RUBELLA INFECTION OF SYNOVIAL CELLS AND THE RESISTANCE OF CELLS DERIVED FROM PATIENTS WITH RHEUMATOID ARTHRITIS*

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Rubella infections commonly cause an acute but limited polyarthritis in man (1, 2). For this reason we have studied the effect of rubella virus on monolayer cultures of cells obtained from explants of human synovial membranes. We find that rubella infection leads to reproducible cytopathic effects in synovial cell cultures obtained from nonrheumatoid membranes, while synovial cells obtained from patients with rheumatoid arthritis are resistant to infection with rubella.

Materials and Methods

Synovial Cells.—Synovial biopsies were obtained from patients with definite or classical rheumatoid arthritis, as judged by the criteria of the American Rheumatism Association (3), who were undergoing reconstructive surgery. Nonrheumatoid synovial membranes were obtained both from patients without inflammatory disease, undergoing orthopedic surgery, or from patients with chronic synovitis of a known cause (e.g., tuberculosis). The method used to grow confluent monolayers of fibroblasts from explants of synovial membrane was that described by Hamerman et al. (4). Cells were maintained at 37°C on Petri dishes in Dulbecco-Vogt modified Eagle's medium (Grand Island Biological Co., Grand Island, N.Y.) with 10% calf serum under 10% CO_2 in room air. Under these conditions, the cells in the primary culture and subsequent subcultures have the appearance of fibroblasts (Fig. 2). Cells from rheumatoid patients show decreased cell density at confluence in a 60 mm Petri dish of 9×10^5 cells compared to 1.2×10^6 cells for nonrheumatoid cultures. The rheumatoid cells in culture also maintain the marked lysosomal enzyme activities characteristic of the rheumatoid synovial membrane (5). Each cell strain was subcultured 2–4 times before infection.

Rubella.—Rubella virus, strain F-8, was obtained from Dr. P. I. Marcus (6). It was passaged three times in the Vero line of African green monkey kidney cells (Microbiological Associates, Inc., Bethesda, Md.) before it was used to infect synovial cultures. Rubella virus was detected and titered by means of the hemadsorption-negative particle assay of Marcus and Carver (6, 7), using a strain of human fibroblasts grown from pleura. The input multiplicity of infection was 1–3 hemadsorption-negative microplaque-forming units per cell, unless otherwise stated. The virulence of this strain of rubella was inadvertently confirmed when one member of the laboratory staff developed clinical rubella, including a polysynovitis, confirmed by reisolation of the virus and the development of a high titer of hemagglutination-inhibiting antibody.

Interferon.—Interferon was induced in nonrheumatoid synovial cells by treating them with poly I:C (Biopolymers Laboratory, Chagrin Falls, Ohio) in a dose of 25 µg per million cells for 20 hr (8). Interferon was detected by the protection it afforded Vero cells against challenge with rubella (9), or synovial cells against infection with vesicular stomatitis virus (VSV). Interferon was measured by a plaque-reduction assay (10) using Vero cells and VSV.

RESULTS

Rubella Infection.—21 rheumatoid (R) and 28 nonrheumatoid (N) strains were infected. Among the nonrheumatoid strains infected were two derived from patients with acute septic synovitis, one from a patient with chronic pyogenic arthritis, the other from a chronic tuberculous synovitis, and three strains derived from patients with chronic synovitis secondary to recurrent dislocation of the patella in one and congenital deformities of the hip in two others. All 28 nonrheumatoid strains demonstrated marked cytopathic effects (CPE)[1] at 12–14 days and usually died by 21 days, while the 21 rheumatoid strains failed to demonstrate such changes even after several subcultures of the infected rheumatoid cells. The CPE was manifested initially by rounded, refractile, and then pleomorphic cells which later detached or degenerated (Fig. 3).

Infectious rubella virus could be recovered from the nonrheumatoid cultures showing CPE by adding the medium from these cultures to Vero cells and isolating rubella virus, or by adding the medium to primary human fibroblasts (pleura) and assaying rubella by the hemadsorption-negative (HAD⁻) single cell test. The titre of rubella recovered from the lysates of nonrheumatoid synovial cells varied between $0.5 - 1.0 \times 10^6$ HAD⁻ particles per ml. In contrast, medium taken from the rheumatoid cultures at intervals up to 28 days after infection failed to produce CPE when added to cultures of nonrheumatoid or Vero cells, nor could any virus be detected by the hemadsorption-negative assay. Finally, human convalescent antisera known to contain neutralizing antibody against rubella prevented the development of CPE in nonrheumatoid cells, while control sera without neutralizing antibody had no effect on the development of CPE.

