

THE EXAGGERATED INFLAMMATORY RESPONSE IN BEHÇET'S SYNDROME: IDENTIFICATION OF DYSFUNCTIONAL POST-TRANSCRIPTIONAL REGULATION OF THE IFN γ /CXCL10 (IP-10) PATHWAY.

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ACKNOWLEDGEMENTS AND STATEMENT OF FINANCIAL SUPPORT

NA was an Arthritis Research UK-funded Fellow. Our study was also made possible by funding through the British Heart Foundation and the NIHR Biomedical Research Centre at Imperial College Healthcare NHS Trust. DOH received professorial core support from the British Heart Foundation. We would like to extend our gratitude to all volunteers who participated, and in particular to the UK Behçet's Syndrome Society. No financial support or benefits were obtained from commercial sources. The authors have no conflicts of interest to declare.

ABSTRACT

The mechanisms underlying the exaggerated inflammatory response in Behçet's Syndrome (BS) remain poorly understood.

Objectives: We investigated the response of CD14⁺ blood monocytes to IFN γ , focusing on the chemokine CXCL10.

Methods: Chemokine synthesis and release were analysed at a protein and mRNA level following stimulation with IFN γ . Findings in BS patients were compared with 25 healthy controls (HC), 15 rheumatoid arthritis (RA) and 15 systemic lupus erythematosus (SLE) disease control patients.

Results: BS monocytes produced significantly more CXCL10 protein than HC monocytes from two hours following IFN γ stimulation, despite equivalent quantities of mRNA, suggesting more efficient translation. This was significantly more pronounced in BS with high disease activity and in those with ocular and neurological clinical manifestations. The imbalance between CXCL10 protein and mRNA expression was not observed in either RA or SLE patients, and was not seen with other chemokines studied (CXCL9, CXCL11 and CCL2). Furthermore, BS monocytes treated with an alternative stimulant (LPS) did not show abnormal TNF α release. Sucrose density gradients to segregate monocyte CXCL10 mRNA into free RNA or polysome-associated RNA showed equal proportions in BS and HC samples, suggesting that the difference between BS and HC may be due to reduced negative control of CXCL10 translation in BS.

Conclusions BS monocytes have dysfunctional post-transcriptional regulation of CXCL10 mRNA, resulting in overexpression of CXCL10 protein upon IFN γ stimulation. As CXCL10 is a chemokine that recruits mononuclear cells, this abnormality may contribute to the exaggerated inflammatory responses that characterises BS.

BACKGROUND

Behçet's Syndrome (BS) is a chronic, multisystem disorder, typically characterised by orogenital ulcerations and uveitis but potentially affecting most organs [1]. Although the pathogenesis of BS remains relatively poorly understood, recent genome wide association studies have highlighted a number of susceptibility loci including HLA B51, IL-10 and IL23R-IL12RB2 [2, 3]. A working model is that BS is a polygenic condition with environmental triggers (possibly viral), being characterised clinically by exaggerated and prolonged inflammatory responses (eg pathergy). A skew towards Th1 cytokine production has been reported [4-6].

Chemokines are low molecular weight cytokines primarily responsible for chemotaxis of either specific leukocytes or groups of leukocytes [7]. CXCL10, along with the closely related chemokines CXCL9 and CXCL11, is an IFN γ -inducible agonist for the CXCR3 chemokine receptor, which is rapidly induced on naive T cells following activation, and remains highly expressed by Th1 CD4 $^{+}$ T cells, effector CD8 $^{+}$ T cells and innate-type lymphocytes, such as NK and NKT cells [8, 9].

The potential contribution of the IFN γ induced chemokine axis to the pathogenesis of BS has been suggested by previous studies. High levels of IFN γ were found in BS aqueous humour fluid compared with other forms of uveitis [10]. Moreover, high levels of CXCL9, 10 and 11 have been observed in serum from BS uveitis patients, and high CXCL10 protein was found in CSF fluid of BS subjects [11, 12]. CXCR3 receptors (the receptor for CXCL9/10/11) have been shown to be highly expressed in BS biopsies of oral ulcers [13]. Moreover, the pathology of pathergy reactions shows increased mRNA transcripts for CXCR3, CXCL10 and CXCL11 [14, 15]. Given the likely importance of CXCL10 in BS, this project investigated the generation of CXCL10 in response to IFN γ in CD14 $^{+}$ blood monocytes.

METHODS

The study was conducted according to the principles of the Declaration of Helsinki and all participants gave written informed consent. Ethical approval was obtained from the local Research Ethics Committee.

Subject recruitment and evaluation

Both male and female individuals aged over 18 years were included. Subjects with significant co-morbidities were excluded. BS subjects were recruited from the Hammersmith Hospital Rheumatology Clinic, and via the UK Behçet's Syndrome Society. All subjects satisfied the 1990 ISG Behçet Diagnostic Criteria [16]. A clinical history including medication history was obtained and disease activity was assessed using the Leeds Behçet's Disease Activity Form, which gives an activity score on a 0-12 point scale. We classified patients as having an inactive / low disease activity score (DAS) (< 4) or higher activity (≥ 4) [17]. Age- and sex-matched healthy control (HC) volunteers were recruited locally and were required to be free of significant acute or chronic inflammatory disease, and not to be on medications (women taking oral contraception were not excluded). Rheumatoid arthritis (RA) subjects fulfilled the 2010 American College of Rheumatology (ACR) / European League Against Rheumatism (EULAR) criteria for RA [18]. A clinical history including medication history was obtained and disease activity was quantified using DAS28 (ESR) scoring which gives a score on a 1-10 point scale (with >5.1 indicating active disease)[19]. Systemic lupus erythematosus (SLE) subjects fulfilled the ACR criteria for SLE [20]. A clinical history including medication history was obtained and disease activity was quantified using the SLE disease activity score (SLEDAI) which gives a score on a 1-105 point scale (with >6 indicating active disease)[20].

