

EXTENDED REPORT

Increased levels of antibodies to cytokeratin 18 in patients with rheumatoid arthritis and ischaemic heart disease

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Objective: To determine whether raised levels of antibodies to CK18 in patients with RA are associated with ischaemic heart disease (IHD).

Methods: IgA, IgG, and IgM antibodies to CK18 were measured by enzyme linked immunosorbent assay (ELISA) in patients with RA with (n=34) or without (n=28) IHD. The relationship between CK18 antibody levels and markers of inflammatory and/or cardiovascular disease was examined.

Results: Initial analysis showed that IgG antibody levels to CK18 were higher in patients with RA with IHD than in those without (50.1 v 34.5 AU, $p=0.047$), although significance was lost after correction for multiple comparisons. Further analysis showed a significant difference ($p=0.015$) between patients with IHD and a positive family history, and patients without IHD and a negative family history (53.7 v 29.0 AU, Kruskal-Wallis multiple comparison Z value test). There was also a significant trend of increasing 10 year cardiovascular risk with increasing CK18 IgG antibody levels ($p=0.01$). No association was found between CK18 antibody levels and conventional markers of inflammation or cardiovascular disease, but an association was found between levels of CK18 IgG and IgG antibodies to cytomegalovirus (CMV) (Spearman's $r_s=0.379$, $p_{corr}=0.04$). No evidence for cross reactivity of CK18 antibodies with CMV antigens was found.

Conclusion: Levels of IgG antibodies to CK18 are raised in patients with RA with IHD, particularly if they also have a positive family history. This may reflect damage to CK18 containing cells in the cardiac vasculature and/or in atherosclerotic plaques, and may be a useful additional marker for the identification of patients with, or likely to develop, IHD.

A number of studies have shown that rheumatoid arthritis (RA) is associated with increased cardiovascular (CV) mortality (reviewed by Goodson¹ and van Doornum *et al*²). This is predominantly due to ischaemic rather than rheumatoid heart disease.³ Indeed, there is an increased incidence of ischaemic CV events in patients with RA,⁴ and atherosclerosis, the underlying cause of ischaemic heart disease (IHD), appears to be accelerated in patients with RA.^{1–3} The reason for this is still unclear but it may be related to clustering of classical cardiac risk factors such as dyslipidaemia,^{6,7} a prothrombotic state,⁸ and other processes. However, classical risk factors, although important, do not appear to be sufficient to explain the accelerated atherosclerosis associated with RA.⁴ This is possibly due to the systemic inflammation associated with RA, which may make RA itself (like diabetes) an independent risk factor for the development of IHD.⁹ Accumulating evidence suggests that systemic inflammation indeed has an important role in the development of atherosclerosis,¹⁰ and markers of inflammatory activity such as C reactive protein (CRP) are predictive of CV risk in the general population.¹¹ In seropositive RA, the extent of inflammation has been shown to predict CV disease and overall mortality.^{12,13}

A number of predictors for risk of CV disease are well known for the general population, but identification of new markers is still required to help with better characterisation of patients at risk of IHD. This may be particularly important for patients with disability, who, owing to their reduced exercise capacity, may not elicit symptoms of cardiac ischaemia, or whose symptoms may be wrongly attributed to musculoskeletal causes.¹⁴

Circulating autoantibodies to various cytoskeletal proteins have been described in primary coronary artery disease, and in coronary artery disease associated with transplants.^{15,16}

