Replication of Rubella, Newcastle Disease, and Vesicular Stomatitis Viruses in Cultured Rheumatoid Synovial Cells[1] (35981)

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A number of reports have detailed biochemical abnormalities in cultured synovial cells derived from rheumatoid as compared with nonrheumatoid patients (1–5). The recent report that rubella virus failed to replicate in rheumatoid derived synovial cells (6) and the suggestion that rheumatoid synovial cells were less susceptible to infection with Newcastle disease virus than cells derived from nonrheumatoid patients (5) suggested more fundamental biological differences between rheumatoid and nonrheumatoid cells. In an attempt to confirm and extend these findings, it was found that rubella, Newcastle disease, and vesicular stomatitis viruses all replicate in nonrheumatoid as well as rheumatoid derived synovial cells in culture.

Materials and Methods. Cell culture and media. Synovial membranes obtained at operation were transported to the laboratory in Eagle's medium supplemented with sodium bicarbonate, 0.75 g/liter and fetal bovine serum (FBS), 20%. Fat and fibrous connective tissue were removed as completely as possible, and the synovial membrane was minced into 0.5–2.0 mm pieces and explanted into plastic tissue culture flasks (25 cm^2, Falcon Plastics, Oxnard, CA). All specimens were explanted within a few hours of operation. Growth media contained sodium bicarbonate, 0.75 g/liter; FBS, 10%; penicillin, 100 units/ml; streptomycin, 100 mg/ml; and Mycostatin, 50 units/ml. In maintenance medium, 1.5 g/liter of bicarbonate and FBS, 2% were substituted. In general, outgrowth of fibroblast-like cells was sufficient, within 10–20 days, to allow trypsinization (0.25% trypsin) of the monolayer and transfer into 4-oz glass prescription bottles. By the second transfer, 16-oz prescription bottles were seeded with the synovial derived cells. Eleven cell cultures were obtained from six patients with rheumatoid arthritis and five patients with nonrheumatoid disease (posttrauma). Additionally, six cell cultures (three rheumatoid, three nonrheumatoid) were kindly provided by Drs. Carol A. Smith, Arthur I. Grayzel, and David Hamerman (Montefiore Hospital and Medical Center, Bronx, NY).

Rabbit kidney cells (RK), chick embryo fibroblasts (CEF), and a continuous line of green monkey kidney cells (BSC-1) were used in the assays for infectious viruses. Details concerning the preparation, maintenance, and handling of these cells have been described (7–9).

Viruses and virus assays. Rubella virus, strain R-1, isolated from the thyroid of a congenitally infected infant (9) and propagated in BHK-21 and Vero cells was used throughout. Newcastle disease virus (NDV) (California strain, American Type Culture Collection) passaged a number of times in the allantoic sac of embryonated hen eggs, and vesicular stomatitis virus (VSV), Indiana strain, propagated in green monkey kidney cells, were also used to challenge the synovial cell cultures.

The 16-oz cell cultures were trypsinized and split (1:2) and constricted glass culture tubes were seeded (10). When confluent mo-

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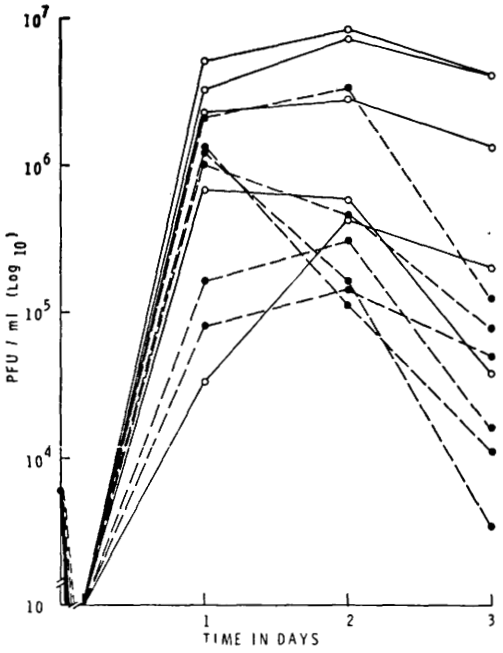

