Rubella virus and rheumatoid arthritis

HELENA HART AND B. P. MARMION

From the Department of Bacteriology, Edinburgh University Medical School, Teviot Place, Edinburgh EH8 9AG

SUMMARY A collection of synovial fibroblasts from 19 patients with rheumatoid arthritis (RA) and 12 patients with osteoarthritis or other non-RA disease has been examined for rubella virus antigens by immunofluorescence and radioimmunoassay with negative results. Eluates of synovial membrane prepared under conditions likely to dissociate antigen-antibody complexes have shown no rubella antibody. A serological survey of RA patients and those with other forms of arthritis has shown no differences in the frequency or levels of rubella haemagglutination-inhibiting antibody. These results provide little support for various hypotheses that a persistent infection with rubella virus underlies or initiates the rheumatoid process.

The phenomenon of persistent infection with rubella virus is familiar from the congenital rubella syndrome and from some patients with a variety of subacute sclerosing panencephalitis (Townsend *et al.*, 1975; Weil *et al.*, 1975), and also from persistent infection of cultured cells *in vitro*. The tendency, taken with the observation of a self-limiting synovitis as an occasional complication of acute rubella, lends attraction to a hypothesis that a persistent infection of synovial cells with rubella virus might be the ultimate antigenic stimulus in juvenile (JRA) or adult rheumatoid arthritis (RA).

To test this hypothesis numerous workers have compared the frequency, or mean titre, of rubella antibody in rheumatoid patients with that in control groups of healthy persons, or patients suffering from other forms of arthritis (Chandler *et al.*, 1971; Laitinen *et al.*, 1972; Simsarian *et al.*, 1970; Deinard *et al.*, 1974). These studies have generally failed to show a difference except for those described by Patterson *et al.*, (1973 and personal communication) and Deinard *et al.* (1974). Other workers have examined antibody from RA synovial fluid cryoprecipitates, or eluates from RA synovial membranes without, however, detecting striking amounts of rubella antibody (Cremer *et al.*, 1974; Zvaifler, 1973).

However, the resolution of rubella virus infection may be protracted in some rheumatoid patients. Thus Ogra, *et al.* (1975) observed a prolonged infection with rubella virus in some JRA patients,

as judged by high and persistent levels of IgM and IgG antibody to rubella virus and the demonstration of rubella antigen by immunofluorescence in smears of synovial fluid cells. These effects may be related to the higher prevalence of histocompatibility antigen HLA-B27 in JRA patients (Rachelefsky *et al.*, 1974), with its postulated immunological implications.

An association of persistent rubella infection and adult RA appears to be much less common. A serologically documented case of rubella arthritis progressing to, or associated with, classical adult RA was described by Martenis *et al.* (1968), who mentioned other patients. More recently, in Edinburgh, a 26-year-old woman has been observed who had an illness resembling rubella and subsequently developed classical RA with positive latex and SSCT tests and a raised erythrocyte sedimentation rate. A raised titre (1/2048-1/4096) of rubella antibody by haemagglutination-inhibition in both the IgG and IgM fractions of her serum was detected early in the illness. The rheumatoid state persisted for 18 months after onset of symptoms when there was a resolution of the symptoms and signs accompanied by a fall in the level of IgM rubella antibody (Duthie *et al.*, unpublished).

Other investigators have approached the problem by trying to show rubella virions or other gene products, or interference by rubella virus, in RA synovial cells, usually fibroblasts. Thus Grayzel and Beck (1970), Smith and Hamerman (1969), and Ford and Oh (1965) found that RA synovial fibroblasts were more resistant to infection with rubella or Newcastle disease viruses than non-RA fibroblasts. This was construed as an intrinsic viral interference, but

Patterson *et al.* (1975) and Clarris *et al.* (1974) have shown that with rubella virus and Newcastle disease virus the apparent resistance of RA synovial fibroblasts is due to a thicker capsule of hyaluronic acid which hinders viral absorption; it can be removed by pretreatment with hyaluronidase.

