PATHOLOGY

Use of Non-biological Particles in Detecting Anti-immune Blood Diseases

In spite of the many methods for detecting anti-immune diseases affecting leucocytes, there is still need for simpler and more specific methods.

From a suspension of leucocytes, prepared by separation of the leucocytes in donor blood with saline[1], an extract is produced. This extract consists mainly of γ-globulins. It has been proved, by immunoelectrophoretic analysis on serum from rabbits, immunized with leucocyte extract, to contain antigens specific only to human leucocytes[2]. Stored in a deep-freeze, the leucocyte extracts do not lose their antigen strength for months. Acryl particles with a diameter of 0·5μ can be charged with the leucocyte extract by using the same technique as when these particles are charged with γ-globulin for demonstrating RAS factor[3,4].

Charging of the particles with leucocyte extract can be controlled by mixing a suspension of particles on a microscopic slide in room temperature with serum from rabbits immunized with leucocyte extract. Then an aggregation of the particles takes place within 5 min. The same happens in many cases, when the charged particles are mixed with inactivated human serum containing antibodies against leucocytes. It will not happen, however, when the serum is free from leucocyte antibodies. When aggregation occurs it is always macroscopically visible; no intermediate forms of aggregation, visible only under microscope, have been seen. The method has so far been tested on 43 human sera which contained antibodies against leucocytes according to accepted methods[5]. In 21 cases the acryl particle test was positive ; in the remaining it was definitely negative. So the method is not as sensitive as the more complicated methods now in use. It has been tested, too, against 100 sera from healthy blood donors and simultaneously the same particles were tested against inactivated serum from rabbits, immunized with human leucocyte extract. In all cases the test was negative against the blood donor sera and positive against the rabbit sera, indicating that the acryl particle method gives no false positive results.

Acryl particles have also been charged with a thrombocyte extract produced in accordance with the leucocyte extract. Such particles are aggregating spontaneously. This will not happen if the particles are suspended in isotonic solution of EDTA when they are charged with thrombocyte extract. In three cases aggregation of the charged particles was noted when human sera containing antibodies against thrombocytes were added.

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Quantitative Relationships in Viral Oncolysis and the Possibility of Artificial Heterogenization of Tumours

Since the classical papers of Levaditi *et al.*[1,2] it has been shown that most viruses reproduce well in malignant tumours although some of them exert rather an oncolytic action[3]. An attempt to use viruses for treatment of malignant tumour in human beings has so far proved unsuccessful[3]. However, in this problem the major factors are still largely obscure.

Apart from a certain sensitivity-level of tumour cells to viruses at least three factors define viral oncolysis: (1) quantitative ratio of viral particles and tumour cells in dynamics; (2) production of immunity to viruses; (3) selection of tumour cells resistant to the destroying action of the virus.

The quantitative relationship of viral particles and tumour cells in viral oncolysis *in vivo* was investigated under as simplified conditions as possible: different quantities of trypsinized cells of mouse tumours (sarcoma 180, hepatoma C_3HA) or rat tumours (sarcoma M-I and Walker sarcoma) were mixed *in vitro* with various dilutions of the vaccinia virus or of the virus of lymphogranuloma inguinale and exposed for 15 min to 22°. Afterwards large amounts of the virus-treated cells (5×10^5–2×10^6) were administered to mice and rats respectively while control animals received equal numbers of non-treated cells.

With a ratio in the inoculum of 0·5–5 vaccinia pock-forming units per tumour cell neither of four tumours tested developed as against 100 per cent development in the control. 0·2–2 pock-forming units caused oncolysis of sarcoma 180 in half of the animals while lesser doses caused no oncolysis. Frequently such doses caused stimulation of tumour growth (occurrence of the infected tumours increased, as well as their weight). The virus of the lymphogranuloma inguinale previously regarded as non-oncolytic[2] at a ratio of 0·1–1 brain mouse LD_{50} or more per one tumour cell likewise elicited complete oncolysis of the sarcoma 180.

