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Keywords
Rubella virus, physiology#. Cell line#. Cytopathogenic effect, viral#. Virus replication. Epithelial cells, virology. Rubella, patology.

Abstract

Objective
The rapid growth of the rubella virus in RC-IAL[2] with development of cytopathic effect, in response to rubella virus infection, is described. For purposes of comparison, the rubella virus RA-27/3 strain was titered simultaneously in the RC-IAL, Vero, SIRC and RK_{13} cell lines.
Methods
Rubella virus RA-27/3 strain are inoculated in the RC-IAL cell line (rabbit Kidney, Institute Adolfo Lutz). Plates containing $1.5x10^5$ cells/ml of RC-IAL line were inoculated with 0.1ml s RA-27/3 strain virus containing $1x\ 10^4 TCID_{50}$/0.1ml. A 25% cytopathic effect was observed after 48 hours and 100% after 96 hours. The results obtained were compared to those observed with the SIRC, Vero and RK_{13} cell lines. Rubella virus was detected by immunohistochemistry.
Results
With the results, it was possible to conclude that the RC-IAL cell line is a very good substrate for culturing rubella virus. The cells inoculated with rubella virus were examined by phase contrast microscopy and showed the characteristic rounded, bipolar and multipolar cells. The CPE in RC-IAL was observed in the first 48 hours and the curve of the increased infectivity was practically the same as observed in other cell lines.
Conclusions
These findings are important since this is one the few cell lines described in the literature with a cytopathic effect. So it can be used for antigen preparation and serological testing for the diagnosis of specific rubella antibodies.

Descritores
Vírus da rubéola, fisiologia#. Linhagem celular#. Efeito citopatogênico viral#. Replicação viral. Células epiteliais, viarologia. Rubéola (sarampo alemão), patologia.

Resumo

Objetivo
Descreve-se o crescimento do vírus-padrão da rubéola RA-27/3 na linhagem celular RC-IAL, com desenvolvimento de efeito citopático em resposta à infecção viral. Para este propósito, o vírus-padrão foi titulado simultaneamente nas linhagens celulares Vero, SIRC e RK_{13}.
Métodos
O vírus-padrão da rubéola RA-27/3 foi inoculado na linhagem celular RC-IAL (rim de coelho, Instituto Adolfo Lutz). Placas contendo $1,5x10^5$ células/ml foram inoculadas com 0,1 ml do vírus contendo $1x10^4$ $DICT_{50}$/0,1 ml. O efeito citopático correspondente a 25% foi observado após 48 h e 100% após 96 h. Os resultados

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obtidos foram comparados com o crescimento do vírus nas linhagens celulares SIRC, Vero e RK_{13}. O vírus da rubéola na linhagem celular RC-IAL foi detectado por imuno-histoquímica.

Resultados

As células inoculadas com o vírus da rubéola apresentaram efeito citopático nas primeiras 48h. As células apresentaram aspecto arredondado, com formação de alguns prolongamentos citoplasmáticos e sincícios, produzindo células multinucleadas. A curva do crescimento da infectividade do vírus foi praticamente a mesma que a observada nas outras linhagens celulares.

Conclusão

Os resultados obtidos mostram que a linhagem celular RC-IAL é um ótimo substrato para crescimento do vírus da rubéola, pois poucas linhagens celulares descritas na literatura apresentam efeito citopático considerável e podem ser utilizadas para preparação de antígenos e nos testes de diagnóstico sorológico para o vírus da rubéola.

INTRODUCTION

Rubella virus (RV) replicates in a variety of cell culture systems, primary cells and continuous cell lines.[1,3,4,8] Usually, rabbit Kidney (RK_{13}) and rabbit cornea (SIRC) are used for rubella virus isolation.

