and immune organs, such as the SH cells within the PLN. In the previous report [3], we made a suggestion about the origin of the SH cells, that is, that the cells are probably differentiated from mesenchymal cells in the capsule of the PLN as the result of a stimulation with ovarian exudate via directly connected lymphatics, following ovulation. Recently, Kochhar [23] reported that steroid hormones induced a sister-chromatid exchange in mammalian cells, suggesting that the environment in the cells was more mutagenic. Thus it may be that steroid hormones produced by the SH cells play an important role in the rearrangement of immunoglobulin genes due to antigen-stimulated B lymphocyte maturation in the follicle of the PLN. Although we were able to demonstrate 3β-HSD activity in the SH cells within the PLN, no direct evidence linking the PLN immune functions and the physiological role of SH cells was obtained. For the present, the biological significance of the SH cells remains unknown; further studies are needed to elucidate the problem of function, especially concerning the effect of the steroidogenic SH cells on the immune cells of the PLN.

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Effect of human serum on alkaline phosphatase induction in cultured human tumor cells

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Summary. The continuous cell lines T 24 and HT-29, derived from human bladder and colon carcinomas, produce term-placental and intestinal alkaline phosphatase, respectively. Growth in hyperosmolar medium or exposure to prednisolone or sodium butyrate induces increased enzyme levels, and combinations of inducers elicit synergistic activity increases. The effect of the inducing agents is strikingly diminished when cells are grown in the presence of high concentrations of human serum, and the synergistic increases are essentially abolished. Major human serum protein fractions do not affect alkaline phosphatase induction.
Key words. Alkaline phosphatase; human serum; cultured tumor cells; enzyme induction.

Alkaline phosphatases (ALPs) (orthophosphoric-monoester phosphohydrolase (alkaline optimum) EC 3.1.3.1) are glycosylphosphatidylinositol-anchored cell membrane metalloenzymes [1]. At least three major, easily distinguishable enzyme forms (term-placental, intestinal and liver/bone/kidney or tissue-unspecific) have been identified in humans [2]. ALPs are among the substances produced ectopically by many human cancers [3] and are synthesized by certain tumor-derived cell lines [4]. Previous studies [5] have shown that primate sera, but not sera from other mammals reversibly depress ALP activity in HeLa cells and their derivatives in a concentration-dependent fashion, without affecting the enzyme characteristics. Here we compare the effect of human serum (HS) on

term-placental and intestinal ALP produced by cultured human bladder[6] and colon[7] cancer cells, respectively, and show that at high concentrations it inhibits enzyme induction by glucocorticoids, hyperosmolality and sodium butyrate. As the factors which directly or indirectly affect tumor cells in vivo are usually blood-borne, these observations may represent an in vitro reflection of the ability of cells to respond to in vivo microenvironmental fluctuations.

Materials and methods

T 24 and HT-29 cells were provided by Drs Carol O'Toole and Jørgen Fogh. Cells were routinely grown as monolayers in plastic T 75 flasks using Eagle's basal medium (BME) + 10% HS for T 24[6] and Eagle's minimum essential medium (MEM) + 10% fetal bovine serum (FBS) for HT-29[7]. The cultures were tested periodically for mycoplasma and were found to be free of contamination. Trypsin (0.25%)[6] and a mixture of trypsin (0.05%) and EDTA (0.02%)[7] were used for the weekly transfer of T 24 and HT-29, respectively. To study the effect of HS on ALP induction, replicate sets of cultures were initiated in regular media and 24 h later the attached cells were washed five times with Earle's balanced salt solution (EBSS). One set of T 24 cultures was covered with BME + 10% HS, another with BME + 50% HS and a third with HS alone. Immediately after the medium change, 50 mM NaCl, 0.5 µg/ml (1.4 µM) prednisolone and a mixture of 50 mM NaCL + 1.4 µM prednisolone were added to duplicate cultures of the 3 sets[6]. Ethanol (0.05%), the solvent for the steroid, was added to the controls. For HT-29, one set was covered with MEM + 10% FBS, another with MEM + 10% FBS + 50% HS and a third with HS.