Monocyte isolation and culture

Mononuclear cells were isolated from peripheral venous blood using Histopaque (Sigma Aldrich, UK). They were re-suspended and incubated in 80 μ L buffer (PBS with 0.5% BSA and 0.5M EDTA (Sigma-Aldrich)) and 20 μ L CD14 microbeads (Miltenyi, Germany) per 10 7 cells. CD14 $^{+}$ monocytes were then positively selected on a MS column (Miltenyi). Cells were plated at 10 6 /ml in DMEM

(Invitrogen, UK) with 10% fetal calf serum (FCS) (Biosera, UK) and 1% penicillin-streptavidin (Sigma-Aldrich, UK). Cell purity was confirmed as 98% by flow cytometry.

Enzyme-linked immunosorbent assays (ELISA)

Concentrations of inflammatory mediators were determined by capture ELISA using matched antibody pairs (DuoSet; R&D Systems, Abingdon, UK) according to the manufacturer's recommendations. Optical Densities (OD) were determined using a microplate reader set to 450nm. Standard curves for each cytokine (in duplicate) were generated by using the reference cytokine concentrations supplied in the kits. All samples were measured in duplicate and the results were calculated against the standard curve, and expressed as the mean $\pm$ SEM cytokine concentration (pg or ng/ml).

RNA extraction, cDNA generation and quantitative real-time PCR

An RNeasy easy kit (Qiagen), Trizol RNA, or Trizol LS RNA extraction methods were used to isolate RNA. For Trizol RNA extraction, cells were homogenized with 1mL of Trizol Reagent (Invitrogen, UK). The samples were incubated for 5 min at room temperature. Chloroform was then added and samples vortexed for 15 secs. Following incubation for 2-3 min, samples were centrifuged at 12,000g for 15 min at 4 °C to separate the mixture. The upper aqueous phase, containing RNA, was separated into a fresh tube and RNA precipitated by adding isopropyl alcohol. Samples were then incubated at room temperature for 10 min and centrifuged at 12,000 x g for 10 min at 4°C. The supernatant was completely removed and the RNA pellet was washed twice with 75% ethanol followed by drying at 37°C for 5-10 min. RNA was then dissolved in 20µL RNase free water (Sigma). The purity and the concentration of the RNA were analysed using a Nanodrop spectrophotometer (Thermo Scientific). DNA-Free (Ambion, UK) was used to purify the sample. cDNA was synthesised using the SuperScript® III RT kit (Invitrogen) following manufacturer's instructions. Quantitative real-time PCR was undertaken using SYBR green fastmix IQ (Quanta perfecta, Invitrogen); and the following primers: CXCL9 F5' TTG CTG GTT CTG ATT GGA GTG 3'; R 5' CCT TGG TTG GTG CTG ATG C 3'; CCL-2 F5' AGT CAC CTG CTG TTA T 3', R 5' TCA AGT CTT CGG AGT T 3'; CXCL10 F5' GCT ATG TTC TTA GTG GAT GTT C3', R 5' AGG ATG GCA GTG GAA GTC 3'; HPRT F 5' TTG GTC AGG CAG TAT ATT CC 3', R GGG CAT ATC CTA AA CA AC 3', Oligo dt 5' CGA CTC GAG TCG ACA TCG ATT TTT TTT TTT TTT TT 3'. Real-time PCR were performed using the iCycler thermocycler (Biorad). On completion of the first qRT-PCT run for each new primer set, the product was validated with an agarose gel. Results were expressed as a ratio of the test signal to the house-keeper gene HPRT. During set up experiments results were also validated using other housekeepers (GAPDH and actin).

Sucrose density gradient testing

A 50-20% discontinuous sucrose gradient was prepared in Polyallomer tubes (Beckman Coulter, USA). Cell lysates were added and the sample was then ultracentrifuged at 150,000g for 90 min at 4° C. The material was then extracted in 1 ml fractions, after which each fraction was 'spiked' with a known quantity of GFP mRNA before RNA purification, cDNA conversion and RT-PCR analysis. The GFP spike was used to normalise values to account for any loss of product during processing.

Statistical methodology

Analyses were carried out using PRISM software. The data are presented as medians and inter-quartile ranges unless otherwise stated. Mann-Whitney non parametric testing was used to compare two outcomes. Kruskal-Wallis was used for non parametric testing for multiple groups, or where required, Dunn's test was used to correct for multiple non-parametric comparisons.

RESULTS

Demographics

Twenty seven BS subjects, 25 HC, 15 RA and 15 SLE subjects donated samples for *in vitro* work. There were three rounds of subject recruitment to answer consecutive questions. The demographic data, clinical characteristics, disease activity and medication use of these three cohorts is listed in Supplementary Tables 1-3.