These include antibodies to actin, desmin, vimentin, and cytokeratin 18 (CK18). We have shown previously that autoantibodies to CK18 are raised in patients with RA compared with patients with osteoarthritis (OA) and normal controls.¹⁷ They are also raised in patients with psoriatic arthritis.¹⁸ We originally proposed that such antibodies may arise as a consequence of damage to synovial endothelial cells during inflammation, and/or from damage to epithelial cells (for example, in the skin, gut). The synovial endothelium differs from most human endothelium in expressing CK18 as well as vimentin intermediate filaments.^{19–21} However, CK18 has also been found in some endothelial cells of the cardiac microvasculature.²² In addition, this cytokeratin (together with cytokeratin 8) becomes expressed in vascular smooth muscle cells (VSMCs) associated with atherosclerotic plaques.²³ Cytokeratins are not found in normal adult VSMCs, but during development of atherosclerotic lesions these cells undergo changes in their cytoskeleton composition and their phenotype. The pattern of expression of CK18 in VSMCs of these lesions is similar to that found in some fetal and/or neonatal SMCs, so their state is that of a dedifferentiated fetal type. Furthermore, the expressed cytokeratins display characteristic phosphorylation patterns in coronary artery lesions, which appear to be associated with DNA fragmentation and apoptosis.²⁴

Abbreviations: AU, arbitrary units; CK18, cytokeratin 18; CRP, C reactive protein; CMV, cytomegalovirus; CV, cardiovascular; ELISA, enzyme linked immunosorbent assay; ESR, erythrocyte sedimentation rate; IHD, ischaemic heart disease; MPI-SPECT, myocardial perfusion imaging with single photon emission coupled tomography; OA, osteoarthritis; PBS, phosphate buffered saline; RA, rheumatoid arthritis; VSMC, vascular smooth muscle cells

This study aimed at investigating whether levels of antibodies to CK18 are raised in patients with RA and ischaemic heart disease (IHD), and whether there is any relationship between antibodies to CK18 and classical cardiovascular risk factors and/or inflammatory markers in RA.

PATIENTS AND METHODS

Patients

Eighty consecutive Northern European patients (aged 35–75) with a diagnosis of RA, attending the Dudley group of hospitals rheumatology clinics, were recruited into a study investigating the prevalence of IHD in RA. All patients fulfilled the 1987 American College of Rheumatology criteria for RA.²⁵ Sixty seven patients completed the full cardiovascular investigation protocol, of whom 62 patients were also investigated for antibody levels to CK18. Table 1 shows the characteristics of the 62 patients with RA with and without IHD. The study had local research ethics committee approval.

Clinical evaluations

Standardised history and examination were performed by a single observer (MJB). Cardiovascular symptoms and risk factors were assessed using the Rose questionnaire.²⁶ Hypertension was assessed by previous diagnosis, resting systolic and diastolic blood pressure,²⁷ and fundoscopy for hypertensive retinopathy (\geq grade II). Height and weight were recorded and body mass index calculated. Absolute baseline risk for a cardiovascular event was calculated for each patient using the "Cardiac Risk Assessor" version 98.02 (University of Manchester), a computer program designed to calculate the absolute risk of cardiovascular events over the next 10 years based on the Framingham equations,^{28, 29} and validated for use with "The Joint Recommendations of the British Cardiac Society, British Hyperlipidaemia Association, British Hypertension Society, and British Diabetic Association on the prevention of Coronary Heart Disease in Clinical Practice".³⁰ A family history of IHD was defined as a male or female first degree relative sustaining an acute cardiovascular event before the age of 55 or 65 years, respectively. RA disease duration, comorbidity, extra-articular features, past and

present use of non-steroidal, steroidal, or disease modifying treatment were recorded in all patients.