To test the effect of major HS protein fractions, cultures of HT-29 were also covered with regular medium containing α- + β-globulins (Cohn Fraction IV-4, 5 mg/ml); γ-globulin (20 mg/ml) and albumin (25 mg/ml). Following the medium change, a mixture of 50 mM NaCl and 2 mM sodium butyrate was added to duplicate cultures of each set[7]; the mixture was omitted from the controls. T 24 cells were not exposed to sodium butyrate and HT-29 was not treated with prednisolone, because their ALPs are not inducible by these agents[7]. Experimental and control cultures were incubated for 72 h.

T 24 cells were harvested with trypsin, washed three times with 0.15 M NaCl and disrupted by ultrasonication[6]. HT-29 monolayer cultures were washed thrice with 0.15 M NaCl and lyzed with 0.25% sodium deoxycholate[7]. ALP activity was measured by the hydrolysis of p-nitrophenyl phosphate[7] using 2-amino-2-methyl-1-propanol-HCl buffer at pH 10.6 and 37 °C. Acid phosphatase activity was assayed with the same substrate at pH 4.8 using 0.1 M acetate buffer[7]. The variation of duplicate assays was less than 10%. Specific activity was expressed as µmoles of p-nitrophenol liberated in 1 min at 37 °C per mg protein, the latter determined according to Lowry et al[8]. The ALP form produced by each cell line has been established previously[6,7].

Results and discussion

T 24 cells grown in regular medium (BME + 10% HS) have low levels of heat-stable, term-placental ALP[6]. Increasing the medium osmolality from 284 mOsm/kg to 384 mOsm/kg by addition of 50 mmol/kg NaCl or by addition of 100 mmol/kg sucrose[9], or growing these bladder cancer cells with prednisolone, induces increased levels of ALP activity, and an over 100-fold augmentation occurs when steroid-containing hyperosmolar medium is used (table 1). Supplementing BME with 50% HS inhibits enzyme induction by either stimulus, and the synergistic effect elicited by their combination is reduced by 97%. When T 24 is grown in HS alone, no ALP activity is detectable in the controls and the inducing effect of hyperosmolality and/or prednisolone is abolished (table 1).

Previous studies have shown that acid phosphatase is not influenced by agents affecting ALP[7]. This is also the case in the present experiments: Acid phosphatase activity is essentially the same in control and experimental cultures (table 1). Growing T 24 with increasing amounts of HS does not significantly affect cell proliferation. Total cell protein content is indeed somewhat higher in BME + 50% HS than in BME + 10% HS, but it is slightly lower in serum alone (table 1). These findings indicate that the

Table 1. Effect of human serum on alkaline and acid phosphatases of cultured human bladder cancer cells.
T 24 cells were inoculated into BME containing 10% HS. After 24 h, cells were washed five times with EBSS and covered with regular medium, regular medium containing 50% HS and HS alone. NaCl and/or prednisolone were added immediately. Cells were harvested 72 h later. Enzyme activity was assayed in duplicate on replicate cultures. Specific activities expressed as µmoles of p-nitrophenol liberated per min/mg protein. ND = non-detectable.

Additions	BME + 10% human serum			BME + 50% human serum			Human serum		
	Alkaline phosphatase ($\times 10^{-4}$)	Acid phosphatase ($\times 10^{-2}$)	Cell protein (mg/flask)	Alkaline phosphatase ($\times 10^{-4}$)	Acid phosphatase ($\times 10^{-2}$)	Cell protein (mg/flask)	Alkaline phosphatase ($\times 10^{-4}$)	Acid phosphatase ($\times 10^{-2}$)	Cell protein (mg/flask)
None	1.4	2.9	0.75	1.2	2.9	1.19	ND	2.7	0.62
50 mmol of NaCl/kg	10.0	2.8	0.94	2.0	3.5	1.16	1.7	2.7	0.78
Prednisolone (1.4 µM)	6.2	3.4	0.88	1.9	3.9	1.21	1.4	2.9	0.79
NaCl + prednisolone	171.2	3.4	0.94	5.2	3.0	0.98	2.6	3.5	0.62

Table 2. Effect of human serum and serum protein fractions on the synergistic induction of alkaline phosphatase in HT-29 cells.
HT-29 cells were inoculated into MEM containing 10% FBS. After 24 h, cultures were washed five times with EBSS and covered with the media and serum indicated. NaCl + sodium butyrate were added immediately to duplicate flasks of each set. No additions were made to the other flasks. Cells were harvested 72 h later. All determinations were done in duplicate. Specific activity expressed as micromoles of p-nitrophenol liberated per min/mg protein.

