Case report

Sequential studies on synovial lymphocyte stimulation by rubella antigen, and rubella virus isolation in an adult with persistent arthritis

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SUMMARY The response of synovial lymphocytes from a 65-year-old lady with persistent polyarthritis, to rubella antigen and a number of other microbial agents was studied over a period of 11 months by [³H]thymidine incorporation. The results were correlated with the ability to isolate rubella virus from both peripheral blood and synovial fluid during the same period. The patient showed initially a maximal stimulation index to rubella antigen assayed on five occasions over a five-month period. Rubella virus was detected in both peripheral blood and synovial fluid samples on three occasions during this period. Five months later the lymphoproliferative response of her synovial lymphocytes to rubella antigen had dropped to low levels, and virus could no longer be isolated from synovial exudates. At this time the patient's arthritis had become much less active, indicating that a good correlation existed between the presence of rubella virus, local lymphocyte sensitisation, and the inflammatory reaction.

In a recent review ¹ the involvement of a number of common viruses, including Epstein-Barr virus, Coxsackie B virus, and rubella virus (RV), in provoking acute polyarthritis was discussed. In particular, rubella virus was mentioned as the virus most frequently associated with joint diseases after both natural infection or immunisation of adults.²⁻⁵ In addition to this known association of RV with acute transient joint inflammation several recent reports have indicated that RV persistence may accompany chronic synovitis in patients with no recent history of rubella infection.⁶⁻⁸ Moreover, rubella-associated arthritis has been reported on several occasions to progress to a chronic erosive polyarthritis, clinically indistinguishable from rheumatoid arthritis (RA).⁹⁻¹⁰ These fragments of information provide tantalising evidence that RV may be one of the aetiological agents in classical rheumatoid arthritis.

This study is a follow up of previous results on the responsiveness of synovial lymphocytes from 15 patients with RA to a number of viral antigens.^{11, 12} Two patients were identified who showed maximal response to rubella antigen and these were both subsequently shown to harbour persistent infections with RV.¹² In this paper a sequential analysis over an 11-month period of the involvement of RV in a patient with persistent polyarthritis complicated by Hashimoto's thyroiditis is presented.

Patients and methods

CASE STUDY

The patient was a Caucasian housewife, aged 65 who had had an episode of 'German measles' at the age of 41. She contracted this from her 12-year-old son, but though her illness was characteristic of rubella, with fever, malaise, rash, and enlarged cervical lymph nodes, it was not observed medically. There were no complications, and she was well again within two weeks. At the age of 53 she began

Accepted for publication 4 March 1985.

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to complain of pain and swelling of her knees, which had persisted and fluctuated up to the present over the succeeding 12 years. At the age of 55 a diagnosis of Hashimoto's thyroiditis was made, and she was subsequently maintained on thyroxine. At the age of 60 she became severely ill, and it was discovered that she had idiopathic retroperitoneal fibrosis with ureteric obstruction. She was treated both surgically and with prednisone without recurrence and with restoration of adequate renal function.

Her arthritis was first treated by one of us (GDR) in 1980. While she had been taking prednisone for the retroperitoneal fibrosis, her arthritis had been suppressed, but it recurred on cessation of the prednisone in April 1980. The arthritis involved both hands, both ankles, and the proximal interphalangeal (PIP) joint of the right little finger. When seen again in July 1983 there was active arthritis of both elbows, both knees, both ankles, and the PIP joint of the right little finger. The clinical state of her arthritis did not change over the first five months of studies from August to December 1983, but subsequently during the spring of 1984 the arthritis became less active, and the patient found she could play golf again. Both knees were persistently swollen during the nine-month study period with variable warmth, and the aspiration of 50–100 ml fluid was always accomplished with ease. During the time of the study she was taking variable amounts of Entrophen and Clinoril.

