of a lack of LPO antibodies or about the physiological role of these antibodies.

Lactoperoxidase (LPO) is an enzyme present in saliva, tears and milk from many different mammals including man. Human colostrum and breast milk have low LPO activity compared with cow's milk (8). There is also a slight decrease in enzyme activity with time after delivery (8). The importance of the LPO system *in vivo* has never been completely clarified in adults or in newborn infants. It is supposed that lactoperoxidase and thiocyanate of the salivary secretions play an important part in the protection of the oral mucosa against hydrogen peroxide-induced lesions (10). The LPO system may be of importance in preventing clinical infections during the bacterial colonization of newborn infants (8).

Antibodies to bovine milk proteins have been reported to be present in a high percentage of men (5, 12). These antibodies are directed against lactalbumin. In a previous study we

have demonstrated endogenous IgG antibodies to bovine LPO in 89 % of healthy adults (6). As LPO is present in the newborn infant, we wanted to delineate the occurrence of LPO antibodies in children and adolescents.

MATERIAL AND METHODS

Patients

Sera were obtained from 146 consecutive patients (76 boys and 70 girls; median age 6 years; range 8 months-18 years) admitted to hospital. In 50 of the patients information on the duration of breast-feeding could be obtained. The length of the breast-feeding period varied from 4 weeks to 8 months. LPO antibodies in the serum of children up to the age of 6 months may be derived from the mother

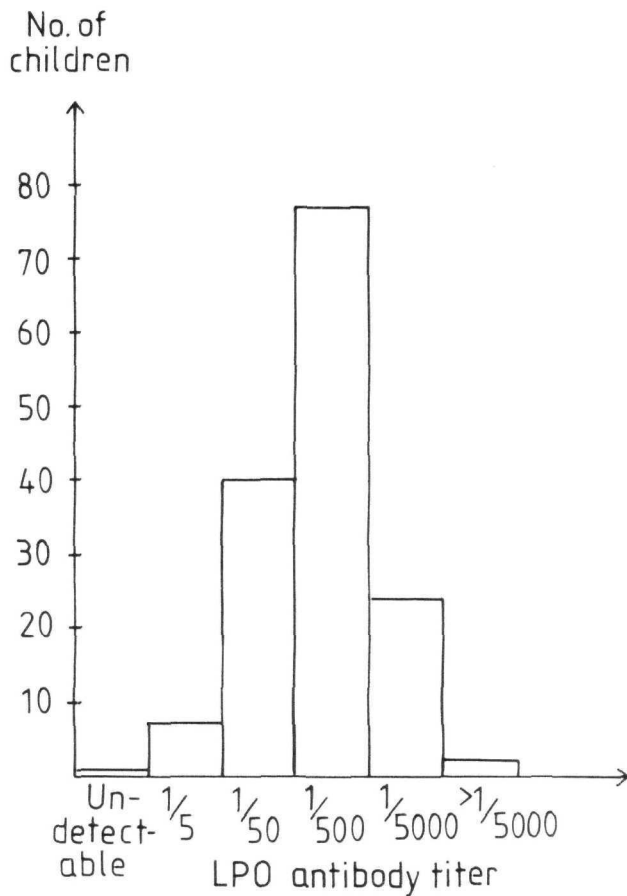


Fig. 1. The distribution of the lactoperoxidase antibody titres.

through placental transfer or through breast-feeding. Therefore only children from 8 months were included in the study.

Assay

The LPO antibodies were measured with a solid-phase immunosorbent radioassay as described in detail elsewhere (6). The LPO was labelled with Na^{125}I by self-iodination. One hundred microlitres of serum was mixed with 400 μl of the ^{125}I -labelled antigen and incubated over night at $+4^\circ\text{C}$. Fifty microlitres of the incubate was mixed with 250 μl of a Sepharose-anti-human-IgG suspension and incubated 2 h at room temperature. To each tube 1 ml barbital buffer, 0.075 mol/l, pH 8.6, with bovine serum albumin, 20 g/l, was added and the tubes were centrifuged 15 min at $2400 \times g$ and $+4^\circ\text{C}$, decanted and the radioactivity of the precipitate measured. The results were expressed as the bound ^{125}I anti-