	Additions to cultures			
	None		NaCl (50 mM) + sodium butyrate (2 mM)	
Cell grown in:	Specific activity ($\times 10^{-4}$)	Cell protein (mg/flask)	Specific activity ($\times 10^{-4}$)	Cell protein (mg/flask)
MEM + 10% FBS	1.8	0.72	2210.0	0.56
MEM + 10% FBS + 50% human serum	1.8	0.70	800.0	0.61
Human serum	2.0	0.71	17.5	0.52
MEM + 10% FBS + α- and β-globulin (5 mg/ml)	1.7	0.75	1777.0	0.63
MEM + 10% FBS + γ-globulin (20 mg/ml)	2.4	0.59	2470.0	0.59
MEM + 10% FBS + albumin (25 mg/ml)	1.8	0.78	1937.0	0.60

HS-mediated reduction in ALP activity is not due to an environment which is detrimental for cell growth.

HT-29 cells express low levels of heat-labile, L-homoarginine-insensitive, intestinal ALP[7], but growth of these colon cancer cells in sodium butyrate (2 mM)-containing hyperosmolar medium (388 mOsm/kg) causes an over 1000-fold increase in specific activity. In contrast to T 24, ALP activity is still detectable when HT-29 cells are grown with 50% HS or in HS alone. However, the enzyme-inducing effect of sodium butyrate + hyperosmolality is significantly depressed; in the presence of 50% HS, induction is reduced by 64% and in HS alone by more than 99% (table 2). As with HeLa S3 cells[5], medium containing 60% FBS dose not affect enzyme levels (not shown). Previous studies have demonstrated that at concentrations of 1 mg/ml, human albumin, α-, β-, and γ-globulin, either singly or in combination, do not duplicate the effect of HS on HeLa ALP[5]. Because it has been reported that human γ-globulin affects enzyme activity in rat hepatoma cells[10], we have re-evaluated the effect of these HS fractions at higher concentrations using HT-29 cells. However, as shown in table 2, none of the substances tested depresses ALP activity or prevents enzyme induction, as in each case the activity of the experimental cultures is over 1000 times greater than in the respective controls.

The mechanism by which primate sera or a thermostable, nondialyzable factor[5] therein affect ALP activity remains unknown. However, it has been shown that the effect is not due to the removal from the growth medium of components essential for ALP production and activity, to enzyme leakage into the medium or to extrinsic or intrinsic inhibitors in cells with depressed activity[5]. To rule out the unlikely possibility that HS inhibits ALP activity directly, purified placental (Type XXIV, Sigma Chemical Co.) and intestinal (from induced HT-29 cells)[6] enzyme solutions were exposed to HS for up to 48 h at 37 °C. Subsequent assays done in the presence of serum revealed neither inhibition nor activation (not shown), indicating absence of an ALP inhibitor. In addition, the lack of enzyme leakage into the medium would suggest that the effect of HS is not due to an ALP-releasing phosphatidylinositol-specific phospholipase C[1].

Our results demonstrate that ALP depression by HS is not restricted to HeLa cells; that the HS-mediated reduction in ALP induction, a consequence of an increase in specific mRNA[11,12] and an increase in enzyme molecules[13–15], is independent of the inducer used; and that these effects are not duplicated by major serum proteins. Although these findings are in sharp contrast to the reported ALP stimulation or induction by serum[10,16] or serum-derived agents[17,18] they suggest that blood-borne factors may control the ALP levels of cells in vivo.

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