The patient had negative tests for rheumatoid factor and antinuclear factors. Her serum antibody level of antirubella IgG antibody, as measured by the enzyme-linked immunosorbent assay employed in the BC Provincial Health Rubella Virus Laboratory, showed a reading of 1.19, which indicated a moderately high level of antibody.

[³H]THYMIDINE UPTAKE PROCEDURES

The synovial lymphocytes were obtained by Ficoll-Hypaque separation and cultured in 10% human AB plasma (Red Cross). They were incubated at 37°C in 5% CO₂ for seven days for antigen stimulation and for four to five days for phytohaemagglutinin (PHA) stimulation. Terasaki plates were used with 10 000 lymphocytes per well, and the plates were inverted to give hanging-drop cultures. [³H]Thymidine (New England Nuclear) was added for 4–6 h before harvesting the cells onto filter discs. Scintillation counting was performed with toluene-omnifluor (New England Nuclear). The data were expressed as the stimulation index.

ANTIGENS FOR LYMPHOCYTE STIMULATION

The preparation of the ureaplasma and chlamydial antigens has been described previously.¹³ All the

antigens were crude preparations and standardised against previous batches. Three twofold, or more recently threefold, dilutions were employed with triplicate wells for each antigen dilution. The ureaplasma antigen was a centrifuged deposit of 20 h broth cultures of serotype four or five. The chlamydial antigen was a frozen and thawed brie of McCoy cells infected with *Chlamydia trachomatis* serotype 595/8 or GF 726. The enteric antigens were harvests of trypticase agar slant cultures, which were boiled for 2–5 h to give an O-antigen preparation as previously described.¹⁴ The *Candida albicans* antigen was prepared from boiled scrapings of trypticase soy slant cultures. The viral antigens employed were all obtained from Microbiological Associates, with the exception of the rubella antigen which was obtained from Flow Laboratories (Gilchrist HA (BHK-21) lyophilised antigen).

VIRUS ISOLATION PROCEDURES

Attempts to isolate RV were carried out on both peripheral blood and synovial fluid samples as described previously.^{7 15} Mononuclear cells (lymphocytes and monocytes) were purified on Ficoll-Hypaque gradients and were stimulated for 48 h with PHA in RPMI + 10% fetal calf serum. They were then cocultivated with subconfluent monolayers of RK13 or VERO cells at a ratio of approximately 1:1 (5×10^6 peripheral blood mononuclear cells (PBMC) or synovial fluid mononuclear cells (SFMC): 5×10^6 RK13 or VERO cells). The cocultures were incubated in DMEM + 5% heat-inactivated fetal calf serum and were split 1:2 by trypsinisation at weekly intervals for three passages. During each subculture the supernatant medium was stored at –70°C for subsequent plaque assays, and one cell sheet was washed with phosphate-buffered saline and air dried for immunoperoxidase staining. The cultures were examined frequently for signs of cytopathology (CPE), which has previously been found to be a useful primary indicator of the presence of RV.^{7 15} In the absence of gross CPE the final culture fluid from the third passage was stored and cocultivation discontinued.

CHARACTERISATION OF ISOLATES

Several techniques were used to identify virus in the samples. Plaque assays were carried out on the supernatant fluids from the second passage of the cocultured cells as described previously.¹⁶ Immunoperoxidase staining of RV antigens in the stored cell sheets was performed with a 1:20 dilution of rabbit anti-RV prepared against our laboratory strain of RV (a plaque-purified wild isolate) and a 1:40 dilution of peroxidase-conjugated goat rabbit IgG (Kirkegaard and Perry Labs, Inc.). The

peroxidase substrate 3,3'-diaminobenzidine tetrahydrochloride, was obtained from Sigma. After counterstaining in haematoxylin/eosin, the cell sheets were examined under a Zeiss photomicroscope II. Further characterisation of the isolates was carried out by examining the polypeptides induced by the virus isolates in normal human lymphocytes (PBMC). For these experiments $5-10 \times 10^6$ normal PBMC were infected with 2 ml of supernatant fluid from the cocultures at second or third passage. These infected PBMC were incubated for 48 h in RPMI + 5% heat-inactivated fetal calf serum and were then labelled with 10 $\mu\text{Ci/ml}$ [^{35}S]methionine in (1/10 Met) RPMI medium. The intracellular polypeptides were then analysed on 5-20% gradient polyacrylamide sodium dodecyl sulphate gels in the Laemmli buffer system as described previously.¹⁶