The mechanism of chronic cellular infection by rubella virus is perhaps not completely understood but presumably involves persistence of infectious virions or viral RNA as cytoplasmic entities transmitted at cell division. Attempts to show infectious rubella virus in RA synovial fibroblasts have been negative by conventional cell culture inoculation, or co-cultivation, as have attempts to show incorporation of radiolabelled uridine into components of fibroblasts with the sedimentation characteristics of rubella RNA or virions (Grayzel, 1973; Person *et al.*, 1973; Norval and Marmion, 1976). Although there appear to be no analogies from cell systems persistently infected with rubella virus, the formal, if slightly heterodox possibility remains that rubella infection of RA synovial cells might take the form of the expression of viral antigens without infectious virions, given, for example, persistence of genetic information in the form of an integrated DNA copy of the RNA viral genome (Sato *et al.*, 1976).

In this context a recent, brief preliminary report by Patterson *et al.* (1973) was therefore of much interest. These workers, using ^{51}Cr release from cells, complement, and a rubella antiserum from a hyper-immunized rhesus monkey showed (presumptive) rubella antigens on 4 of 6 strains of RA synovial fibroblasts and on a chronically infected cell strain from an infant with the rubella syndrome. Control fibroblasts were negative. This important observation clearly required confirmation, if possible, by other serological techniques. In this paper we report the negative results of attempts to show rubella virus-coded antigens on RA and non-RA synovial fibroblasts by immunofluorescence or radioimmunoassay, and also summarize our own surveys of sera and synovial membrane eluates from RA and non-RA patients for rubella antibody.

Materials and methods

SYNOVIAL FLUID AND MEMBRANE CULTURES FROM RA OR NON-RA PATIENTS

Fibroblasts from synovial fluids were established by mixing equal volumes of fluid and Eagles Complete Medium (1959 modification). No serum was used at this stage. After 5 days the fluid was replaced by Eagles Complete Medium containing 100 IU/ml penicillin and 100 $\mu\text{g/ml}$ streptomycin, supplemented with 10% fetal calf serum (Tissue Culture Services).

Some of the primary cultures were also incubated in the presence of sodium aurothiomalate 10 $\mu\text{g/ml}$ (Myocrisin, 45% metallic gold, May and Baker, Dagenham, England) and hydrocortisone 36 $\mu\text{g/ml}$ (Sigma). Sodium aurothiomalate is concentrated within phagocytic cells and inhibits their lysosomal enzyme activity (Persellin and Ziff, 1966); it has also been shown to inhibit the cytotoxic and antitumour cell activity of macrophages (McBride *et al.*, 1975; Ghaffar *et al.*, 1976). Synovial fibroblasts with 'nonself' or neoantigens destined for destruction by macrophages might thereby be preserved for examination. Glucocorticosteroids have been reported to increase proliferation of adult human fibroblasts *in vitro* and reduce the rate of hyaluronic acid formation (Castor, 1965).

Fibroblasts from synovial membrane were obtained by overnight trypsinization of synovial tissue (0.25% trypsin in Dulbecco solution) at 37°C and transfer of the free cells into Eagles Complete Medium containing penicillin and streptomycin with 10% fetal calf serum. The monolayers of fibroblasts from either source were then propagated by trypsinization. Some of the features of these fibroblast cultures, marked A to N, in particular their examination for the presence of leucoviruses, are described elsewhere (Norval and Marmion, 1976). Additional fibroblasts have been used in this study and are labelled II-XXI.

VIRUSES

The HPV-77 rubella virus, vaccine strain and a recent isolate strain 'Thomas', were kindly supplied by Drs. Gould and Freestone of the Wellcome Research Laboratories (Beckenham).

INFECTED AND NONINFECTED CELLS FOR CONTROLS IN SEROLOGICAL TESTS

A persistent infection was established in the LCC-MK₂ cell line (American Cell Type Collection) with the HPV-77 rubella virus strain and kept on 199 medium (Wellcome) with 2% heat inactivated (56°C) fetal calf serum (FCS). The persistence and growth curve of rubella virus in these cells has been described (Maassab *et al.*, 1964) and their use for indirect immunofluorescence has been evaluated (Brown *et al.*, 1964). The infected cells change morphology, becoming sparser, fatter, and more elongated compared with the uninfected parent cells. After an initial 3 weeks they were subcultured every 2 weeks. The presence of rubella virus was confirmed by indirect immunofluorescence (Lennette *et al.*, 1967) and by labelling with ^3H -uridine (Norval and Marmion, 1976). For lytic infection the Thomas or HPV-77 strain of rubella virus was absorbed on to BHK-21 cells in suspension as described by Lennette

