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Growth of Newcastle Disease Virus and Rubella Virus in Rheumatoid and Nonrheumatoid Synovial Cell Cultures

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Rheumatoid and nonrheumatoid synovial cell cultures were challenged with Newcastle disease virus and rubella virus in an attempt to confirm reports that rheumatoid synovial cells are relatively resistant to infection with these viruses. Newcastle disease virus caused complete cell destruction by day 7 in both rheumatoid and nonrheumatoid cultures, and peak virus titers were similar. Rubella virus replicated in both rheumatoid and nonrheumatoid synovial cell cultures, and no consistent differences in virus titers were detected. Rubella-infected cell lines were observed for up to 28 days and no virus-specific cytopathic effect was seen.

Chronic or noncytopathic viral infection of cells may be manifested as cellular resistance to infection with other viruses (5, 6, 8). Thus, reports that rheumatoid synovial cells were less susceptible than normal synovial cells to infection with Newcastle disease virus (C. Smith and D. Hamerman, Arthritis Rheum. **11**:842-843, 1968, Abstract) or rubella virus (4) have been interpreted as suggesting the presence of a virus in rheumatoid synovial cells (4).

Recently, Person et al. (7) and Runge and Allison (9) were unable to detect a difference in susceptibility between normal and rheumatoid synovial cells to infection with Newcastle disease virus, vesicular stomatitis virus, or rubella virus. Because of the conflicting reports in the literature, we would like to record our studies of the comparative sensitivity of normal and rheumatoid synovial cells to rubella virus and Newcastle disease virus.

MATERIALS AND METHODS

Synovial cell cultures. Synovium obtained at autopsy or during surgery was immediately transported to the laboratory in Leibovitz medium (L-15). The synovial intima was dissected away, cut into 2 to 5 mm^2 fragments, and transferred into etched 25-cm^2 disposable plastic flasks (Falcon, Div. of BioQuest). Explants were incubated at 37 C in L-15 media supplemented with 10% heat-inactivated fetal bovine serum (FBS), penicillin (100 units/ml), gentamicin (100 μg/ml), and Amphotericin B (2.5 μg/ml). Medium was changed every 3 to 5 days initially and every 7 to 10 days when the monolayers became established. Confluent monolayers were transferred using 0.25% trypsin.

Synovial cell cultures were assayed for the production of hyaluronic acid by the sensitivity of acid-induced precipitates to hyaluronidase (1). A 2-ml amount of 7-day-old culture media was incubated for 2 hr at 37 C with 175 IU of bovine testicular hyaluronidase (B grade, Calbiochem, Los Angeles). A 0.05-ml amount of 7 N acetic acid was then added, and the resultant precipitate was compared with that of a tube not treated with hyaluronidase. The test was considered positive if the enzyme-treated tube was completely clear and the untreated tube had a definite precipitate; this result was considered presumptive evidence that hyaluronic acid was present and that there were not significant quantities of other mucopolysaccharides.

Rubella virus. The M-33 strain of rubella virus was supplied by Paul Parkman (Division of Biologic Standards, National Institutes of Health [NIH]). Prior to use in our studies the virus had been passed three times in BSC-1 cells, four times in primary African green monkey kidney cells (AGMK), and twice in Vero cells. Neutralization studies with pre- and postvaccination equine rubella antisera (lot no. H860131N and no. H860131, Flow Laboratories, Rockville, Md.) confirmed the identity of the virus.

Newcastle disease virus. Newcastle disease virus (NDV, F-8) was obtained from A. Grayzel (Montefiore Hospital, Bronx, N.Y.) and passed five times in Vero cells. The identity of this virus was confirmed by both neutralization and hemadsorption inhibition tests using NDV antisera (Research Reference Reagents, NIH).

Infection of synovial cell cultures. Synovial cell cultures were inoculated with 10 fifty percent tissue

culture infectious doses ($TCID_{50}$) of NDV. Virus was adsorbed for 1 hr at 4 C after which the medium was changed. Medium was assayed for virus at varying times after infection by detection of cytopathic effect (CPE) on Vero cells.

Cultures were inoculated with rubella virus in virus to cell input ratios (multiplicities) of 6:1, 2:1, 1:25, and 1:250. The virus was adsorbed at 35 C for 3 hr, after which the monolayers were washed twice, and then 5 ml of L-15 medium with 2% FBS was added (time zero). At various time intervals medium was withdrawn and stored at −100 F before assay for rubella virus. Medium was replaced to keep the volume constant.

Rubella virus was assayed by the enteroviral interference technique using Echo-11 virus (NIH) and AGMK cells (6).