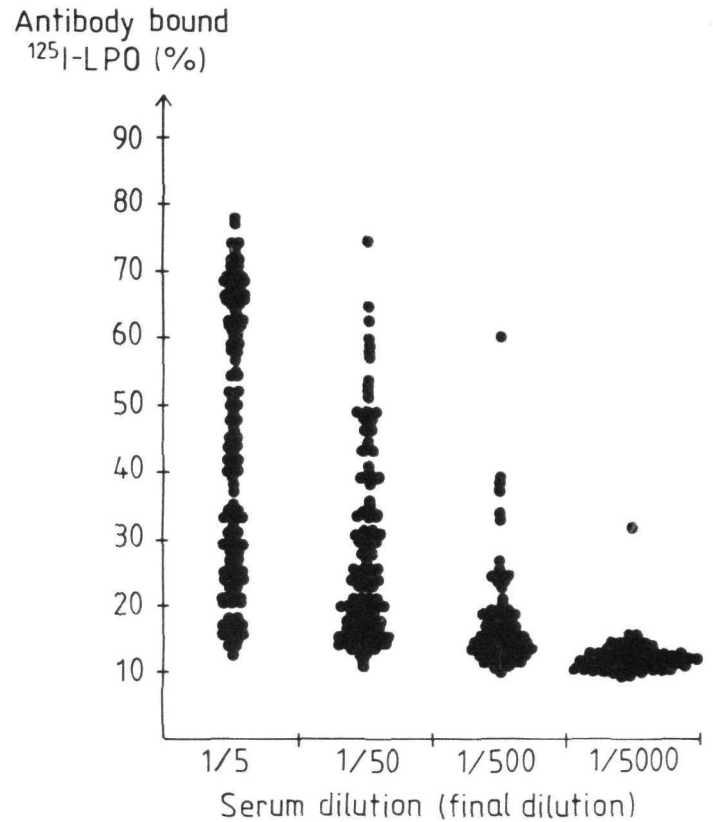


Fig. 2. Antibody binding of ^{125}I -labelled lactoperoxidase in different dilutions of the same serum samples (final dilutions) from 146 children.

gen in per cent. All samples were assayed in serial dilutions.

Statistical methods

The Mann-Whitney's test was used for unpaired observations and the co-variation was calculated with the use of the product-moment correlation coefficient.

RESULTS

The distribution of the LPO-antibody titres among the 146 children is illustrated in Fig. 1. LPO antibodies were detected in the serum of all but one patient. A low titre (1:5, final dilution) was observed in seven individuals (5%). Most children had an LPO-antibody titre from 1:50 to 1:5000. Two subjects had a very high antibody titre ($> 1:5000$). The results of the serial dilutions in each individual are illustrated in Fig. 2. There was no correlation between the LPO-antibody titre and age or sex, nor was

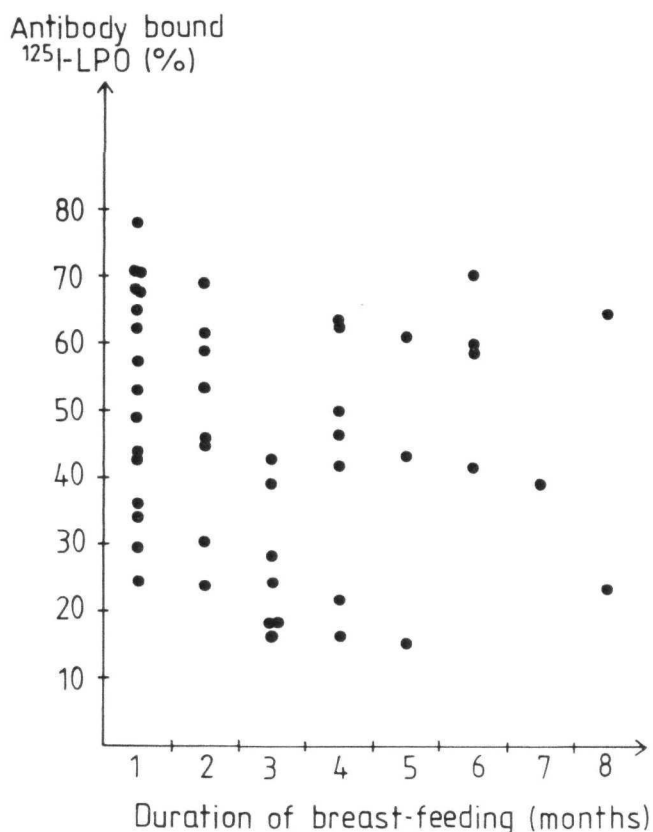


Fig. 3. Correlation between the antibody bound ¹²⁵I-labelled lactoperoxidase and the duration of breast-feeding.

there any correlation to the duration of breast-feeding (Fig. 3). Forty children had a diagnosis of allergy. The median LPO-antibody titre was the same in these children as in the 106 without known allergy (median titre 1:500 in both groups).