Results

LYMPHOCYTE STIMULATION

Fig. 1 shows the [^3H]thymidine uptake responses of the synovial lymphocytes of the patient, as shown by the stimulation indexes to 14 microbiological antigens, obtained on eight occasions between 9 August 1983 and 28 June 1984. Comparison with PHA stimulation was made on the first two studies. The greatest stimulation index on the first six occasions was to rubella antigen, though significant responses were seen to salmonella, mumps, and cytomegalovirus (CMV), the latter equalling the rubella response on the sixth test. The stimulation index of peripheral lymphocytes was also examined during the first test (9 August 1983) and was found to be 1 as opposed to 17 for the synovial cells.

The stimulation index to PHA on both the initial two tests was less than that to rubella antigen. The test antigens included the candida antigen, but the responses were much less than to the rubella antigen. Although there was some variation in the degree of response between the tests, with the synovial lymphocytes being most reactive on 11 October 1983, the relative responses between antigens were consistent. The seventh and eighth tests were done in May and June of 1984 when the patient's arthritis was significantly improved, and this clinical improvement seemed to coincide with lessened reactivity of the synovial lymphocytes and also with a reduced number of lymphocytes in the synovial fluid.

VIRUS ISOLATION

At the same time as synovial samples were obtained for the lymphoproliferative studies a duplicate sample was processed for virus isolation. In addition, peripheral lymphocytes prepared from hepari-

nised blood were also examined on three occasions for RV persistence. The results are summarised in Table 1. The isolation system which we have found to be successful involves PHA stimulation of the lymphoid cells for 48 h followed by their cocultivation with subconfluent monolayers of RK13 or VERO cells for three passages.

In previous studies we have found that distinct cytopathology of the monolayer appeared in the coculture and that this could be correlated with the presence of plaque-forming virus in the culture.

Fig. 1 Stimulation indexes of synovial lymphocytes of the patient against up to 14 microbial antigens and PHA on eight occasions between 9 August 1983 and 28 June 1984.

Table 1 Detection of rubella virus and rubella antigen in lymphocytes from peripheral blood and synovial fluid

Date of sample	Sample	CPE*	PFU*	Immuno- peroxidase staining	PAGE*
24 Aug 1983	(1) PB*	—	3.6×10^4	NT*	+
24 Aug 1983	(2) SF*	—	1.2×10^4	NT	+
11 Oct 1983	(3) PB	—	2.0×10^3	+	+
11 Oct 1983	(4) SF	—	3.0×10^6	++	+
6 Dec 1983	(5) PB	±	10^2	+	—
	(6) SF	±	10^2	+	—
6 Mar 1984	(7) SF	±	10^2	—	NT
28 Mar 1984	(8) SF	—	10^2	—	NT
7 May 1984	(9) SF	—	10^2	—	NT
28 June 1984	(10) SF	±	10^2	—	NT

*CPE=cytopathology in culture; PFU=plaque-forming units;
PAGE=polyacrylamide gel electrophoresis; PB=peripheral blood;
SF=synovial fluid; NT=not tested.

fluid. In the case of the patient no cytopathology was seen. However, fixed cell sheets stored from each passage were subsequently shown by immunoperoxidase staining to contain foci of RV antigen. Furthermore, the culture fluids were found to contain plaque-forming virus, which was inhibited by anti-RV serum. Final confirmation that the isolates were RV was obtained by examining the polypeptides induced by the putative isolates in cultured cells. These were found to be identical to the intercellular polypeptide pattern induced by our laboratory strain RV.