RESULTS

Synovial tissue cell cultures were obtained from 14 persons with rheumatoid arthritis and 10 controls with illnesses other than rheumatoid arthritis (Table 1). Most of the rheumatoid arthritis specimens were from patients with active disease and were obtained at the time of therapeutic synovectomy. Two rheumatoid cell cultures were provided by A. Grayzel. Two of the control subjects had degenerative joint disease, and the others had normal joints. Cell cultures were examined qualitatively for hyaluronic acid in the media, and 9 of the 12 rheumatoid specimens and 6 of 8 control specimens were positive.

Replication of NDV in synovial cells. The growth of NDV was assessed in four rheumatoid and four normal synovial cell lines (Fig. 1). There was no appreciable difference in the rate of virus growth or peak titer of virus between normal and rheumatoid cells. CPE was observed by day 5 and progressed uniformly to complete cell destruction by 7 days in all virus-infected cultures.

TABLE 1. *Source of synovial tissue cell cultures*

Case no.	Age–sex	Known arthritic condition	Means tissue obtained	No. of tissue culture passes when infected	Duration of cell cultures when infected (months)	Hyaluronic acid presumptively demonstrated in tissue culture media
Patients with RA[a]						
35	66–F	RA	Synovectomy	P_6	8	NT[b]
37	56–F	RA	Synovectomy	P_6	7	NT
39	54–F	RA	Synovectomy	P_{11}	14	+
42	28–F	RA	Synovectomy	P_2	5	+
45	58–F	RA	Synovectomy	P_8	12	+
46	22–M	RA	Synovectomy	P_2	5	−
47	57–M	RA	Synovectomy	P_{6-8}	9–12	+
108	58–F	RA	Synovectomy	P_4	8	+
110	59 F	RA	Synovectomy	P_{3-6}	5–8	+
115	42–F	RA	Synovectomy	P_5	5	+
117	36–F	RA	Synovectomy	P_5	8	+
120	50–M	RA	Synovectomy	P_2	2	+
R-24[c]	60–F	RA	Synovectomy	P_{13}	5	−
R-33[c]	14–F	RA	?	?	>4	−
Patients without RA						
N-13	58–F	None	Autopsy	?	5	NT
N-14	52–M	None	Autopsy	P_5	11	+
N-15	46–F	None	Autopsy	P_2	4	+
N-16	58 M	None	Autopsy	?	3	NT
101	52–F	None	Autopsy	P_{5-6}	8–11	+
107	Newborn-M	None	Autopsy	P_6	6	+
118	88–M	None	Autopsy	P_{3-5}	3–6	−
119	80–F	None	Autopsy	P_3	2	+
122	63–M	DJD[d]	Autopsy	P_{3-4}	2	+
127	61–F	DJD[d]	Total hip replacement	P_4	4	−

[a] RA, Rheumatoid arthritis.
[b] NT, Not tested.
[c] Provided by A. Grayzel.
[d] DJD, Degenerative joint disease.

Replication of rubella virus in synovial cells. Four experiments were conducted with different virus to cell multiplicities: 6:1, 2:1, 1:25, and 1:250. No differences related to multiplicity were observed, and the results are hereafter combined.

Ten rheumatoid arthritis cultures and seven nonrheumatoid arthritis cultures were observed for CPE due to rubella virus by serial observation of inoculated and uninoculated flasks for as long as 28 days (Table 2). No specific cytopathic changes could be attributed to rubella virus infection. Degeneration of cells was first noted in the second week of culture of nonrheumatoid cells, and in the third week of culture of rheumatoid cells (Table 2). This rapid rate of degeneration was not seen in synovial cell cultures maintained on 10% serum.

Synovial cell monolayers (50 to 75% confluent) were inoculated with a 6:1 or 1:25 multiplicity of rubella virus, and the subsequent replication of rubella virus was followed by serial determinations of the virus titer in the media. The results with eight rheumatoid and six nonrheumatoid cell lines are presented in Fig. 2.

Virus titers on individual specimens showed wide fluctuation; but upon combining the data,

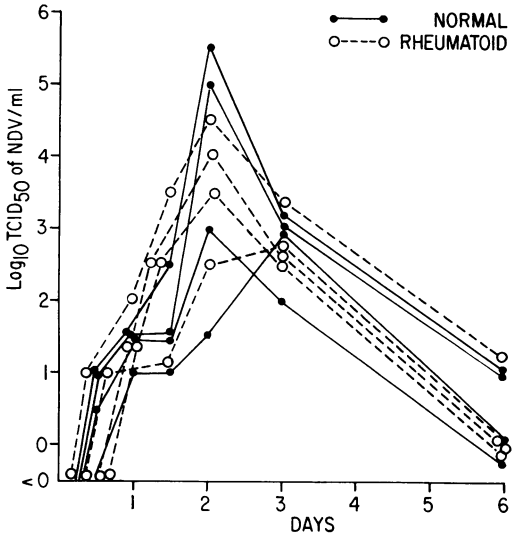

FIG. 1. *Growth of Newcastle disease virus in normal and rheumatoid synovial cell cultures. Inoculum was 10 $TCID_{50}$ per culture. Normal synovial tissues used were no. N-13, N-15, and N-16; rheumatoid cultures used were no. 35, 37, 42, and 46.*

