Serum placental type alkaline phosphatase in cigarette smokers

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SUMMARY By means of enzyme immunoassays based on two monoclonal antibodies with specificities for distinct forms of placental type alkaline phosphatase (PI-ALP), together with L-leucine inhibition studies, it has been possible to distinguish the Nagao type carciino-placental enzyme from other placental type alkaline phosphatases. This approach has shown that it is the Nagao type (placental like) enzyme which is detectable in small amounts in the plasma of healthy individuals, particularly cigarette smokers.

Human placental type alkaline phosphatase (PI-ALP) is a thermostable genetically polymorphic isoenzyme of placental trophoblast membranes.12 It was designated a carcinoplacental protein when identified also in the circulation of certain patients with cancer.13-14 PI-ALPs derived from tumour cells form at least two distinct groups: the Regan type enzyme is closely similar or identical to placenta derived PI-ALP,13 and the Nagao type enzyme is a parallel but antigenically and biochemically distinct grouping.13-15 Nagao type PI-ALP is distinguishable from placenta derived PI-ALPs and the Regan enzyme by its sensitivity to inhibition by L-leucine and different antigenic profile revealed with monoclonal antibodies.16-17 Recently, trace amounts of Regan like PI-ALP have been found in non-malignant lung, cervical, ovarian, and mammary tissues,18 whereas Nagao like PI-ALP has been detected in non-malignant testicular as well as cervical tissues.19-21 This latter PI-ALP has also been termed placental like alkaline phosphatase.7

There has been recent interest in the evaluation of PI-ALP as a tumour marker using monoclonal antibodies specific for this isoenzyme. Circulating PI-ALP is found in low concentrations in some healthy individuals, however, especially cigarette smokers;6-11; the most obvious origin would be from lung tissue, and the serum enzyme would thus be expected to be of the Regan type.7 By means of sensitive and specific immunoassays based on monoclonal antibodies to PI-ALP,7,12 together with L-leucine inhibition, it has been possible to discriminate accurately between Regan like and Nagao like PI-ALP in the sera of healthy smoking and non-smoking subjects as well as in non-smoking patients with non-malignant inflammatory lung disease.

Patients and methods

SUBJECTS Twenty eight healthy individuals were divided into two groups: 16 smokers (>10 cigarettes/day) and 12 non-smokers. Their ages ranged from 20 to 34 years (mean ± SD, 24 ± 3.5 years). A third group consisted of 11 non-smoking patients with non-malignant chronic inflammatory lung disease who had fibrosing alveolitis (one patient), bronchiectasis (two patients), asthma (seven patients), and asthma with pneumonia (one patient). The age range of these patients was 19 to 65 years (mean ± SD, 42 ± 14 years). None of these subjects had experienced a recent pregnancy.

Solid phase enzyme immunoassays for PI-ALP based on two separate murine IgGl monoclonal antibodies (H317 and H17-E2) were performed. The monoclonal antibody H317 reacts with most human placenta derived PI-ALPs, but not with any intestinal alkaline phosphatase or liver/bone/kidney alkaline phosphatase isoenzymes.7,13 It is reactive with tumour derived Regan enzyme and PI-ALP found in normal lung, cervix, ovary, and breast tissue, but is unreactive with tumour derived Nagao enzyme and the PI-ALP from normal testicular tissue.7 The monoclonal antibody H17-E2 reacts with all forms of PI-ALP from whatever source, but not...
Serum PI-ALP concentrations, estimated by enzyme immunoassay with H17-E2, in relation to smoking and lung disease. Open symbols represent L-leucine resistant PI-ALP and closed symbols represent L-leucine sensitive PI-ALP. ◦ female, □ male. A = cigarette smokers (n = 12); B = non-smokers (n = 16); C = inflammatory lung disease (n = 11).

with other tissue alkaline phosphatase isoenzymes.7-14

The enzyme immunoassay was performed as described previously.7-12 Briefly, each monoclonal antibody was immobilised on to MicroElisa plate wells using rabbit antimouse immunoglobulin (Dakopatts A/S), and then test plasma samples were added at 1/3 dilution; any bound PI-ALP was subsequently detected by colour change in a phosphatase substrate. This assay has a lower limit of sensitivity of 0.1 U/l PI-ALP.12 All tests were performed in quadruplicate with alternate duplicates having 0.5 mM L-leucine included in the substrate solution so that parallel estimations of L-leucine sensitivity could be made.

