Influence of DEAE-D on the growth characteristics and the number of chromosomes in L-cells

Duration of action of DEAE-D (500 µg/ml)	Relative plating[a] (%)	Generation time (h)	S-phase[b] (h)	G2 phase[b] (h)	Mitotic index (%)	Number of chromosomes	
						Modal	Range
0	100	17.5	7.5	3.5	5.4	54	50–58
30 min	62–80	18.5	6.5	4.5	4.4	51 and 53	48–60

[a] Absolute plating efficiency 75–86%. [b] Determined by autographic technique in asynchronous population[9].

exogenous DNA in mammalian cells cultivated in vitro. For the experiments we used ^{3}H-DNA isolated from L-cells[11], as host cells L-cells cultivated in monolayer in minimal Eagle's medium supplemented with calf serum and carrier thymidine. After the application of DEAE-D, the incorporation of ^{3}H-DNA determined at several intervals by the specific activity of L-cell DNA[12], was 20 times higher than in the DEAE-D-deficient series[7].

The kinetics of the incorporation of exogenous DNA and its localization in the host cell in connection with the function and the mechanism of the effect of DEAE-D on the uptake of this DNA are the subject of further detailed studies.

Zusammenfassung. Wachstum, morphologische Eigenschaften und Karyotypie kultivierter L-Zellen wurden nach Applikation von DEAE-Dextran im Inkubationsmedium untersucht. In einer Konzentration von 500 µg/ml (was die Aufnahme von exogener DNS fördert) und bei einer Einwirkung während 30 min verändert DEAE-Dextran die untersuchten Eigenschaften nicht.

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In vitro Response of Human Leukocytes to Anti-Human-Thymocyte Globulin

Heterologous antilymphocyte serum and its globulin derivative (ALG) prolong homograft survival in vivo in man and animals[1–3]. However, there are variable and often conflicting reports on its mitogenic effects in vitro. Several laboratories[4–8] have reported its stimulatory effect on DNA synthesis, while others[9, 10] have reported its inhibitory effects on synthesis of DNA.

The present report shows that within a narrow dose range antilymphocyte globulin has a stimulatory effect on DNA synthesis by cultured human leukocytes, but if the concentration of ALG is increased, DNA synthesis is inhibited, at least partly because of the cytotoxic properties of these globulins for peripheral blood leukocytes.

Materials and Methods. The antilymphocyte serum (ALS) and ALG used in this study were made in a horse repeatedly immunized with human thymic cells[11]. The ALG, purified by DEAE-cellulose batch chromatography, contained a single IgG band detectable by immunoelectrophoretic techniques. Complement was removed from all antisera by heat inactivation at 56 °C for 30 min. The lymphocytotoxic titer of ALS was 1:6400 and of ALG, 1:3200, against human thymocytes in the presence of guinea-pig complement.

Blood samples were obtained from normal adults. The separation of leukocytes (by sedimentation method), leukocyte culture and determination of the degree of stimulation of DNA synthesis were done according to the methods used by PRASAD et al.[12]. Dilutions of NHS, ALS and ALG were made in Hank's balanced salt solution. Phytohemagglutinin M (PHA) was reconstituted with triple distilled water. The diluted sera and the undiluted PHA were added to the medium (0.1 ml per tube) prior to the addition of the cell suspension. 0.5×10^6 leukocytes were suspended in 2.5 ml of culture medium for each culture. Thymidine-methyl-H^3 (specific activity, 12 ci/m mole) was used to determine the degree of stimulation of DNA synthesis. 5 h prior to harvest 0.5 µci of the isotope was added to each culture tube. Experiments were repeated 3 times with similar results with leukocytes from 6 different individuals.