TABLE 2. *Cytopathic changes in synovial cells following inoculation with rubella virus*

Cells	Proportion of cultures with cytopathic changes			
	0-7[a]	8-14	15-21	22-28
Rheumatoid (10 cell lines)[b]				
Control	0/12	0/12	1/12	2/6
Rubella virus inoculated	1/13	1/13	2/13	2/7
Nonrheumatoid (7 cell lines)[b]				
Control	0/10	4/10	3/7	5/5
Rubella virus inoculated	0/10	3/10	3/7	4/5

[a] Days postinoculation.

[b] Some cell lines were challenged more than once with different multiplicities.

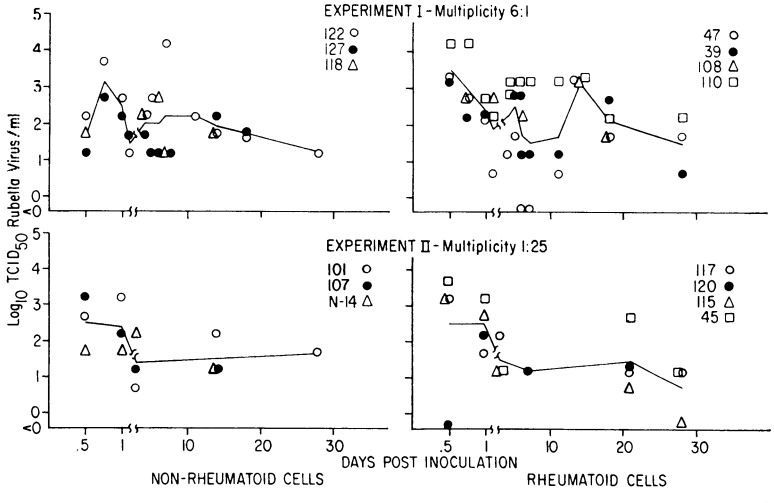

FIG. 2. *Titer of rubella virus in the medium after inoculation of rheumatoid and nonrheumatoid cell cultures.*

no major differences were apparent between rheumatoid and nonrheumatoid cells. With one exception, the highest titers were seen within the first 24 hr after inoculation, and thereafter titers remained stable or declined slightly. Virus was detectable at 28 days in five of six rheumatoid and two of two nonrheumatoid cell lines. A 150-fold difference in input multiplicity appeared to have only a minor effect on the magnitude of subsequent titers.

DISCUSSION

The possibility that rheumatoid arthritis may have an infectious etiology has been the object of many investigations (2). The report that rheumatoid synovial cells were relatively resistant to infection with rubella virus (4) suggested the presence of an interfering virus in rheumatoid synovial cell cultures and encouraged others to investigate the relative sensitivity of rheumatoid and normal synovial cells to virus infection. Runge and Allison (9) were unable to detect any resistance of rheumatoid cell lines to rubella virus replication or cytopathic effect; and Person et al. (7) were unable to detect differences in replication of rubella virus, NDV, or vesicular stomatitis virus in rheumatoid versus normal synovial cells. Our own observations are similar to those of Runge and Allison (9) and Person et al. (7) in that we were unable to detect differences in the sensitivity of rheumatoid and nonrheumatoid synovial cells to infection with rubella virus or NDV.

It is unlikely that our inability to confirm the original observations of Grayzel (4) is due to differences in the challenge strain of rubella virus, since Grayzel (*personal communication*) has been able to repeat his observations with the M-33 strain of rubella virus. In addition, it is unlikely that the conflicting results are due to differences in the cells tested since the methods of establishing cultures of synovial cells are similar, and cell lines established by Grayzel were included in the present study and in Person's study (7).

If the differences in reported findings are not due to differences in technique, they may be due to differences in susceptibility of synovial cells to viral infection. Ford and Oh (3) reported considerable variation in the sensitivity of both normal and rheumatoid synovial cells to infection with NDV, and we noted similar variability in sensitivity to rubella virus. As the number of synovial lines tested for sensitivity to rubella virus infection by various laboratories increases, it appears that relative resistance to infection with rubella virus may be an occasional but probably not a general property of rheumatoid synovial cells.

ACKNOWLEDGMENTS

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