Comparison of CXCL10 expression by blood monocytes in BS and HC

In pilot experiments, 20 ng/ml was found to be the lowest concentration of IFN γ that elicited a robust expression of CXCL10. Monocytes from cohort 1 BS subjects were found to produce significantly more CXCL10 protein in response to IFN γ than those from HC at both 2 hours ($p=0.005$) and 4 hours ($p<0.001$) (Figure 1A). In contrast, no difference was observed between groups in the quantity of mRNA measured (Figure 1B). To test whether the increased CXCL10 protein production was due to immediate or delayed release, we performed a second experiment using cohort 2, examining earlier time-points. This confirmed the increased CXCL10 protein expression from BS monocytes, and showed that this was not evident before two hours following IFN γ treatment ($p=0.027$) (Figure 1C). As before, there was no difference between BS and HC in CXCL10 mRNA expression (Figure 1D). Pooling data from the 2 hour time-points in these two experiments, we plotted CXCL10 protein production against CXCL10 mRNA and found the slopes to be significantly different, as analysed by linear regression ($F=12.5829$, $DFn=1$, $DFd=65$, $p<0.001$, linear regression analysis) (Figure 1E,F). Taken together, these data suggest that the mechanism responsible for increased CXCL10 protein expression in BS is post-transcriptional.

Relation between CXCL10 expression and BS disease activity and disease characteristics

A significant difference was observed in CXCL10 protein between HC and BS, regardless of BS disease activity ($DAS <4$, $p <0.05$; $DAS \geq 4$, $p <0.05$) (Figure 2A). Whilst there was no significant difference in CXCL10 protein expression by monocytes from BS with low *versus* high activity, we were able to demonstrate a significant difference between the low *versus* high activity groups when the protein to mRNA expression was compared ($p<0.05$) (Figure 2B). This indicated that the CXCL10 protein to mRNA ratio may relate to disease activity. Connections between clinical manifestations and CXCL10 expression were then assessed. Those in the mucocutaneous group (MC) were free of cardiovascular (CV), ocular (OC) or neurological (NEU) involvement. Analysis of variance of CXCL10 protein concentration showed a significant variance between the groups ($p<0.001$), with a NEU and OC BS subjects having significantly higher CXCL10 expression than HC ($p<0.05$) (Figure 2C). The sizes of the clinical subgroups were not sufficient to allow statistical evaluation of differences in protein to mRNA ratio between specific groups. Nevertheless, there was a trend towards having a higher protein to mRNA ratio with the OC and NEU groups, and, in contrast, the HC and the limited mucocutaneous groups closely resembled each other (Figure 2D). These results indicate that BS patients with neurological and ocular manifestations have exaggerated CXCL10 protein expression in contrast to those with limited mucocutaneous subjects.

Comparison of CXCL10 expression by blood monocytes in BS *versus* RA and SLE

To determine whether the increase in CXCL10 protein secretion was particular to BS patients, we compared a further BS cohort (cohort 3) with patients with RA or SLE. CXCL10 protein concentrations were greater following IFN γ stimulation in the supernatant of BS samples than those of RA ($p<0.005$) or SLE ($p<0.005$) subjects, whereas no differences were observed at the mRNA level (Figure 3).

Comparison of CXCL10 expression by blood monocytes in BS *versus* other chemokines

We evaluated CXCL9, CXCL11 (chemokines closely related to CXCL10) and CCL2 protein production following IFN γ stimulation in HC and BS subjects using samples from cells stimulated for two hours with IFN γ from the pooled cohorts, as in Suppl. Table 4. We were unable to detect significant CXCL11 protein production in these samples. Both HC and BS monocyte supernatants contained CCL2, with no difference between groups (data not shown). Supernatants from BS patients contained significantly more CXCL9 than those from HC (Figure 4A), but in contrast to CXCL10 results, there was also a statistically significant increase the mRNA level in the BS samples, suggesting a closer transcription-translation relationship (Figure 4B). When the CXCL9 and CXCL10 protein to mRNA plots were compared (Figure 4C,D), CXCL10 showed greater protein:mRNA disparity ($p<0.05$).

Comparison of TNF α expression by blood monocytes in BS compared to other groups following LPS stimulation

We also tested whether monocytes from BS subjects secrete more TNF α than those from other groups following stimulation with LPS (20ng/ml) for two hours. Monocytes from most individuals demonstrated a robust response, but there were no differences either in TNF α protein or mRNA expression between groups (Figure 5).

Polysome profiling to assess post-transcriptional regulation of IFN γ - CXCL10

To further investigate translational efficiency in BS and HC, sucrose density gradient analysis was undertaken to assess CXCL10 mRNA polysomal association. For this experiment, we re-recruited the three BS subjects with the highest CXCL10 protein to mRNA ratios and the three HCs with the lowest CXCL10 protein to mRNA ratios. There was no statistically significant difference between HC and BS CXCL10 mRNA polysomal association despite higher expression of CXCL10 protein in the BS subjects (Supplementary Figure 1), indicating that the more efficient translation was not dependent on increased polysomal association, and therefore involved translational regulation at the post-initiation level.

