EXTENDED REPORT

Aberrant TNF secretion by whole blood in healthy subjects with a history of reactive arthritis: time course in adherent and non-adherent cultures

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Background: The pathogenesis of reactive arthritis (ReA) apparently involves aberrations in innate immune functions such as monocyte tumour necrosis factor (TNF) generation.

Objective: To investigate TNF production in healthy subjects with previous yersinia triggered reactive arthritis.

Methods: The study comprised HLA-B27 positive subjects with previous reactive arthritis (B27+ReA+), and B27+ReA– and B27–ReA– subjects (n=15 each). Whole blood TNF production was induced by lipopolysaccharide (LPS), which binds to CD14/TLR4 on the monocyte surface, or by a combination of phorbol 12-myristate 13-acetate (PMA) and Ca^{2+} ionophore A23187, which activates monocytes independently of cell surface receptors. To further evaluate the possible role of adhesion mediated signalling on TNF production, blood samples were incubated in adherent or non-adherent conditions. TNF levels in culture supernatants were measured using an automated immunoassay analyser. The *CD14*(–159)C/T genotype was determined by a cycle minisequencing method.

Results: B27+ReA+ supernatants had higher TNF levels than B27+ReA– supernatants in PMA/A23187 wells in two hour (p=0.004) and four hour cultures (p=0.001). Rapid initial TNF release took place in adherent but not in non-adherent conditions. This adhesion associated difference was greater in the B27+ReA+ group than in the B27+ReA– or B27–ReA– group in response to PMA/A23187 (p values <0.001), and greater in the B27+ReA+ group than in the B27–ReA– group in response to LPS (p=0.021). *CD14*(–159)T was associated allele dose dependently with an increase in the LPS induced TNF secretion allele (p=0.030).

Conclusions: Subjects who have recovered from yersinia arthritis show enhanced TNF production, which may be regulated at the level of monocyte adhesion.

Reactive arthritis (ReA) is a sterile joint inflammation, complicating an extra-articular infection, most commonly gram negative chlamydia urethritis, or yersinia, salmonella, campylobacter, or shigella enteritis, especially among HLA-B27 positive individuals.¹ The pathogenesis of reactive arthritis is unclear but may involve aberrations in the adaptive immune response—for instance, presentation of arthritogenic self peptides to CD8⁺ T cells by HLA-B27, or formation of HLA-B27 dimers recognised by CD4⁺ T cells.² However, evidence has accumulated to show that innate immune reactions, which represent early stages of the host defence against the arthritogenic microbes,³ may also play a role in the development of reactive arthritis. Innate immune system receptors, such as CD14/Toll-like receptor complexes on monocytes and macrophages, recognise lipopolysaccharides (LPS) and other pathogen associated molecular patterns. Binding of LPS activates intracellular signalling, which leads to activation of transcription factors and the generation of immunoregulatory cytokines, such as tumour necrosis factor (TNF). Low TNF secretion by blood mononuclear cells may be related to chronic reactive arthritis,⁴ and, in general, may interfere with eradication of bacterial infection in its early stages.^{5–7}

Several factors may affect monocyte TNF production. First, circulating monocytes are heterogeneous in terms of function and maturity.⁸ In healthy subjects, most monocytes are strongly positive for CD14 and negative for the Fcγ-III receptor CD16. However, in patients with generalised inflammation, up to 50% of monocytes express CD14 weakly and co-express CD16. These CD14⁺CD16⁺ monocytes show

enhanced TNF production capacity⁹ and are major TNF producers in the blood.¹⁰ Second, signalling through the CD11b/CD18 complex—a β_2 integrin, which mediates monocyte adhesion to endothelium and non-endothelial surfaces^{11 12}—may modify TNF production and monocyte survival.^{13 14} Finally, the *CD14*(–159)C→T polymorphism has been reported to increase monocyte CD14 expression.^{15 16}

Because leucocyte migration from the circulation into the joint requires leucocyte adherence to vascular endothelium and extracellular matrix proteins, we reasoned that leucocyte activation might be regulated at the level of CD11b/CD18 complex signalling. Furthermore, in patients with systemic inflammation, phagocytes are activated in the blood but do not necessarily adhere to the endothelium.¹⁷ To mimic these adherent and non-adherent *in vivo* conditions we used concurrently adherent cell culture wells and non-adherent tubes, respectively. Using this setting we studied first, whether the induction of TNF release is abnormal in healthy subjects with a history of yersinia triggered reactive arthritis, and second, the effect of cell adherence on TNF production. Monocytes in whole blood samples were stimulated by a combination of Ca^{2+} ionophore A23187 and phorbol 12-myristate 13-acetate (PMA), cell surface receptor independent activators of the protein kinase C pathway, or by lipopolysaccharide (LPS).

