Search for viral nucleic sequences in rheumatoid cells

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SUMMARY DNA and RNA were extracted from synovial membranes, synovial fibroblast cells, peripheral blood lymphocytes, and synovial fibroblast cells strains derived from patients with rheumatoid arthritis and other joint conditions. They were hybridised after immobilisation on nitrocellulose filters with iodinated viral nucleic acids extracted from measles, rubella virus, SV-40, and a retrovirus, RD-114. In addition, in-situ hybridisation was carried out on sections of synovial membranes by means of iodinated measles and rubella virus RNA. In no case did any hybridisation occur. Positive control systems included synovial fibroblast strains transformed with SV-40, LLC-MK₂ cells chronically infected with rubella virus and RD cells infected with RD-114. It was concluded that the synovial cells did not contain viral genomes of measles, rubella virus, SV-40, or RD-114, or at least at a level equivalent to the positive control cells.

In recent years many attempts have been made to isolate a virus from tissues of rheumatoid patients and there have been no positive results; for reviews see Marmion and Mackay (1977) and Hamerman (1975). However, the immunological abnormalities apparent in this disease could be associated with a virus which is not fully expressed and which therefore would not be detectable by virus culture methods. Such a virus might be present within the cells of the synovial membrane or synovial fluid and might alter the surface properties of these cells. Some work has been done lately to examine this possibility and again the results are predominantly negative.

Hart and Marmion (1977) studied fibroblast strains which grew in culture from synovial membranes or synovial fluids for retroviral and rubella viral antigens by immunofluorescence and radio-immunoassay and none were found, though there is evidence from Patterson *et al.* (1973) that by means of a cytotoxic assay the rheumatoid cells may be shown to contain rubella virus membrane antigens. Similarly the lymphocytes from rheumatoid synovial fluids and cells in sections of synovial membranes showed no sign of possessing retroviral or rubella viral antigens by immunofluorescence, immunoperoxidase, and radioimmunoassay (Hart and Marmion, 1977).

Attempts to rescue viruses from synovial fibroblast strains by such procedures as chemical induction and cocultivation were not successful (Norval and

Marmion, 1976). Recently synovial lymphocytes were fused and cocultivated with cell lines permissive for primate retrovirus but with no virus being induced (Norval *et al.*, 1978, to be published).

Thus in the aetiology of rheumatoid arthritis there is little to implicate viruses, whether being produced from infected cells or even in a non-productive form so far. However, consideration should be given to those viruses which may be present within cells and which may alter cell function or induce new proteins at the cell surface. Such hypotheses, among others, have been reviewed recently by Marmion and Mackay (1977). For example, adenovirus may be considered as an oncogenic virus in a transforming situation where a part of the viral genome may integrate into the host cell chromosome. This cannot be recovered as infectious virus and will be detectable only by nucleic acid hybridisation or perhaps by synthesis of a few early proteins like T-antigen, although in some cell lines even these may not be expressed; for review, see Flint (1977).

We decided therefore to examine nucleic acids from rheumatoid and nonrheumatoid sources for homology with various viral nucleic acids. DNA and RNA were extracted from synovial membranes, synovial fluid cells, peripheral blood lymphocytes, and synovial fibroblasts grown in culture. These were hybridised with iodinated viral nucleic acids from measles, rubella, SV-40, and RD-114, an endogenous feline retrovirus, which is antigenically related to viruses from certain nonhuman primates, by a

simple method of fixing the cellular nucleic acid on nitrocellulose filters.

Materials and methods

PREPARATION OF CELLULAR NUCLEIC ACIDS

Specimens from synovectomies were received in the laboratory less than 2 hours after operations. The synovial membranes were dissected out and cut into small pieces (2–5 mm). They were disaggregated in serum-free medium either with trypsin or with a mixture of collagenase (1 mg/ml) and hyaluronidase (80 units/ml) at 37°C for 3 hours with stirring. The dispersed cells were washed in serum-free medium (Earles-based Eagles), then in extraction buffer (0.15M NaCl, 0.1M EDTA in 0.1M Tris, pH 8.0).

Synovial fluids, removed by syringe from various effusions, were treated with hyaluronidase (80 units/ml) for 30 mins at 37°. The cells were deposited by centrifugation and washed in serum-free medium, then in extraction buffer.

Peripheral blood lymphocytes were prepared from 100 ml heparinised blood by separation on Triosil-Ficoll gradients. They were washed in serum-free medium, then in extraction buffer.