DISCUSSION

The IFN γ – CXCL10 pathway is of interest to us as IFN γ appears to be an important cytokine in BS and CXCL10 has been found in excess in uveitis, CSF and histological samples of BS [10-15]. We found increased CXCL10 protein expression by IFN γ -stimulated monocytes in repeated experiments comparing BS and HC. As this difference was not matched by differences in CXCL10 mRNA, the abnormality is likely to be post-transcriptional. It is notable that its detection required functional analysis of monocytes, and the abnormality could not readily have been predicted by genomic investigations.

The increased expression of CXCL10 by BS monocytes was only observed at 2 hours and after, indicating that it relates to more efficient new protein synthesis rather than increased release from intracellular stores. This is consistent with previous reports that CXCL10 is not stored intracellularly, but is rapidly secreted following synthesis [21]. By performing sucrose density centrifugation, we found that there was no difference between BS and HC in the proportion of CXCL10 mRNA in free versus polysome fractions. This suggests that the reason for the increased CXCL10 protein expression is not due to differential translational initiation, but more likely due to more efficient translation within polysomes. We hypothesise that this is due to reduced presence of a regulatory microRNA species normally suppressing CXCL10 mRNA translation [22, 23], but this obviously requires further study.

To investigate the clinical relevance of our findings we correlated CXCL10 expression with disease activity scores (DAS) and clinical characteristics. There was a significant difference between high DAS and other groups when results were plotted as protein to mRNA ratio. Additionally, we were interested to find that CXCL10 protein expression correlated most strongly with neurological or ocular manifestations. This is in keeping with high CXCL10 levels previously observed in such patients [12, 24].

Our study has some limitations. First, all patient groups consisted of individuals taking a wide variety of medications. However, if medications had accounted for the observations, we might have seen a suppressed rather than exaggerated response in patients compared to non-medicated healthy controls. Furthermore, patients with RA and SLE were on as much medication as those with BS, if not more. Secondly, our patient numbers were not powered to find statistical significance in correlations with DAS or clinical subtypes and it would be interesting to undertake a larger study to properly address this question. Thirdly, we did not include a HC group in our comparison of BS with RA and SLE, and so we are unable to state whether or not CXCL10 expression by monocytes is normal in these disease control groups.

To our knowledge, this approach to investigating cytokine protein synthesis has not been undertaken in an inflammatory disease before. Our findings show an ‘uncoupling’ in the mRNA to protein ratio in BS, and highlight CXCL10 as an affected chemokine. It will be interesting to determine what other

genes are similarly overexpressed at the protein synthesis level in BS, and to find out whether they collectively contribute to the exaggerated inflammatory response that characterises this disorder.

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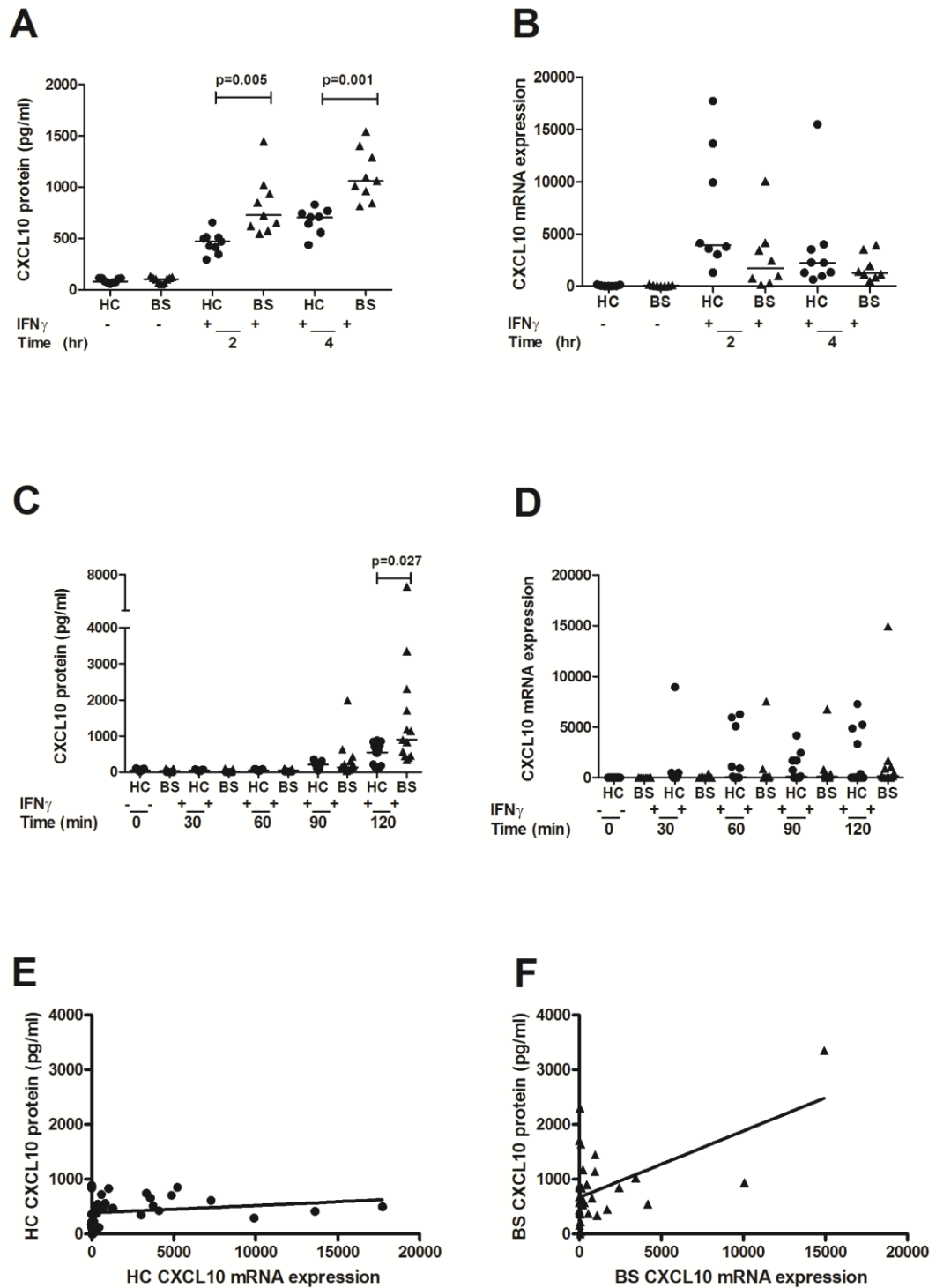