and Schmidt (1968) and maintained in Eagles Complete Medium with 2% FCS. For the detection of rubella membrane antigens the virus strains 'Thomas' and HPV-77 were propagated in BHK-21 with Complete Eagles Medium, supplemented with 1% FCS. The cells were used 4 days after being infected.

LYMPHOCYTES

Peripheral blood and synovial fluid lymphocytes were separated on Triosil-Ficoll gradients. Synovial fluids were treated with 80 units/ml hyaluronidase for 30 min at 37°C before separation on the gradient. Lymphocytes were used at a concentration of 10⁷ cells/ml for the immunofluorescence test.

MYCOPLASMA TESTS

All cultures of synovial fibroblasts or continuous lines were mycoplasma-free except for A from which *Mycoplasma orale* was cultured.

ANTISERA

Antisera to HPV-77 rubella were produced by multiple injections of 10× concentrated supernatant (polyethylene glycol) from RK₁₃ culture (199 medium supplemented with 5% rabbit serum free of rubella antibody) into New Zealand White rabbits. Initially 5 ml of fluid was given subcutaneously and 1 ml intravenously, followed by 4-weekly injections of 1 ml intravenously (Plotkin, 1969). 2 weeks after the last inoculation the rabbits were bled and their sera tested by the haemagglutination-inhibition test (HAI) for rubella antibodies; this schedule was repeated until their sera had HAI titres of 1/1024-2048. These high titre sera were then absorbed with human liver powder (acetone-treated) and Bentonite particles coated with fetal calf serum (Goodman and Bozicevich, 1964).

CONJUGATES

For indirect immunofluorescence sheep antirabbit immunoglobulin conjugated with fluorescein isothiocyanate (Wellcome Reagents) was used, diluted 1/10 in 20% beef brain saline to reduce nonspecific staining. Goat antirabbit immunoglobulin from Nordic Diagnostics Ltd. was used in the radioimmunoassay. It was fractionated on DEAE-cellulose DE52 (Reif, 1969); the resulting goat IgG was labelled with ¹²⁵I by the chloramine-T method (Hunter, 1974). The ¹²⁵I was added at a concentration of 500 µCi/50 µg protein.

IMMUNOFLUORESCENCE

The indirect immunofluorescence test was performed on acetone-fixed (10 min at room temperature) synovial cells grown on coverslips (6×22 mm)

(Lenette *et al.*, 1967; Schmidt *et al.*, 1966). Some were treated to remove the hyaluronic acid produced by fibroblasts, by including 80 units/ml bovine hyaluronidase (Sigma, Type I) in the complete medium as described by Clarris *et al.* (1974). Each test included persistently infected LLC-MK₂ cells as a positive control.

To detect rubella virus membrane antigens the virus infected cells were used unfixed, either in suspension (lymphocytes) (Yoshiki *et al.*, 1974), or as fibroblasts grown on coverslips (M. Haire, personal communication). Later, to minimize cell detachment, fixation in 4% formol-saline for 10 min was adopted (K. B. Fraser and M. Haire, personal communication), followed by a 30-min wash in phosphate-buffered saline (PBS). After washing, and without drying, the antigen test was carried out using the same method as described for the acetone fixed cells.

SOLID PHASE RADIOIMMUNOASSAY

In the solid phase radioimmunoassay for antigen (described by Forghani *et al.*, 1974) the virus infected cells and synovial fibroblasts were grown on the bottom of glass vials (controlled neck shell vials, 9.5/0.5×48/50, Johnsen and Jorgensen, London) in 0.4 ml of media. The concentration of the cell seed was 20 000/ml media. After 4 days' incubation at 37°C the cultures contained approximately 50 000 cells per vial. After removal of the growth medium the cells were rinsed with 1 ml of PBS, and then fixed in cold acetone for 10 min while still wet. The anti-serum dilutions were made in PBS supplemented with 2% FCS. 0.1 ml was then added to each vial and incubated for 2 hours at 37°C. The contents of the vials were then aspirated and they were washed 4 times in PBS. The ¹²⁵I conjugate was diluted to contain 60 000 counts/100 s in 0.1 ml. This quantity was added to each vial and they were incubated for 80 min at room temperature. The contents were aspirated and the vials were again washed 4 times. The residual radioactivity was assayed on a gamma counter (Wallac-LKB counting system).