Role of Interferon.—The following experiments demonstrated that interferon played no role in protecting the rheumatoid cells. Medium taken from rheumatoid cells, or medium from rheumatoid cells removed 20 hr after pretreatment with poly I: C in an effort to induce interferon, failed to protect nonrheumatoid cells from infection with vesicular stomatitis virus (VSV) or to protect Vero cells from infection with rubella. Similarly conditioned medium from nonrheumatoid cells pretreated with poly I: C completely protected the recipient cells in both test systems (Table I). Rheumatoid cells pretreated with poly I: C remained more sensitive to VSV infection than nonrheumatoid cells so treated (Table II).

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[1] *Abbreviations used in this paper:* CPE, cytopathic effects; HAD⁻, hemadsorption negative; NDV, Newcastle disease virus; VSV, vesicular stomatitis virus.

To quantitate the interferon induced by poly I:C we measured the protection afforded Vero cells against VSV infection by a plaque-reduction assay. Vero cells were used in this test because they are easier to maintain under agar than our strains of human fibroblasts, and human interferon has been shown to protect simian cells (9). A 50% reduction in the number of plaques was obtained with a 1:80 dilution of medium from nonrheumatoid cells pretreated with poly

TABLE I

Failure of Conditioned Medium from Rheumatoid Cells Pretreated with Poly I:C to Protect against Virus Infection

Source of medium		Challenge	
Cell strain	Treatment	N cells with VSV (20 pfu/cell)	Vero with rubella (1-3 pfu/cell)
N 25	Poly I:C	No CPE (5 days)	No CPE (5 days)
R 28	None	CPE day 1	CPE day 1
" "	Poly I:C	CPE day 1	CPE day 1
" "	Rubella	CPE day 1	CPE day 1

The nonrheumatoid (N) and rheumatoid (R) cells were treated with poly I:C 25 μg per 10^6 cells, for 20 hr, or infected with rubella for 24 hr.

TABLE II

VSV Infection* of Synovial Cells Pretreated with Poly I:C (25 μg per 10^6 Cells) for 20 hr

Cell strain	Treatment	CPE
Nonrheumatoid:		
N 25	None	2 days
" "	Poly I:C	12 days
N 26, N27, N28, N29	Poly I:C	10-12 days
Rheumatoid:		
R 28	None	2 days
" "	Poly I:C	4 days
R24, R27, R29	Poly I:C	2 days

* 1-2 pfu/cell

I:C. By comparison, a two-fold dilution of medium from pretreated rheumatoid cells was needed to afford a similar reduction in the number of plaques. The presumed interferon produced by nonrheumatoid cells was stable at pH 3 for 24 hr at 4°C and failed to protect cells pretreated with actinomycin D.

These experiments indicated that the resistance of rheumatoid cells to rubella infection was not mediated by interferon, and that rheumatoid cells, in fact, produced less interferon than nonrheumatoid cells when stimulated by the synthetic polynucleotide poly I:C.

Fate of Rubella Virus in Synovial Cells.—To see if rubella virus could attach

and penetrate into rheumatoid synovial cells, the following experiment was performed. A total of 1.6×10^6 rubella particles were mixed with 10^6 synovial cells and incubated for 90 min at 37°C. The cells were then washed 8 times with phosphate-buffered saline, allowed to incubate in nutrient medium for an additional 60 min and lysed. Virtually equal titers of rubella were obtained from lysates of two nonrheumatoid and two rheumatoid cell strains representing 74–78 % of the input virus. Fig. 1 shows a growth curve of rubella in a strain of nonrheumatoid synovial cells, and, for comparison, the titers at two time points, of rubella in a
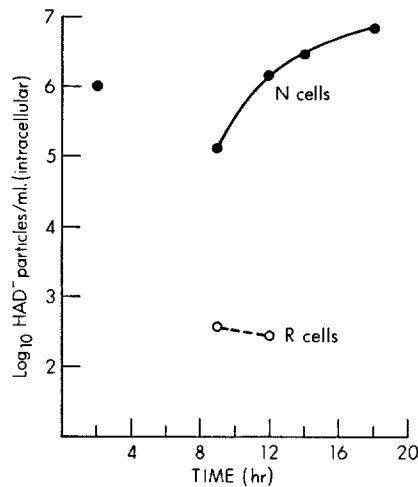