Cardiovascular investigations

Twelve lead ECG recordings were taken supine at rest using a Marquette Mac 8 ECG machine (GE Medical Systems) and analysed by a single "blinded" cardiologist using GE Medical software; in cases of disagreement, the cardiologist's opinion was considered correct. Trans-thoracic echocardiography was performed by a single expert technician and validated by a cardiologist, using a GE Vingmed digital ultrasonoscope with a 1.5–2.5 MHz phased array transducer probe, and the images were stored in a computer based storage and analysis system (Echopac). Left ventricular mass was calculated as previously described.³¹ Detection of cardiac ischaemia was carried out using adenosine stressed thallous-201 chloride myocardial perfusion imaging with single photon emission coupled tomography (MPI-SPECT), using a Siemens Diacam single headed SPECT gammacamera incorporating a 180° circular orbit to acquire 32 images. Scans were processed with Siemens ICON software, with a 180° orbit back projection using Butterworth's correction filter, without attenuation correction. IHD was defined as: (a) reversible perfusion defect on MPI-SPECT or (b) fixed perfusion defect plus either previous myocardial infarction confirmed by typical ECG changes and/or serial cardiac enzyme rise or typical symptoms of angina or documented occlusion >70% of at least one epicardial coronary artery or 50% of the left main stem. A normal stress scan excluded diagnosis of IHD. High risk IHD was defined as reversible perfusion defects in more than one epicardial vessel territory, or reversible perfusion defect of the anterior, septal, or apical wall of the left ventricle implying left anterior descending artery involvement. "Silent" ischaemia was defined as reversible ischaemia on MPI-SPECT in the absence of Rose angina and/or ECG changes of either a bundle branch block or QRS-T abnormality consistent with IHD.

Serology and biochemistry

Serum and plasma from all patients were separated within 30 minutes of venesection and stored at -70°C . The following were assessed on entry into the study using routine

Table 1 Characteristics of the patients with RA with and without ischaemic heart disease (IHD)

Characteristic	RA with IHD (n = 34)	RA without IHD (n = 28)	p Value
Age (years)	60.9 (7.7)	53.6 (11.2)	0.001
Male sex	19 (56%)	8 (29%)	0.03
Rheumatoid factor	29 (85%)	25 (89%)	0.7
Current smoking	9 (26%)	9 (32%)	0.6
Diabetes	4 (12%)	0	0.1
Family history	20 (59%)	12 (43%)	0.08
Past medical history of IHD	12 (35%)	4 (14%)	0.06
Body mass index	28.4 (4.6)	25.5 (3.4)	0.01
Hypertension			
Past diagnosis	13 (38%)	9 (32%)	0.7
Systolic BP	156.5 (24.9)	140.6 (22.6)	0.03
Diastolic BP	86.9 (11.9)	81.3 (7.3)	0.04
ECG			
LVH	7 (21%)	5 (18%)	0.8
Bundle branch block	6 (18%)	2 (7%)	0.2
QRS abnormality	13 (38%)	9 (32%)	0.6
Lipids			
Total cholesterol (mmol/l)	5.8 (0.9)	5.6 (1.0)	0.6
HDL (mmol/l)	1.41 (0.42)	1.47 (0.26)	0.2
Triglycerides (mmol/l)	1.72 (0.69)	1.41 (0.59)	0.07
Absolute 10 year risk of a cardiac event	0.22 (0.12)	0.11 (0.10)	0.0003

Continuous data are expressed as means (SD). Frequencies are given as actual number with (%). Continuous data were analysed using Student's *t* test or the Mann-Whitney U test depending on normality distribution, and frequencies were compared by χ^2 test.

laboratory procedures: full blood count, biochemical profile, serum folate, ferritin, fibrinogen, homocysteine, erythrocyte sedimentation rate (ESR), CRP, immunoglobulins (IgG, IgM, IgA), rheumatoid factor, complement (C3 and C4), von Willebrand factor, total cholesterol, high density lipoprotein and triglycerides. ESR and CRP values taken at regular three-monthly intervals of monitoring over the past 4 years before entry into the study were used to calculate mean ESR and CRP and area under the curve of ESR and CRP for each patient. IgG and IgM antibodies to *Helicobacter pylori*, *Chlamydia pneumoniae*, and cytomegalovirus (CMV) were assessed in serum using commercially available enzyme linked immunosorbent assay (ELISA) kits: RADIM (Via del Mare, 125-00040 Pomezia (Roma) Italy), Immunocomb, Organics (PO Box 360, Yavne 70650, Israel), IMxSystem, Abbott Laboratories (Diagnostic Division, Abbott Park, IL 60064), respectively.

