Growth of rubella virus into SIRC (rabbit cornea cell line) using various culture media

Cultivo do vírus da rubéola em SIRC (linhagem cellular de cornea de Coelho) em vários meios de cultura

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ABSTRACT

The present study evaluated the efficacy of various culture media for performing the isolation and growth of the rubella virus inoculated into SIRC cells. Rubella virus RA-27/3 strain and RVi/SãoPaulo/BRA99 wild type strain (GenBank number DQ458965) were inoculated into SIRC cell line and cultivated in 199, DMEM, MEM and RPMI media. The inoculated cells when examined on phase contrast microscopy showed the characteristic rounded and multipolar cells. The CPE was observed at the first 48 hours cultivation in the respective tested media. The curve of the infectivity increase was higher in the cultures maintained in DMEM and RPMI media. Hence, the SIRC cellular lineage cultivated in DMEM or RPMI media is an excellent substratum for performing the rubella virus isolation. These findings are relevant since the SIRC has been one of the few cell lines described in the literature which presents a cytopathic effect, and on that account it can be useful for carrying out the virus isolation from clinical specimens.

Key words. rubella virus, SIRC cells, virus isolation, cytopathic effect.

RESUMO

O presente estudo avaliou a eficiência de vários meios de cultura no isolamento e crescimento do vírus da rubéola inoculado na linhagem celular SIRC. O vírus da rubéola padrão RA27/3 e o vírus selvagem RVi/SãoPaulo/BRA99 (GenBank número DQ458965) foram inoculados na linhagem celular SIRC e cultivada nos meios de cultura 199, DMEM, MEM e RPMI. As células inoculadas observadas em microscopia de fase apresentaram aspecto arredondado, com produção de células multinucleadas. O efeito citopático foi observado após 48 horas de cultivo nos respectivos meios de cultura testados e a curva do crescimento da infectividade do vírus foi maior nas células cultivadas em meios DMEM e RPMI. Os resultados obtidos indicam que SIRC é um ótimo substrato para crescimento do vírus da rubéola, uma vez que poucas linhagens celulares descritas na literatura apresentam efeito citopático considerável e mostram que o potencial dessa linhagem celular ser utilizadas para efetuar o isolamento do vírus da rubéola em amostras clínicas.

Palavras-chave. vírus da rubéola, células SIRC, isolamento viral, efeito citopático.

INTRODUCTION

The discovery in the early 1900s that human cells could be propagated in vitro provided virologists with an alternative to embryonated eggs and laboratory animals for in vitro isolation of viruses. Cell cultures, which are derived from dispersed cells taken from original tissue and disaggregated by enzymatic, mechanical, or chemical means, provided large numbers of cells suitable for virus isolation[1]. Although virus isolation in cell cultures was employed by research laboratories by the early 1960s, diagnostic services were very limited, varying from laboratory to laboratory and often not available at all, except in major medical centers. For viral diagnosis, isolation is sensitive because a positive result can be obtained with a single infectious virion[2.] In addition, viral isolation permits the detection of unexpected viruses, new viruses, or multiple viruses in a specimen.

Rubella was initially known as "German Measles", as it was first described by two German physicians. It is generally a mild disease and received little attention until 1941, when Norman MacAlister Gregg recognized its association with congenital defects [3]. The risk of congenital defects was underestimated until the 1960s, when tecniques were developed to identify the virus. In São Paulo, Brazil the rubella immunization program began in 1992 with the introduction of MMR (measles/mumps/rubella) vaccine or MR (measles/rubella) vaccine to the basic immunizations schedule. In our laboratory the routine diagnosis of rubella virus (RV) infections is based on detection of RV-specific IgM in serum, isolation of rubella virus in cell culture and PCR[5,6,7]. RV replicates in a variety of cell culture systems, primary cells and continuous cell lines[1,2,7,8,9]. The virus isolation permits the detection of a wide variety of virus types including new or antigenic variants of known viruses. However, the presence of rubella virus indicated by the typical cytopathic effect (CPE) in infected cells, is difficult and laborious to detect. The objective this study study was to evaluate the importance of culture medium for the isolation and growth of the rubella virus inoculated in SIRC cells

MATERIAL AND METHODS

▪ Media

The SIRC cells were grown in: (1) 199; (2) Dulbecco Minimum Eagle Essential Medium (DMEM, Gibco); (3) MEM; (4) RPMI 1640) media (Invitrogen/Life, Carlsbad, CA). All media were supplemented with 10% inactivated fetal bovine serum (FBS, Invitrogen/Life, Carlsbad, CA), 20mM L-glutamine and contained 0.1% gentamicin.