FIG. 1. The initial growth of rubella in nonrheumatoid (N) and rheumatoid (R) synovial cells at a multiplicity of one particle per cell. The titer of virus in lysates of washed cells was determined by a hemadsorption-negative (HAD⁻) particle assay (6) on primary green monkey kidney cells (Microbiological Associates).

strain of rheumatoid cells infected in an identical manner. Some rubella virus could be recovered from within rheumatoid cells but the titers were very low compared with those obtained in the nonrheumatoid cells. We could not, however, recover rubella virus from lysates of infected rheumatoid cells at 2, 10, or 28 days after infection.

DISCUSSION

Our experiments demonstrate that rubella virus is able to infect nonrheumatoid synovial cells, yielding a moderately high titer of infectious particles and causing cell damage and death. Arthritis is a prominent manifestation of acquired rubella (1, 2) and, while the virus has been isolated from synovial fluid in at least one patient (11), it has not been established that the virus proliferates in the synovial membrane. Our findings increase the liklihood that rubella syno-

vitis does represent virus multiplication within synovial cells, rather than a secondary immune response. Although the synovitis during rubella is self-limited and usually persists for less than 2 wk, the possible relationship of rubella arthritis to the subsequent development of rheumatoid arthritis has been raised (12). The tendency of rubella to establish a persistent infection, as in the congenital rubella syndrome (13), and to lead to abnormalities of lymphocyte function (14, 15) suggest that the role of rubella and other viruses in the etiology of rheumatoid arthritis deserves investigation.

The resistance of the rheumatoid synovial cells to rubella is not easily explained. We did not detect infectious virus in the medium taken from rheumatoid cells 4–28 days after infection with rubella, nor in lysates of these cells at 2, 10, or 28 days postinfection. We could recover small numbers of virus particles from lysed rheumatoid cells 9–12 hr after infection, indicating that the virus is able to attach and to penetrate rheumatoid cells and, perhaps, even to replicate in a very limited way or in a small portion of these cells. The persistence of one or more virus particles within the rheumatoid cells could not be ascertained directly by a hemadsorption-negative technique because rheumatoid cells are more than 50% hemadsorption-negative in the absence of added virus (16).

We could not demonstrate that the resistance of rheumatoid cells to rubella was mediated by interferon. On the contrary, the rheumatoid cells were poor producers of interferon even when stimulated by poly I:C. These findings are not unique. One example of non–interferon-mediated resistance, called intrinsic interference, has been described by Marcus and Carver (6). This interference is specifically directed against Newcastle disease virus (NDV) and is induced by a variety of viruses. Intrinsic interference is lost upon serial subculture, whereas our rheumatoid cells do not lose their resistance to rubella upon repeated subculture. In a more closely related system, Rawls and Melnick (13) have shown that cells chronically infected with rubella, cultured from infants with the congenital rubella syndrome, are resistant to superinfection with VSV and herpes simplex virus, in the absence of demonstrable interferon. The tantalizing question is whether the resistance of rheumatoid cells reflects the acquisition of genetic information by virtue of a latent or defective virus, rather than some alteration in their metabolic or immune function as a consequence of the disease. The resistance does not result from changes induced merely by chronic inflammation, since several cell strains derived from severe and chronically inflammed synovial membranes not caused by rheumatoid arthritis are sensitive to rubella.

Smith and Hamerman (16) have recently reviewed the characteristics that distinguish rheumatoid synovial cells in culture from nonrheumatoid cells, including the finding that nonrheumatoid cells are highly susceptible to NDV, whereas the rheumatoid cells are partially resistant. Our studies with rubella provide further evidence that there are differences between rheumatoid and

nonrheumatoid synovial cells that can be propagated through many generations in culture. This evidence further justifies the use of synovial cells in culture to investigate the etiology of rheumatoid arthritis.