In the past, RV has been identified by interference techniques in primate African green monkey kidney cells (AGMK).[9,10] As these cells are rarely available, the continuous kidney African green monkey (Vero) cell line is a more suitable alternative. As Vero cells do not produce interferon, RV can therefore replicate more rapidly and reach higher titers in these cells. Baby Hamster kidneys (BHK-21) and Vero cell cultures are used extensively in the production of high titers of RV required as antigens in serological tests.[4]

Rubella virus has been cultivated in a variety of continuos mammalian cell cultures where cytopathic effects (CPE) are seen as a consequence of viral infection.[4] The search for CPE in inoculated cultures is, however, difficult and laborious. When the conditions are carefully controlled, a certain degree of cytopathic effects is observed in some continuous RK_{13},[7] SIRC[11] cell lines and some Vero sub-lines.[6]

The present report describes the rapid growth of the rubella virus in RC-IAL[1] with the development of cytopathic effect in response to rubella virus infection. For purposes of comparison, the rubella virus RA-27/3 strain was titered simultaneously in the RC-IAL, Vero, SIRC and RK_{13} cell lines.

METHODS

Virus stock

The rubella virus RA-27/3 strain (Meruvax II, Merck, Sharp and Dohme) was seeded in Vero cells and titered as previously described by Kaerber. The results are in $TCID_{50}$/0.1ml.

Cells and virus

The rabbit cornea — ATCC CCL-60 (SIRC) —, African green monkey kidney — ATCC CCL-81 (Vero) —, rabbit kidney — ATCC CCL-37 (RK_{13}) —, and rabbit kidney Institute Adolfo Lutz (RC-IAL) cell lines were grown in 75cm^2 plastic cell culture flasks, in 50% Eagle's minimum essential medium (MEM) and Leibovitz medium (MEM/L-15) supplemented with 10% inactivated fetal bovine serum (FBS).

The confluent monolayers were dispersed with 0.2% trypsin and 0.02% versene, and resuspended in MEM/L-15 growth medium with 100 IU/ml penicillin G and 100 µg/ml streptomycin. For the preparation of 24 well plates, the cell suspension was diluted to $1.0x10^5$ cells/ml. Plates were seeded with 1 ml of suspension and incubated at 37^{0}C in a humidified 5% CO_2 atmosphere. RA 27/3 stock virus was quantified by medium tissue – culture infections with 0.01 moi. (multiplicity of infection) on cell cultures. For the virus adaptation in each cell line, six passages of the rubella virus RA27/3 strain were made in SIRC, Vero, RK_{13} and RC-IAL. The confluent cell cultures were inoculated with a 100 µl diluted virus in quadruplicates, for each different cell lines. After 1 hour adsorption at room temperature, each well received 1ml of medium with 2% FBS. The medium was replaced every three days. Uninfected cultures were also prepared and treated identically as controls. Plate cultures were observed for CPE daily during 5 days, at which point the test was interrupted. The virus titers for SIRC, Vero, RK13 and RC-IAL were calculated using Kaerber method, and the results were in $TCID_{50}$/0.1 ml.

Optic microscopy

RC-IAL infected cultures and controls on cover slips were routinely examined by phase-contrast microscopy and photographed.

For staining cell cultures with toluidine blue, RC-IAL 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.

Immunoperoxidase staining of RV antigen

RC-IAL cells were grown in microtissue culture chamber slides (Nunc Inc., Naperville, IL) at a concentration of $1x10^5$ cells/ml and incubated at 37^0C in 5% CO_2. After 48 hours, the cells were infected with 0.01 moi. rubella virus. After 1-hour adsorption at room temperature, 1.0ml was added as a maintenance medium. Uninfected rubella cells were included as negative controls.

After 3 days, the cell monolayers were rinsed with phosphate-buffered saline and fixed with 4% paraformaldehyde in phosphate buffer. The cells were then treated with 1.8% hydrogen peroxide in absolute methanol to remove endogenous peroxidase activity. Indirect immunoperoxidase staining was carried out with monoclonal antibody (CDC/USA), followed by incubation with peroxidase-labeled goat anti-mouse IgG. Both primary and secondary antibody incubations were performed at room temperature for 1 hour. The peroxidase substrate, 3,3'diaminobenzidine tetrahydrochloride, was obtained from Sigma.

After counterstaining in hematoxylin/eosin, the cells were examined in a light microscope. Immunostaining negative controls were included by replacing the primary antibody with PBS.

RESULTS

The RC-IAL line morphologically consists of elongated fibroblastic-like cells, which form good, well maintained monolayers.