▪ Virus stock

The rubella virus RA-27/3 strain (Meruvax II, Merck, Sharp and Dohme) and RVi/SãoPaulo/BRA99 wild type strain isolated in our laboratory (GenBank number DQ458965) was seeded in Vero cells (African green monkey kidney — ATCC CCL-81) and tittered, as previously described by Kaerber[8].

▪ Cells and Virus

The SIRC (Statens Serum Institute Rabbit Corneal Epithelial Cell line - ATCC CL 60) were adapted to 199, DMEM, MEM and RPMI media, through five serial passages. Cells were cultivated in 75cm2 plastic cell culture flasks contained 5x105 cells/mL. After five passage, SIRC cells were grown in 24 well plates in each media supplemented with 2mM L-glutamine and 10% FBS. Plates were incubated at 37°C in a humidified 5% CO2 atmosphere. A serial tenfold dilution of the rubella virus (RA 27/3 and RVi/SãoPaulo/BRA99) was prepared in medium 1, 2, 3 and 4. The confluent cells were inoculated with 200mL of the virus dilutions in quadruplicates, for each medium. After one hour of adsorption at room temperature, each well received 2mL of medium with 2% FBS. Uninfected cultures were also prepared and treated identically as controls. Plate cultures were observed for CPE daily during seven days, when the test was concluded. Fifty per cent infectivity end points were calculated by the method of Karber(8).

▪ Optic microscopy

SIRC cells infected cultures and controls were routinely examined by phase-contrast microscopy and photographed. For staining cell cultures with toluidine blue, SIRC infected cultures and controls on coverslips were washed with PBS, fixed with 1% glutaraldehyde in 0.15M phosphate buffer at pH 7.2 for 1 hour and then stained with 1% toluidine blue, pH 3.5 for 5 minutes. After fixation, a brief water rinse was carried out and the cultures were then immediately examined and photographed.

RESULTS

The SIRC cells are fibroblasttic type and form a dense monolayer (Figure 1a). After changing to the various

