ROTAVIRUS AND POLIOVIRUS CO-INFECTION IN HT-29 CELLS

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Summary. – The effect of a mixed poliovirus-rotavirus infection in HT-29 cells, a gut tumour derived cell line highly susceptible to both viruses, has been analyzed. The obtained results showed an increase of poliovirus multiplication in cells super-infected or co-infected with rotavirus, whereas the pre-infection with poliovirus had an interfering effect on rotavirus replication.

Key words: rotavirus; poliovirus; co-infection; vaccine

The establishment of a virus infection in cultured cells can often affect their sensitivity to a second virus infection (Marsh and Helenius, 1989). Many mechanisms of interference are known (Fuller *et al.*, 1985) and range from effects at level of entry (Marsh and Helenius, 1989) to those at level of replication or propagation (Fenner *et al.*, 1974).

It has also been described that infection with one virus increases the susceptibility of cells to a second virus replication (Fuller *et al.*, 1985; Khelifa and Menezes, 1983).

Viral replication can also influence *in vivo* the subsequent infection by other viruses. It is well known that enteric viruses can induce cell modifications which allow the adhesion and the invasion of intestinal mucosa epithelial cells by other pathogens, such as bacteria or viruses, with a negative influence on the course of the disease (Sweet and Smith, 1990). Rotaviruses play an important role in the aetiology and epidemiology of diarrhoeal diseases in children between 6 and 24 months of age, in developed as well as underdeveloped countries (Kapikian and Chanock, 1990) in which the administration of trivalent live oral poliovirus vaccine is increasing. An interference between type 2 and types 1 and 3 poliovirus vaccine (Patriarca *et al.*, 1991) as well as between oral poliovirus vaccine and oral rotavirus vaccine (Giammanco *et al.*, 1988) have already been described. Therefore, it appears relevant to analyze interactions between these enteric naked RNA viruses in gut epithelial cultured cells.

In this paper we report results of an *in vitro* investigation on the effect of a mixed poliovirus type 1 and rotavirus SA-11 infection in HT-29 cells, a gut tumour derived cell line highly susceptible to both viruses (Patel *et al.*, 1985; Superti *et al.*, 1991).

LLC-MK2 cells, a monkey Kidney cell line, were grown at 37 °C in mixture (1:1) of Eagle's Minimum Essential medium (MEM) and 199 medium (Superti and Donelli, 1991). Vero cells, a monkey kidney cell line, were cultured at 37 °C in MEM as previously described (Marchetti *et al.*, 1992). HT-29 cells, a human colon adenocarcinoma cell line (obtained from American Type Culture Collection, Rockville, MA) were grown in RPMI 1640 medium (Superti *et al.*, 1991).

Simian rotavirus SA-11 was grown in LLC-MK2 cells (Superti and Donelli, 1991), and poliovirus type 1, Mahoney strain, was grown in Vero cells. Confluent cell monolayers were infected at a MOI of 1 PFU/cell as previously described (Marchetti *et al.*, 1992).

HT-29 cells were infected with poliovirus and rotavirus (10 and 1.5 PFU/cell, respectively) at different times. Intracytoplasmic rotavirus antigen synthesis was determined by indirect immunofluorescence 8, 16 and 24 hrs post infection (p.i.) Poliovirus relased into the supernatant of infected cell culture was measured by plaque titration in Vero cells 8, 16 and 24 hrs post infection (p. i). Rotavirus antigen synthesis was measured by immunofluorescence as previously described (Superti *et al.*, 1992).

In a first set of experiments, HT-29 cells were co-infected with poliovirus and rotavirus or, alternatively, rotavirus was added to the cell monolayers 1 or 3 hrs after poliovirus infection. In Figs 1 and 2 poliovirus release into the supernatant and intracytoplasmic rotavirus antigen synthesis, respectively, are shown. An increased poliovirus replication was observed in all experimental conditions, mainly when rotavirus was added simultaneously with poliovirus (Fig. 1).