CK18 ELISA

Flat bottomed 96 well microtitre plates (high binding; Costar, Corning Inc) were incubated overnight at 4°C with 100 µl per well of purified CK18 from bovine liver (1.0 µg/ml; Progen Biotechnik, Heidelberg, Germany) in carbonate buffer, pH 9.6. Plates were coated with 1% bovine serum albumin in phosphate buffered saline/0.05% Tween 20 (PBS/Tween) for 1 hour at 37°C. The plates were then incubated with test samples, diluted 1:100 in PBS/Tween, for 1 hour at 37°C. Blank (background) wells were incubated with PBS/Tween only. Plates were washed three times in PBS/Tween and incubated at 37°C for 1 hour with 100 µl/well of antihuman immunoglobulin (IgA, IgG, or IgM) conjugated with alkaline phosphatase (Sigma, Poole, UK) at a dilution of 1:1000. Plates were again washed and incubated with 100 µl/well of 1 mg/ml *p*-nitrophenyl phosphate in diethanolamine buffer at 37°C for 30 minutes. The reaction was stopped by addition of 50 µl of 3M NaOH to each well.

The absorbance (optical density) of the reaction mixture was read at 405 nm in a Titertek Multiskan Plus MkII Microplate Reader (Labsystems). Concentrations of antibodies were expressed in arbitrary units (AU), as defined by a standard serum sample from a patient with high levels of antibodies to CK18 (100 AU). A standard series of dilutions of this serum sample was assayed with every ELISA in order to construct a standard curve. Blank (background) wells were used in each assay, together with sera from previous assays that had both high and low levels to ensure inter- and intra-assay reliability. The background readings were subtracted from the optical density readings before calibration.

To determine whether antibodies to CK18 were cross reactive with CMV antigens we carried out a competitive binding ELISA in which we added CMV antigens (strain AD169; Biogenesis Ltd, Poole, England) to 10 patients' sera before measuring levels of antibodies to CK18. All patients had been shown previously to have detectable levels of IgG antibodies to both CK18 and CMV. Each serum sample was diluted 1:100 in PBS/Tween and divided into two sets of aliquots. One set of aliquots was preincubated with CMV antigens overnight at room temperature. A range of concentration (0.625–40 µg/ml) of CMV antigens was used. The other set of aliquots was incubated without any CMV antigens. The reactivity of both sets of samples with CK18 was then measured in a standard CK18 ELISA. As a positive control we also tested the binding of the same sera preincubated with CK18 (50 µg/ml).

Statistical analysis

Comparisons between patients with and without IHD were carried out using Student's *t* test for parametric data or the Mann-Whitney U test for non-parametric data. Where

appropriate, the analyses were corrected for multiple comparisons using the Bonferroni procedure. Analysis of differences between subgroups of patients was carried out by Kruskal-Wallis analysis of variance for non-parametric data, and corrected for multiple comparisons with the Kruskal-Wallis Z value test. Analysis of trend was performed using the Cuzick test. Spearman's rank correlation was used to examine the relationship between CK18 antibody levels and other variables. A value of $p < 0.05$ was considered significant. All statistics were calculated using the Number Cruncher Statistical System for Windows (NCSS 2000).

RESULTS

CK18 antibody levels

Measurement of IgA, IgG, and IgM levels of CK18 antibodies showed a weak but significant difference in CK18 IgG antibody levels between patients with RA with or without evidence of IHD (50.1 v 34.5 AU, $p = 0.047$) (table 2), which was lost after correction for multiple comparisons ($p = 0.14$). There was no significant difference in IgA or IgM antibody levels. In subsequent analyses only the levels of CK18 IgG antibodies were compared between different subgroups of patients.

Of the 34 patients with evidence of IHD, 13 were classed as having clinically silent IHD. The levels of CK18 IgG antibodies in these patients were similar to those in patients with Rose angina and/or ECG changes (53.8 v 47.8 AU), and were significantly higher than in patients without evidence of IHD (53.8 v 34.5 AU, $p = 0.008$).