Figure 1. Behçet's Syndrome monocytes overexpress CXCL10 protein but not mRNA following IFN γ . (A,B) Monocytes from healthy controls (HC) and Behçet's Syndrome subjects (BS) (cohort 1) were stimulated with IFN γ (20ng/ml): (A) CXCL10 protein secretion and (B) CXCL10 mRNA after 2 and 4 hours. Significantly more CXCL10 protein was produced at both 2 hours ($p < 0.005$) and 4 hours ($p < 0.001$) by BS monocytes but there was no difference in CXCL10 mRNA between the two groups.

(C, D) The experiment was repeated using cohort 2 BS and HC, with measurements at earlier time-points over 2 hours. A significant difference was again observed at the 2 hour time point in CXCL10 protein levels ($p = 0.027$) but not in CXCL10 mRNA. (E, F) CXCL10 protein production plotted against mRNA using the 2 hour time-points in both experiments: (E) HC and (F) BS. The slopes are significantly different ($p < 0.001$).

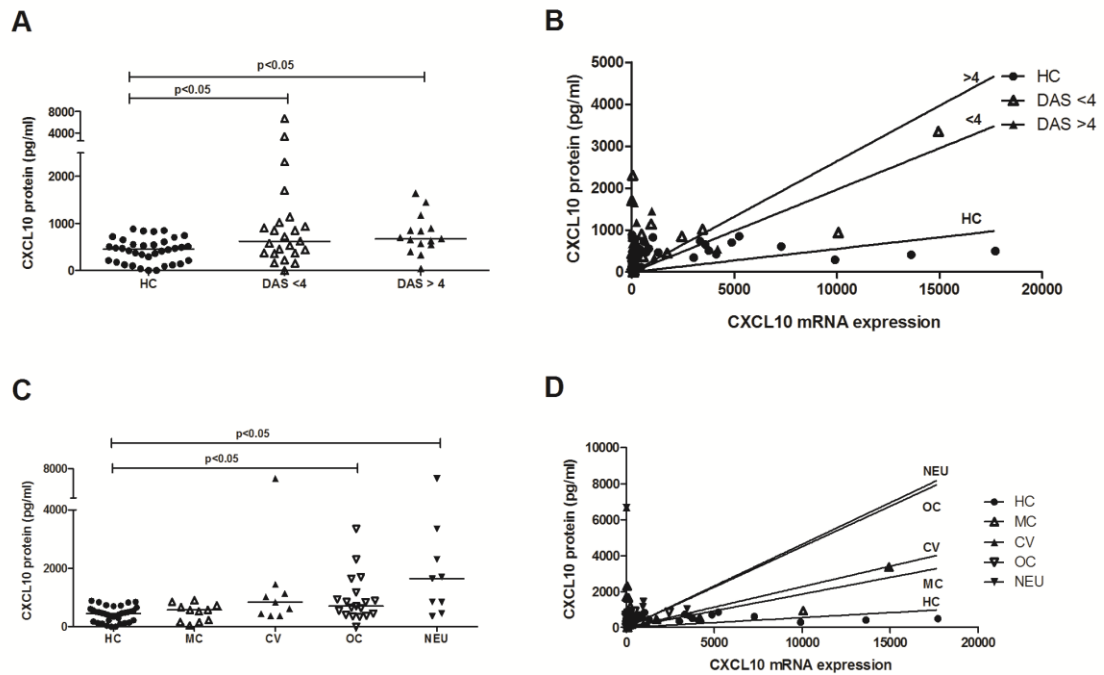


Figure 2. Relation between monocyte CXCL10 expression and BS disease activity CXCL10 data from BS monocytes stimulated with IFN γ (20 ng/ml) for 2 hours were assessed in relation to disease activity score (DAS). (A) Shows that CXCL10 protein expression was similarly increased in those with low versus high DAS, whereas in (B) the disparity between protein and mRNA expression is greater in patients with high DAS ($p < 0.05$). (C, D) CXCL10 expression was related to clinical manifestations. There was a significant difference between CXCL10 protein in HC versus ocular (OC) ($p < 0.05$) and neurological (NEU) ($p < 0.05$) BS samples, and the same trend was seen when CXCL10 protein to mRNA levels were plotted. In contrast no significant difference was observed between CXCL10 expression in HC and mucocutaneous (MC) or cardiovascular (CV) BS samples.