Abbreviations: ABC, antibody binding capacity; LPS, lipopolysaccharide; PE, phycoerythrin; PMA, phorbol 12-myristate 13-acetate; ReA, reactive arthritis; RPMI, Roswell Park Memorial Institute medium; TNF, tumour necrosis factor

Table 1 Subjects with previous yersinia triggered reactive arthritis

Characteristics at the time of acute disease and at follow up visit	
Sex (women/men)	10/5
Age at study (years)	57 (39 to 71)
<i>Acute arthritis</i>	
Age at onset (years)	31 (15 to 45)
Patients positive for the HLA-B27 antigen	15
Inflamed joints	6 (2 to 15)
Patients with extra-articular symptoms	11
Urethritis	9
Iritis	4
Conjunctivitis	1
Erythema nodosum	1
Duration (months)	3 (1 to 8)
White blood cell count $\times 10^{-9}/l^*$	10.8 (5.6 to 15.2)
Erythrocyte sedimentation rate (mm/h) [†]	91 (16 to 140)
Lowest Hb (g/l) [‡]	
Women	116 (99 to 139)
Men	118 (103 to 140)
Reciprocal agglutination titre for <i>Yersinia enterocolitica</i>	
O:3 or O:9s	2560 (640 to 20 480)
<i>Follow up visit</i>	
Period since diagnosis of acute arthritis (years)	18 (15 to 25)
Fully recovered patients	15

Values are n or median (range).

*Normal range 4 to 10.

[†]Normal range 1 to 10.

[‡]Normal range 125 to 160 in women, 135 to 180 in men.

§Normal <160.

METHODS

Subjects

Forty five native Finnish volunteers were recruited. Fifteen HLA-B27 positive subjects had had yersinia triggered reactive arthritis with complete recovery (B27+ReA+ group, table 1). They had participated in clinical studies of acute reactive arthritis¹⁸ and disease outcome.¹⁹ Yersinia antibody titres were measured after recovery, during follow up for a mean of 8.5 years after the acute disease, and in all patients they had decreased to normal levels. Fifteen of the healthy reference subjects (10 women, five men, median age 47 years, range 32 to 64) were HLA-B27 positive (B27+ReA− group), and 15 (10 women, five men, median age 53, range 43 to 58) were HLA-B27 negative (B27−ReA− group). Individuals on anti-inflammatory drugs or with signs of infection at the time of donation were excluded from the study. Lack of subclinical systemic inflammation was verified using a high sensitivity C reactive protein assay: median serum C reactive protein concentration (range) was 0.75 µg/ml (0.30 to 5.80) for the B27+ReA+ group, 0.80 µg/ml (0.20 to 8.40) for the B27+ReA− group, and 0.50 µg/ml (0.20 to 1.80) for the B27−ReA− group. The subjects gave their informed consent.

Reagents

The following reagents were used: pyrogen-free heparin (Lövens, Ballerup, Denmark); Dulbecco's phosphate buffered saline (PBS) and RPMI 1640 medium (both from Life Technologies, Paisley, UK); *Escherichia coli* O111:B4 lipopolysaccharide (LPS); phorbol 12-myristate 13-acetate (PMA) and Ca^{2+} ionophore A23187 (all from Sigma, St Louis, Missouri, USA); TNF sample diluent (Diagnostic Products, Los Angeles, California, USA); sterile AB serum (Finnish Red Cross, Helsinki, Finland); fluorescein isothiocyanate (FITC) conjugate of mouse anti-CD11b mAb (IgG1, clone BEAR 1), phycoerythrin-CY5 (PC5) conjugate of anti-CD16 mAb (IgG1, clone 3G8), and PC5 conjugate of irrelevant mouse mAb IgG1 (clone 679.1Mc7) (all from Immunotech, Marseille, France);

R-phycoerythrin (RPE) conjugate of anti-CD14 mAb (IgG2a, clone TÜK4) and RPE conjugate of irrelevant mAb (IgG2a, clone DAK-GO5) (both from Dako, Glostrup, Denmark); FACS lysing solution and QuantiBRITE phycoerythrin (PE) standards (Becton Dickinson, San José, California, USA); dNTPs (Pharmacia, Uppsala, Sweden); AmpliTaq Gold DNA polymerase and polymerase chain reaction (PCR) buffer (Perkin Elmer, Boston, Massachusetts, USA); shrimp alkaline phosphatase and exonuclease I (USB, Cleveland, Ohio, USA); DyNAzyme DNA polymerase II and DyNAzyme buffer (Finnzymes, Espoo, Finland); ³H labelled dCTP (specific activity 53 Ci/mmol) and dTTP (96 Ci/mmol) (Amersham Life Sciences, Buckinghamshire, UK); and primers (Sigma-Genosys, Cambridgeshire, UK).