ELUTION OF IMMUNOGLOBULINS FROM SYNOVIAL MEMBRANES

The method followed that of McCormick *et al.* (1971). Synovial membranes obtained at operation were finely chopped and were repeatedly washed with PBS pH 7.2. The fluids were checked for adsorption at 280 nm; when this indicated minimal protein, glycine buffer pH 2.5 10 ml/g wet tissue was added and stirred at 4°C for 2 hours. The 'eluates' were centrifuged immediately at 100 000 g for 1 hour to remove any dissociated 'antigen'. pH was then adjusted to 7.2 with solid TRIS buffer and the

fluid dialysed for 72 hours against PBS pH 7.2. Sodium azide at 0.1% concentration was added and the 'eluates' stored at 4°C until required for rubella HAI tests. The fluid from the last wash was kept and tested in parallel.

Results

ATTEMPTS TO DETECT RUBELLA VIRUS ANTIGEN BY IMMUNOFLUORESCENCE

(a) Acetone fixed cells grown on coverslips

The LLC-MK₂ cells persistently infected with HPV-77 virus showed a granular cytoplasmic staining that sometimes extended round the nucleus, but was mainly located on one side, in the form of bright cytoplasmic inclusions. This type of staining was detected in about 80% of the persistently infected cells; the number differed according to the number of days after trypsinization and the antiserum dilution used. Such granular cytoplasmic staining

was not observed in synovial fibroblasts from any source, either with or without hyaluronidase pretreatment (Table 1). The synovial fibroblasts were prepared from 8 classical or definite cases of RA, 7 of osteoarthritis, and one other form of inflammatory joint disease (non-RA).

(b) Membrane antigens

It was possible to detect membrane antigens on unfixed BHK-21 cells, infected with rubella virus and grown on glass coverslips and also on virus infected cells fixed in formol-saline (Fig. 1). The latter technique was used on the collection of synovial fibroblasts from different sources and all gave negative results irrespective of pretreatment with hyaluronidase (Table 1). It will be noted from Table 1 that whereas cytoplasmic and membrane staining was readily shown with acute infection of BHK fibroblasts, the LLC-MK₂ cells chronically infected with HPV-77 showed only a weak or no membrane staining.

Table 1 Examination of synovial fibroblasts by immunofluorescence for the cytoplasmic or membrane antigens of rubella virus

Culture	Diagnosis	Source	Tissue	Treatment of fibroblasts	Immunofluorescence cytoplasmic/membrane
A { P10 P10	RA for 3 yr	R70 (Dr. Hamerman)	SM	—	— —
D { P24 P24	OA	N104 "	SM	H	— —
E { P12 P14	RA for 14 yr	17/74 (W. Neill)	SM	H	— —
F { P14 P14	RA for 14 yr	20/74 "	SM	H	— —
G { P11 P11	OA for 4 yr	23/74 "	SM	H	— —
H { P20 P20	RA for 4 yr	24/74 "	SM	H	— —
I { P15 P15	Polyarthrititis (non-RA)	25/74 "	SM	—	— —
J { P19 P19	RA for 12 yr	27/74 "	SM	H	— —
K { P16 P16	OA for 8 yr	33/74 "	SM	H	— —
M { P10 P10	RA	42/74 "	SM	H	— —
N { P13 P13	OA for 3 yr	— (Virus research group)	SM	H	— —
II { P4 P4	OA	—	SF*	H	— —
III { P6 P6	RA	—	SF**	H	— —
IV { P6 P6	RA	—	SF**	H	— —
VI { P4 P4	OA	—	SF**	H	— —
VII { P3 P3	OA	—	SF*	H	— —
HPV-77-LLC-MK2	P14	..	Monkey	..	++
LLC-MK2	P12	..	kidney	..	—
Thomas-BHK-21	P8	..	Hamster	..	+++
BHK-21	P8	..	kidney	..	—