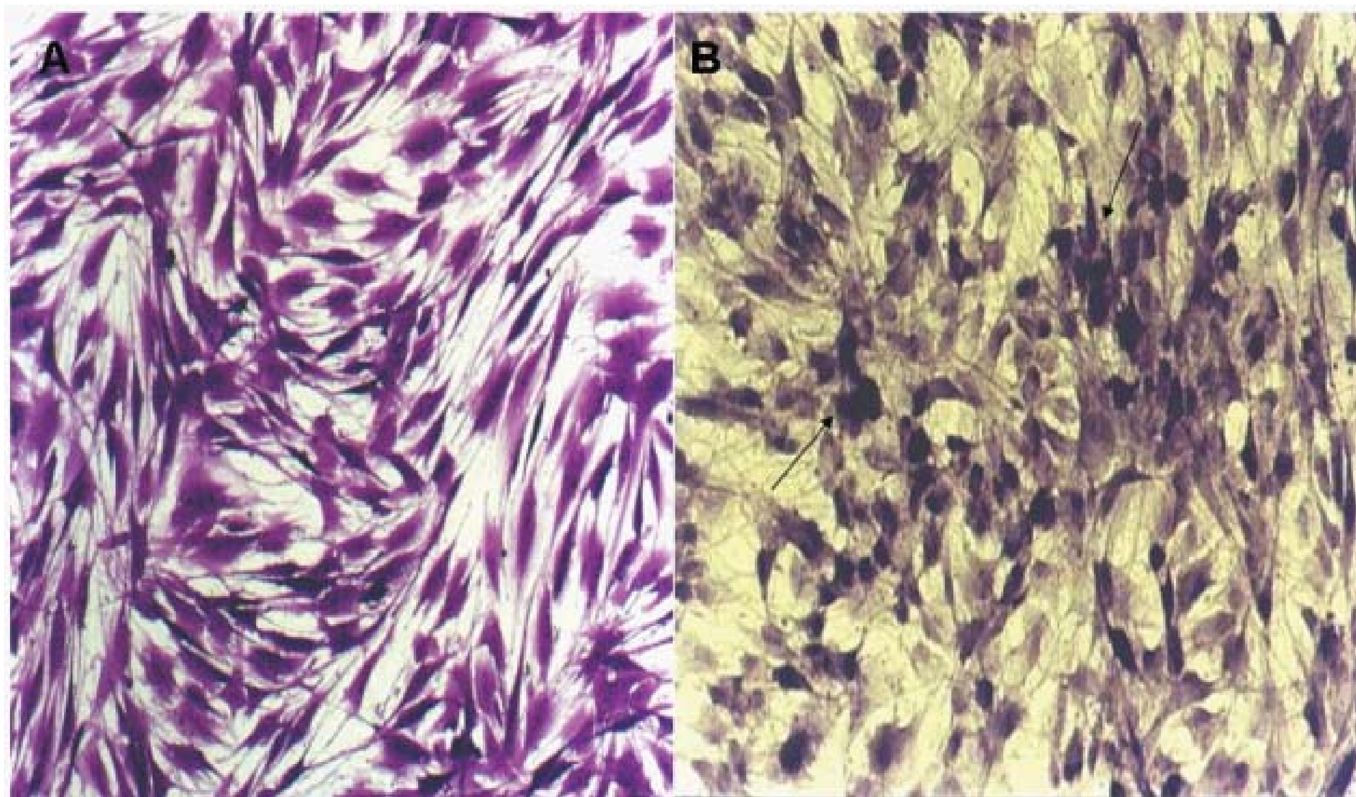

Figure 1. Cytopathic effect of rubella virus in SIRC cell line. A: Uninfected; B: Cells infected after 8 days with wild type strain (DQ4589) cultivated in DMEM. Observe the cytopathic effect is characterized by a formation of foci consisting of small, circular cells, (100x),

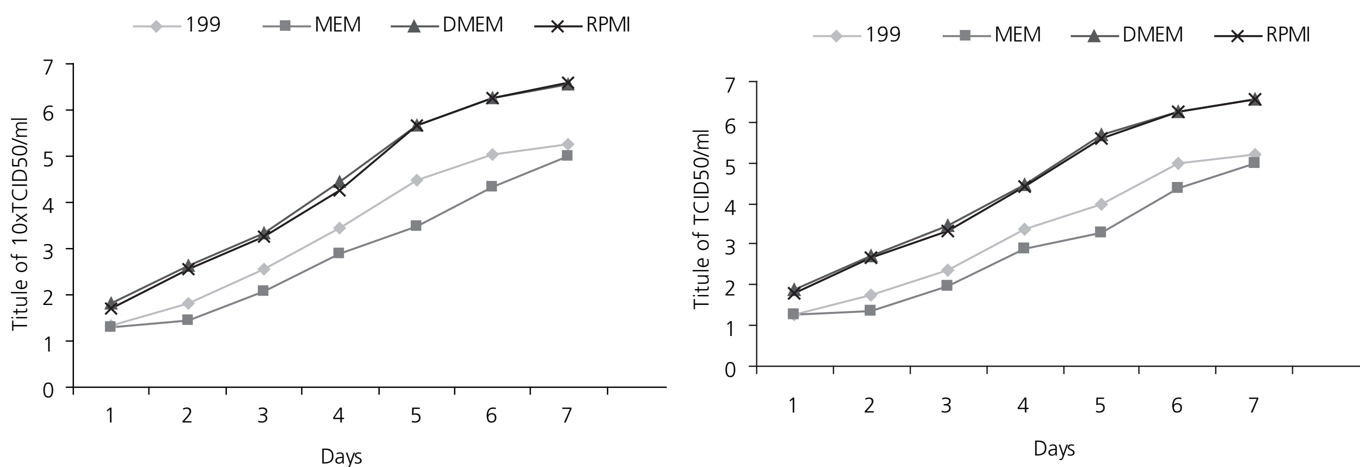

Figure 2. A- Curve of increased infectivity of SIRC cells infected with the strain RA27/3 of the rubella virus during 7 days and B- Curve of increased infectivity of SIRC cells infected with strain. DQ458965