**Results**

H317 reactive PI-ALP was not detected in the sera of any of the 16 healthy non-smokers or 12 healthy smokers using a lower limit in enzyme immunoassays of 0.1 U/l PI-ALP. In contrast, H17-E2 reactive PI-ALP activity of >0.1 U/l was found in the sera of 5/7 (71%) male smokers and 3/5 (60%) female smokers at concentrations of up to 1.7 U/l (Figure). This activity (mean ± SD, 0.39 ± 0.49 U/l) was clearly inhibited by L-leucine (that is, Nagao type) in 4/5 (80%) of the male smokers and in 2/3 (67%) of the female smokers. In one male smoker and one female smoker the circulating PI-ALP activity was of an unusual type; it was unreactive with H317 (and hence not Regan type enzyme) and reactive with H17-E2 but L-leucine resistant (and hence clearly not Nagao type enzyme) (Figure). This synplacental alkaline phosphatase was also found in the circulation of one of the male non-smokers, but none of the other healthy non-smokers had detectable concentrations (>0.1 U/l) of H17-E2 reactive PI-ALP.

None of the non-smokers with chronic inflammatory lung disease had detectable amounts (>0.1 U/l) of circulating PI-ALP reactive with H317, but 4/11 (36%) did have trace amounts (mean ± SD, 0.10 ± 0.08 U/l) of PI-ALP reactive with H17-E2 and sensitive to L-leucine inhibition (that is, Nagao type enzyme) (Figure).

**Discussion**

Small amounts of circulating PI-ALP may be found in some non-pregnant individuals without malignancy,10-11 and this was clearly shown in 6/9 PI-ALP positive cases to be of the carcinoplacental Nagao or closely related form of placental like alkaline phosphatase. This serum PI-ALP is characterised by being H317 unreactive, H17-E2 reactive, and sensitive to L-leucine inhibition, and is the form that is often found in raised concentrations in the sera of cigarette smokers. PI-ALP with such characteristics has previously been found only in malignant tissues and normal testicular and cervical tissue.5-7,9 Hence, the source of this normal circulating enzyme is unclear since tissue extracts from normal lung (of both smokers and non-smokers) (Williams and Johnson, unpublished observations) contain PI-ALP which is predominantly, if not all, of the Regan type.7 Nevertheless, coexpression of both Nagao and Regan type enzyme has been reported in other tissue of both malignant and non-malignant origin, sometimes in widely differing proportions.7,8,12 Thus, it is possible that small amounts of a Nagao type enzyme may be readily released from lung tissues. This is partly supported by the observation that occasional non-smoking patients with chronic inflammatory disease of either bronchial or parenchymal lung tissue had slightly raised serum PI-ALP concentrations, although the alternative explanation that cigarette smoking may promote release of a Nagao type (placental like) enzyme from another body organ remains a possibility. It will be of interest to determine, with sensitive enzyme immunoassays based on monoclonal antibodies, whether variations in serum PI-ALP reflect changes in any clinical disorders associated with cigarette smoking.
The trace amounts of serum PI-ALP found in three of the other healthy individuals may represent an unusual form related to the Nagao type enzyme. This was H317 unreactive and H17-E2 reactive, and hence antigenically resembled the Nagao (placental like) enzyme, but was not inhibited by L-leucine (unlike the Nagao type enzyme). This PI-ALP was heat stable at 65°C for 1 h. The only heat stable PI-ALP known to have this antigenic and biochemical profile is the homozygous 2-2 (FF) genetic phenotype of placenta derived PI-ALP. This is found in about 9% of pregnancies. Hence, it is possible that the similar PI-ALP found in trace amounts in occasional healthy non-pregnant sera may be a corresponding allotypic form of a Nagao like enzyme.

Cigarette smoking seems to have a pronounced effect on the production or cellular release, or both, of a carcinoplastic Nagao like PI-ALP. Indeed, the presence of this form of PI-ALP in the sera of healthy individuals restricts its use as a tumour marker. However, a sensitive assay able to identify accurately Regan type enzyme separately from Nagao type enzyme, such as that based on the H317 monoclonal antibody, may be a more specific carcinoplastic parameter.

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References


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