Synovial fibroblast strains, grown in culture (Norval and Marmion, 1976) were harvested by trypsinisation and washed in extraction buffer. These were normally around pass 5.

In all cases a minimum of 2×10^7 cells were obtained.

Nucleic acid was extracted by the method outlined in Simpson and Iinuma (1975), a 10% solution of sodium dodecyl sulphate (SDS) in extraction buffer being added to make the final concentration 1%. The precipitated nucleic acid after chloroform/isoamyl alcohol extraction was treated with RNase (50 µg/ml, pretreated at 80° for 10 min, Sigma) for DNA preparations or with DNase (20 µg/ml, Sigma) for RNA preparations. The final precipitation of nucleic acid was with ethanol, and it was dissolved in SSC buffer (0.15M NaCl, 0.015 M Na citrate). The ratio of absorbance at 260 to 280 nm was at least 1.85, and from 10^8 cells the yield varied from around 100 µg in the case of synovial membrane cells to around 500 µg from cultured synovial cells. The nucleic acid was stored at -70°C until use.

In addition RNA and DNA were extracted from BHK-21 cells 3 days after infection with rubella virus (Thomas strain), and from LLC-MK₂ cells chronically infected with rubella virus (Hart and Marmion, 1977). DNA was extracted from RD virus and RD cells chronically infected with RD-114 (McAllister *et al.*, 1972). Also DNA was extracted

from synovial fibroblast strains, 2 rheumatoid and 1 non-rheumatoid, transformed by SV-40. This transformation was achieved by pretreating the cells with hyaluronidase (1 mg/ml) for 1 h at 37°C, then infecting with virus at a multiplicity of 100:1 or 300:1. Transformation became apparent by morphological changes after 4 to 16 weeks, and the presence of T antigen was confirmed by immunofluorescence.

PREPARATION OF VIRUSES AND VIRAL NUCLEIC ACID

Purified SV-40 virus was a kind gift from Dr Ching-Juh Lai (National Cancer Institute).

Measles virus (Schwartz strain) was grown in Vero cells in roller bottles. The medium was Earles-based Eagles containing 2% calf serum and 100 IU/ml penicillin and 100 µg/ml streptomycin. The culture supernatant (3 litres) was harvested 3 days after infection and clarified. The virus was concentrated by centrifugation at 30 000 r.p.m. for 1 hour in a SW 40 rotor, and resuspended in 0.01 M Tris-HCl, 1 mM EDTA, pH 7.1 buffer (Grayzel, 1973). It was layered on to sucrose density gradients (20% and 70%) and centrifuged at 25 000 r.p.m. for 18 hours in a SW 40 rotor. Drops were collected from the base of the tube, and fractions with density 1.19 to 1.23 g/cm³ were pooled and pelleted at 40 000 r.p.m. for 1 hour in an SW 50.1 rotor. The pellet was suspended in 3 ml SSC and stored at -70°C.

Rubella virus (Thomas strain) was also grown in Vero cells in roller bottles. The culture supernatant (2 litres) was harvested after 5 days and 8 days and clarified. It was purified as for measles except that the 1.18 to 1.21 g/cm³ band was taken from the sucrose density gradients.

RD cells chronically infected with RD-114 virus were grown in roller bottles in Eagles medium containing 5% fetal calf serum. Culture supernatants were harvested every second day (2 litres) and clarified. The virus was purified as for measles except that the 1.15 to 1.17 g/cm³ band was taken from the sucrose density gradients.

The nucleic acid was then extracted from each virus preparation. The volume was made up to 10 ml in TNES buffer (0.1M Tris pH 9.0, 0.1M NaCl, 1mM EDTA, and 0.1% SDS) and extracted 3 times with TNE (0.1M Tris pH 9.0, 0.1M NaCl, 1mM EDTA)-saturated phenol containing 10% chloroform. The nucleic acid was precipitated with ethanol and the final precipitate dissolved in 0.3 ml sterile SSC and stored at -70°C.