Influence of family history and sex

We stratified patients with or without IHD into groups with or without a family history of IHD (table 3). Analysis of CK18 IgG levels by Kruskal-Wallis analysis of variance showed that patients with both IHD and a positive family history had the highest levels, while patients without IHD and a negative family history had the lowest (53.7 v 29.0 AU). This was significant after correction for multiple comparisons by the Kruskal-Wallis Z value test ($p = 0.015$). Interestingly, the levels in patients with a family history but no current evidence of IHD were similar to those in patients with IHD but no family history (41.8 v 43.6 AU).

Further stratification of patients by sex as well as family history indicated that male patients with IHD and a family history ($n = 10$) had higher levels than women with IHD and a family history ($n = 12$) (67.6 v 41.9 AU), although this difference was not significant ($p = 0.2$). The lowest levels were found in women with no IHD and no family history (26.8 AU).

Table 2 Cytokeratin 18 (CK18) antibody levels in patients with rheumatoid arthritis (RA) with or without ischaemic heart disease (IHD)

Variable	IHD positive (n = 34)	IHD negative (n = 28)	p Value
CK18 IgG	50.1 (35.8)	34.5 (21.6)	0.047
CK18 IgM	20.7 (13.9)	25.0 (21.0)	0.5
CK18 IgA	29.3 (28.4)	22.2 (16.9)	0.9

Results are expressed as the means (SD). Data were analysed by the Mann-Whitney U test for non-parametric data. The p values are shown uncorrected for multiple comparisons. Significance for the difference in CK18 IgG levels was lost after correction by the Bonferroni procedure ($p = 0.14$).

Table 3 CK18 IgG antibody levels in patients with or without IHD stratified by family history of IHD

Family history	IHD positive	IHD negative
Positive	53.7 (40.1) (n=22)*	41.8 (27.0) (n=12)
Negative	43.6 (26.7) (n=12)	29.0 (15.2) (n=16)

Results are expressed as the means (SD). Data were analysed by Kruskal-Wallis analysis of variance for non-parametric data.

*Patients with IHD and a positive family history had significantly higher levels of CK18 IgG antibodies than patients without IHD and a negative family history ($p=0.015$, Kruskal-Wallis multiple comparison Z value test).

Relationship between absolute cardiovascular risk and CK18 antibody levels

We investigated whether there was any relationship between absolute 10 year cardiovascular risk and CK18 IgG antibody levels. Division of cardiovascular risk into quartiles revealed a significant trend of increased risk with increasing levels of CK18 IgG antibodies (table 4).

Relationship between CK18 antibody levels and markers of inflammatory and cardiovascular disease

No relationship was found between CK18 antibody levels and markers of disease activity such as ESR and CRP (table 5). There were also no associations with rheumatoid factor or antinuclear antibody levels. Systolic blood pressure and cholesterol levels showed no relationship with CK18 IgG levels. Diastolic blood pressure and left ventricular mass showed weak associations ($r_s = 0.29$, $p = 0.02$, and $r_s = 0.35$, $p = 0.02$, respectively), although significance was lost after correction for multiple testing.

Relationship between CK18 and CMV antibody levels

Interestingly, of all the other markers examined the only significant correlation was found with IgG antibody levels to CMV (Spearman $r_s = 0.38$, $p_{corr} = 0.04$) (table 5). Patients who were seropositive for IgG antibodies to CMV ($n = 34$) had significantly higher levels of IgG antibodies to CK18 than those who were seronegative ($n = 14$) (51.5 v 28.4 AU, $p = 0.006$).

To investigate whether antibodies to CK18 were cross reactive with CMV antigens we carried out competitive binding ELISAs. We found that in sera preincubated with different concentrations of CMV antigens there was no reduction in binding of IgG anti-CK18 antibodies to the plates, while preincubation with CK18 (50 $\mu\text{g/ml}$) reduced the binding by at least 50% in most cases (data not shown). In a similar experiment we found that preincubation of patients' sera with CK18 antigen (50 $\mu\text{g/ml}$) caused no reduction in binding of IgG CMV antibodies to CMV antigens in a commercial CMV ELISA (Sigma, Poole, UK).