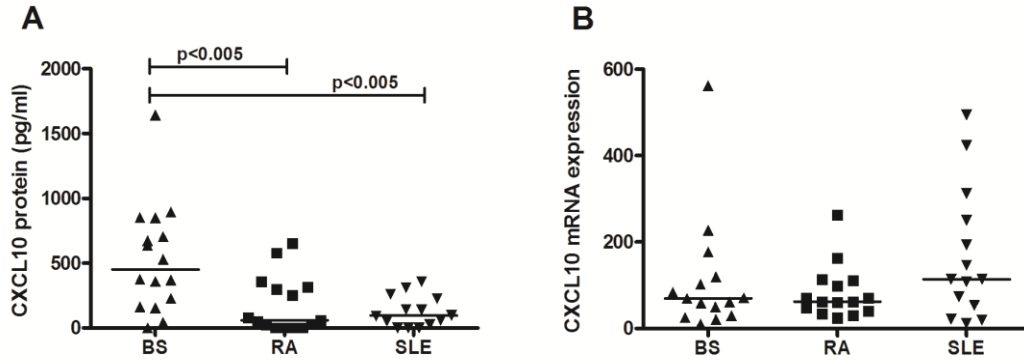


Figure 3. Behçet's Syndrome monocytes express more CXCL10 protein than those from patients with RA and SLE. Monocytes from patients with BS (cohort 3), RA or SLE were stimulated with IFN γ (20ng/ml): **(A)** CXCL10 protein, and **(B)** CXCL10 mRNA. CXCL10 protein expression was greater in BS monocytes than in those from RA ($p < 0.005$) or SLE ($p < 0.005$) subjects. There were no significant differences in mRNA levels.

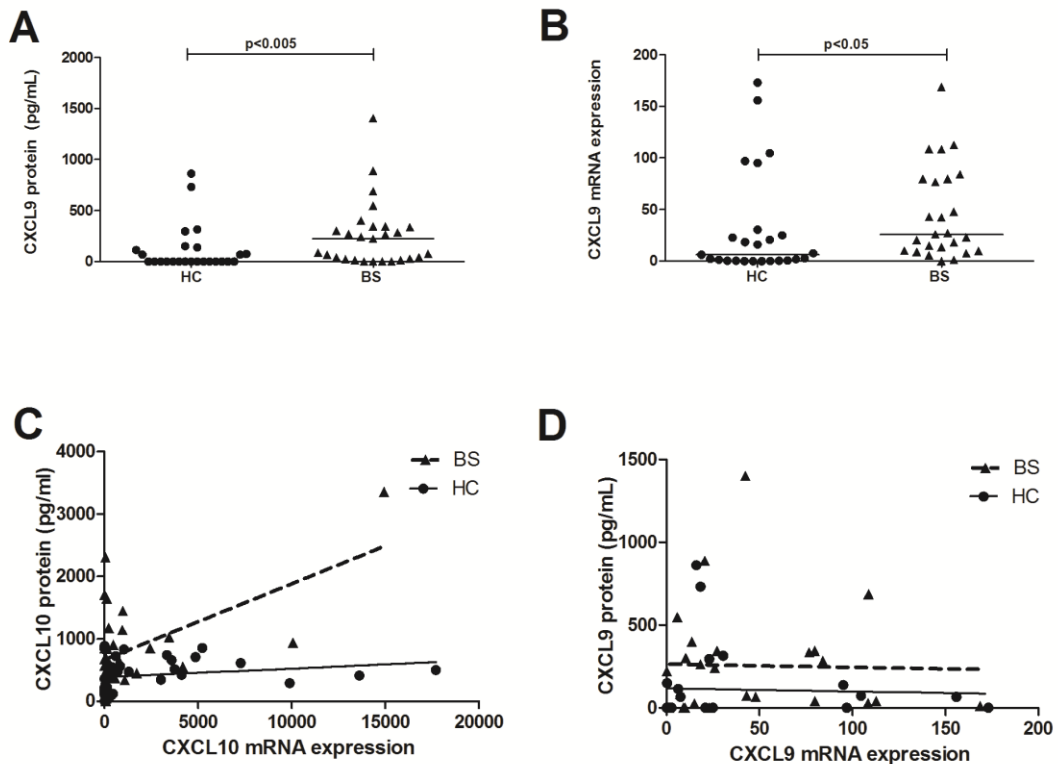


Figure 4. Expression of CXCL9 protein and mRNA from IFN γ -stimulated monocytes. Monocytes from patients with BS or HC were stimulated with IFN γ (20ng/ml): **(A)** CXCL9 protein in BS was significantly greater than HC ($p < 0.005$), **(B)** CXCL9 mRNA expression was also significantly greater ($p < 0.05$). **(C, D)** CXCL9 **(C)** and CXCL10 **(D)** protein production plotted against mRNA. For CXCL9, the slopes for BS and HC were similar, and the slope for BS CXCL10 was significantly greater than for CXCL9 (BS dashed lines, HC solid lines; $p < 0.05$).

