VARIABILITY OF ALKALINE PHOSPHATASE ACTIVITY IN HUMAN DIPLOID CELLS *IN VITRO*

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More publications have been devoted to the group of enzymes classified as alkaline phosphatase (AlP) than to any other enzyme system. Their wide distribution in nature, including bacteria, plants, and animals, is evidence that AlP participates in fundamental biological processes; however, the biological role of these isozymes has not yet been explained.

In man there are two tissue-specific AlP isozymes, namely placental and intestinal, which differ in their electrophoretic properties, the degree of their inhibition by various factors, and their antigenic determinants. A third isozyme, also called tissue-specific, has been found in the liver, kidneys, bones, lungs, and other tissues. This is more likely to be a group of isozymes without any marked tissue specificity, sharing some common properties, and differing from the first two isozymes [12].

It is considered that there are at least three different genes coding for AlP, but their chromosomal localization in man is unknown [6]. AlP activity is modified in many diseases. Some isozyme variants of AlP are related to oncofetal antigens [12]. It has also been suggested that an "induction of AlP activity" test be used in the prenatal diagnosis of hereditary diseases [7]. However, most investigations into the study of AlP have been undertaken on permanent lines of transformed cells (HeLa, for example). It is not yet known whether these data are applicable to human diploid cells.

The aim of the present investigation was accordingly to study manifestations of AlP activity in human diploid cells *in vitro*. A cytochemical method of detection of activity was chosen to enable AlP to be determined in individual cells, because biochemical data are the result of analysis of the "gross" cell biomass. The well documented fact of heterogeneity of a population of cultured cells is ignored in this case.

EXPERIMENTAL METHOD

Nineteen strains of human diploid cells were used. Sixteen of them were obtained from different tissues (skin, lung, muscle tissue) of 8-12-week fetuses obtained from medical abortions, three strains were obtained by skin biopsy from healthy donors aged 25-35 years. The usual method was used to obtain primary cultures. Separation of muscle tissue into myoblasts and fibroblasts was based on the different rate of adhesion of the cells to the surface of a collagen-coated Petri dish [1]. The conditions of culture and of the cloning experiments were described previously [4]. AlP was determined cytochemically by the azo-coupling method [2]. The numerical results were subjected to statistical analysis by the usual method [3].

EXPERIMENTAL RESULTS

The number of AlP positive cells depended largely on the stage of growth of the culture. It was small in actively proliferating cultures and correlated positively with cell density and length of stay in hospital. At all stages of culture heterogeneity of strains for AlP was observed (only some cells stained positively).

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TABLE 1. Frequency of Colonies Positive for AlP in Strains Obtained from Different Fetal Tissues and in Strains of Skin Fibroblasts from Healthy Donors

Tissue	No. of strains	Frequency of positive colonies, %
Skin of healthy adult donor	3	2
Fetal skin	4	5
Muscle tissue fibroblasts	5	75
Muscle tissue myoblasts	4	91
Lung	3	0

Legend. At least 300 colonies were counted for each strain.

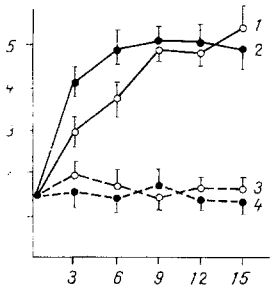

Fig. 1

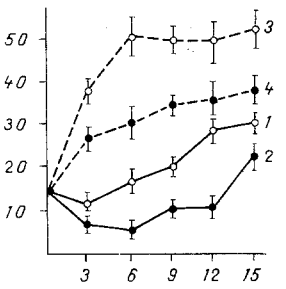

Fig. 2

Fig. 1. Effect of conditions of culture of multiplication of cells. 1) Eagle's medium +15% serum +10^{-6} M dexamethasone, 2) Eagle's medium +15% serum, 3) Eagle's medium +0.5% serum +10^{-6} M dexamethasone, 4) Eagle's medium + 0.5% serum; vertical lines indicate 95% confidence interval. Abscissa, time of culture (in days); ordinate, number of cells/cm^2 (×10^4).