IODINATION OF VIRAL NUCLEIC ACIDS

200 µg measles RNA, 50 µg SV-40 DNA, 200 µg rubella RNA, and 280 µg RD-114 RNA were

iodinated by modification of the method of Commerford (1971). DNA, when used, was heated at 80°C for 20 min just before iodination. A mixture was made of 0.3 ml of 0.5 M Na acetate buffer, pH 5.0; 0.3 ml SSC containing the nucleic acid to be iodinated; 0.3 ml thallic chloride, 2.5 mM in 0.1 M acetate buffer, pH 5.0; and 0.3 ml potassium iodide 0.125 mM, which had previously been added to 600 $\mu\text{Ci}^{125}\text{I}$. It was incubated at 60° for 1 hour, then cooled to room temperature. The labelled nucleic acid and ^{125}I were separated on Sephadex G50. The nucleic acid peak was dialysed exhaustively against SSC buffer. The specific activity obtained was 6×10^4 counts per minute (cpm) per μg nucleic acid for measles RNA, 3×10^5 for SV-40 DNA, 3×10^4 for rubella RNA, and 6×10^4 for RD-114 RNA.

FILTER HYBRIDISATION

Essentially the method of Gillespie and Spieglemann (1965) was used in which the cellular nucleic acid derived from the synovial membranes, synovial fluid cells, peripheral blood lymphocytes, synovial fibroblasts, and various cell lines plus calf thymus DNA were immobilised on Millipore nitrocellulose filter discs. The filters each contained 20 μg nucleic acid. Hybridisation was carried out in small sealed vials containing iodinated viral nucleic acid (in the case of viral DNA it was preheated for 15 min at 80°C), 20 μl 5% SDS, and 4 μg cold carrier DNA (calf thymus DNA, except for calf thymus control vial in which 4 μg human DNA was used as carrier), and 3 $\times$ SSC to make the final volume 1 ml. The vials contained up to 5 filters with nucleic acid plus 2 blanks. Incubation was carried out at 62°C for 22 hours. The filters were washed in 3 $\times$ SSC with several changes of buffer, digested with S_1 nuclease (Biochemicals Inc.) to remove unhybridised nucleic acid, and rewashed. They were counted in a Wallace-LKB gamma counter.

IN-SITU HYBRIDISATION

The method of Pardue and Gall (1975) was followed in outline. Frozen sections were prepared of synovial membranes, and various cell lines were grown on glass slides until semiconfluent. Two methods of fixation were used: (1) 0.1% Glutaraldehyde at 4°C for 30 min followed by dehydration in 50%, 70%, 90%, and 100% ethanol. (2) Acetic acid: ethanol, 1:3 for 2 min, followed by dehydration in 95% ethanol. These slides were treated with 200 μl pancreatic RNase (100 $\mu\text{g}/\text{ml}$ in 2 $\times$ SSC, Sigma) for 1 hour at 37°C, washed in 2 $\times$ SSC, then dehydrated again. They were denatured in 0.07N NaOH for 2–3 min and dehydrated.

With all slides 10 μl hybridisation mix was added which consisted of 100 μl 42% formamide, 20 μl

10X acetate buffer (3M NaCl, 0.1 M Na acetate, pH 5), and 80 μl iodinated viral nucleic acid (about 25 000 cpm) or SSC (control). Hybridisation was carried out at 46°C for 22 hours under cover-slips sealed with Cowgum. The slides were washed in acetate buffer, treated with S_1 nuclease for 1 hour at 37°C, rewashed, dehydrated in ethanol, and covered with Ilford K2 emulsion. They were developed with Kodak D 19 after periods of 4 to 8 weeks.

Results

sv-40

1.7 μg (500 000 cpm) iodinated SV-40 DNA was used in hybridisation with nitrocellulose filters containing 20 μg DNA from calf thymus, from peripheral blood lymphocytes of 4 normal people, from 2 rheumatoid synovial membranes, and from 2 rheumatoid synovial fibroblast strains. As a positive control for the method DNA was extracted from 2 rheumatoid synovial strains and 1 non-rheumatoid strain transformed by SV-40. These should contain an integrated copy (or copies) of the viral genome. The cpm of the filters after treatment with S_1 nuclease are shown in Table 1. These represent an average of 2 to 4 determinations done within 4 weeks of iodinating SV-40.

It can be seen that the cpm of the filters containing DNA from the SV-40 transformed synovial cells are 2 to 3 times more than the other synovial preparations. The latter were considered therefore not to contain SV-40 viral sequences.