SM=synovial membrane; SF*=synovial fluid culture was isolated with hydrocortisone (36 µg/ml); SF**=synovial fibroblasts were isolated in the presence of hydrocortisone and sodium aurothiomalate (10 µg/ml); H=hyaluronidase (80 units/ml). ± to +++=increasing degrees of immunofluorescence. —=negative immunofluorescence. ..=not tested or no treatment. P=passage level

Fig. 1 (a) Cytoplasmic accumulations of rubella virus antigen in BHK-21 cells, fixed with acetone and stained with rubella antiserum (R3/HPV-77/6-14.3.75) at 1/5 dilution. (b) Membrane immunofluorescence of rubella virus in BHK-21, fixed with 4% formol-saline and stained with the same serum.

Separated lymphocytes from synovial fluid and peripheral blood were treated with hyperimmune rabbit rubella antiserum (R3/HPV-77/6-14.3.75) at 1 : 10 dilution as for membrane antigens. There was no membrane immunofluorescence with 8 lots of synovial lymphocytes from RA patients and one JRA patient. In 2 instances the peripheral blood lymphocytes from the same patient were also treated, but with negative results.

ATTEMPTS TO DETECT RUBELLA VIRUS ANTIGEN BY RADIOIMMUNOASSAY

Solid-phase radioimmunoassay will detect viral antigens in infected cells (Hayashi *et al.*, 1972, 1973; Forghani *et al.*, 1974) and its sensitivity has been shown to be greater than that of immunofluorescence (Hayashi *et al.*, 1973-74). So far as we

are aware it has not been applied to the detection of rubella antigens; it was felt that a test of greater sensitivity than immunofluorescence should be used in further attempts to validate the results of Patterson *et al.* (1973), particularly in view of the failure to show membrane antigens on HPV-77-LLC-MK₂ by immunofluorescence (Table 1).

Calibration of radioimmunoassay

The rubella (HPV-77) antiserum (R3/HPV-77/6-14.3.75) from the hyperimmunized rabbit R3 and its preimmunization serum (R3/N2), were titrated by radioimmunoassay on BHK 21 cells acutely infected with the strain 'Thomas' and LLC-MK₂ cells chronically infected with HPV-77. The preimmunization and the antiserum were absorbed with human liver powder, LLC-MK₂ cells, and human diploid fibroblasts; half-log dilutions from 1/30 to 1/100 000 of the sera were layered on monolayers on the bottom of glass vials. Goat IgG labelled with ¹²⁵I and containing the antibody against rabbit IgG was used at a dilution giving 60 000 counts/100 s in 100 μ l. The resulting titration curves of the two sera on infected and uninfected BHK 21 cells is shown in Fig. 2 and on infected and uninfected LLC-MK₂ in Fig 3. In each instance the number of counts bound to infected cells treated with antiserum R3/HPV-77/6-14.3.75 was substantially in excess, over the range of dilutions from 1/30-1/3000, when compared with the same serum on uninfected cells, or the preimmunization serum on

Fig. 2 Effects of a concentration of intermediate serum on binding with rubella antigens. ●, Thomas-rubella infected BHK-21 cells; △, control BHK-21 cells; —, R3/HPV-77/6-14.3.75 hyperimmune rubella antiserum; ---, R3/N2 preimmunization serum.

Fig. 3 Radioimmunoassay with hyperimmune anti-rubella rabbit serum. ^{125}I -labelled goat antirabbit IgG was used at 60 000 counts/100 s. ●, chronically infected LLC-MK2 cells with HPV-77 rubella; △, control LLC-MK2 cells; —, hyperimmune antirubella rabbit serum R3/HPV-77/6-14.3.75; ----, preimmunization serum R3/HPV-77/N2.

infected or uninfected cells. Inspection of the curves suggests a titration end point in the region 1/1000–1/3000 which approximates to the titre of 1/2048 of the serum measured by HAI before absorption.

