

CONCISE REPORTS

Absence of Epstein-Barr virus carrying cells in synovial membranes and subcutaneous nodules of patients with rheumatoid arthritis

Pierre Brousset, Michel Caulier, Alain Cantagrel, Carole Dromer, Bernard Mazières, Georges Delsol

Abstract

Objectives—To determine whether the Epstein-Barr virus is present in synovial membranes and subcutaneous nodules of patients with rheumatoid arthritis.

Methods—A sensitive *in situ* hybridisation technique was applied to tissue sections of 11 synovial membranes and five rheumatoid nodules.

Results—Cells carrying the Epstein-Barr virus were not detected using EBER and BHLF1 oligonucleotides in the tissue samples investigated here.

Conclusions—Although it has been suggested that the Epstein-Barr virus could play a part in the aetiology of rheumatoid arthritis, it was not detected in synovial membranes and subcutaneous lesions in this study.

(*Ann Rheum Dis* 1993; 52: 608-609)

On the basis of serological and molecular studies, the Epstein-Barr virus was strongly suspected to play a part in the aetiology of rheumatoid arthritis (RA). Previous studies clearly showed that patients with RA have high titres of antibodies to the Epstein-Barr virus/capsid antigen^{1,2} associated with an increased frequency of cells infected with the Epstein-Barr virus in peripheral blood.³ Additional evidence was provided by the description of the RA nuclear antigen, corresponding to EBNA1, which appeared to be a specific target for antibodies in RA.⁴⁻⁶ The link between the Epstein-Barr virus and RA has been further reinforced by the isolation of a common epitope for HLA-DR4 (subtype HLA-Dw4 DR β 1 chain) and the Epstein-Barr virus induced glycoprotein gp110 (viral capsid antigen, BALF4 open reading frame), corresponding to the QKRAA determinant.⁷ Thus Epstein-Barr virus infection could induce a T cell reactivity to gp110 and HLA-Dw4. The location and characterisation of cells infected with the Epstein-Barr virus which could be implicated in the production of such cross linking determinants are still the subject of controversy, however. To address this question, we investigated tissue samples from synovial membranes and rheumatoid nodules using *in situ* hybridisation with EBER and

BHLF1 oligonucleotides which detect Epstein-Barr virus associated RNAs.

Patients and methods

All patients had definite or classic RA according to the 1958 American Rheumatism Association criteria⁸ and met the 1987 revised criteria for RA.⁹ Eleven tissue samples of synovial membranes and five rheumatoid nodules were routinely processed—that is, fixed in Duboscq-Brasil's fluid and embedded in paraffin for histopathological examination. In all instances fresh tissue was available for cryopreservation and immunostaining on frozen sections.

Details of the *in situ* hybridisation technique have been published elsewhere.¹⁰ This procedure was performed with the DAKO hybridisation kit (Dako, Copenhagen, Denmark) using a cocktail of EBER oligonucleotides (one oligonucleotide corresponding to EBER1 and one corresponding to EBER2, both base length 30) (Dako code No Y. 017) and a cocktail of BHLF1 oligonucleotide probes (three different oligonucleotides of base length 30) (Dako code No Y. 018). The two groups were labelled with fluorescein isothiocyanate (FITC) and were visualised with alkaline phosphatase and new fuschin for chromogen. The EBER and BHLF1 RNAs are the most abundant transcripts detected during the latent and replicative (lytic) phases respectively.^{10,11}

Five synovial membranes from patients with non-specific inflammatory lesions were tested for comparison. Three lymph nodes affected by infectious mononucleosis served as positive controls for the EBER oligoprobes and one patient with hairy leucoplakia of the mouth was used as a positive control for BHLF1 oligonucleotide hybridisation.¹⁰ A control for RNA preservation was provided by the use of two cocktails of FITC labelled oligonucleotides (Dako) which hybridise transcripts encoding for immunoglobulin κ and λ light chains.