MEASLES

0.45 μg (27 000 cpm) and 0.045 μg (2700 cpm) iodinated measles RNA were used in hybridisation with nitrocellulose filters containing 20 μg DNA from calf thymus, from 3 synovial membranes of RA

Table 1 Hybridisation of SV-40 ^{125}I -DNA with cellular DNA

	cpm per filter
No nucleic acid	238
Calf thymus DNA	275
Peripheral blood lymphocytes, 2	199
Peripheral blood lymphocytes, 3	194
Peripheral blood lymphocytes, 4	188
Peripheral blood lymphocytes, 5	186
Rheumatoid synovial membrane, 1	220
Rheumatoid synovial membrane, 8	202
Rheumatoid synovial fibroblast strain, 2	263
Rheumatoid synovial fibroblast strain, 7	222
SV-40 transformed rheumatoid synovial fibroblast strain, 5	442
SV-40 transformed rheumatoid synovial fibroblast strain, 7	408
SV-40 transformed non-rheumatoid synovial fibroblast strain, 10	666

patients and 1 juvenile RA, from 1 synovial membrane of an OA patient, and from 1 synovial fluid from an RA patient. The results can be seen in Table 2; they represent an average of 2 determinations. The counts obtained were all lower than the filter with no nucleic acid or with DNA from calf thymus, and it was concluded that no hybridisation had taken place.

Moreover, in-situ hybridisation was carried out on frozen sections cut from 4 synovial membranes. These came from 2 patients with RA and 1 each with OA and a popliteal cyst. The sections were all fixed in acetic acid/ethanol and treated with RNase before denaturing the DNA. After 4 and 8 weeks the slides were developed and did not contain more grains over the cells than the control slides.

These hybridisations were set up to detect measles-specific DNA in synovial cells, and no positive control was included for this system. Some evidence has been produced to suggest that measles in a chronic type of infection of chick embryo fibroblasts goes through a DNA proviral stage (Zhdanov, 1975), but this has not been substantiated subsequently by Holland *et al.* (1976) using hybridisation techniques and transfection.

RUBELLA

2 µg (60 000 cpm) and 0.2 µg (6000 cpm) iodinated rubella RNA were used in hybridisation with DNA and RNA from 10 synovial sources as listed in Table 3. No sample was considered to show hybridisation. DNA and RNA were extracted from BHK-21 cells lytically infected with rubella and from LLC-MK₂ cells chronically infected with rubella to act as controls for the system. These results are shown in Table 4. It may be seen that there are higher counts associated with RNA from both the chronically infected and lytically infected cells as compared to the RNA from the uninfected cells, while the DNA does not show this. This is perhaps surprising as rubella is a positive strand RNA virus, and one would imagine only small quantities of the negative strand being present in the infected cells at any one time. Also from this there is no evidence for a DNA intermediate in the replication, even in a chronic infection, as has been postulated recently by Sato *et al.* (1977). Work is continuing on this chronically infected cell line and will be published shortly (Norval, 1978, to be published).

In-situ hybridisation was also carried out with

Table 2 Hybridisation of measles ¹²⁵I-RNA with cellular nucleic acid

Nucleic acid	Specimen	Diagnosis	cpm per filter	
			0.45 µg ¹²⁵ I-RNA	0.045 µg ¹²⁵ I-RNA
None			1906	303
DNA	Calf thymus		2570	373
DNA	Synovial membrane A	Rheumatoid arthritis	1683	258
DNA	Synovial membrane B	Rheumatoid arthritis	1610	234
DNA	Synovial membrane C	Rheumatoid arthritis	1467	280
DNA	Synovial membrane D	Juvenile rheumatoid arthritis	—	257
DNA	Synovial membrane E	Osteoarthritis	1960	—
DNA	Synovial fluid F	Rheumatoid arthritis	2065	272

A dash means not done.

Table 3 Hybridisation of rubella virus ¹²⁵I-RNA with cellular nucleic acid

Nucleic acid	Specimen	Diagnosis	cpm per filter	
			2 µg ¹²⁵ I-RNA	0.2 µg ¹²⁵ I-RNA
None			3035	315
DNA	Calf thymus		4644	438
DNA	Synovial membrane A	Rheumatoid arthritis	2428	283
DNA	Synovial membrane B	Rheumatoid arthritis	2086	261
DNA	Synovial membrane C	Rheumatoid arthritis	3192	360
DNA	Synovial membrane E	Osteoarthritis	3225	291
DNA	Synovial fluid F	Rheumatoid arthritis	3530	350
RNA	Synovial membrane G	Rheumatoid arthritis	—	386
RNA	Synovial fluid H	Reiter's disease	—	404
RNA	Synovial fluid I	Polyarthritis after rubella	—	216
RNA	Synovial fibroblast strain J			
	derived from synovial membrane	Osteoarthritis	3626	—
RNA	Synovial fibroblast strain K			
	derived from synovial membrane	Rheumatoid arthritis	2979	—

A dash means not done.