Blood samples

Three venous blood samples were obtained from each subject. The first was collected into a Falcon polypropylene tube (Becton Dickinson, Lincoln Park, New Jersey, USA) containing heparin 10 IU/ml blood, and placed in an ice water bath. Twelve 100 µl aliquots were used immediately for the whole blood culture assay, and two 25 µl aliquots for flow cytometry. The second sample was collected into an EDTA(K₃) tube (Vacutainer No 367657, Becton Dickinson) aliquoted into Cryo tubes (Greiner GmbH, Solingen, Germany), and stored at −70°C for nucleic acid analysis. The third was collected into a EDTA(K₃) tube for a differential cell count.

Methods

Experimental design

In preliminary experiments, TNF dose responses to LPS and combinations of PMA and A23187 were tested on whole blood samples diluted 1:10 in RPMI 1640. LPS at 1 µg/ml, and PMA plus A23187 both at 5 µM were found as convenient and comparable stimuli and chosen for the actual experiments.

Preliminary studies showed that TNF levels reached the plateau by four hours of incubation. This agrees with previous studies indicating that in whole blood assay, TNF mRNA is induced in response to LPS in one to two hours²⁰ and the initial TNF levels in the culture supernatants peak at two to six hours.^{21–24} Later waves of cytokine production may occur, dependent on the cytokines released within the first one to four hours of incubation.²⁵ We decided to have two time points, at two and four hours, to observe the time course of LPS dependent or PMA/A23187 dependent TNF production.

LPS was from *Escherichia coli*, and not from *Yersinia enterocolitica*, to exclude the possibility that the patients have low levels of yersinia specific antibodies which can respond to the polysaccharide moiety of yersinia-LPS.²⁶ The proinflammatory properties of the lipid A moiety of LPS from *Yersinia enterocolitica* or *Escherichia coli* appear comparable.²⁷

Whole blood culture assay

Stock solutions of LPS (400 µg/ml PBS), PMA, and Ca^{2+} ionophore A23187 (both at 5 mM in 99.5% ethanol) were stored at −20°C. The 100 µl aliquots of heparinised blood were placed in polypropylene tubes or cell culture wells presupplemented with either 800 µl of RPMI 1640 and 100 µl of LPS (finally at 1 µg/ml), or with 900 µl RPMI, 1 µl of PMA, and 1 µl of A23187 (both finally at 5 µM), or with 900 µl of RPMI 1640 alone. Two identical sets of cultures, one for a two hour incubation and the other for a four hour incubation, were made in both test tubes and cell culture wells, and incubated at +37°C in 5% CO₂. The culture supernatants were snap frozen and stored at −70°C. TNF levels were determined

Figure 1 Tumour necrosis factor (TNF) in whole blood culture supernatants of HLA-B27 negative subjects without reactive arthritis ($n = 15$). TNF levels after incubation in bacterial lipopolysaccharide $1 \mu\text{g/ml}$, (A) in cell culture wells, (B) in test tubes; TNF levels after incubation in the combination of phorbol 12-myristate 13-acetate $5 \mu\text{M}$ and Ca^{2+} ionophore A23187 $5 \mu\text{M}$, (C) in cell culture wells and (D) in test tubes.

within two months. The cell pellet was suspended in $50 \mu\text{l}$ of ice cold AB serum and used for flow cytometry.

Cell surface staining of leucocytes

The aliquots of heparinised blood and cell pellet in AB serum were stained with pretitrated amounts of FITC-anti-CD11b, RPE-anti-CD14, and PC5-anti-CD16, as described previously.²⁸ Flow cytometric data acquisition was within four hours.

Flow cytometry

A FACSort flow cytometer (Becton Dickinson) and CellQuest software were used. QuantiBRITE PE bead standards (Becton Dickinson) were run weekly.