An optimal dilution of 1/300 of the antiserum was evident on the basis that the distance separating the antiserum and control curves was greatest at that dilution. Activity at that antiserum dilution on the BHK 21 cell system could be expressed either (i) as a binding ratio of counts/100 s of serum dilution on virus infected cells divided by counts/100 s on uninfected cells—giving values, respectively, of 6.3 for the immune serum and 1.3 for the preimmune serum—or (ii) as a binding ratio, counts per 100 s antiserum/counts per 100 s preimmune serum on the same cells, with values respectively of 4.5 and <1.0. The chronically infected HPV-77-LLC-MK₂ cells and the controls gave binding ratios of 4.9 and 1.2 by method (ii). These cells had been infected for one year at that time and were in the 22nd passage (3.5% of cells showed immunofluorescence at that stage compared with about 80% after the initial infection).

Synovial fibroblasts were grown on the bottom of glass vials and exposed to 1/100, 1/300, and 1/1000 dilutions of the antiserum, or of the corresponding preimmunization serum, and the binding ratios were calculated by method (ii). Fig. 4 shows the distribution of binding ratios for 10 RA synovial fibroblasts

Fig. 4 The binding ratios for each serum dilution (c/100 s for hyperimmune serum R3/HPV-77/6-14.3.75 divided by c/100 s for the preimmunization serum) are plotted in 2 groups: RA fibroblasts (●), and non-RA fibroblasts (○) include osteoarthritis and other forms of inflammatory joint diseases.

and 10 non-RA fibroblasts (A–N and II–VII, Table 1), at the 3 dilutions of serum R1/HPV-77/4-7.1.75, and also the ratios obtained with rubella virus infected BHK cells. There was little difference in the distribution of counts bound between RA synovial fibroblasts on the one hand and non-RA fibroblasts on the other. However, it was known that despite attempts to use a homologous system the antiserum had some activity against calf serum due to problems of residual contamination with calf serum of the antigen for immunizing the rabbit. Because of this and also because the collection of synovial fibroblasts was at a high passage level, some fresh isolates (VIII–XXI) of fibroblasts were established and tested with R29/N1 (preimmunization serum) and R29/Thomas/2-23.9.75 (hyperimmune) rabbit antiserum (HAI titre 1:2048) (see Table 2).

Five samples were used at each dilution and standard deviations were also calculated. Of 111 cultures of fibroblasts from patients with definite RA only one culture had slightly raised binding ratio (XIX P4). However, a raised binding ratio was also found in one culture out of six non-RA fibroblasts (N P17). These results do not support the view that rubella antigens are expressed in synovial fibroblasts from rheumatoid patients.

Table 2 Examination of hyaluronidase-treated synovial fibroblasts from patients with rheumatoid arthritis, and other joint conditions, for rubella antigens detectable by radioimmunoassay with pre- and postimmunization sera from rabbit 29 given the 'Thomas' strain of rubella virus

