Disclosure statement: The authors have declared no conflicts of interest.

Srilakshmi M. Sharma1, Tammy M. Martin2, Carlos D. Rosé3, Andrew D. Dick4 and Athimalaipet V. Ramanan5

1Bristol Eye Hospital, University Hospitals Bristol NHS Foundation Trust, Bristol, UK, 2Casey Eye Institute, Oregon Health & Science University, Portland, OR, USA, 3Department of Paediatric Rheumatology, DuPont Hospital for Children, Wilmington, DE, USA, 4Department of Cellular and Molecular Medicine, School of Medical Sciences, University of Bristol, Bristol, UK and 5Department of Paediatric Rheumatology, Bristol Royal Hospital for Children, Bristol University Hospitals NHS Foundation Trust, Bristol, UK

Correspondence to: Srilakshmi M. Sharma, Department of Neuroophthalmology, Wilmer Eye Institute, Johns Hopkins Hospital, 600 N Wolfe Street, Baltimore, MD 21287, USA. E-mail: ssharm28@jhmi.edu

References


Advance Access publication 27 January 2011

Potential biomarkers of monocyte/macrophage activity in a patient with sarcoidosis, treated with infliximab

Sir, Sarcoidosis is a granulomatous disease of unknown, and probably heterogeneous, aetiology. The histological resemblance between lesions in sarcoid and mycobacterial infection suggests that some cases of sarcoidosis may be driven by unidentified intracellular bacteria [1]. Most patients can be managed with CSs and/or conventional immunosuppressants. However, TNF-α blockade has been proposed for patients whose disease is resistant to conventional strategies [2–5]. This therapy has proven efficacious only in a minority of steroid-resistant patients [6, 7], and presents the risk of reactivating latent microbial infection. There is, therefore, a need for biomarkers of efficacy of anti-TNFα therapy (or indeed other immunosuppressive therapies). Previous work has revealed peripheral blood monocyte hyperactivity in sarcoidosis [8]. Here, we report that several markers of monocyte hyperactivity rapidly normalized following successful anti-TNF-α therapy of steroid-resistant, advanced sarcoidosis.

Our patient was a 44-year-old Afro-Caribbean male who was considered to be in the terminal stages of multisystem sarcoidosis. As a retired United States Air Force serviceman, he had a history of exposure to beryllium, depleted uranium and hydrazine. He had presented with breathlessness 16 years earlier. Bilhar lymphadenopathy was noted on plain chest radiograph and a transbronchial biopsy revealed granulomatous inflammation consistent with sarcoidosis. His disease progressed despite CSs and AZA, to involve renal, rhinological and cutaneous sites; severe lung involvement necessitated use of a wheelchair and continuous domiciliary oxygen.

Investigations revealed a systemic inflammatory response (ESR: 75 mm/h; CRP: 33 mg/ml) but normal renal and liver function, serum angiotensin-converting enzyme and autoantibody titres. Sputum microscopy and culture, T-spot and beryllium function tests were negative. High-resolution CT of the chest revealed bilateral hilar lymphadenopathy (Fig. 1A, arrow), widespread ground glass and fibrosis (Fig. 1A). Due to his pulmonary compromise, lung function tests were impractical; he had an saturation of 92% (PaO2 10.3 kPa) on oxygen 4 l/min.

Following discussion of risks and benefits, our patient opted for a trial of infliximab 3 mg/kg/month and replacement of AZA with MMF 3 g/day. There was rapid subjective improvement and he was able to perform his first 6-min walk test (6MWT; Fig. 1B). Since the contributions of MMF and infliximab to this improvement were unclear, MMF alone was continued. A repeat 6MWT at 6 months revealed increased walk distance, decreased rest time and reduced desaturation (Fig. 1B). Unfortunately, this improvement was not sustained and our patient reported a return of his systemic symptoms. Therefore, we reintroduced...
infliximab, at a dose of 5 mg/kg/month. This improved systemic symptoms, 6MWT results (Fig. 1B) and inflammatory markers (ESR 35 mm/h; CRP 9 mg/ml).

