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AN ATTEMPT TO CULTIVATE HUMAN ROTAVIRUS IN HUMAN LEUKOCYTES CULTURE (*)

(PRELIMINARY REPORT)

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Human rotavirus is an important causal agent of diarrhoea in children [5]. Conventional tissue cultures for virus isolation has demonstrated ineffective although some isolates have shown a limited number of passages [2]. It is well know that some viruses grow in animal or human leukocytes either in fresh preparation or in mitogen-stimulated cultures [3]. Some viruses, e.g. polio grow in both preparation but their yields are higher in mitogen-treated cultures [4]. The reason why mitogen-treated leukocytes cultures allow or even increase virus production is still unknown. It is thought that some metabolic mechanisms would be activated to produce macromolecules needed for the replication of viruses. It is also suggested that stimulated leukocytes would have an increased capacity to adsorb viruses due to new receptors at cell surface or increased phagocytic activity [1].

The present experiment was performed in leukocytes cultures obtained from healthy donors. Mononuclear cells were separated from whole blood by Histopaque 1077 (Sigma Chem. Co.) gradient and stimulated with phytohemagglutinin (PHA) (Sigma Chem. Co.) at final concentration of 20 μg/ml before infection with human rotavirus obtained from diarrhoeic stool by conventional technique. The cultures were washed three times in maintenance medium after adsorption and kept at 37°C under 5% CO_2 in growth medium (199) supplemented with 20mM Hepes (Flow Lab.) and 10% foetal calf serum (Gibco Co.). Cell samples harvested 12, 24, 36 and 48 hours post infection were pelleted and embedded for electron microscopy. The 12 and 24 hours samples showed no intracellular virus particles, although morphological change which may have been due to PHA stimulation was observed. However samples harvested 36 hours p.i. showed many cells containing rotavirus particles in the cytoplasm (Fig. 1). We also found many cells in degeneration, cell debris-associated virus particles and a few intact small lymphocytes. We found no virus in untreated infected cultures. This preliminary result suggests that human rotavirus may infect human leukocytes under mitogen stimulation.

RESUMO

Uma tentativa de cultivo de rotavírus humano em cultura de leucócitos humano

(Nota Prévia)

Experimentos preliminares da permissividade de cultura de leucócitos humanos estimulados com mitógeno frente a infecção pelo rotavírus humano foram realizados por microscopia eletrônica. Observamos que, células mononucleadas, mantidas em cultura, após estimulação com fitohemaglutinina (PHA) colhidas 36 horas pós-infecção apresentavam muitas partículas virais no citoplasma. Verificamos, também, muitas partículas virais associadas a fragmentos celulares, várias células em degeneração e alguns linfócitos pequenos intactos. Não presenciamos partículas virais em células colhidas previamente (12 e 24 horas p.i.) e nas culturas controle (sem tratamento com PHA). Sugerimos que o rotavírus humano pode se replicar em culturas de leucócitos humanos estimulados com PHA.

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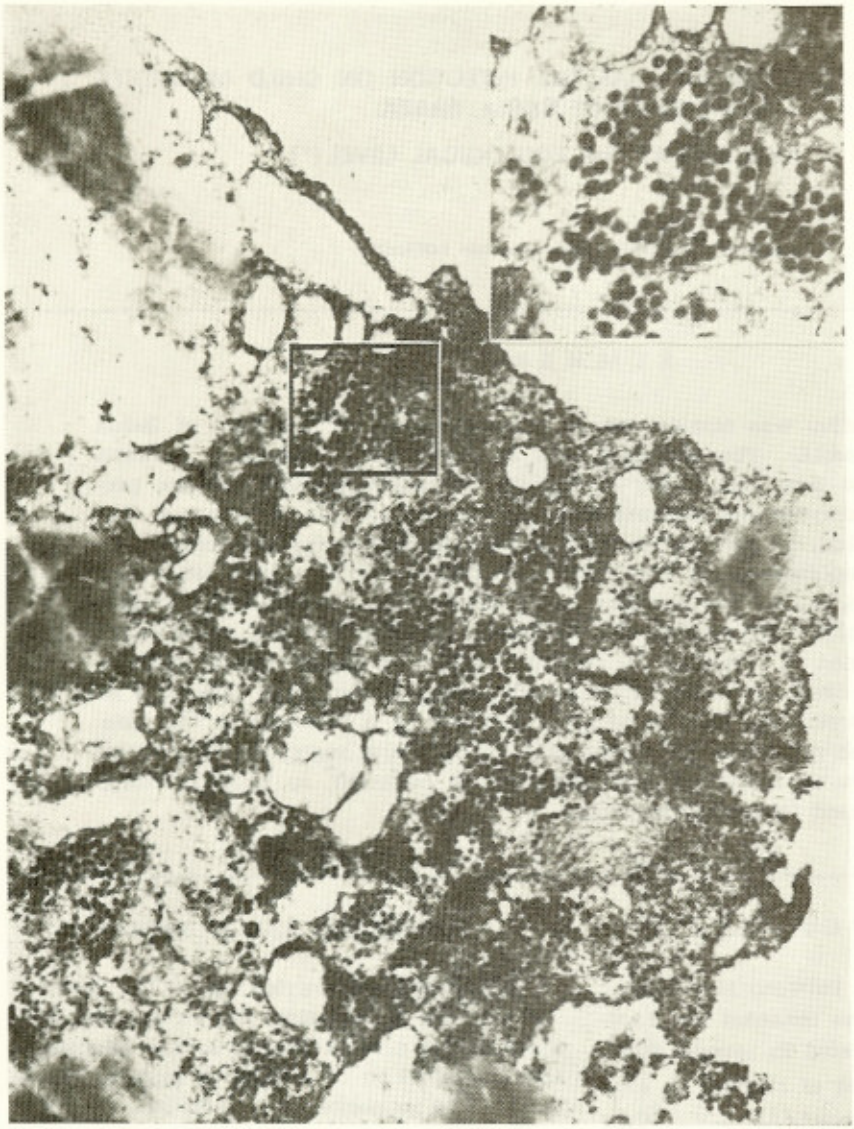

Fig. 1 — PHA-stimulated leukocyte sampled 36 hours p.i. It shows human rotavirus particles widely spread in its cytoplasm (X 17.500). Inset: complete and empty particles surrounded by membranous structure are shown (X 37.000)

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