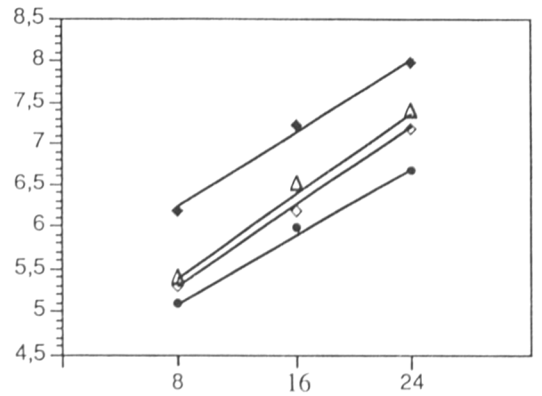

Fig. 1
Poliovirus yield in HT-29 cells co-infected or post-infected with rotavirus
Absicissa: hrs p. i.; ordinate: poliovirus yield (log PFU/ml). Poliovirus (●). Co-infection of poliovirus and rotavirus (◆). Rotavirus 1 hr after poliovirus (Δ). Rotavirus 3 hrs after poliovirus (◇).

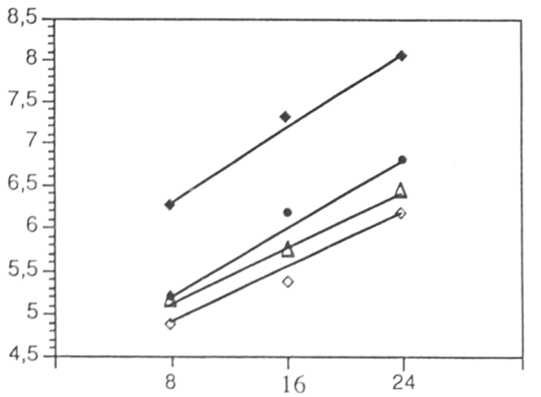

Fig. 3
Poliovirus yield in HT-29 cells pre-infected or co-infected with rotavirus
Abscissa: hrs p.i.; ordinate: poliovirus yield (log PFU/ml). Poliovirus (●). Co-infection of poliovirus and rotavirus (◆). Rotavirus 1 hr before poliovirus (Δ). Rotavirus 3 hrs before poliovirus (◇).

What concerns rotavirus multiplication, viral antigen synthesis was inhibited by pre- or co-infection with poliovirus (Fig. 2). The reduction of the percentage of fluorescent (rotavirus infected) cells was related to the time of poliovirus infection; it was more marked when poliovirus was added to the cells 3 hrs before rotavirus.

In other experiments HT-29 cells were co-infected with poliovirus and rotavirus or, alternatively, poliovirus was added to cell monolayers 1 or 3 hrs after rotavirus infection. Poliovirus released into the supernatant and intracytoplasmic rotavirus antigen synthesis are reported in Figs 3 and 4, respectively. A slight inhibition of poliovirus production was observed in cell monolayers pre-infected with rotavirus, whereas co-infection caused an increase of poliovirus yield (Fig. 3). Under the same experimental conditions, no variations of intracytoplasmic rotavirus antigen synthesis were observed (Fig. 4).

Virus infection can facilitate a secondary infection by an other virus *in vitro* (Fuller *et al.*, 1985; Khelifa and Menezes, 1983), and a similar phenomenon can occur also *in vivo* (Sweet and Smith, 1990). Both poliovirus and rotavirus *in vivo* infect gastrointestinal tract and *in vitro* replicate in HT-29 cells (Patel *et al.*, 1985; Superti *et al.*, 1991).

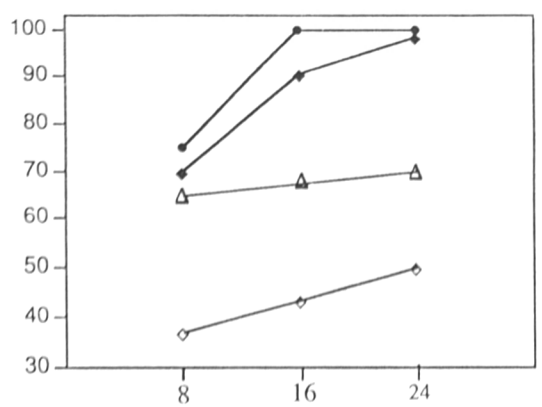

Fig. 2
Rotavirus antigen synthesis in HT-29 cells pre-infected or co-infected with poliovirus
Abscissa: hrs p.i.; ordinate: fluorescence (%). Rotavirus (●). Co-infection of rotavirus and poliovirus (◆). Poliovirus 1 hr before rotavirus (Δ). Poliovirus 3 hrs before rotavirus (◇).