Sensitivity and specificity of CK18 antibodies

To test the utility of IgG antibodies to CK18 as a marker for IHD in patients with RA, we determined the sensitivity and

specificity of this marker. We used the mean + 2SD of the levels found in patients without IHD and no family history of IHD as the positive cut off point (59.4 AU). When this cut off figure was used the sensitivity of the assay in detecting IHD in these patients with RA was only 23.5%, while the specificity was 82.1%. Increasing the cut off point to the mean + 3SD improved specificity (92.9%), but reduced sensitivity (11.8%). Similar levels of specificity and sensitivity were found in patients with clinically silent IHD.

DISCUSSION

We provide evidence that IgG antibodies to CK18 are raised in patients with RA with IHD, particularly if they also have a family history of IHD. There was some indication that the highest levels were found in men with IHD and a family history of heart disease, while the lowest levels were found in women without a family history and no current evidence of IHD. However, the numbers of patients in these subgroups were small, so these findings will need to be confirmed in larger studies.

The cardiac cytoskeleton has an essential role in maintaining cellular integrity and function of the myocardium. The network of microtubules and intermediate filaments may be disrupted by the inflammatory response associated with heart disease. Thus, in patients with IHD, the presence of antibodies to CK18 may indicate increased exposure of CK18 antigen to the immune system as a result of injury to CK18 containing cells within the myocardium. We proposed previously that antibodies to CK18 in patients with RA may arise as a consequence of damage to synovial endothelial cells during inflammation.¹⁷ Unlike the present study we compared anti-CK18 levels in patients with RA and OA, and demonstrated a difference in IgA anti-CK18 levels. However, we did not investigate the presence of IHD in those patients, so it is unclear whether the increased levels of IgA anti-CK18 antibodies had any relationship with IHD in that case.

The level of IgG anti-CK18 antibodies in the patients with RA was not associated with markers of disease activity such as ESR and CRP, or with levels of rheumatoid factor or antinuclear antibodies. IgG anti-CK18 antibodies do not therefore appear to be markers of RA inflammation or general autoantibody production. There were no associations with conventional cardiac risk factors such as cholesterol levels or systolic blood pressure, although diastolic blood pressure and left ventricular mass were weakly correlated in analyses uncorrected for multiple testing.

Although IHD is highly prevalent in RA, its identification is often difficult on clinical grounds alone, especially where reduced physical exertion and symptoms of IHD may be attributed to musculoskeletal rather than cardiac causes.¹⁴ Furthermore, it may be clinically silent in many patients, emphasising the need for a high degree of awareness.^{32 33} Procedures such as angiography or exercise ECG may be employed in the detection of IHD. However, angiography would not be suitable as a general screening tool because of its invasive nature, risk, and cost, and many patients with RA

Table 4 Relationship between CK18 IgG antibody levels and absolute 10 year risk of a cardiovascular event

	Risk quartiles (%)			
	0-25	26-50	51-75	76-100
Absolute risk	0-6.1	6.2-13	13.1-23.5	23.6-45.4
Number	16	17	17	17
CK18 IgG	27.8 (16.7-43.6)	34.9 (17.5-51.5)	35.0 (18.6-69.7)	57.9 (29.5-73.2)

CK18 IgG levels are shown as median values (interquartile range). There is a significant relationship between absolute cardiovascular risk quartiles and CK18 IgG antibody levels: $p = 0.01$ (Cusick's test for trend).

Table 5 Correlation between serum levels of CK18 IgG antibody levels and markers of inflammatory and cardiovascular disease

	ESR	CRP	Diastolic BP	LVM	α -CMV(IgG)
α -CK18(IgG)	-0.209	-0.025	0.293*	0.348*	0.379†
ESR		0.513‡	-0.170	-0.154	0.078
CRP			-0.094	0.001	0.107
Diastolic BP				0.013	
LVM					0.038

ESR, erythrocyte sedimentation rate; CRP, C reactive protein; BP, blood pressure; LVM, left ventricular mass; α -CMV, anticytomegalovirus.