FIG. 1. Replication of VSV in synovial cell cultures: (○ —) nonrheumatoid; (● - -) rheumatoid; input 6 × 10^3 PFU of VSV.

nolayers had developed, the cells were challenged with 0.1 ml of stock rubella virus containing 1.5 × 10^4 hemadsorption-negative plaque-forming units (HAd-PFU; as assayed in BSC-1 cells); NDV containing 1 × 10^4 PFU/0.1 ml (as assayed in CEF under agar overlay); or VSV containing 6 × 10^3 PFU/0.1 ml (as assayed in BSC-1 cells under agar overlay). Comparable numbers of cells (~0.5–1 × 10^5) both rheumatoid and nonrheumatoid were challenged with the three viruses. All stock viruses were diluted in maintenance medium and all adsorptions were carried out for 1 hr at 37°. Following adsorption, the monolayers were washed twice with 2 ml of maintenance media, and 1 ml of medium was added to each tube. Three constricted tubes of each cell strain with each challenge virus were harvested postadsorption and on days 1, 2, and 3 in the case of NDV and VSV, and additionally on days 4 and 5 in the case of rubella virus. The inoculated cell cultures and uninoculated controls were observed daily for cytopathic effects (CPE). Prior to assay for infectious virus, the harvested tubes were frozen (dry ice–alcohol bath) and thawed three times and their contents were pooled.

The plaque assays for NDV in CEF and VSV in RK have been described (7). The agar overlay in both assays contained the same constituents as the growth medium with the addition of Bacto-agar, 1.2%, and neutral red, 25 mg/liter. Confluent monolayers of CEF and RK were inoculated with 0.2 ml of serial 10-fold dilutions of NDV and VSV harvested from the synovial cell cultures, adsorbed for 1 hr at 37°, and overlayed with 4 ml of agar medium. The overlay was allowed to solidify, and the cultures were incubated at 37° in a 5% CO_2 atmosphere. The sodium bicarbonate content of all cultures incubated in the 5% CO_2 was increased to 2.25 g/liter. After 3 days incubation, plaques were counted. Assay for rubella virus in BSC-1 cells utilizing the hemadsorption-negative

TABLE I. Replication of Rubella Virus in Synovial Cell Culture.[a]

Cell strain/ passage level	Diagnosis	Maximum virus produced (log_{10})
A[b,c]	RA	4.23, 3.17[d]
B/11[c]	RA	3.33, 2.61
Wyn/9	RA	3.45, 3.03
Bri/9	RA	3.00, 1.83
Blu/4	RA	4.07, 3.30
Val/9	RA	3.33, 2.81
E/15	RA	3.40, 3.27
Haw/4	RA	3.17, 2.97
Gar/4	RA	2.82, 2.99
Mean ± SD		3.15 ± 0.52
C/9	NR	3.44, 2.79
D[c]	NR	4.70, 3.42
G/9	NR	2.99, 2.51
Tay/3	NR	3.03, 3.42
Sch/3	NR	2.65, 3.26
Bak/3	NR	3.57, 3.29
She/3	NR	3.39, 3.29
Bar/3	NR	2.95, 3.03
Mean ± SD		3.23 ± 0.50
Muscle	Control	3.23, NT

[a] RA, rheumatoid arthritis; SD, standard deviation; NR, nonrheumatoid; and NT, not tested.

[b] All cell strains with letter designation were kindly provided by Drs. Smith, Grayzel, and Hamerman.

[c] Epithelioid cell type.

[d] Separate determinations.