The cells inoculated with rubella virus were examined by phase-contrast microscopy and revealed an increased cellular refractability and rounding of the infected cells. The first morphological changes were seen in 48 hours after inoculation and they spread to produce complete destruction by the 5^{th} day. The cytophatic effect (CPE) was found to decrease significantly if infected cultures had more than one medium replacement during the observation period.

Uninoculated control cultures of RC-IAL cell lines stained with blue toluidine showed monolayer of densely packed fibroblastic cells, fairly even in size, and with sharp, clear intercellular boundaries (Figure 1). In cells inoculated with rubella virus and stained with toluidine blue the first manifestations of the cytopathic effect was vacuolization of the cytoplasm at the cell poles. Close to the nucleus, there was a circular shape seen with the contraction of the cytoplasm around the altered nucleus. The infected cells ultimately appeared rounding which uses their detachment from the surface (Figure 2).

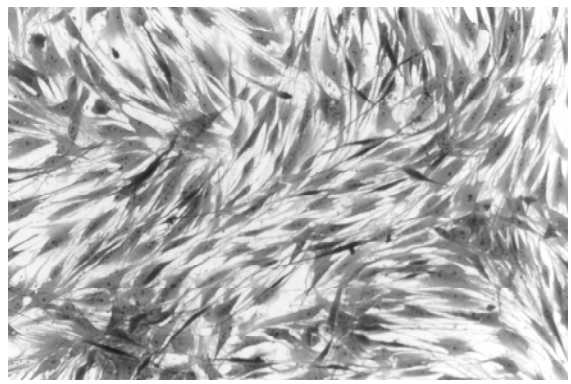

Figure 1 – RC-IAL cell line strain by toluidine blue after 48 hours in monolayers (100x).

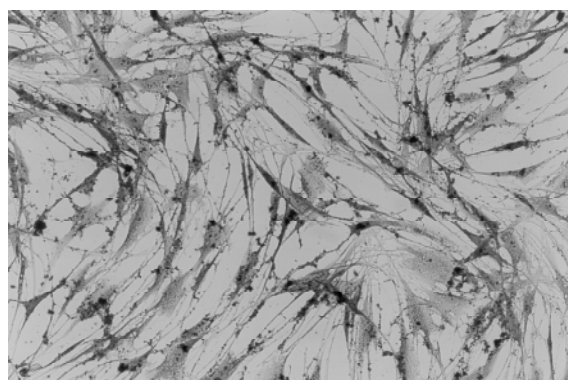

Figure 2 – RC-IAL cell line 5 days after infection with rubella virus RA27/3 strain by toluidine blue after 48 hours of inoculation. Note cells showing extensive degeneration of the cell monolayer (100x).

The CPE in RC-IAL, Vero and RK_{13} cell lines was observed at 25% in the first 48 hours and at 50% after 96 hours By day 5 and 6 extensive degeneration was observed and there was about 75% of the cells altered by CPE.

The infectivity curve increased during the five days of observation. SIRC showed any CPE only after 96 hours, but in day 5 titers were practically the same as observed in other cell lines. The titers were: $10^{5.25}$ $TCID_{50}$/0.1 ml RK_{13}, $10^{5.77}$ $TCID_{50}$/0.1 ml RC-IAL, $10^{5.72}$ $TCID_{50}$/0.1 ml in SIRC and $10^{5.51}$ $TCID_{50}$/0.1 ml in Vero (Figure 3).

Rev Saúde Pública 2000;34(4):353-7
www.fsp.usp.br/rsp

Immunoperoxidase staining of RC-IAL cells infected for 3 days with rubella virus strain. Antigens appear as a dark-brown stain in the cells and are evidently not present in uninfected control cells (Figure 4A and B).

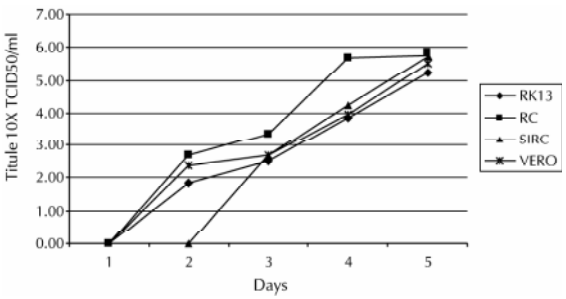

Figure 3 - Curve of the increased infectivity of cells Vero, SIRC, RK_{13} and RC-IAL cells there infected with the strain RA27/3 of the rubella virus and observed during 5 days.