Table 4 Hybridisation of rubella ^{125}I -RNA with cellular nucleic acid

	cpm per filter		
	2 μg ^{125}I -RNA	0.4 μg ^{125}I -RNA	0.2 μg ^{125}I -RNA
No nucleic acid	2168	476	347
DNA from BHK cells	2425	462	265
DNA from BHK cells lytically infected with rubella virus	2005	495	283
RNA from BHK cells	2709	630	394
RNA from BHK cells lytically infected with rubella virus	4242	1065	467
DNA from LLC-MK ₂ cells	1586	456	281
DNA from LLC-MK ₂ cells chronically infected with rubella virus	1313	410	371
RNA from LLC-MK ₂ cells	2075	474	347
RNA from LLC-MK ₂ cells chronically infected with rubella virus	2961	797	538

synovial membrane sections from the same patients as in the measles experiments above, except that these were fixed and pretreated in 2 ways, firstly, to degrade RNA and denature DNA, and, secondly, to leave the RNA intact. They were developed after 4 and 8 weeks and showed no more grains over the cells than the control slides. In a similar manner LLC-MK₂ cells and LLC-MK₂ cells chronically infected with rubella were grown on glass slides and fixed by the 2 methods. The ones treated with RNase and denatured did not contain more grains after an 8-week exposure than the control slides. The results for the set fixed in glutaraldehyde are shown in Table 5.

It may be seen that there are more grains per cell in the LLC-MK₂ cells chronically infected with rubella virus and hybridised with ^{125}I -rubella RNA than in the uninfected cells. Thus it may be assumed that the synovial cells contain either no rubella nucleic acid or less than is synthesised in the chronically infected system used here as a control for the method.

RD-114

As with the other viral nucleic acids, 1.5 μg (40 000 cpm) iodinated RD-114 RNA was used in filter

Table 5 Hybridisation of rubella ^{125}I -RNA in situ

	Average number of grains per cell (20 cells counted)
LLC-MK ₂ cells hybridised with SSC buffer	9
LLC-MK ₂ cells hybridised with ^{125}I -rubella virus RNA	8
LLC-MK ₂ cells chronically infected with rubella virus and hybridised with SSC buffer	14
LLC-MK ₂ cells chronically infected with rubella virus and hybridised with ^{125}I -rubella virus RNA	46

hybridisation with DNA from various synovial sources. The results are shown in Table 6, and each count represents an average of 3 determinations. In addition DNA extracted from RD cells and RD infected with RD-114 were used, and the results given in Table 6. None of the filters containing nucleic acid from synovial cells was thought to show hybridisation to the viral probe, but the counts on the RD-114 DNA filter were about twice that of the RD filter, demonstrating that the RD cells infected with RD-114 contained viral sequence(s) in the DNA.

Discussion

Molecular hybridisation has been widely used as a tool in recent years to probe for viral nucleic acid sequences in human tissues, especially in various malignancies such as lymphomas (Hehlmann *et al.*, 1972) and leukaemias (Larsen, *et al.*, 1975). These experiments are difficult to do satisfactorily, being dependent, among other things, on obtaining nucleic acid preparations that are pure enough plus good control material, and on labelling the viral nucleic acid sufficiently. Despite these inherent difficulties it seemed worthwhile in view of the negative results from virus isolations and in screening for viral antigens on synovial cells to look for viral nucleic acid sequences in rheumatoid material.

The method used here was a simple one which consisted of immobilising the denatured cellular

Table 6 Hybridisation of RD-114 ^{125}I -RNA to cellular DNA

Nucleic acid	Specimen	Diagnosis	cpm per filter
None			492
DNA	Synovial membrane B	Rheumatoid arthritis	349
DNA	Synovial membrane C	Rheumatoid arthritis	346
DNA	Synovial membrane L	Rheumatoid arthritis	493
DNA	Synovial fluid F	Rheumatoid arthritis	478
DNA	Synovial fluid M	Rheumatoid arthritis	348
DNA	Synovial fluid N	Inflammatory polyarthritis	489
DNA	RD cells		473
DNA	RD cells infected with RD-114		835

DNA on nitrocellulose filter discs and then hybridising in solution with iodinated viral nucleic acids from 4 viruses—SV-40, measles, rubella, and RD-114. Owing to the relative scarcity of clinical material, especially of non-RA control material, the kinetics of hybridisation were not measured. A comparison was made between the counts on filters containing nucleic acid from RA patients with those from non-RA patients at one time. In addition, the technique of iodination should give ideally a specific activity of around 10^7 cpm/ μ g nucleic acid but, here, only about 10^5 was achieved, and this will obviously lead to a distinct loss in sensitivity.