TABLE II. Induction of Interference in Synovial Cell Cultures.[a]

Cell strain/ passage level	Diagnosis	Pretreatment	VSV titer (PFU/ml) Postad-sorption	Day: 1	2	3
Bak/6	NR	None	0	1.3×10^5	3.6×10^5	1.4×10^5
		Poly I:C[b]	0	0	0	0
C/14[c]	NR	None	0	1.0×10^5	1.6×10^5	2.1×10^5
		Poly I:C	0	0	0	0
Bri/14	RA	None	0	1.2×10^5	1.2×10^6	2.5×10^5
		Poly I:C	0	0	0	0
Blu/6	RA	None	0	1.2×10^6	2.3×10^6	1.4×10^5
		Poly I:C	0	0	0	0
Wyn/15	RA	None	0	4.1×10^5	1.8×10^6	3.4×10^5
		Poly I:C	0	0	0	0

[a] NR, nonrheumatoid; RA, rheumatoid arthritis.
[b] 25 μg/10^6 cells $\times$ 18 hr.
[c] Cell strain kindly provided by Drs. Smith, Grayzel, and Hamerman.

plaque assay has been described in detail (8).

Interference. Three rheumatoid and 2 nonrheumatoid derived cell strains were exposed to polyriboinosinic-polyribocytidylic acid (Poly I:C; Microbiological Associates, Bethesda, MD) for 18 hr at a concentration of 25 μg/10^6 cells. The medium was removed, the cell monolayers were washed twice with 2 ml of maintenance medium, and the cells were challenged with 6×10^3 PFU of VSV. The cells were observed daily for CPE and were harvested and assayed for infectious VSV as described above.

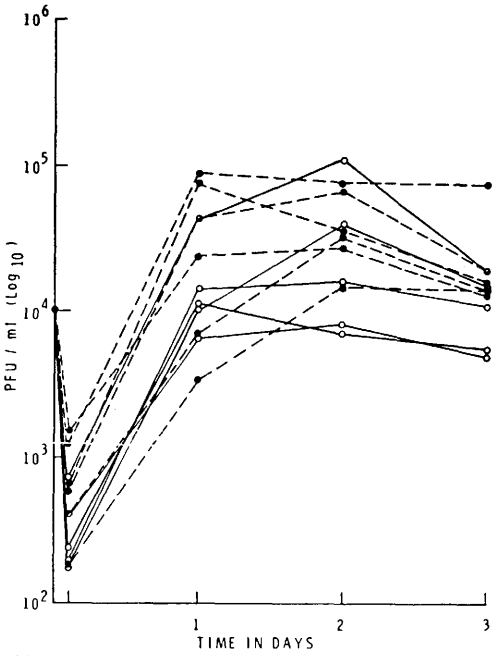

FIG. 2. Replication of NDV in synovial cell cultures: (O—) nonrheumatoid; (●- -) rheumatoid; input 1×10^4 PFU of NDV.

Inactivation of viruses by extracellular fluids. In another series of experiments, rheumatoid and nonrheumatoid cells were seeded into constriction tubes. After monolayers developed, the growth medium was replaced with maintenance medium. The cell cultures were incubated for 48 hr at 37° and the extracellular fluids were harvested. Virus stocks were diluted 1:10 in the extracellular fluid medium and incubated at 37°. Aliquots were removed and assayed for surviving virus at 0, 12, and 24 hr for rubella virus; and at 0, 24, and 48 hr for NDV and VSV.

Results. Rubella virus produced no detectable CPE in synovial cell cultures during the 5 day observation period. Significant and characteristic CPE was produced in VSV and NDV inoculated cultures, such that essentially total destruction of the monolayers was observed within 3 days with VSV and 50–75% involvement of the monolayers was observed with NDV-infected cells during the same period. No differences in the character

or extent of the CPE was observed when rheumatoid and nonrheumatoid cell strains were compared. Assay for infectious VSV (Fig. 1) indicated that following total eclipse the virus replicated significantly over the period of 3 days. There were no significant differences in the replication of VSV in rheumatoid *versus* nonrheumatoid cells. Likewise, NDV replicated well in both cell types (Fig. 2). Rubella virus replicated in all cells tested, and no significant differences obtained between cell types (Table I).

Interferon induction with Poly I:C was evaluated in 2 nonrheumatoid and 3 rheumatoid cell strains. Interference was manifested in all cell strains when challenged with 6×10^3 PFU of VSV. No CPE developed in pretreated cell cultures and infectious virus could not be recovered from the pretreated cell cultures (Table II).