Viral isolations were obtained from both peripheral blood and synovial fluid obtained on two occasions, in August and October 1983. A third sample, in December 1983, showed evidence of intracellular viral antigens in both PBMC and SFMC cocultivations, by immunoperoxidase staining, but no infectious virus was detected in these cultures, suggesting that the virus had become cell associated. Subsequent samples in May and June 1984 were negative. At this time the patient's arthritis was improved, and her synovial lymphocytes no longer responded to rubella antigen.

Discussion

In a previous report we described the synovial mononuclear cell responses of 10 patients with rheumatoid arthritis and eight patients with persistent knee arthritis to rubella antigen.¹² One patient in each category was found to respond maximally to RV, and in both cases RV was isolated from synovial mononuclear cells. The present paper is an extension of the previous results, representing

repeated analyses of a patient's mononuclear cells during an exacerbation of her symptoms and through to a remission phase. The results quite clearly link the period of increased inflammation with a high synovial lymphocyte reactivity to rubella antigen, and the presence of infectious RV. As in other studies¹²(JKC, unpublished data) which we have conducted on patients with arthritis associated with rubella virus persistence, RV was isolated from both peripheral and synovial mononuclear cell populations. However, the high lymphoproliferative response to rubella antigen was detected only in synovial lymphocytes (not in peripheral blood lymphocytes), indicating that accumulation of RV-sensitised mononuclear cells in the joint had occurred. A high stimulation index of synovial lymphocytes to cytomegalovirus was also found in the present study, though at no time was the CMV response as high as that for rubella. The significance of this is unclear at the present time.

Since a previous report¹² describing RV persistence in association with a high stimulation index to rubella antigen in a patient with RA, lymphoproliferative studies on a large group of adult arthritics have not singled out RV as a likely aetiological agent. The present patient represents the first individual in approximately two years in whom maximum sensitisation to RV was found, indicating the virus might have a role. Thus it appears that RV sensitisation occurs in association with adult RA in only approximately 5% (2/30) of cases. This is in contrast to our recent findings in patients with juvenile rheumatoid arthritis (JRA), where joint symptoms in association with RV persistence have been found in over 30% (7/19) of children studied (Chantler *et al.*, unpublished data), a result in agreement with the earlier findings of Ogra and collaborators which showed that RV antigen could be detected in one third of synovial smears from JRA patients by indirect immunofluorescence.¹⁷

During the last five years synovial lymphocyte responses to a number of microbiological antigens have been tested by one of us (DKF) in 45 cases of rheumatoid arthritis, in 40 patients with persistent or recurrent arthritis confined to the knees, in 41 patients with enteric or sexually transmitted reactive arthritis, and in 46 cases with miscellaneous types of arthritis. In those patients with a 'rheumatoid-like' arthritis particular attention has been given to the synovial lymphocyte response to viral antigens. To date about 20 patients have been identified in whom the synovial lymphocytes showed significantly increased responses to one or other of the tested viruses. The viruses involved have been mumps, adenovirus, parainfluenza virus, respiratory syncytial virus, and human cytomegalovirus, in addition

to rubella virus. However, only in the case of rubella virus have the lymphoproliferative responses been correlated with the presence of infectious virus in the joint. A similar association between a high synovial lymphoproliferative response to a specific agent and infection by the agent can be made in Reiter's syndrome, where there is both clinical and epidemiological data to indicate a role for a specific enteric or genitourinary pathogen in the induction of joint disease. Moreover, at the 1984 American Rheumatism Association meeting in Minneapolis, Minnesota it was reported¹⁸ that in patients with Lyme arthritis synovial lymphocytes were stimulated by antigen prepared from Lyme spirochaetes to a much greater degree than peripheral blood lymphocytes. It is our belief that the synovial lymphocyte responses may be an indication of the microbiological agent involved in a wide array of chronic human arthritides.

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