SUMMARY

Cells from explants of synovial membranes obtained from patients with and without rheumatoid arthritis and grown in tissue culture were infected with rubella virus. All 28 nonrheumatoid cell cultures showed marked cytopathic effects by 14 days, while none of the 21 rheumatoid cultures were visibly affected. This resistance was not mediated by interferon.

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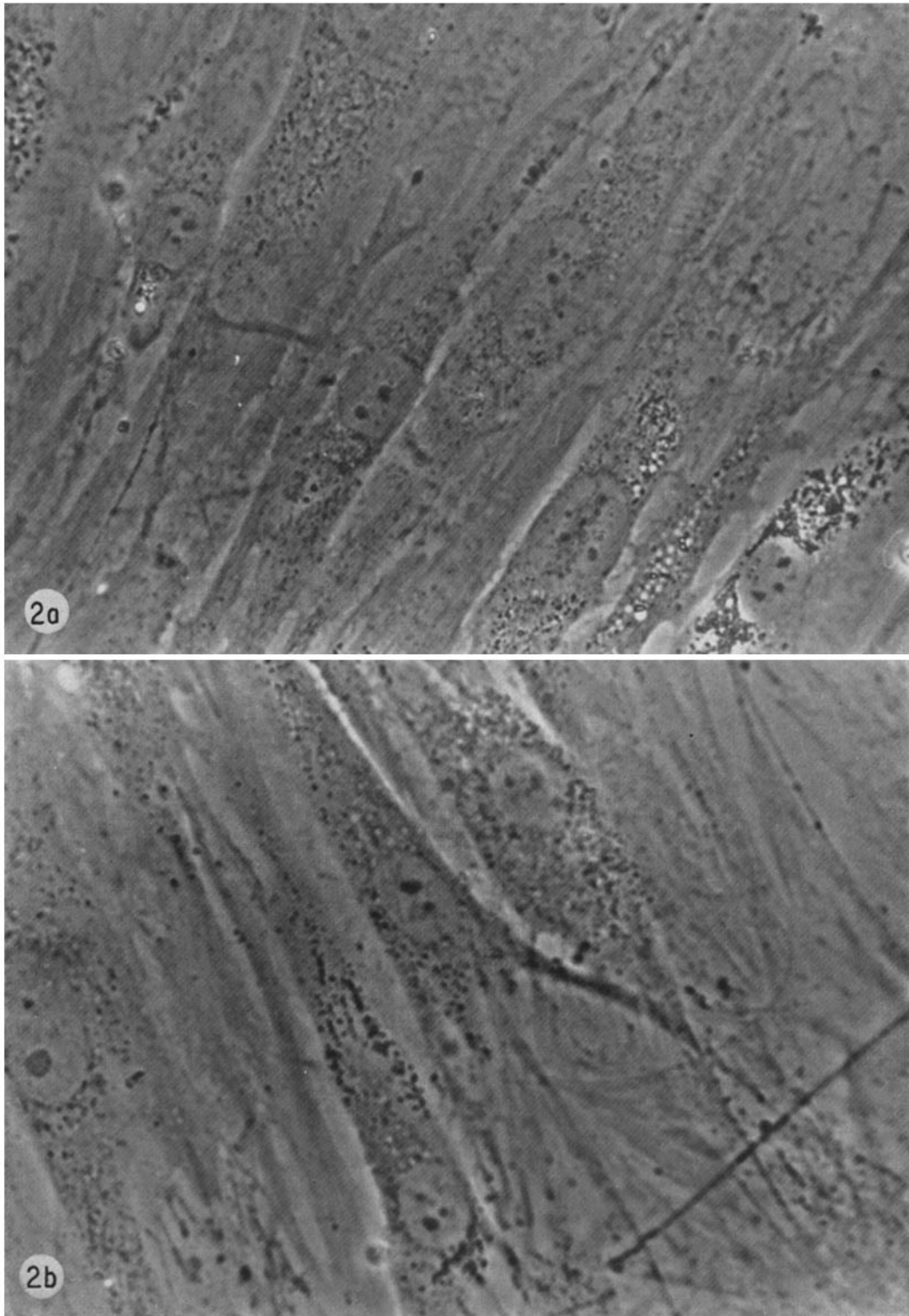
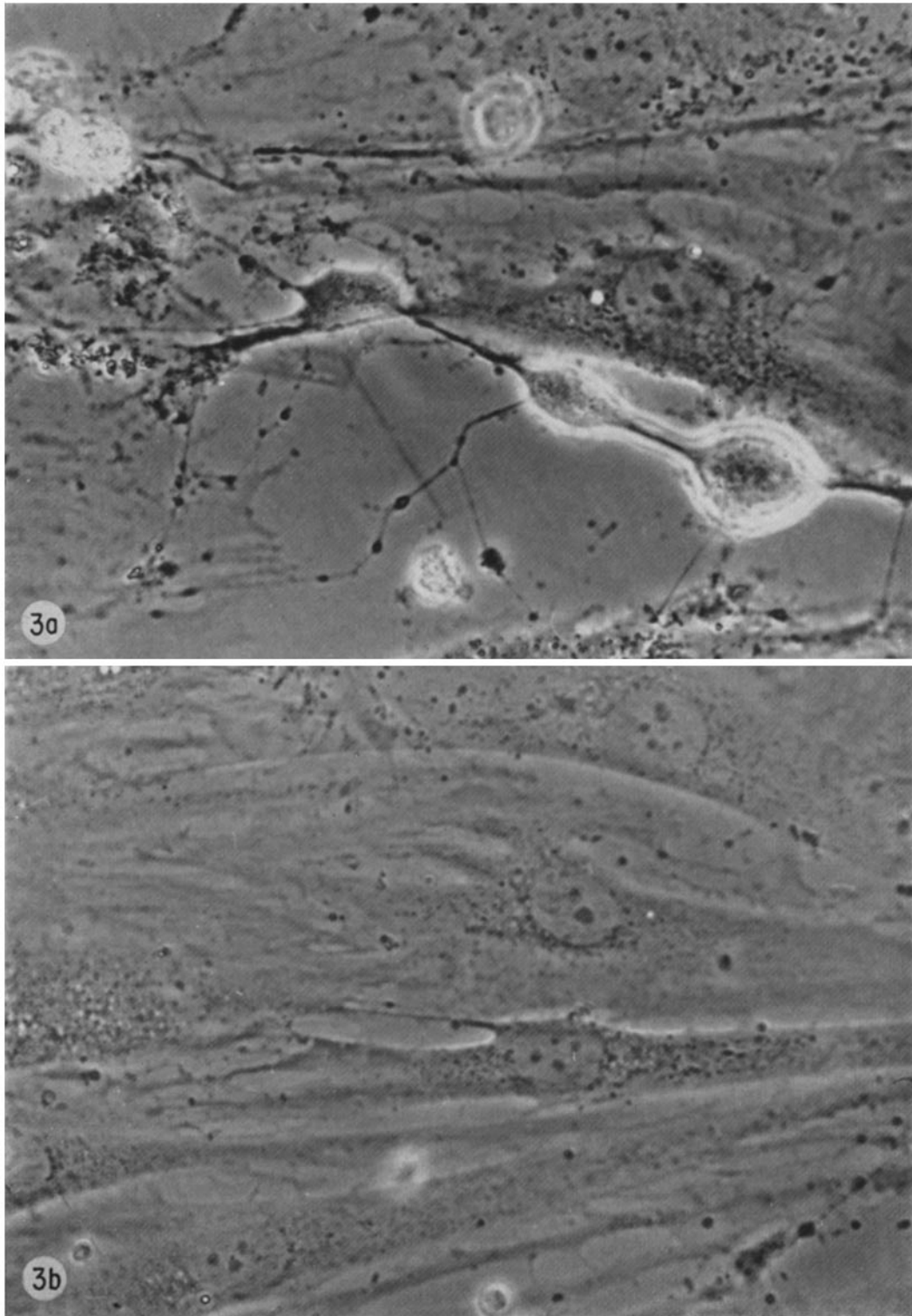Fig. 2. Nonrheumatoid (a) and rheumatoid (b) synovial cells subcultured three times. × 760.

FIG. 3. Nonrheumatoid (a) and rheumatoid (b) synovial cells, replicate cultures of those in Fig. 2, 14 days after infection with rubella virus demonstrating the marked CPE seen in infected nonrheumatoid cells × 760.