Preliminary data suggested that inactivation of VSV by rheumatoid and nonrheumatoid lysates was comparable. Studies on inactivation of VSV, NDV, and rubella virus by extracellular fluids are listed in Table III. It is clear from these data, and in spite of a suggestion of enhanced inactivation of VSV between days 2 and 3 (Fig. 1) that, at least with the extracellular fluids tested, there was no significant difference in inactivation by rheumatoid *versus* nonrheumatoid fluids.

Discussion. The preliminary data suggesting a relative insensitivity of rheumatoid synovial cells to NDV (5) and the report that rubella virus failed to replicate in rheumatoid synovial cells (6) required an explanation. It is clear from the present results that, employing the virus strains, cell strains, quantitative assays for infectious virus production, and conditions as defined, rubella virus, NDV, and VSV all replicate in synovial cell cultures, irrespective of the origin (rheumatoid or nonrheumatoid). Further, the synthetic interferon inducer, poly I:C, protected both rheumatoid and nonrheumatoid cells from challenge with VSV.

These results are in general agreement with a previous report by Ford and Oh (11), who could demonstrate no significant difference between the multiplication of NDV in rheumatoid and nonrheumatoid synovial cell

TABLE III. Inactivation of VSV, NDV, and Rubella by Extracellular Fluids.[a]

Virus	Time (hr)	Cell strain/passage level							
		Nonrheumatoid				Rheumatoid			
		Sch/11	Bak/16	Tay/12	Bar/9	Cha/7	Haw/9	Haw/14	Val/15
VSV	0	5.6×10^5	1.6×10^6	5.3×10^5	3.0×10^5	1.3×10^6	2.8×10^5	5.0×10^5	3.3×10^5
	24	1.6×10^5	1.6×10^4	1.3×10^4	6.5×10^4	2.8×10^4	1.1×10^5	4.2×10^4	5.3×10^4
	48	3.7×10^3	3.0×10^3	2.6×10^2	1.4×10^4	4.9×10^3	2.3×10^3	NT	3.3×10^3
NDV	0	7.2×10^7	7.7×10^7	6.9×10^7	NT	5.5×10^7	NT	4.4×10^7	NT
	24	2.0×10^7	1.4×10^7	2.1×10^7	NT	1.6×10^7	NT	1.2×10^7	NT
	48	1.0×10^7	3.0×10^6	1.2×10^7	NT	2.4×10^5	NT	7.3×10^6	NT
Rubella	0	1.1×10^5	1.0×10^5	1.3×10^5	NT	9.5×10^4	NT	6.8×10^4	NT
	12	$<10^2$	$<10^2$	$<10^2$	NT	$<10^2$	NT	$<10^2$	NT
	24	$<10^2$	$<10^2$	$<10^2$	NT	$<10^2$	NT	$<10^2$	NT

[a] NT, not tested.

cultures, but are at variance with other reports (5, 6).

It has been established that cell cultures derived from rheumatoid synovial membranes differ metabolically from cell cultures derived from nonrheumatoid synovial membranes (1–5). Rheumatoid synovial cells have been reported to have an increased rate of glucose uptake (2), increased rate of hyaluronate synthesis (2), increased lysosomal enzyme activity (3, 5) and an increased rate of lactate production (2) when compared to nonrheumatoid synovial cell cultures.

We favor the view that major metabolic differences between rheumatoid and nonrheumatoid cells in culture may very well alter the apparent sensitivity of the cells to viral infection. We were, however, unable to demonstrate enhanced inactivation of the viruses by rheumatoid extracellular fluids. This confirms and extends previous work with NDV (11).

The possibility remains that viruses, cell strains, and techniques were sufficiently different (5, 6) to explain the divergent results. None of these results excludes a possible infectious etiology for rheumatoid arthritis. It is suggested, however, that newer, more innovative methods will be required to elucidate the etiology of rheumatoid arthritis.

Summary. Rubella virus, VSV, and NDV replicate as well in rheumatoid as in nonrheumatoid derived synovial cells, in culture. Interference to VSV challenge is produced in both cell types following treatment with poly I:C. Extracellular fluids from both rheumatoid and nonrheumatoid cells inactivate the three viruses to approximately the same degree.

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