Results

The table summarises the results. None of the 11 synovial membranes and the five rheumatoid nodules contained cells carrying the Epstein-Barr virus. These results were

Laboratoire Central
d'Anatomie
Pathologique,
Centre Hospitalier
Universitaire de
Purpan,
Toulouse, France
P Brousset
G Delsol

Service de
Rhumatologie,
Centre Hospitalier
Universitaire de
Purpan,
Toulouse, France
M Caulier
C Dromer

Service de
Rhumatologie,
Centre Hospitalier
Universitaire de
Ranguell,
Toulouse, France
A Cantagrel
B Mazières

Correspondence to:
Dr Pierre Brousset,
Laboratoire d'Anatomie
Pathologique,
Centre Hospitalier
Universitaire de Purpan,
Place du Docteur Baylac,
31059 Toulouse Cedex,
France.

Accepted for publication
24 March 1993

Results (number positive/number tested) of *in situ* hybridisation using EBER, BHLF1, κ , and λ fluorescein isothiocyanate labelled oligonucleotides on tissue specimens from patients with rheumatoid arthritis, lymph nodes affected by infectious mononucleosis (positive control for EBER oligoprobes), and hairy leucoplakia of the mouth (positive control for BHLF1)

Probes	Synovial membranes	Rheumatoid nodules	Lymph nodes with infectious mononucleosis	Hairy leucoplakia of the mouth
EBER1/2	0/11	0/5	3/3	0/1
BHLF1	0/11	0/5	0/3	1/1
κ^*	11/11	5/5	NT†	NT†
λ^*	11/11	5/5	NT†	NT†

*Cytoplasmic staining of plasma cells.

†NT=not tested.

similar for the five additional synovial membranes used as controls.

All positive controls for EBER or BHLF1 oligoprobes showed strong nuclear labelling. In all samples *in situ* hybridisation performed with the oligoprobes specific for κ and λ transcripts yielded strong cytoplasmic staining of almost all plasma cells present in the infiltrate. This staining was indicative of a good preservation of RNA.

Discussion

The mechanisms affecting the lesions of RA are still unclear and several causative agents have been suggested.¹² Among the possible aetiological factors, the Epstein-Barr virus is one of the most likely influences because of its ubiquity and ability to persist in a latent state in B lymphocytes and some epithelial cells of infected subjects.^{11 13} Disruption of latency may occur in these cells, however, particularly in epithelial cells, and is responsible for an active production of virions.¹¹ This replicative phase is associated with the expression of several antigens which are different from those of the latent phase. Thus EBNA1 (Epstein-Barr nuclear antigen 1), expressed during latency, and gp110 (viral capsid antigen), expressed during the lytic cycle, have been found to contain epitopes possibly affecting the B cell immune responses in patients with RA.^{6 7} As already shown in salivary glands from patients with Sjögren's syndrome,¹³ cells carrying the Epstein-Barr virus (B lymphocytes or synovial cells) may be expected to be found within specific lesions of RA. Regarding the expression of the CD21/C3d-receptor, also known as the Epstein-Barr virus receptor, the best target should be B lymphocytes as the CD21 molecule is not expressed by synovial cells (personal unpublished data).

We have been unable to detect the Epstein-Barr virus in lymphoid cells or synovial cells using *in situ* hybridisation with EBER oligoprobes, a technique which is now considered to be as sensitive as the polymerase chain reaction to detect the latent Epstein-Barr virus

in infected cells.^{14 15} Indeed, EBER encoded non-polyadenylated RNAs, localised to the nucleus as ribonucleoprotein complexes resistant to formalin fixation, are the most abundant Epstein-Barr virus RNAs in latently infected cells (10^7 copies/cell).^{10 11 14 15} Similar results were obtained with the oligoprobes detecting BHLF1 transcripts, which are known to be most abundant during the replicative phase.^{10 11}

Whether the Epstein-Barr virus is one of the causative agents of RA, putative viral cross linking antigens do not appear to be released from specific synovial lesions or cutaneous rheumatoid nodules. Thus other reservoirs of Epstein-Barr virus infected cells, such as circulating small B lymphocytes (10^6 circulating B lymphocytes), epithelial cells of the cavum, mouth cavity, and salivary glands, could be the main sites of production of these immunogenic molecules in patients with RA.