However, the control systems used, which were synovial fibroblasts transformed with SV-40, LLC-MK₂ cells chronically infected with rubella virus and RD cells infected with RD-114 virus, were all positive by the methods used. Various alternative methods of labelling the viral nucleic acid were tried. These included adding ^3H -uridine during measles infection of Vero cells, but the specific activity of the extracted nucleic acid was not as high as with iodination. Further, a ^3H -cDNA was made to RD-114 by the method of Rothenberg and Baltimore (1976), but again the extracted and purified cDNA was not labelled sufficiently. It would have been preferable to use cDNA, as it will hybridise to viral mRNA in cells containing the viral genome, and in most cases this will amplify the viral information within the cells and allow easier detection by hybridisation.

Thus, despite these reservations about the hybridisation assay, it proved sufficiently sensitive to detect viral sequences in the control cells. The negative results obtained with the rheumatoid cells are valid at this level of sensitivity, that is, they would have to contain at least the same number of genome equivalents as in the positive control systems. If, on the other hand, only a few cells from the population of synovial cells contain viral information, such as a certain class of lymphocytes perhaps, then this would probably not be detectable in the assay system used. Nucleic acids were extracted from synovial membranes, which consist of a mixed population of cells, mostly synovial cells lining with some lymphocytes, polymorphs, monocytes, and macrophages, and from synovial fluids, which again consist of a mixture of cells, largely polymorphs with some lymphocytes, monocytes, macrophages, and a few lining cells. As these specimens came directly from the patient without prior culturing, it was hoped that the total mass would contain the relevant population of cells. All the nucleic acids extracted from these different sources, however, showed no homology with the viral probes prepared from SV-40, measles, rubella, and RD-114.

Another method tried for measles and rubella probes, in addition to filter hybridisation, was in-situ hybridisation, but, again, no homology was found with the nucleic acids in synovial membrane sections from RA patients and patients with other conditions. This technique has been used successfully to detect viral nucleic acids in several systems, such as viral mRNA in adeno infected and transformed cells (Moar and Jones, 1975). One clinical situation of interest in this context is that of detecting viral DNA in sheep with visna (Haase *et al.*, 1977). Sections of choroid plexus were taken, and, while only 0.025% cells showed the presence of membrane p30 antigen by immunofluorescence, 18% contained viral DNA by in-situ hybridisation using ^3H -cDNA of visna virus as probe. It was calculated from the number of grains over the nucleus that there were 100–200 copies of the viral genome per cell, which is a high level of gene reiteration and very helpful, obviously, in detection.

The 4 viruses used here as probes were selected for various reasons. SV-40 can be a transforming virus, and other closely related members of the same genus, *Polyomavirus*, have been described in human beings, especially when immunosuppressed. RD-114 is an endogenous cat retrovirus which has a very close relationship to the baboon endogenous virus, and it was hoped that it would also show some homology with a putative human retrovirus. In systemic lupus erythematosus (SLE) an antigen cross-reacting to a retrovirus, SP104, has been described on blood lymphocytes of human and canine cases (Schwartz, 1975). SP104 was first produced by mouse tumour cells in culture after the tumour was induced by a cell free filtrate of canine SLE spleen. However, very recently Quimby *et al.* (1978) have reported that there is no homology using techniques of competitive hybridisation between the cDNA of this virus and RNA from cells of dogs and human beings with SLE. Measles was chosen because of its association with subacute sclerosing panencephalitis in man, a slow chronic progressive condition (Hall and ter Meulen, 1976), and also structures like paramyxovirus nucleocapsids have been described in synovial lymphoreticular cells in rheumatoid arthritis (Neumark and Farkas, 1973). Finally rubella was selected for the reasons outlined in Hart and Marmion (1977) and McCormick *et al.* (1978).

We are continuing this study by using adeno-2 DNA as a probe and looking at in-situ hybridisation to RNA in sections of synovial membranes and synovial fibroblasts grown on slides. Also the kinetics of hybridisation to RNA extracted from rheumatoid membranes and from rheumatoid and non-rheumatoid synovial fibroblasts are being examined.

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