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Influence of ALS, ALG and/or PHA on viability of an uptake of ^{3}H-Thymidine by peripheral blood leukocytes at 72 h of culture

Dil'n of ALS or ALG	ALS alone			ALG alone				ALG + PHA				PHA alone			Untreated control		
	^{3}H-thymidine uptake	Viable (%)	Cell viability: No. of viable cells	^{3}H-thymidine uptake	Viable (%)	Cell viability: No. of viable cells	Horse serum protein per tube (mg)	^{3}H-thymidine uptake	Viable (%)	Cell viability: No. of viable cells	Horse serum protein per tube (mg)	^{3}H-thymidine uptake	Viable (%)	Cell viability: No. of viable cells	^{3}H-thymidine uptake	Viable (%)	Cell viability: No. of viable cells
1:10	138	49	0.37×10^6	170	20	0.08×10^6	5.25	83	16	0.16×10^6							
1:25	177	47	0.22×10^6	2519	46	0.11×10^6	2.1	2628	45	0.31×10^6							
1:50	5879	70	0.80×10^6	414	59	0.29×10^6	1.05	3252	58	0.27×10^6							
1:100	235	91	0.48×10^6	143	71	0.37×10^6	0.525	3113	51	0.22×10^6							
1:200	204	87	0.32×10^6	172	87	0.50×10^6	0.263	2703	51	0.23×10^6							
None	–	–	–	–	–	–	–	–	–	–		3486	67	0.31×10^6	128	84	0.35×10^6

	CPM/mg HSP																
1:10	7.4	32.4															
1:25	23.6	1199.5															
1:50	1567.7	394.3															
1:100	125.3	272.3															
1:200	217.5	650.2															
None	–	–															

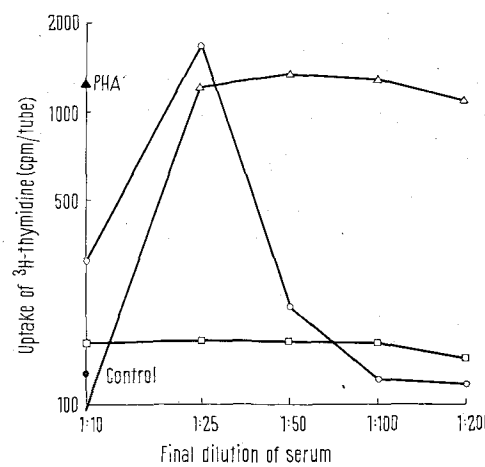
One tube from each treatment of the experiment (without pulse labelling) was taken and the absolute number of viable cells was determined using the trypan blue dye-exclusion technique at 72 h of culture.

Results. In all the repeated experiments the general pattern of DNA synthesis in human peripheral blood leukocytes stimulated by ALG was as shown in the Figure. The stimulation of ^{3}H-thymidine uptake by ALG was dose-dependent and occurred only within very narrow limits of dose (Table). Maximum stimulation occurred at a final dilution of 1:25 and was approximately 20 times that of the control. DNA synthesis decreased sharply at concentrations of ALG above and below 1:25. Similar curves were obtained with ALS. Peak stimulation was obtained at a final dilution of 1:50 (Table), which contained the same number of cytotoxic antibody units as the 1:25 dilution of ALG.

Optimal doses of ALS or ALG appeared to be as effective a mitogen as the non-specific stimulant PHA. The immunological mediation of the increased ^{3}H-thymidine uptake by ALS or ALG was indicated by the failure of NHS to produce a similar stimulation in DNA synthesis (Figure). No additive synthetic capacity was found when cells were incubated in the presence of both ALG and PHA.

The failure of cells to incorporate ^{3}H-thymidine in the presence of high concentration of ALG was unexpected. However, large amounts of ALG produced predominantly nonviable cultures as measured by dye uptake (Table). The highest concentration of ALG, in the presence of PHA, also inhibited the stimulation of DNA synthesis and reduced cell viability. High concentrations of ALS also reduced cell viability, although not as greatly as did ALG.

Discussion. The results of this study indicate that horse anti-human thymocyte globulin can stimulate DNA synthesis in cultured human peripheral leukocytes. Similar observations have previously been made[4,13]. Our dose-response curves for ALG and ALS were quite sharp as compared to some other reports. The differing effective-



Stimulation of DNA synthesis in human peripheral blood leukocytes by ALG. –○–○–, ALG; –△–△–, ALG with PHA; –□–□–, NHS.

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ness of these antisera in stimulation of DNA synthesis might be related to the methods of immunization and the sources of the antigens employed. A steep dose response curve for DNA stimulation by ALG with some reduction of response at the highest ALG concentration has also been reported[14] using guinea-pig anti-rabbit-lymphocyte serum on lymph node cell cultures.