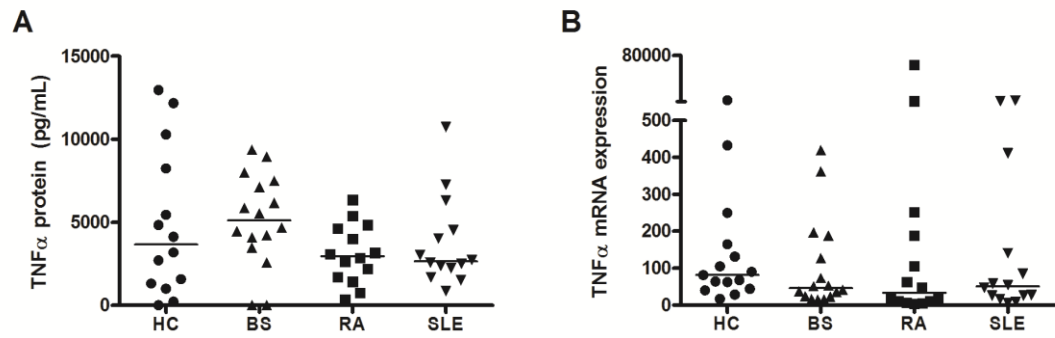


Figure 5. Expression of TNFα in monocytes stimulated with LPS. Monocytes from patients with BS, RA or SLE or from HC were stimulated with LPS (20ng/ml) for two hours. There were no differences in either TNFα protein secretion or mRNA expression between groups.

Supplementary table 1: Cohort demographics

Cohort 1 demographics	Healthy Controls		Behçet's Syndrome	
Cohort size	9		9	
Median age (Interquartile range)	34	(32-36)	35	(26-53)
Male sex (%)	6	66.7%	5	55.6%
Clinical Characteristics				
Mucocutaneous	-		9	100%
Erythema nodosum			5	56%
Pathergy			6	67%
Musculoskeletal - arthralgias	-		8	89%
Arthritis			1	11%
Cardiovascular	-		3	33%
Aneurysm			2	22%
Thrombosis			2	22%
Ocular	-		3	33%
Posterior/panuveitis			2	22%
Gastrointestinal inflammation	-		1	11%
Neurological	-		2	22%
Disease Activity Score *	-		3	
Current medications				
Colchicine	-		2	22%
Azathioprine	-		1	11%
Mycophenolate	-		1	11%
Anti-TNF therapy	-		5	56%
Cyclosporine	-		0	
Methotrexate	-		2	22%
Steroids **	-		2	(5.5mg)

* Behçet's Syndrome: Leeds Behçet's Disease Activity Form (2002), score 0-12 (>4 indicates active disease)

** Number of subjects taking steroids, and the median prednisolone dose taken by those subjects

Supplementary table 2: Cohort demographics

Cohort 2 demographics	Healthy Controls		Behçet's Syndrome	
Cohort size	13		13	
Median age (Interquartile range)	34	(24-39)	46	(27-58)
Male sex (%)	7	53.8%	5	38.5%
Clinical Characteristics				
Mucocutaneous	-		13	100%
Erythema nodosum			6	46%
Pathergy			10	77%
Musculoskeletal - arthralgias	-		13	100%
Arthritis			9	69%
Cardiovascular	-		1	8%
Aneurysm				
Thrombosis			1	8%
Ocular	-		9	69%
Posterior/panuveitis			5	38%
Gastrointestinal inflammation	-		3	23%
Neurological	-		2	15%
Disease Activity Score *	-		3	
Current medications				
Colchicine	-		1	8%
Azathioprine	-		4	31%
Mycophenolate	-		0	
Anti-TNF therapy	-		4	31%
Cyclosporine	-		0	
Methotrexate	-		0	
Steroids **	-		7	(6mg)

* Behçet's Syndrome: Leeds Behçet's Disease Activity Form (2002), score 0-12 (>4 indicates active disease)

** Number of subjects taking steroids, and the median prednisolone dose taken by those subjects

Supplementary table 3: Cohort demographics

Cohort 3 demographics	BS		RA		SLE	
Cohort size	16		15		15	
Median age (IQR)	46	(37-64)	59	(52-67)	52	(36-53)
Male sex (%)	6	37.5%	2	13.3%	3	20.0%
Clinical Characteristics						
Mucocutaneous	16	100%	0		7	47%
Erythema nodosum	7	44%	0		0	
Pathergy	14	88%	0		0	
Arthralgias	14	88%	15	100%	14	93%
Arthritis	7	44%	15	100%	4	27%
Cardiovascular	3	19%	0		3	20%
Aneurysm	2	13%	0		0	
Thrombosis	1	6%	0		3	20%
Ocular	8	50%	0		1	7%
Posterior/panuveitis	5	31%	0		0	
Gastrointestinal	4	25%	0		1	7%
Neurological	3	19%	0		2	13%
Renal	0		0		11	73%
Disease Activity Score *	2.6		4.3		5.2	
Current medications						
Colchicine	3	19%	0		0	
Azathioprine	1	6%	0		1	7%
Mycophenolate	5	31%	0		9	60%
Anti-TNF therapy	5	31%	3	20%	0	
Cyclosporine	1	6%	0		0	
Methotrexate	2	13%	11	73%	0	
Tacrolimus	0		0		3	20%
Plaquenil	0		4	27%	9	60%
Tocilizumab	0		1	7%	0	
Rituximab	0		0		1	7%
Steroids **	8	(10mg)	6	(5mg)	6	(5mg)

HC=Healthy Controls, BS= Behçet's Syndrome, RA= Rheumatoid Arthritis, SLE= Systemic Lupus Erythematosus

* Behçet's Syndrome: Leeds Behçet's Disease Activity Form (2002),score 0-12 (>4 indicates active disease),

SLE: Sledai score 0-105 (>6 indicates active disease), RA: Disease Activity Score 1-10 (>5.1 indicates active disease). IQR=interquartile range.