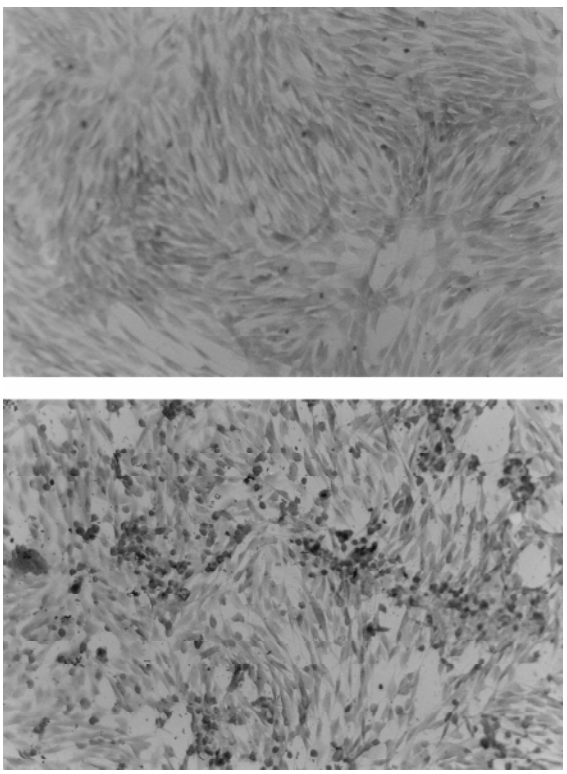

Figure 4 – Immunoperoxidase staining RC-IAL cell culture infected for 3 days with for RV strain RA 27/3(B) and uninfected as control (A). Viral antigens appear as a dark brown stain in the cells. RA27/3 viral strain were found to replicate widely in the cultures, producing large amounts of viral antigen and some cytopathology (100x).

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. 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. A definitive diagnosis of rubella virus infection is achieved with serological tests and virus isolation. However, in all cell culture lines used, the CPE of rubella virus are unpredictable, depending on virus adaptation to the cell line. They are also very sensitive to minor differences in the growth media.[4]

Rubella virus can be isolated from human respiratory tract secretions, urine, blood, cerebrospinal fluid, organ tissues obtained in the autopsy of patients who died with severe and generalized infection.[4] The cell lines most used for virus isolation are RK_{13}, SIRC and Vero.[6, 7,11] In the present study, CPE in RC-IAL cells was demonstrated and compared with those seen in RK-13, SIRC and Vero cell lines. In comparison to the cytopathic effects seen in these cell lines, RK-13 and Vero showed several disadvantages, including the cells appearance and difficulty in detecting CPE. These same observations have been also reported by others authors.[4,5]

The results are in agreement with Rhim & Schell[10] (1967). The rubella virus in SIRC cells inoculated with RA27-3 showed clear and readily detectable CPE and these cell have been shown to be as effective for primary isolation of rubella virus.

The present paper demonstrates that the RC-IAL cell line could be included in the group of cell lines where CPE induced by rubella virus is observed within 2-5 days of inoculation. Infected cells to be rounded, are easily visualized after immunoperoxidase staining in the light microscope. The growth curve for the rubella virus in RC-IAL was practically the same as observed in Vero, SIRC and RK-13 cell lines. This is important because shows that these cell lines can last while rubella virus is replicating.

A rabbit kidney cell line RC-IAL[2] isolated in 1976 has been studied. This is a continuous cell line easily kept in the laboratory and free from contaminating agents which could probably be used in the isolation of rubella virus from biological specimens in regular routine work. This line could also be used for antigen preparation and serological tests for the diagnosis of specific rubella antibodies.

These two advantages are believed to be useful in attempts to isolate the virus in cases suspected of rubella infection, enabling the identification of the etiologic agent and the study of specific antibody responses.

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