The data were acquired and analysed using QuantiCalc software (Verity Software House, Topsham, Maine, USA) as described previously.²⁸ According to the manufacturer, the ratio of fluorochrome to protein is 1. Consequently, the number of PE molecules bound is equal to the anti-CD14 antibody binding capacity (ABC) of the cell.

DNA isolation and CD14 genotyping

The whole blood samples were thawed and DNA was isolated using QIAamp DNA blood midi kit (Qiagen, Hilden, Germany).

The *CD14*(-159)C/T polymorphism was genotyped using a cycle minisequencing technique as described previously.²⁹

Primers flanking the *CD14*-159 polymorphism were 5'-CCTGGAAATATTGCAATGAAGGATG-3' and 5'-CCAGGAGA CACAGAACCCTAGATGC-3'.

The PCR products were diluted 1:2 in DyNAzyme buffer and transferred onto new 96-well PCR plates (Advanced Biotechnologies, Columbia, Maryland, USA) containing reagents for the cycle minisequencing reaction. The primer construct was 5'-biotin-GCAGAATCCTTCCTGTTACGG-3', and the nucleotides were ^3H labelled dCTP and dTTP.

After cycling, the contents of the wells were transferred into plate wells with covalently attached streptavidin (SA cov. Scintiplat 1450-511, Wallac, Turku, Finland). After binding, counts per minute (CPM) of the bound primers were calculated using a beta counter (Microbeta 1450, Wallac). The ratio of the two signals was used for genotyping.

Determination of TNF

The culture supernatants were thawed and diluted 1:5 in TNF sample diluent. TNF levels were measured using Immulite, a chemiluminescent immunoassay system (Diagnostic Products). The detection limit was 4 pg/ml . The intra-assay variation was 9% and the interassay variation 12%. The results were corrected for dilution and background. To evaluate the time course of TNF production, the kinetic ratio was calculated (TNF level at four hours divided by the TNF level at two hours).

Figure 2 Scattergrams of tumour necrosis factor (TNF) levels in whole blood culture supernatants. TNF levels after incubation in the combination of phorbol 12-myristate 13-acetate (PMA) and A23187, (A) in cell culture wells for two hours, (B) in cell culture wells for four hours, and (C) in test tubes, expressed as kinetic ratios. Horizontal lines indicate the medians. Note the logarithmic scale in (A) and (B), and the cutting and scaling of the vertical axis in (C). For explanation of the subject groups and details of the assay conditions see table 2.

Differential cell count

Cell counts were made using an automatic analyser.

Data analysis

To characterise the trends of the whole blood assay, the data obtained with B27-ReA- group were analysed. B27+ReA- group was excluded because of the possibility of clinically

silent sacroiliitis related to B27 positivity.³⁰ Two dependent samples were compared by the Wilcoxon signed ranks test. Second, differences between the three subject groups were evaluated using the Kruskal-Wallis test. Post hoc analyses were done by the Mann-Whitney U test. The effect of *CD14*(-159)T allele dose on TNF production was tested using the Jonckheere-Terpstra test for linear trend, and T allele prevalence in the groups by Fisher's exact test. The analyses were done using SPSS version 10.07 (SPSS, Chicago, Illinois, USA). A probability (p) value less than 0.05 was considered significant.

RESULTS

TNF production of 15 healthy HLA-B27 negative subjects

LPS

Results depicted in fig 1A and table 2 show that in cell culture wells supporting cell adhesion, LPS-induced TNF levels at four hours did not differ from the respective values at two hours, resulting in a median kinetic ratio of 1.0. In test tubes (fig 1B, table 2), which do not support cell adhesion, TNF production was slower, the TNF levels being significantly higher at four hours than at two hours ($p = 0.001$), and the median kinetic ratio was 4.5.

In a comparison of cell culture wells with test tubes, the former showed higher TNF levels at two hours ($p = 0.005$), indicating that adhesion enhanced rapid LPS stimulated TNF release. However, in non-adherent conditions the cells continued producing TNF after the initial release, and TNF levels at four hours ($p = 0.001$) and the kinetic ratio were higher ($p = 0.001$).

Combination of PMA and Ca^{2+} ionophore A23187

In cell cultures stimulated with PMA and A23187 (fig 1C and 1D, table 2), both in adherent and non-adherent conditions, TNF levels at four hours were higher than at two hours, resulting in a median kinetic ratio of 1.7.

In a comparison of cell culture wells with test tubes, the former had higher TNF levels at two hours ($p = 0.015$), indicating again that adhesion enhanced early stage TNF release, whereas four hour levels were comparable, indicating that adhesion did not affect maximum LPS receptor independent TNF production.