Culture no.		Diagnosis	Serum dilution	Counts bound ($\pm$ SD) with		Binding ratio
				Rubella antiserum (R29/THOMAS/ 2-23.9.75)	Preimmunization serum (R29/NI)	
G	P17	OA (4 yr)	1 : 100	529 $\pm$ 238	415 $\pm$ 97	1.27
			1 : 300	449 $\pm$ 48	371 $\pm$ 73	1.35
			1 : 1000	297 $\pm$ 26	326 $\pm$ 43	<1
I	P19	Polyarthrititis (non-RA)	1 : 100	597 $\pm$ 110	403 $\pm$ 36	1.48
			1 : 300	437 $\pm$ 57	408 $\pm$ 58	1.07
			1 : 1000	317 $\pm$ 29	310 $\pm$ 8	1.02
N	P17	OA (3 yr)	1 : 100	628 $\pm$ 84	323 $\pm$ 24	1.94
			1 : 300	381 $\pm$ 62	281 $\pm$ 22	1.36
			1 : 1000	309 $\pm$ 45	235 $\pm$ 32	1.31
V	P8	RA (2½ yrs)	1 : 100	699 $\pm$ 51	918 $\pm$ 54	<1
			1 : 300	512 $\pm$ 39	732 $\pm$ 73	<1
			1 : 1000	387 $\pm$ 42	525 $\pm$ 53	<1
VIII	P7	OA (8 yr)	1 : 100	436 $\pm$ 60	577 $\pm$ 63	<1
			1 : 300	336 $\pm$ 63	457 $\pm$ 46	<1
			1 : 1000	253 $\pm$ 54	335 $\pm$ 31	<1
IX	P6	RA (3 yr)	1 : 100	243 $\pm$ 14	229 $\pm$ 37	1.06
			1 : 300	186 $\pm$ 23	261 $\pm$ 46	<1
			1 : 1000	180 $\pm$ 111	227 $\pm$ 37	<1
X	P7	RA (6½ yr)	1 : 100	465 $\pm$ 64	315 $\pm$ 22	1.48
			1 : 300	315 $\pm$ 39	288 $\pm$ 36	1.09
			1 : 1000	309 $\pm$ 172	273 $\pm$ 32	1.13
XI	P7	non-RA (7 yr)	1 : 100	302 $\pm$ 26	407 $\pm$ 44	<1
			1 : 300	211 $\pm$ 10	260 $\pm$ 19	<1
			1 : 1000	152 $\pm$ 29	170 $\pm$ 10	<1
XII	P3	RA (7 yr)	1 : 100	324 $\pm$ 50	518 $\pm$ 97	<1
			1 : 300	252 $\pm$ 31	359 $\pm$ 33	<1
			1 : 1000	218 $\pm$ 47	209 $\pm$ 46	1.04
XIII	P7	RA (6 yr)	1 : 100	450 $\pm$ 63	760 $\pm$ 67	<1
			1 : 300	316 $\pm$ 67	533 $\pm$ 181	<1
			1 : 1000	275 $\pm$ 49	340 $\pm$ 49	<1
XIV	P5	Prob. RA (6 yr)	1 : 100	527 $\pm$ 131	506 $\pm$ 28	1.04
			1 : 300	331 $\pm$ 31	367 $\pm$ 45	<1
			1 : 1000	277 $\pm$ 41	264 $\pm$ 15	1.05
XV	P4	RA	1 : 100	257 $\pm$ 43	346 $\pm$ 95	<1
			1 : 300	256 $\pm$ 71	313 $\pm$ 44	<1
			1 : 1000	219 $\pm$ 33	227 $\pm$ 60	<1
XVI	P7	RA	1 : 100	494 $\pm$ 14	505 $\pm$ 119	<1
			1 : 300	346 $\pm$ 61	321 $\pm$ 24	1.08
			1 : 1000	252 $\pm$ 18	239 $\pm$ 31	1.05
XVII	P7	RA	1 : 100	1016 $\pm$ 180	1365 $\pm$ 196	<1
			1 : 300	829 $\pm$ 264	1211 $\pm$ 294	<1
			1 : 1000	818 $\pm$ 108	1003 $\pm$ 168	<1
XVIII	P5	RA	1 : 100	454 $\pm$ 80	503 $\pm$ 64	<1
			1 : 300	339 $\pm$ 24	437 $\pm$ 35	<1
			1 : 1000	249 $\pm$ 32	318 $\pm$ 58	<1
XIX	P4	RA (21 yr)	1 : 100	459 $\pm$ 37	295 $\pm$ 30	1.56
			1 : 300	374 $\pm$ 57	218 $\pm$ 20	1.72
			1 : 1000	230 $\pm$ 19	171 $\pm$ 22	1.35
XX	P3	RA (10 yr)	1 : 100	505 $\pm$ 35	721 $\pm$ 43	<1
			1 : 300	361 $\pm$ 78	512 $\pm$ 29	<1
			1 : 1000	261 $\pm$ 40	398 $\pm$ 117	<1
XXI	P5	Psoriatic arthropathy (4 yr)	1 : 100	526 $\pm$ 91	614 $\pm$ 39	<1
			1 : 300	429 $\pm$ 71	435 $\pm$ 46	<1
			1 : 1000	395 $\pm$ 35	423 $\pm$ 132	<1
Control						
BHK-21 infected with Thomas strain of rubella virus			1 : 100	1935 $\pm$ 141	884 $\pm$ 29	2.19
			1 : 300	1895 $\pm$ 129	803 $\pm$ 58	2.36
			1 : 1000	1313 $\pm$ 132	702 $\pm$ 110	1.87