Preliminary active or adoptive immunization of animals against the virus eliminated the oncolytic effect of even very large viral doses (10^2–10^3 pock-forming units per 1 tumour cell). Growth-stimulating action of tumours by vaccinia virus is frequently apparent under these conditions[4].

In the next experiments the lymphatic cells of twice intensively vaccinia-immunized mice C_{57} were administered to a group of mice of the same strain (10^6 cells intraperitoneally and 10^4 intravenously per mouse). The control mice were treated with similar numbers of non-immune lymphatic cells. On the next day mice of both groups were inoculated with 10^5 sarcoma 180 cells per mouse treated at a ratio of 0·2–2 pock-forming units per tumour cell. Tumours arose in the group with non-immune cells in 8 of 19 animals (average weight of the tumours on the tenth day, 1·63 g) and in the group with immune cells in 14 out of 17 mice (average weight 4·9 ($P = 0{\cdot}954$)). Moreover, intensive active vaccinia immunization of mice (simultaneously in 4 paws) on the next day after administration of sarcoma 180 cells infected with vaccinia virus in a ratio of 0·05 pock-forming units to 1 unit per cell prevented oncolysis and enhanced the tumour growth-stimulating effect of virus.

The experiments showed that oncolysis is caused only by a fairly high ratio of viral particles to tumour cells at a certain phase of tumour growth. These results agree with the recently published results of Durr and Briody[5]. However, the technique used by these workers, namely, implantation of pieces of tumour mixed with the vaccinia virus, does not indicate the ratio between the number of viral particles and tumour cells. The fact that immunity to the virus (particularly an active one) inhibits the oncolytic effect even of very large doses of the virus demonstrates that even under simplified conditions of the experiment when the virus is brought into contact with tumour cells prior to inoculation oncolysis is not immediately effected, so that live tumour cells persist in the organism after a considerable time.

Virus immunity is one of the factors interfering with the curative use of viruses. To prevent the development of immunity in the host we have recently used irradiation of the animals with cobalt-60. For example, complete oncolysis occurs in 13 out of 15 mice C_{57} irradiated (300 r.)

24 h before the inoculation of 10^6 cells of sarcoma 180 infected with lymphogranuloma inguinale virus (0·01 LD_{50} per cell), whereas in non-irradiated controls oncolysis occurred in 2 out of 10 mice. Average tumour weight was in these groups correspondingly 0·04 g and 0·272 g (P = 0·988). There was no difference in the growth of uninfected tumours in the irradiated and non-irradiated mice. Irradiation or cortisone treatment increased viral oncolysis in our experiments. The use of both treatments indicates curative oncolytic treatment by biological agents, such as viruses, bacteria or protists.

In addition to the direct oncolytic effect of the virus on the tumour another mode of virus treatment of tumours may be mentioned, namely, artificial induction of new antigens in tumour cells by means of viruses or other agents and followed by exposure of these antigens to actively acquired and passively administered immune lymphatic cells or antibodies. It is maintained since the time of Philibert[8] and Rivers[9] that the primary reaction of the cell to the reproduction of the virus consists in proliferation or destruction. Svet-Moldavsky[10] suggested that the most general type of the primary cell reaction to the virus is heterogenization of the cells with regard to other structures of the organism. The recently described antigens induced in the cells by viruses of the polyoma, SV_{40} and leukosis[11] are a mere manifestation of this general regularity—a property of viruses not only of 'oncogenic' viruses. If heterogenization of the cells is a natural property of the viral processes then viral infection of tumours might be used for artificial heterogenization of tumour cells.

Cause of malignancy involves both antigenic simplification and the appearance of new antigens[11]. These antigens, particularly in cancerogen-induced tumours, proved of low activity. This is why the possibility of immunological treatment in respect of natural tumour antigens is very slight, whereas artificial heterogenization of tumours seems possible.