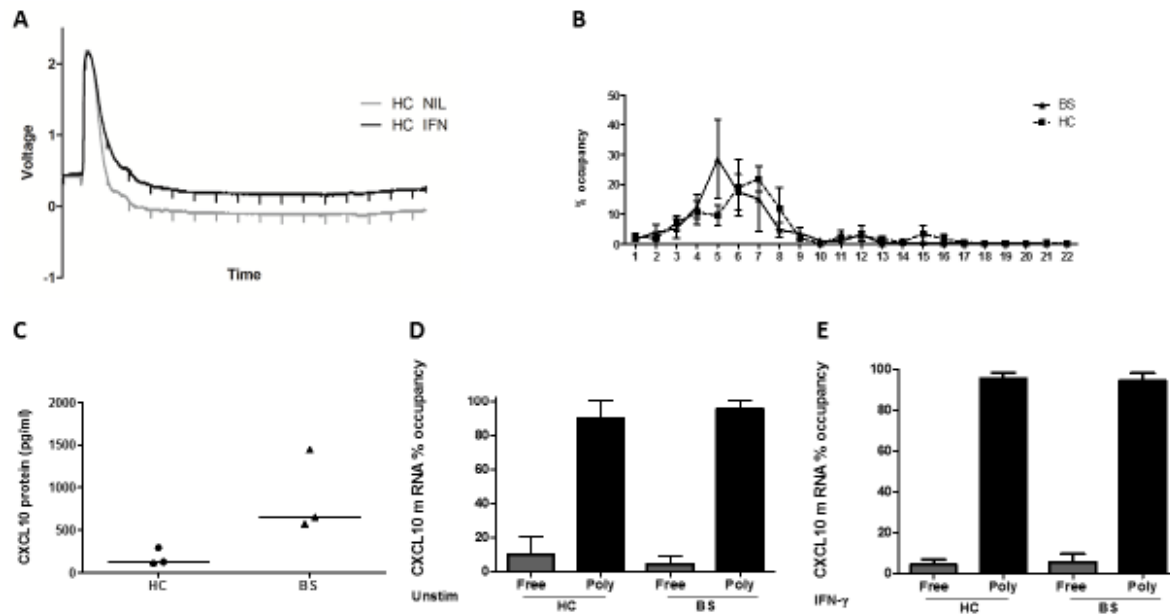
Supplementary table 4: Cohort demographics

Pooled Cohort demographics	Healthy Controls		Behçet's Syndrome	
Cohort size	25		27	
Median age (Interquartile range)	35	(30-38)	45	(33-51)
Male sex (%)	13	52.0%	12	44.4%
Clinical Characteristics				
Mucocutaneous	-		27	100%
Erythema nodosum			12	44%
Pathergy			19	70%
Musculoskeletal - arthralgias	-		24	89%
Arthritis			13	48%
Cardiovascular	-		6	22%
Aneurysm			3	11%
Thrombosis			4	15%
Ocular	-		13	48%
Posterior/panuveitis	-		9	33%
Gastrointestinal inflammation	-		6	22%
Neurological	-		9	33%
Disease Activity Score *	-		3	
Current medications				
Colchicine	-		3	11%
Azathioprine	-		6	22%
Mycophenolate	-		5	19%
Anti-TNF therapy	-		10	37%
Cyclosporine	-		1	4%
Methotrexate	-		4	15%
Steroids **	-		15	(9mg)

* Behçet's Syndrome: Leeds Behçet's Disease Activity Form (2002), score 0-12 (>4 indicates active disease)

** Number of subjects taking steroids, and the median prednisolone dose taken by those subjects

Supplementary figure 1: Polysomal association study of CXCL10 mRNA



Supplementary Figure 1. CXCL10 mRNA shows a similar association with polysomes in BS and HC despite differences in protein production: **(A)** A representative voltage tracing of polysome fractions within a sucrose density gradient passing through under laser over time. The far left of the trace represents free total RNA and later segments represent total RNA associated with ribosomal subunits and polysomes. **(B)** The percentage occupancy of CXCL10 mRNA. CD14⁺ monocytes from BS (n=3) and HC (n=3) stimulated for 2 hours with IFN γ (20 μ L/mL). Samples 1&2 represent free RNA. There was no statistically significant difference between HC and BS. **(C)** CXCL10 protein expression in HC and BS subjects (n=3). **(D-E)** Comparison of free and polysomal mRNA in (a) unstimulated or (b) stimulated monocytes. There was no statistically significant difference between HC and BS, supporting the finding that despite different quantities of protein being produced by the two groups, there was no change in polysomal association between the groups.