With ethical approval by Norfolk Research Ethics Committee (07/H0310/178) and informed consent, we studied monocyte/macrophage activation before restarting infliximab, and 2 weeks after each of the first two doses. Monocytes were enriched to 95% purity from peripheral blood mononuclear cells using magnetic bead selection of CD14+ cells (Miltenyi Biotec, Bergisch Gladbach, Germany). Flow cytometry (FACSCantoII; Becton Dickinson, UK) was used to assess the expression of monocyte activation markers, using antibodies specific for CD14 (allophycocyanin), CD16 (peridinin chlorophyll), CD86 (phycoerythrin) and HLA-DR (FITC) (BD Biosciences, Franklin Lakes, NJ, USA). Macrophages were differentiated by culturing monocytes with GM-CSF (5 pg/ml), TNF-α (10 pg/ml) and IFN-γ (1000 U/ml) for 6 days; supernatants were analysed by ELISA or cytokine bead array for the presence of monokines promoting effector T-cell differentiation: IL-12 (pro-Th1; Lab Reagents) and IL-23 (pro-Th17). We also quantified chemokines previously reported to be elevated in bronchoalveolar lavage fluid from patients with sarcoidosis, interferon gamma-induced protein 10 kDa (IP10) and regulated upon activation normal T-cell expressed and secreted (RANTES) (Bender Medsystems, San Diego, CA, USA) [9, 10]. Data from our patient were compared pre- and post-treatment with infliximab 5 mg/kg, and with healthy controls (n = 7).

Two monocyte subsets were distinguished by the expression level of CD16; CD16 hi monocytes exhibited a more activated phenotype, expressing higher levels of HLA-DR MHC Class II molecules and of the costimulatory molecule, CD86. Our patient’s monocytes contained a markedly higher proportion of CD16 hi monocytes (22%) than those from healthy donors [1.8 (1.3%); n = 7; Fig.
1C and D). Strikingly, the patient’s CD16hi subset declined from 22 to 6.8% 2 weeks following his first infusion of infliximab 5 mg/kg (Fig. 1E) and remained at 6.6% after the second infliximab infusion (data not shown).

Macrophages derived from our patient secreted high levels of IP-10, which declined to near control levels post-infliximab (Fig. 1F). RANTES displayed a similar pattern, but neither IL-12 nor IL-23 was detected in patient or control samples (data not shown).

To our knowledge, this is the first attempt to assess the response of monocyte/macrophage activation to anti-TNF-α therapy of sarcoidosis. A patient with life-threatening sarcoid exhibited increased monocyte/macrophage activity that normalized shortly after clinically successful TNF-α blockade. The findings implicate systemic monocyte activation in the pathophysiology of sarcoidosis and suggest that monocyte activation is maintained by TNF-α in vivo. Monocyte activation markers may thus be useful as a biomarker of therapeutic efficacy in this setting. Further study is required to establish whether these biomarkers correlate with disease activity in sarcoid specifically, or in all granulomatous conditions, and whether these or other activation phenotypes could report on (re)activation of intracellular microbial infection post-TNF-α blockade.

Our patient defied expectations by surviving, and continuing in part-time employment, for ~2 years after the institution of infliximab. He died as a result of a chest infection and secondary pulmonary arterial hypertension, complicating established pulmonary disease. The post-mortem revealed no evidence of active sarcoidosis.

**Rheumatology key message**


**Acknowledgements**

The authors wish to thank the patient and his family for their enthusiasm and commitment.

**Funding:** We would like to thank the NIHR Biomedical Research Centre and CARE (Cambridge Arthritis Research Endeavour), which has funded and supported this study.

**Disclosure statement:** The authors have declared no conflicts of interest.

**Brian Fang1, Shweta Bhagat2, Robert Busch1, Helen Parfrey1 and Frances C. Hall1**

1Department of Clinical Medicine, University of Cambridge, Addenbrooke’s Hospital and 2Rheumatology Department, Cambridge University Hospitals NHS Trust, Cambridge, UK

Accepted 9 December 2010

Correspondence to: Frances C. Hall, Department of Clinical Medicine, University of Cambridge, Addenbrooke’s Hospital, Box 157, Cambridge CB2 0QQ, UK.