For artificial heterogenization of the tumour-affected organism compounds or biological agents are administered which selectively accumulate or multiply in the tumours, thereby providing new antigen determinants in tumour cells. It is precisely on these determinants the immunological effect is produced.

It is possible, in particular, that the cells may become resistant to the course of the direct oncolytic action of the virus when they prove latently infected with the virus and heterogenized.

For this purpose not only 'infectious' viruses are promising but also 'oncogenic' ones as well as bacteria, rickettsias and protists.

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Quantitative Enzyme Histochemistry using Frozen-dried Glomeruli

Methods of obtaining kidney glomeruli for the determination of enzyme content are microdissection of cryostat sections or differential centrifugation of kidney homogenates in sucrose. We wish to report alkaline phosphatase determinations on glomeruli dissected from fragments of frozen-dried rat kidneys. This method of preparation has the practical advantage of being simple and convenient, and may also avoid enzyme destruction by freeze-thawing or solution in aqueous media. Preliminary observations on the effect of nephrotoxic serum are also reported.

Kidneys excised from Wistar rats were sectioned into 2–3-mm cubes and rapidly frozen by plunging into isopentane cooled in liquid nitrogen. They were stored in liquid nitrogen until needed. Blocks were dried *in vacuo* at −32° C for about 18 h over phosphorus pentoxide. The drying tube was brought to room temperature before the vacuum was broken, and the blocks were dissected with jeweller's forceps under a dissecting microscope. Glomeruli appeared as discrete reddish balls which protruded from the surface of cleaved tissue fragments, and could easily be lifted away without any adhering tubules. Several dozen can be obtained in a few minutes.

The glomeruli were weighed in groups of four using a quartz fibre balance[1]. Alkaline phosphatase activity was determined by the method of Dubach[2]. The substrate was nitrophenyl phosphate. Optical density of the released nitrophenol was measured in alkaline solution in a Beckman spectrophotometer at 410 λ. The incubation period was 1 h at 37° C.

Six rats were injected with 1 ml. of nephrotoxic serum prepared in rabbits. Two rats were killed at intervals of 24, 48 and 96 h. Urine was collected every 24 h and protein determinations made by the method of Lowry[3]. Glomerular localization of rabbit globulin was demonstrated in cryostat sections of the injected rat kidneys using a fluorescent antirabbit globulin.

Table 1

	No. of glomeruli	Alkaline phosphatase activity *	Urine protein (mg %)
Normal glomeruli	24	4·62	54
Nephrotoxic serum 24 h	12	3·78	91
" " 48 h	12	3·18	105
" " 96 h	12	2·84	104

* μM nitrophenol released in 1 h at 37° C per mg dry tissue.

The results are summarized in Table 1. The average weight of frozen-dried normal rat glomeruli was 226 mμg. This includes Bowman's capsule. After injection of nephrotoxic serum there was no significant change in weight. The alkaline phosphatase activity of normal glomeruli is somewhat greater than that reported by Dubach and Recant, especially when it is considered that the latter workers used glomerular tufts only. This difference may mean that the freezing and thawing used to prepare cryostat sections cause some loss or inactivation of enzyme. It is seen that alkaline phosphatase activity progressively falls after injection of nephrotoxic serum, which is in agreement with the results of Dubach. The nephrotoxic serum was of low titre. It did not cause a significant proteinuria and no glomerular lesions were seen in the light or electron microscope. Nevertheless, γ-globulin was localized to glomeruli as seen by brilliant fluorescence on staining cryostat sections with fluorescein labelled rat antirabbit globulin serum. No staining occurred in uninjected rat kidneys. It has been proposed that changes in glomerular enzyme in Masugi nephritis are the result of proteinuria[4]. This may not be so. If there was an increase of glomerular permeability in these experiments, protein leakage did not exceed tubular resorption. Normal rats will secrete up to 10 mg of protein in their urine per day. The results indicate that an antigen–antibody reaction can occur in the glomerular tuft accompanied by a marked fall of alkaline phosphatase