maintenance media the cultures not developed differences in your morphology. The SIRC cells showed best adaptation to two media: DMEM and RPMI-1640, not showing any significant signs of degeneration until the 12th day. In the 199 and MEM medium, dead cells were observed in the supernatant, after the 7th day and the monolayer showed signs of loss of confluency. The MEM was the least adequate, once in the 6th passage more than 50% of the cells were dead, the cellular monolayer was not confluent in several points and the monolayer was unfastened. The cells cultivated in DMEM/RPMI had good aspect until the 15th day. After the 15th day , the cells showed a progressive rise in the number of unfastened cell on the supernatant. The cytophatic effect in SIRC cells inoculated with rubella virus was observed after 48 hours of the inoculation in all media tested. The early cytophatic effect in all cultures is characterized by a formation of foci consisting of rounding, along with bipolar and multipolar cells. In media 1and 3, the foci disappear very rapidly and fast degeneration of the cell sheet takes place. In media 2 and 4, various foci can be observed for approximately one week after the inoculation, when a complete degeneration of the cell sheet eventually occurs. However, SIRC cells inoculated in DMEM or RPMI showed about 90% of the cell altered by CPE by day 8 (Figure 1B). Infective titers of RA27/3 strain and RVi/ /Sao Paulo.BRA/99 in SIRC cells cultivated with various medium are shown in Figure 2A and B. The maximum virus titers are reached approximately seven days after inoculation with all maintenance media, nevertheless, the titrations are higher in cultures maintained on media 2, and 4 while titer values in media 1 and 3 cultures are lower.

DISCUSSION

Rubella is a common cause of childhood rash and fever. Its public health importance is related with the teratogenic effects of primary rubella infection in pregnant women[2,3]. Congenital rubella syndrome may be diagnosed through the classic triad of clinical signs, but many infants only have one of these clinical signs or may present neonatal signs earlier. The clinical diagnosis is further more elusive by the fact that maternal infection is often subclinical[3]. A definitive diagnosis of rubella virus infection is achieved with serological tests and virus isolation.

The present study reports the SIRC cultures maintained on different media as well as the influence of these media on the titers of rubella virus. In our results, the morphology of the SIRC cells was not affected by change of media. A difference, however, was observed in the ability of the media to maintain the cells, media 2 and 4 maintaining cultures in good condition for the 15-day period of observation. Furthermore, the cells did not develop non-specific degenerations which are observed in other media tested. The results of the growth of rubella virus in SIRC cells are in agreement with Leerhoy et al., 1965;1968[12,13]. The rubella virus in SIRC cells inoculated with RA27-3 and RVi/SãoPaulo/BRA99 showed clear and readily detectable CPE and these cell have been shown to be as effective for primary isolation of rubella virus and cells cultivated with DMEM or RPMI produced earlier and more extensive cytophatic effect. Titration of rubella virus in SIRC cultures showed differences in the growth of the virus and titer of approximately 0.5-1 $\log^{10}$ depending on maintenance media. The maximal titers 6.40-6.45 log10 were obtained in media 2 and 4. The low replication level of RV in SIRC maintained in media 1 and 3 can be related to the fact that few cell receptors were exposed to virus. The media used can promote easy virus attachment to the cell, by exposing virus receptors. However, other factors, though, could be influencing the viral yield, such as temperature, pH, oxygen concentration etc[11]. The present paper demonstrates that the SIRC cell line cultivated in DMEM and RPMI could be included in the group of cell lines in which CPE induced by RV is observed within 2-5 days after inoculation. The isolation of rubella virus is extremely valuable for molecular epidemiology studies as a rapid and convenient method for obtaining field isolates[14] (WHO,2005).

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