Paradoxical Effect of Freund's Complete Adjuvant upon Transplantation Efficiency of Adenovirus-induced Tumour Cells

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Adjuvants appear to act by creating a depot from which antigen is slowly released and by stimulating antibody-forming cells (Ehrich et al. 1945). Freund's adjuvant consists of an emulsion of water in paraffin oil stabilized by lanolin derivatives (Freund, Casalas-Ariet & Genghof, 1940). Its action is potentiated by adding dead mycobacteria or mycobacterial lipid (complete adjuvant) and Freund attributes the cellular response to the mycobacterial substance (Freund, 1956). Freund's complete adjuvant (FCA) can produce delayed hypersensitivity of extreme severity to a foreign antigen and at times sensitize an animal to its own tissue (Freund, Thompson & Lipton, 1955) (auto-immunity). Transplantation and tumour immunity are chiefly of the cellular type (delayed hypersensitivity) and FCA appears to be an ideal agent to enhance such immunity. However, we observed increased tumour growth when hamsters were treated with FCA before challenge with adenovirus tumour cells. The present report describes this paradoxical enhancing effect.

Random-bred female Golden Syrian hamsters 3-4 weeks of age were obtained from Lakeview Farms, Newfield, N.J. Adenovirus type 12 transformed hamster cells furnished by Dr Aaron Freeman (Microbiological Associates, Inc.) were grown in 32 oz bottles containing Eagle's minimal essential medium (MEM) without calcium, to which a 1% solution of L-glutamine and a 0.2% solution of vitamins and non-essential amino acids were added. Penicillin 100 u./ml., streptomycin 100 µg./ml. and kanamycin 50 µg./ml. were included as well as 10% heat-inactivated foetal calf serum. The cells were allowed to grow until about 80% confluent. The growth medium was then discarded, the cell layer washed twice with 10 ml. of MEM and scraped into 5 ml. of MEM. The cells were counted in a haemocytometer and adjusted to the desired count with 85 to 90% viability as determined by trypan-blue exclusion. Seventy-two hamsters were divided into three groups of 24; one group received 10^6, the second group 10^5 and the third group 10^4 cells in 0.5 ml. of MEM. All injections were subcutaneous. Half of the animals in each group were given 0.2 ml. of FCA * intraperitoneally 17 days before tumour cell challenge; the other half were not pre-treated.

All the animals in the groups injected with FCA developed tumours after injections of 10^6, 10^5 and 10^4 cells; however, of the hamsters receiving no adjuvant, one-third of those inoculated with 10^4 cells, one-half of those with 10^5 cells, and all those with 10^6 cells developed tumours (Fig. 1).

FCA has been reported to increase the incidence of tumours as well as the number of metastases in young chickens treated with small doses of Rous sarcoma virus (Rauscher, Fink & Kvedar, 1963). Emulsions of FCA with polyoma virus and Rous sarcoma virus (killed and dried) 5 mg.

* Arlacel A (mannide mono-oleate) 1.5 ml.; Bayol F (paraffin oil) 8.5 ml.; Mycobacterium butyricum (killed and dried) 5 mg.
sarcoma virus administered to newborn mice and rats also stimulated oncogenesis (Ter-Grigorov & Irlin, 1968). Goldner, Girardi and Hilleman (1965) reported that newborn hamsters inoculated with SV40 followed by the administration of Freund's incomplete adjuvant in the latent period increased tumour development. However, oncogenesis was reduced in hamsters treated neonatally and up to 1 week of age with adenovirus type 12 and FCA (Berman, Allison & Pereira, 1967).

Under the conditions of this experiment, pretreatment with FCA apparently enhanced tumour formation in hamsters challenged with adenovirus type 12 tumour cells. General stimulation of the lymphoreticular system by non-specific materials has been utilized as a means of increasing resistance to tumours (Stjernswärd, 1966). Our results suggest that such a stimulation might produce serious disadvantages to subjects so treated.

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*Laboratory for Virus and Cancer Research*  
The George Washington University  
School of Medicine  
Washington, D.C. 20037, U.S.A.

*Viral Carcinogenesis Branch*  
National Cancer Institute  
National Institutes of Health  
Bethesda, Maryland 20014, U.S.A.
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