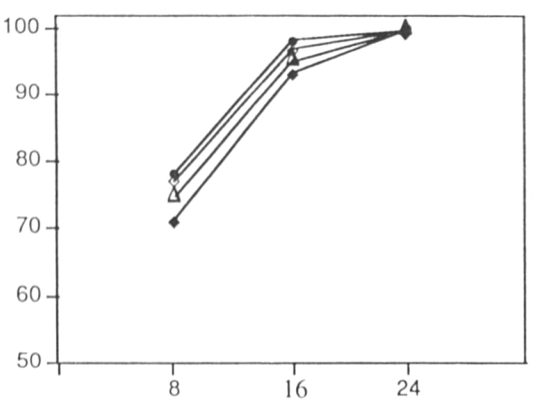

Fig. 4
Rotavirus antigen synthesis in HT-29 cells co-infected or post-infected with poliovirus
Abscissa: hrs p.i.; ordinate: fluorescence (%). Rotavirus (●). Co-infection of rotavirus and poliovirus (◆). Poliovirus 1 hr after rotavirus (Δ). Poliovirus 3 hrs after rotavirus (◇).

The results obtained in co-infection studies, when rotavirus SA-11 and poliovirus type 1 were simultaneously added to HT-29 cells, indicate an interaction between these different naked RNA viruses, which results in an increased poliovirus production.

This enhancement was also observed, although to a lower extent, when rotavirus was added 1 or 3 hrs after poliovirus infection. The mechanism of this increased susceptibility of HT-29 cells to poliovirus multiplication, induced by rotavirus, could be related to the early events of infection. An indirect binding in which rotavirus proteins could act as poliovirus receptors, as postulated for other viral co-infections (Fuller *et al.*, 1985; Khelifa and Menezes, 1983), can be ruled out since outer shell rotavirus proteins do not show similarities with poliovirus binding structures. It is more likely that poliovirus entry is facilitated by the simultaneous entry of rotavirus into HT-29 cells. It has been suggested that rotavirus internalization in host cells occurs by direct penetration of the cell membrane resulting in a specific permeability alteration of plasma membrane (Kaljot *et al.*, 1988). As either endocytosis or direct passage has been proposed for poliovirus entry into susceptible cells (Dimmock, 1982), it is possible that membrane perturbation, induced by rotavirus internalization, facilitates the direct passage of poliovirus particles.

Rotavirus replication remained unaffected when HT-29 cells were super-infected with poliovirus, whereas a reduction of viral antigen synthesis was achieved when cells were co-infected or post-infected with poliovirus.

Since it is well known that rotavirus attachment is mediated by membrane sialoglycoconjugates (Yolken *et al.*, 1987; Keljo and Smith, 1988; Superti and Donelli, 1991) and poliovirus binds to novel members of the immunoglobulin superfamily (White and Littman, 1989), a competition between the two viruses at the level of cell receptors could be excluded.

The inhibition of rotavirus replication can be supposed to be the consequence of the dramatic reduction of cell protein synthesis induced by poliovirus since the first hour of infection (Rueckert, 1990).

These last findings are in agreement with data obtained by Giammanco *et al.* (1988) who demonstrated that the RIT 4237 live attenuated rotavirus strain is immunogenic when administered alone to infants three months old, whereas its capacity to induce seroconversion is highly reduced when administered together with live attenuated poliovirus vaccine.

On the basis of the presented data we can conclude that, in the attempt to produce an effective rotavirus vaccine, which is particularly needed in developing countries, it must also be taken into account the possible interference by other enteric viruses on rotavirus multiplication and the possibility that rotavirus can enhance the virulence of other pathogens, such as enterobacteria (Bukholm, 1988) or enteroviruses.

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