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PROPAGATION OF TICK-BORNE ENCEPHALITIS VIRUS VARIANTS IN SHORT-TERM CULTURES OF NORMAL HUMAN LEUKOCYTES

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Summary. — The cloned variants of tick-borne encephalitis (TE) virus, differing markedly in their neurovirulence for monkeys and in other 6 genetic markers, showed basic differences in their capacity to propagate serially in suspended short-term roller cultures of normal human leukocytes. High in vivo virulence was associated with the ability to multiply in these cells maintained in vitro.

The informations concerning the relationship between human leukocytes and TE virus are as yet limited. It is well known, however, that a variety of viruses multiply in human leukocytes cultivated in vitro, which fact is significant for better understanding of the viraemic period of infection and of the spread of the virus in the organism (for literature see Wheelock, 1966). This particular problem was studied with TE virus in mice by Málková (1960*a*, *b*; 1968).

The investigation on the mutual relationships in the system TE virus — cultured human peripheral leukocytes was stimulated by the question as to whether the susceptibility of these cells with physiology completely different from that of currently used cell types (e.g. chick embryo cells or stable cell lines) is equal for TE virus variants showing different degree of virulence (as judged by their efficiency of killing mice or monkeys — Mayer, 1966). In contrast to most other cells, peripheral leukocytes are end-stage forms, which have no more a mitotic potential (Wheelock, 1966).

The unequal susceptibility of human embryonic cerebellar and retinal tissue in this respect was convincingly demonstrated (Mayer and Mitrová-Bellová, 1969).

The technique used for the isolation of the cells from the white blood fraction was in principle that described by Wheelock (1966). Fresh blood, obtained from healthy donors, with added heparin (1 unit per 10 ml), was incubated for 2 hours at 37° C. The leukocyte-containing plasma was carefully separated from the sedimented erythrocytes and slightly centrifuged for 15 minutes. The sedimented leukocytes were then suspended in Eagle's minimum essential medium, containing 20% of heated newborn calf serum, 4% of tryptoso-phosphate broth and sodium bicarbonate (1.75 g per litre). The cells were then counted in a haemocytometer. The suspension was further diluted so as to contain 2×10^6 cells per ml. The roller tubes were seeded with 2 ml of diluted cell suspension and incubated at 37° C.

The monkey- and mouse-virulent TE virus clone, designated P III-E, and the attenuated clone, designated Hy-HK28"2" (showing the set of 6 genetic markers of completely different character (Mayer, 1966; Mayer and Rajčáni, 1967), were used in these experiment.

To avoid the described virus resistance appearing constantly in freshly prepared leukocyte cultures (Gresser and Chany, 1964; Nahmias *et al.*, 1964; Duc-Nguyen and Henle, 1966; Strander and Cantell, 1967), three types of experiment using cell suspensions of different age were carried out: a) cultures prepared from suspended, freshly isolated cells; b) suspended cell cultures used after one day of cultivation in; the roller tubes; and c) suspended cell cultures after two days cultivation in the roller tubes at 37° C.

The cell suspensions of different age (a, b, c) were inoculated (two tubes each) with approximately 10^4 ic LD_{50}/0.1 ml of the appropriate virus clone and incubated further at 37° C. The leukocyte suspensions were centrifuged at 24-hour intervals and the extracellular virus in the pooled supernatants was titrated by the interference method with Western equine encephalomyelitis virus (Vilček, 1960). Pooled supernatants served simultaneously as inoculum (0.1 ml) for the next virus passage in the appropriate type of culture. A total of 5 serial passages was completed with each virus in each type of suspension culture. Three sets of such experiments withe a, b, c cultures were performed.

Roller tubes, containing only nutrient medium without leukocytes, were inoculated as controls of the virus multiplication and the virus was further passaged by the same technique as in experiments described above.

The suspended human leukocytes, cultivated in short-term cultures, supported only the multiplication of the monkey- and mouse-virulent P III-E virus. Multiplication of the Hy-HK28"2" virus was not detected in any experiment or type of suspension used (Table 1).

The survival of virus from the initial inoculum, as shown in control experiments (nutrient medium without cells, see Table 1), did not influence the values found in the titration experiments.

Further, the human leukocytes (with prevalence of the mature forms, i.e. from donors showing normal cell counts) under the experimental conditions employed, proved to be not very efficient producers of TE virus, as shown by the decreased yields of virus in higher passages. It was not the aim of the present report to analyze all the factors which could have influenced the extent of TE virus multiplication in such a type of cells. The differences observed in TE virus susceptibility (or resistance) of freshly prepared leukocyte suspensions and of those incubated for 1—2 days before infection with TE virus, very probably could be ascribed to the known early interferon response. The typical feature of leukocyte suspensions, the increase of their susceptibility during cultivation (Strander and Cantel, 1967), was confirmed also for TE virus in the present experiments. But the observations mentioned are significant also for the estimation of variability of leukocyte suspensions.

The different behavioural patterns of the P III-E and Hy-HK28"2" viruses were also investigated in pilot tests by the fluorescent antibody technique. After 24 hours of incubation, the leukocytes were infected with either virus in the way described above; after a further 24 hours the cells, together with appropriate controls, were stained by the direct immunofluorescence method. The findings fully confirmed the virological results. In leukocytes infected with the attenuated virus no presence of specific antigen was detected. By contrast, in the smear of cell suspension infected with the virulent virus clone, specific fluorescence was observed in about 40% of cells.

The mechanisms playing a role in the observed resistance of cultivated leukocytes to infection with the Hy-HK28"2" virus (e.g. the inability of the attenuated virus to attach to the surface, or to penetrate into the cell)

Table 1. Propagation of TE virus variants during serial passages in suspensions of normal human leukocytes (roller tubes)

Type of cell suspension	Virus clone	Exp. No.	Titre* of extracellular virus at passage level									
			Cell suspensions					Nutrient medium (Control)				
			I	II	III	IV	V	I	II	III	IV	V
Freshly prepared	P III—E	1	4.3	1.8	0	0	0	2.8	1.5	0	0	0
		2	3.3	1.7	0		0	2.5	0	0	0	0
		3	0	0	0	0	0	1.75	0	0	0	0
24 hr old		1	5.5	4.5	3.3	2.5	1.3	2.7	1.5	0	0	0
		2	4.5	3.4	2.7	2.3	1.5	1.7	0	0	0	0
48 hr old		1	5.3	4.3	3.5	3.3	N.D.	0	0	0	0	0
		2	4.3	3.3	2.3	1.7	1.3	2.7	1.7	0	0	0
		3	3.7	4.3	3.3	1.7	1.5	1.7	0	0	0	0
Freshly prepared	Hy-HK28"2"	1, 2, 3	0	0	N.D.			0	0	N.D.		
24 hr old		1, 2, 3	0	0	N.D.			0	0	N.D.		
48 hr old		1, 2, 3	0	0	N.D.			0	0	N.D.		

* log IfD_{50}/ml of virus at the end of 24 hr passage; 0 = no virus detected in undiluted fluid.
N.D. = not done.

are not yet clear, but the fact that they did not become infected after contact with one of the two viruses used, is essential.

Many limitations of in vitro experiments become obvious when certain analogies for in vivo systems are to be drawn. But in the present case the results obtained are so striking that it is impossible to consider them without accounting a possible relation also to some factors which could participate in, or determine the degree of virulence of certain variants of TE virus in vivo.

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