E-mail: fch22@medschl.cam.ac.uk

**References**


Rheumatology 2011;50:994–996
doi:10.1093/rheumatology/ker117
Advance Access publication 15 March 2011

**Comment on: Usefulness of anti-cyclic citrullinated peptide antibody and rheumatoid factor to detect rheumatoid arthritis in patients with systemic sclerosis**

Sir, We read the recent article by Ueda-Hayakawa et al. [1] with great interest. They reported that the frequency of elevated serum MMP-3 levels was significantly higher in patients with dcSSc than in those with lcSSc. They also stated that there was a significant correlation between the MMP-3 level and the modified Rodnan total skin thickness score (mRTSS) in SSC patients. However, their findings contradict the previous report that the TSS is higher in patients with normal MMP-3 levels than in those with elevation of MMP-3 [2]. Further, serum MMP-3 levels are reported to be normal in steroid-free SSC patients [3]. We would like to make some comments about the relationship between the serum level of MMP-3 and SSC.

References


doi:10.1093/rheumatology/ker117

Advance Access publication 15 March 2011

**Comment on: Usefulness of anti-cyclic citrullinated peptide antibody and rheumatoid factor to detect rheumatoid arthritis in patients with systemic sclerosis**

Sir, We read the recent article by Ueda-Hayakawa et al. [1] with great interest. They reported that the frequency of elevated serum MMP-3 levels was significantly higher in patients with dcSSc than in those with lcSSc. They also stated that there was a significant correlation between the MMP-3 level and the modified Rodnan total skin thickness score (mRTSS) in SSC patients. However, their findings contradict the previous report that the TSS is higher in patients with normal MMP-3 levels than in those with elevation of MMP-3 [2]. Further, serum MMP-3 levels are reported to be normal in steroid-free SSC patients [3]. We would like to make some comments about the relationship between the serum level of MMP-3 and SSC.

References


doi:10.1093/rheumatology/ker117

Advance Access publication 15 March 2011

**Comment on: Usefulness of anti-cyclic citrullinated peptide antibody and rheumatoid factor to detect rheumatoid arthritis in patients with systemic sclerosis**

Sir, We read the recent article by Ueda-Hayakawa et al. [1] with great interest. They reported that the frequency of elevated serum MMP-3 levels was significantly higher in patients with dcSSc than in those with lcSSc. They also stated that there was a significant correlation between the MMP-3 level and the modified Rodnan total skin thickness score (mRTSS) in SSC patients. However, their findings contradict the previous report that the TSS is higher in patients with normal MMP-3 levels than in those with elevation of MMP-3 [2]. Further, serum MMP-3 levels are reported to be normal in steroid-free SSC patients [3]. We would like to make some comments about the relationship between the serum level of MMP-3 and SSC.

References


doi:10.1093/rheumatology/ker117

Advance Access publication 15 March 2011

**Comment on: Usefulness of anti-cyclic citrullinated peptide antibody and rheumatoid factor to detect rheumatoid arthritis in patients with systemic sclerosis**

Sir, We read the recent article by Ueda-Hayakawa et al. [1] with great interest. They reported that the frequency of elevated serum MMP-3 levels was significantly higher in patients with dcSSc than in those with lcSSc. They also stated that there was a significant correlation between the MMP-3 level and the modified Rodnan total skin thickness score (mRTSS) in SSC patients. However, their findings contradict the previous report that the TSS is higher in patients with normal MMP-3 levels than in those with elevation of MMP-3 [2]. Further, serum MMP-3 levels are reported to be normal in steroid-free SSC patients [3]. We would like to make some comments about the relationship between the serum level of MMP-3 and SSC.

References


doi:10.1093/rheumatology/ker117

Advance Access publication 15 March 2011

**Comment on: Usefulness of anti-cyclic citrullinated peptide antibody and rheumatoid factor to detect rheumatoid arthritis in patients with systemic sclerosis**

Sir, We read the recent article by Ueda-Hayakawa et al. [1] with great interest. They reported that the frequency of elevated serum MMP-3 levels was significantly higher in patients with dcSSc than in those with lcSSc. They also stated that there was a significant correlation between the MMP-3 level and the modified Rodnan total skin thickness score (mRTSS) in SSC patients. However, their findings contradict the previous report that the TSS is higher in patients with normal MMP-3 levels than in those with elevation of MMP-3 [2]. Further, serum MMP-3 levels are reported to be normal in steroid-free SSC patients [3]. We would like to make some comments about the relationship between the serum level of MMP-3 and SSC.