Fig. 2. Effect of conditions of culture on frequency of cells positive for AlP. Abscissa, time of culture (in days); ordinate, frequency of cells positive for alkaline phosphatase (in %).

The results showing the effect of conditions of culture and of dexamethasone (10^{-6} M) on cell proliferation are shown in Fig. 1, and their effect on the number of cells positive for AlP in the strain of fibroblasts obtained from fetal muscle tissue in Fig. 2. Blocking cell growth by contact inhibition (when the culture reached saturation density) or during "hunger block" (0.5% serum) led to a significant increase in the number of AlP-positive cells.

The presence of dexamethasone affected both the dynamics of these changes and the number of cells positive for AlP.

The increase in AlP activity stimulated by dexamethasone has been shown to be connected with the effect of the hormone on mevalonate metabolism [9]. Mevalonate (a plasma membrane glycoprotein) is a precursor of cholesterol and takes part in the regulation lying at the basis of the increase in AlP activity during contact inhibition and during "hunger block" is the same.

Data on the prevalence of positive (over 50% of cells stained) colonies for AlP in strains obtained from different fetal tissues and in strains of skin fibroblasts from healthy adults are given in Table 1. The colonies were stained on the 18th day of culture.

It must be concluded from these results that manifestation of AlP activity exhibits marked tissue specificity. The highest values were obtained for strains of muscular origin. It is an interesting fact that when myoblasts fused together (terminal differentiation) their AlP activity disappeared.

On the basis of the results we consider that AlP activity, because of its considerable variability within the strain and between strains, and its marked dependence on external conditions, cannot be recommended as a sufficiently reliable indicator of hereditary pathology in prenatal diagnosis. The prevalence of colonies positive for AlP may evidently provide an indication (at least for fetal cells) of the tissue to which the strain belongs.

To study inherited AlP activity a strain giving 98% of colonies positive for AlP on cloning was selected. These colonies consisted entirely of cells positive for AlP. The remaining 2% contained single unstained cells. From this particular strain five clones were isolated, and immediately after isolation they were subcloned. AlP activity in the resulting subclones was found to be variable. Three types of subclones were observed: 1) positive, consisting entirely of stained cells; 2) negative, consisting entirely of unstained cells; 3) mixed subclones. On the whole the intensity of staining and, because of this, clarity of the "AlP activity" trait, were depressed. Nevertheless, these results, together with those obtained by subcloning of clones negative for AlP, indicate that cells negative for AlP remain negative, whereas cells positive for AlP, on division, give rise to both positive and negative descendants.

A decrease in AlP activity during long-term culture was found to be characteristic not only of clonal lines of diploid cells, but also of the original strains. A decrease in AlP activity during long-term culture also was observed by other workers [13]. This is particularly characteristic of strains obtained from fetal muscle tissue, which has very intensive AlP activity in most cells exhibiting such activity at early passages (5th-10th) but hardly perceptible in cells at late passages (45th-55th).

The study of inheritance of the "AlP activity" trait in permanent lines of transformed cells *in vitro* led to the following conclusions: 1) clones inheriting this trait consistently in all cells can be distinguished; 2) in some clones there is constant segregation into positive and negative cells; 3) clones negative for AlP produce only negative descendants, but there are exceptions to this rule; 4) hybrids between cells positive and negative for AlP are positive, evidence of dominance of the "AlP activity" trait [6, 10].

Comparison of these data with results obtained on human diploid cells shows that the spectrum of variability of the "AlP activity" trait is wider for transformed cells.

Results obtained by Baskin [5], who studied mechanisms of crossed drug resistance to adriamycin, vincristine, maitenin, and Baker's antifol (triazinate) on mutant neuroblastoma lines, are extremely interesting in our view. A decrease in activity of most lysosomal enzymes was found in cells of all lines, except AlP, whose activity was 20-150 times higher than normally. It was concluded that crossed drug resistance is due to amplification of AlP genes.

If it is assumed that amplification of AlP genes lies at the basis of control of activity of the enzyme, and that during cell division there is an unequal distribution of the number of gene copies among daughter cells (and the grounds for this last hypothesis also do exist), the inheritance of the "AlP activity" trait as described above in a series of cell generations obtains an acceptable explanation.

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