EXAMINATION OF RA SYNOVIAL MEMBRANES FOR BOUND ANTIBODY TO RUBELLA VIRUS

If the antigen-antibody reaction initiating the rheumatoid process were a combination between rubella virus-coded or induced antigens in synovial cells, and locally produced antibody, it might be expected that material eluted from RA membranes under conditions that dissociate antigen-antibody complexes would contain substantial amounts of antibody. This method has been used for example to demonstrate antibody to nucleic acid or Gross leukaemia virus in the kidneys of NZB mice or those chronically infected with lymphocytic choriomeningitis virus (Mellors *et al.*, 1971; Oldstone and Dixon, 1971). This aspect has been investigated in collaboration with Dr. J. McCormick. Synovial membrane eluates from 14 patients (13 RA and 1 non-RA) which contained protein demonstrable by adsorption at 280 nm were tested for a variety of viral antibodies, including those against rubella. All were negative at a dilution of 1/4 in the standard rubella HAI test. This approach is being pursued further with estimations of total immunoglobulins, rheumatoid and antinuclear factors, as well as other specific viral antibodies in the eluates (J. McCormick and H. Hart, to be published).

Table 3 *Distribution of haemagglutination-inhibiting (HAI) antibody to rubella virus among patients with rheumatoid arthritis and other arthritic conditions. (Patients with antibody titres ≥ 64 were regarded as positive)*

Category	No in category with rubella HAI antibody titres of		Total
	≤ 32	≥ 64	
Rheumatoid arthritis (RA)	49	31	80
non-RA	42	20	62
Total	91	51	142

EXAMINATION OF RA AND NON-RA PATIENTS FOR CIRCULATING ANTIBODY TO RUBELLA VIRUS

In view of the differences in prevalence rates and geometric mean titres of antibody to rubella virus found in RA patients and healthy individuals by Patterson *et al.* (1973 and personal communication), we record in Table 3 the results of testing RA and non-RA patients for rubella HAI antibody by standard methods; these figures are extracted from a larger survey done by Susan Baker and workers at the Northern General Hospital and covering a variety of viruses and immunoglobulin fractions. As in numerous previous investigations there was no significant difference between the frequency or levels of rubella antibody in the two groups ($P=0.72$).

Discussion

There are several ways in which (hypothetically) rubella virus (or other viruses) might be involved in RA; (i) persistence in the A or B lining cells, vascular endothelium of the synovium, or in cartilage, (ii) generation of antibody cross-reacting with synovial antigens as a result of viral infection at distant site, (iii) lodgement in the synovium of immune complexes of virus and antibody generated elsewhere in the body, and (iv) viral infection of synovial lymphocytes or macrophages with impairment of homeostatic mechanisms limiting macrophage action (see also Marmion, 1976; Morley, 1974).

The results reported in this paper, together with those of Person *et al.* (1973) and Norval and Marmion (1976), cover a number of aspects of (i) and (ii) for rubella virus, but as the virus has been found in the cartilage chondrocytes of congenitally infected rabbits (London *et al.*, 1970; Smith *et al.*, 1973) and as this tissue has not been covered in our studies of human RA, investigations of the joint *per se* are not complete. Possibility (iii) would probably not be covered for rubella virus by the examination of synovial membrane eluates as the complexes could be inside phagocytic cells, or, if free in interstices of the membrane, lost during the washing process. As for possibility (iv), rubella infected lymphocytes have been shown to have a decreased response to phytohaemagglutinin stimulation (McMorrow *et al.*, 1974; Vesikari and Blumovici-Klein, 1975) and decreased responsiveness to phytohaemagglutinin and other antigens has been observed in RA synovial lymphocytes (G. S. Panayi, personal communication; Hedberg *et al.*, 1971; Astorga and Williams, 1969). However, our limited observations on synovial lymphocytes do not indicate that this anergy is due to the presence of rubella virus coded antigens in the lymphocyte membrane.

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