Spearman rank correlation (r_s values shown in table).

* $p=0.02$ ($p_{\text{corr}}=0.1$); † $p=0.008$ ($p_{\text{corr}}=0.04$); ‡ $p<0.0001$ ($p_{\text{corr}}=0.0005$).

p Values for correlation of anti-CK18 IgG with other markers are shown uncorrected and corrected for multiple testing (Bonferroni procedure).

may be unable to have an exercise ECG owing to musculo-skeletal disability. MPI-SPECT or echocardiography under pharmacological stress and more advanced cardiac imaging may be useful but are not widely available.³ Single measurements of CRP or other markers of inflammation may not be as useful or specific in RA as they appear to be in the general population, as they are likely to be raised in most patients with RA. In this study, IgG antibodies to CK18 seem to be a relatively specific marker for IHD (including silent IHD) in patients with RA, but the assay is not very sensitive. Thus many patients with IHD would not be detected using a cut off point defined by the mean + 2SD of levels found in patients without IHD and no family history of IHD. However, such an assay may be useful in combination with other possible measures of cardiovascular risk (for example, in male patients with a family history of IHD).

Of particular interest was our finding of a significant correlation between levels of IgG antibodies to CK18 and CMV. Infection with human CMV has been implicated in the development of atherosclerosis and coronary heart disease in some studies, although this is still a controversial area.³⁴⁻³⁵ We carried out a comparative BLAST search of protein sequence databases to investigate whether any sequence homology existed between CK18 and human CMV antigens used to coat the plates in the ELISA test for CMV antibodies. The CMV antigens comprised the following proteins: pp150(UL32), pp65(UL83), pp52(UL44), and pp38(UL80a). No significant regions of sequence identity were found between CK18 and any of the CMV proteins, although a span of 13 amino acids in CK18 (position 56–68) demonstrated a 61.5% homology with CMV DNA polymerase accessory protein (pp52, UL44) at position 324–336. This is a region containing repeating glycine units, where six of the eight amino acids common to both UL44 and CK18 were glycines. UL44 is a major antigenic protein of human CMV³⁶ that produces a strong, early, and lasting antibody response in a majority of people after infection.³⁷

Although the similarity of CK18 and UL44 sequences in one small region was interesting, we found no evidence for cross reactivity of CK18 antibodies with human CMV antigens, or of human CMV antibodies with the CK18 antigen. The reason for the correlation between levels of anti-CK18 and anti-CMV antibodies is therefore unclear. It may represent a more general autoantibody response in CMV infected patients. However, we found no association of rheumatoid factor or antinuclear antibody levels with antibodies to CMV in these patients. Conflicting data have previously been presented on autoantibody production in CMV infected patients. CMV infection has been associated with increased production of rheumatoid factor, antiphospholipid antibodies, antimyosin antibodies, antiendothelial cell antibodies, and antiganglioside antibodies.³⁸⁻⁴² Endothelial cells are one of the main reservoirs for CMV

infection⁴³ and it has been speculated that CMV-induced endothelial damage may be a potent antigenic stimulus, which leads to the production of antiendothelial cell autoantibodies. Such autoantibodies may represent not only a marker of cell injury but also contribute to the progression of the inflammation, leading to the exposure of tissue privileged self antigens and induction of other autoantibodies.⁴⁴

In conclusion we have shown that levels of IgG antibodies to CK18 are raised in patients with RA with IHD, particularly if they also have a family history of IHD. We suggest that this may reflect damage to CK18 containing cells in the cardiac vasculature or in atherosclerotic plaques, or both. Although IgG antibodies to CK18 are not sensitive markers for IHD, they appear to be relatively specific and may be useful in combination with other markers for the identification of patients with RA with, or likely to develop, IHD.

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