The narrow limits of stimulating activity expressed by ALG probably relate to an inherent complement-independent cytotoxic activity. The low number of viable leukocytes found in the presence of high concentrations of ALG indicated that these concentrations did not simply inhibit DNA per se, but instead destroyed the viability of the cells. This is probably why the non-specific stimulation of DNA synthesis by PHA is also prevented in high concentrations of ALG. After dilution of the ALG to a point that cell viability as measured by dye exclusion remained at 45% or above, PHA was again active in the presence of ALG. This confirms the findings of LUNDGREN et al.[15], SIMONS et al.[8], and WOODRUFF et al.[13] that the stimulatory effect of PHA may be inhibited by ALG. However, the inhibition may reflect cell death or injury rather than a more specific biochemical effect.

In the case of ALS, the lack of ^{3}H-thymidine uptake at the 2 highest concentrations was associated with a cell viability which ,while reduced, was as good or better than that at the peak DNA response to ALG alone. This suggests that either the dye exclusion test is an inadequate measure of cell injury or an additional mechanism is involved in the failure of response to the highest concentrations of ALS.

Peak DNA synthesis occurred with only 2.1 mg of ALG protein as compared to 3.75 mg of ALS protein, suggesting a higher specific activity of ALG. Whereas the ALS contains all of the serum proteins, the ALG is the purified 7S IgG fraction, which is known to contain the specific antilymphocyte activity. Since the stimulation of DNA synthesis occurs in such a narrow dilution range of ALS or ALG, this in vitro technique appears not to be an ideal assay for antilymphocyte activity[16].

Résumé. La faculté qu'ont le sérum (ALS) antithymocyte du cheval et son dérivé IgG (ALG) de stimuler la synthèse du DNA avec les leukocytes périphériques humains a été contrôlée in vitro. Sauf déviation même faible de dosage, l'ALS et l'ALG stimulent la synthèse du DNA. Une forte concentration de ALG inhibe l'effet stimulant de la phytohémagglutinine. Cela peut contribuer en partie à la mort de la cellule ou à sa dégradation à cause de la grande concentration de l'ALS ou de l'ALG.

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Immunological Blockade of the Adenohypophysis and its Possible Application in Prophylaxis and Therapy of Neoplasia

Introduction. Immunological intervention provides one method to block organ function. Since antisera to organs can be prepared, it should be possible, therefore, to inhibit specifically or to modulate function by varying doses of antisera or immunoglobulins. In particular, we wish to discuss the inhibition of the adenohypophysis. This gland, besides secreting stimulating factors for various target glands, such as adrenocorticotropic hormone (ACTH), thyrotropic hormone (TTH) and gonadotropic hormones (GH), is also secreting daily a large amount of somatotropic or growth hormone (STH). We wish to propose in this paper that STH is probably involved in the growth of many types of tumours and that consequently blocking its production should lead to tumour growth inhibition.

The hormone dependence or the sensitivity to hormones of many malignant or benign tumours in humans is well known. In the attempt to control growth of many tumours, hormones are therefore used extensively. Hormones have been and are administered even in very high doses, the so-called 'pharmacological dosage' in many types of neoplasia such as the localized or systemic tumours of the lymphatic and haematopoietic systems, carcinoma of the breast, carcinoma of the prostate, myelomatosis and many others. However, no serious attempt has been made to try to interfere in a specific manner with the function of the hypophysis which receives the inhibiting or secretory stimuli both from the periphery and from the hypothalamus and which regulates the entire endocrine system.

Role of growth hormone. STH is a phylogenetically old, polypeptide molecule, which, like prolactin, is present even in primitive species. It has probably acquired new functions in higher organisms but preserved its former functions present in the lower species. One of these acquired functions in mammals is presumably that of controlling cell proliferation either alone or in chronological synergism with other hormones. These actions of STH become evident when the target cells, as for example in the epiphyseal cartilage of the long bones, appear during growth in postnatal life. It is therefore important to establish in man which tumour cells have acquired or maintained the characteristics of STH dependence of certain tissues for their growth. This concept of hormone dependence may be useful for adopting the appropriate hormone therapy for certain tumours.

STH has a structural similarity with another hormone, prolactin, which is seemingly secreted by the same or very similar so-called acidophilic cells of the adenohypophysis. Both of them have a 'trophic' action as shown in many experimental systems. Another main point to be considered is that the acidophilic cells secreting somatotropic hormone, constitute to a large extent the cell population of the adenohypophysis. The reason for this is unknown. It is