DISCUSSION

We have for the first time demonstrated that endogenous antibodies to bovine LPO are present in most human beings from early years throughout life. The antibody titres in the children and adolescents are of the same order as those previously described in healthy adults (6). Allen & Morrison (1) have shown that bovine and human LPO are immunologically related. Therefore, the LPO antibodies detected by us may be directed against the endogenous LPO and perhaps constitute an integrated part of the LPO system. Absence of LPO antibodies is a rare phenomenon (one of 146 individuals, 0.7%). The significance of the lack of LPO

antibodies is not known. The LPO system is thought to play an important role in the protection of the oral mucosa against infections (8, 10). It has been shown to inhibit several *Streptococci* (11), *Staphylococcus aureus*, *Escherichia coli*, *Candida tropicalis* (9) and some viral species (2).

The role of the LPO antibodies in this system is not known. In some instances specific antibodies to an enzyme may potentiate the effect of the enzyme or prolong its effect by causing alterations in structural configuration or by preventing enzymatic degradation (3, 4, 7). If the effect of the LPO antibodies in the system really is to potentiate the effect of LPO, then a lack of antibodies to LPO may result in an increased sensitivity to certain infectious agents. This is, however, purely speculative. Further work has to establish the physiological role of the LPO antibodies in the LPO system and to evaluate the clinical importance of a lack of LPO antibodies.

REFERENCES

1. Allen, P. Z. & Morrison, M.: Lactoperoxidase. VI. Immunochemical studies on lactoperoxidase from the milk of several species. *Arch. Biochem. Biophys.* **113**, 540-547, 1966.
2. Belding, M. E., Klebanoff, S. J. & Ray, C. G.: Peroxidase-mediated virucidal systems. *Science* **167**, 195-196, 1970.
3. Butler, Jr., V. P.: Antibodies as specific antagonists of toxins, drugs, and hormones. *Pharmacol. Rev.* **34**, 109-114, 1982.
4. Cinader, B.: Antibodies to enzymes - A discussion of the mechanisms of inhibition and activation. In Cinader, B. (ed): *Antibodies to biologically active molecules*, pp. 85-137. Pergamon Press, Oxford, 1967.
5. Davies, D. F., Rees, B. W. G., Johnson, A. P., Elwood, P. C. & Abernethy, M.: Food antibodies and myocardial infarction. *Lancet* **1**, 1012-1014, 1974.
6. Ericsson, U.-B. & Larsson, I.: Interference of endogenous lactoperoxidase antibodies in a solid-phase immunosorbent radioassay for antibodies to protein hormones. *Clin. Chem.* **30**, 1836-1838, 1984.
7. Goodfriend, T. L., Webster, M. E. & McGuire, J. S.: Complex effects of antibodies to polypeptide hormones. *J. Clin. Endocrinol. Metab.* **30**, 565-572, 1970.
8. Gothefors, L. & Marklund, S.: Lactoperoxidase activity in human milk and in saliva of newborn infants. *Infect. Immunol.* **11**, 1210-1215, 1975.
9. Hamon, C. & Klebanoff, S. J.: A peroxidase mediated, streptococcus mitis-dependent antimicrobial system in saliva. *J. Exp. Med.* **137**, 438-450, 1973.

10. Hännström, L., Johansson, A. & Carlsson, J.: Lactoperoxidase and thiocyanate protect cultured mammalian cells against hydrogen peroxide toxicity. *Med. Biol.* 61, 268-274, 1983.
11. Morrison, M. & Steele, W. F.: Lactoperoxidase; the peroxidase in the salivary gland. In: *Biology of the mouth*, pp. 89-110. American Association for Advancement of Science, Washington D.C., 1968.
12. Toivanen, A., Viljanen, M.K. & Savilahti, E.: IgM and IgG anti-milk antibodies measured by radioimmunoassay in myocardial infarction. *Lancet* 2, 205-207, 1975.

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