TAILORING OF AN ANTI-HUMAN PLACENTAL ALKALINE PHOSPHATASE IMMUNOGLOBULIN USING GENETIC ENGINEERING.

A. VAN DE VOORDE, V. FEYS, P. DE WAELE, P. CASNEUF, W. FIERS.


1. HUMAN PLACENTAL ALKALINE PHOSPHATASE.

Human placental alkaline phosphatase (hPLAP; E.C. 3.1.3.1.) is a member of a group of enzymes named according to the organ in which they predominate. Other members of this group include an intestinal isoenzyme (IAP) and a tissue non-specific isoenzyme found in liver, bone and kidney (LAP).

hPLAP normally occurs in the microvilli of the syncytiotrophoblast (1) and can be detected in sera of pregnant women in rising concentrations starting from the 13th week of pregnancy (2, 3). The enzyme acts as a dimer with a M.W. of 130 kDa and has a subunit M.W. of 67 kDa. It is a metalloenzyme containing 4 Zn\(^{2+}\) atoms per molecule and it appears to be glycosylated (for reviews see 4, 5). Its partial amino acid sequence has been determined by amino acid sequencing (6). Its complete amino acid sequence was deduced from the nucleotide sequence of the cloned cDNA (7).

hPLAP is a highly polymorphic protein for which more than 20 allelic variants were described (8), but only 3 alleles make up 6 homozygotic and heterozygotic phenotypes that occur in 98% of all phenotypes found, while the remaining 2% are made up of rare variants (9, 10).

The renewed interest in hPLAP stems from the fact that apart from its eutopic expression in the serum of pregnant women it was also found in sera and tumor tissues of various cancer patients (11-19). The enzyme found in tumor tissues displays the same pattern of phenotypic variation, although historically 3 major ectopic variants were described: the "Regan" variant, later identified as the normal placental enzyme (20), the "Nagao" variant, shown to closely resemble the rare D-variant (20, 21) and the "Kasahara" variant, thought to be a heterodimer composed of hPLAP and the fetal form of hIAP (22). hPLAP or its Nagao variant (recently renamed PLAP-like AP) has also been detected in very low amounts in various types of normal tissues, including thymus, cervix, endometrium, Fallopian tube and testis (23-26). The hPLAP-like AP can regularly be found in the serum of smokers (27, 28).

Previously, the detection of hPLAP was severely hampered by the close resemblance of the biochemical and biophysical properties of hPLAP and IAP. Usually, the distinction between the 3 isoenzymes (PLAP, IAP, LAP) was based on
characteristics such as heat-inactivation (29), sensitivity to uncompetitive inhibitors (4, 30), differential migration in acrylamide, agarose or starch gels (4) and differentiation of these isoenzymes was also attempted by immunological means (31, 32). The latter method is seriously hampered by the cross-reactivity between the intestinal and the placental isoenzymes, due to the fact that the two enzymes share a number of tryptic peptides (33) and on the level of the nucleotide sequence almost 90% homology in the translated regions is found between the two enzymes (34). Until recently, the cross-reactivity between hPLAP and hIAP could only be eliminated by intensive adsorption of the polyclonal sera used (35). With the advent of the hybridoma technology, monoclonal antibodies were prepared that not only differentiate completely between hPLAP and hIAP but also enable the partial discrimination of hPLAP and the PLAP-like AP variant (2, 21, 28, 36, 37). Monoclonal antibodies directed against hPLAP thus allowed the accurate and sensitive detection of the enzyme. Assays, developed in various laboratories, used the fact that anti-hPLAP monoclonal antibodies do not interfere with the activity of the enzyme and as such the antigens own enzymatic activity could be used for quantification with sensitivities as low as 50 micrograms per liter using p-nitrophenylphosphate and 0.5 micrograms per liter using 4-methyl-umbelliferylphosphate (2, 32, 38).

In order to evaluate hPLAP as a tumor associated protein a multicenter study was set up under supervision of Prof. M. De Broe (Dept. Nephrol., Univ. Hospital Antwerp, Belgium) in which 17 institutions in 8 countries were engaged. In this study the monoclonal antibody 327 was used (39) to determine the amounts of hPLAP found in the sera of 506 cancer patients. The results showed that elevated hPLAP levels (>0.1 U/l) occurred in 90% of all seminomas, 73% of testicular cancers, 48% of ovarian cancers, 20% of lung cancers, 15% of gastro-intestinal cancers and in 10% of breast cancers. A comparison of tumor marker distribution in several types of cancer allowed a rating according to sensitivity (40). It was shown that hPLAP is a first choice marker for testicular cancers and a strongly advised marker for ovarian cancers, mainly due to its low frequency of false positives which usually can be ascribed to interference from smoking (27, 38, 41, 42). hPLAP was also detected in butanol extracted, homogenised tumor tissues and in histochemical paraffin sections of tumor tissues. Here a serious discrepancy was found between the number of seropositive patients as compared to tissue positive patients (17, 19). In all cases substantially more tissue-positivity was detected than seropositivity (for breast cancer: 5.2% seropos. versus 43% tissue pos.; for ovarian cancer: 25 to 54% seropos. versus 68 to 94% tissue pos.; pooled results from ref. 17, 19, 25, 38).

In view of these findings, monoclonal antibodies directed against hPLAP have opened new therapeutic possibilities, such as the possible use of tumor-directed monoclonal antibodies for radioimmunolocalization and radioimmunotherapy, and as such have spurred numerous studies. At first these were performed on nude mice xenografted with human tumors, but now