This work was supported by grants from INSERM (CRE).

- Alspaugh M A, Henle G, Lennette E T, Henle W. Elevated levels of antibodies to Epstein-Barr virus antigens in patients with rheumatoid arthritis. *J Clin Invest* 1981; **67**: 1134-40.
- Ferrel F B, Aitchison C T, Pearson G R, Tan E M. Seroepidemiological study of relationships between Epstein-Barr virus and rheumatoid arthritis. *J Clin Invest* 1981; **67**: 681-7.
- Tosato G, Steinberg A D, Yarchoan R. Abnormally elevated frequency of Epstein-Barr virus-infected cells in the blood of patients with rheumatoid arthritis. *J Clin Invest* 1984; **73**: 1789-95.
- Venables P J W, Smith P R, Maini R N. Rheumatoid nuclear antigen and Epstein-Barr virus. *Arthritis Rheum* 1984; **27**: 476-7.
- Billings P B, Hoch S O, White P J, Carson D A, Vaughan J H. Antibodies to Epstein-Barr nuclear antigen and to the rheumatoid arthritis nuclear antigen identify the same polypeptide. *Proc Natl Acad Sci USA* 1983; **80**: 7104-8.
- Venables P. Epstein-Barr virus infection and autoimmunity in rheumatoid arthritis. *Ann Rheum Dis* 1988; **47**: 265-9.
- Roudier J, Petersen J, Rhodes G H, Luka J, Carson D A. Susceptibility to rheumatoid arthritis maps to a T-cell epitope shared by the HLA-Dw4 DR B-1 chain and the Epstein-Barr virus glycoprotein gp110. *Proc Natl Acad Sci USA* 1989; **86**: 5104-8.
- Ropes M W, Bennett G A, Cobb S, Jacox R, Jessar R A. 1958 revision of diagnostic criteria for rheumatoid arthritis. *Bull Rheum Dis* 1958; **9**: 175-6.
- Arnett F C, Edworthy S M, Bloch D A, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum* 1988; **31**: 315-24.
- Brousset P, Butet V, Chittal S, Selves J, Delsol G. Comparison of *in situ* hybridisation using different nonisotopic probes for detection of Epstein-Barr virus in nasopharyngeal carcinoma and immunohistochemical correlation with anti-latent membrane protein antibody. *Lab Invest* 1992; **67**: 457-64.
- Kieff E, Liebowitz D. Epstein-Barr virus and its replication. In: Fields B N, Knipe D M, eds. *Virology*. 2nd ed. New York: Raven Press, 1990: 1889-958.
- Fox R, Luppi M, Pisa P, Kang H. Potential role of Epstein-Barr virus in Sjögren's syndrome and rheumatoid arthritis. *J Rheumatol Suppl* 1992; **32**: 18-24.
- Mariette X, Gozlan J, Clerc D, Bisson M, Morinet F. Detection of Epstein-Barr virus DNA by *in situ* hybridisation and polymerase chain reaction in salivary gland biopsy specimens from patients with Sjögren syndrome. *Am J Med* 1991; **90**: 286-94.
- Weiss L M, Chen Y Y, Liu X F, Shibata D. Epstein-Barr virus and Hodgkin's disease: a correlative *in situ* hybridization and polymerase chain reaction study. *Am J Pathol* 1991; **139**: 1259-65.
- Randhawa P S, Jaffe R, Demetris A J, et al. Expression of Epstein-Barr virus-encoded small RNA (by EBER-1 gene) in liver specimens from transplant recipients with post-transplantation lymphoproliferative disease. *N Engl J Med* 1992; **327**: 1710-4.