TNF production of HLA-B27 positive subjects with previous yersinia arthritis

Time course of TNF production

The median monocyte count of the blood samples was $0.4 \times 10^9/\text{l}$ in each subject group. In adherent cell cultures treated with PMA and A23187 (table 2, fig 2), the B27+ReA+ group had higher TNF levels than the B27+ReA- group at both two hours ($p = 0.004$) and four hours ($p = 0.001$).

In non-adherent cultures containing PMA and A23187, median TNF levels did not differ between the groups, but the kinetic ratio was higher in the B27+ReA+ group than in the B27+ReA- group ($p = 0.002$) or the B27-ReA- group ($p = 0.008$) (table 2, fig 2).

Effect of adhesion on TNF production

To explore whether the effect of adhesion on TNF release differed between the B27+ReA+ group and the reference groups, the difference between TNF levels in cell culture well and respective test tube was calculated. Median difference between the groups was significant in two hour PMA/A23187 cultures ($p < 0.001$, Kruskal-Wallis test), and post hoc analysis showed that the difference was significantly higher in the B27+ReA+ group than in the B27+ReA- group or the B27-ReA- group (p values < 0.001 , Mann-Whitney U test).

Table 2 Tumour necrosis factor levels in whole blood culture supernatants

Stimulus	TNF level (pg/ml)		Kruskal-Wallis test	Kinetic ratio*		Kruskal-Wallis test
	B27+ReA+	B27+ReA−		B27+ReA+	B27+ReA−	
Adherent culture wells						
LPS†						
2 hours	426.5 (165.5 to 822.0)	257.0 (126.0 to 685.0)	297.0 (138.5 to 1440.0)	1.0 (0.4 to 1.9)	1.0 (0.6 to 2.5)	p=0.912
4 hours	334.0 (164.0 to 1185.0)	335.5 (151.0 to 880.0)	278.5 (175.0 to 1050.5)			p=0.395
PMA‡ and A23187§						
2 hours	1122.5 (644.5 to 3535.0)	660.0 (328.0 to 2290.0)	745.0 (366.5 to 1810.0)	1.7 (1.0 to 5.8)	1.7 (0.8 to 2.7)	p=0.017†
4 hours	1780.0 (1070.0 to 20430.0)	1115.0 (729.5 to 1889.0)	1210.0 (664.0 to 3125.0)			p=0.005**
Non-adherent test tubes						
LPS†						
2 hours	196.0 (69.0 to 540.0)	183.5 (59.0 to 490.0)	170.0 (73.0 to 895.0)	5.3 (2.9 to 14.4)	4.5 (3.1 to 12.9)	p=0.749
4 hours	1134.5 (575.0 to 2503.5)	855.0 (457.5 to 2039.5)	765.0 (91.0 to 3510.0)			p=0.316
PMA‡ and A23187§						
2 hours	480.0 (24.0 to 1365.0)	500.0 (100.5 to 2235.0)	615.0 (222.0 to 1150.0)	3.0 (0.9 to 46.4)	1.5 (0.2 to 3.0)	p=0.491
4 hours	1430.0 (347.0 to 4690.0)	725.0 (110.0 to 2790.0)	1200.0 (0 to 3270.0)			p=0.066
						p=0.003††, ‡‡

Comparison of HLA-B27 positive subjects with previous reactive arthritis (B27+ReA+, n = 15), and HLA-B27 positive (B27+ReA+, n = 15) and negative (B27-ReA-, n = 15) reference subjects. Values are median (range).

*TNF level at 4 hours/TNF level at 2 hours.

†Bacterial lipopolysaccharide 1 µg/ml.

‡Phorbol 12-myristate 13-acetate 5 µM.

§Ca²⁺ ionophore A23187 5 µM.

Post hoc (Mann-Whitney U test): *p = 0.004, B27+ReA+ > B27+ReA-; **p = 0.001, B27+ReA+ > B27+ReA-; ††p = 0.002, B27+ReA+ > B27+ReA-; ‡‡p = 0.008, B27+ReA+ > B27-ReA-.

LPS, lipopolysaccharide; PMA, phorbol 12-myristate 13-acetate; TNF, tumour necrosis factor.

Figure 3 Tumour necrosis factor (TNF) production/100 000 monocytes in whole blood in relation to *CD14*(-159) genotype. Whole blood samples were incubated in bacterial lipopolysaccharide 1 µg/ml in test tubes for four hours.

In LPS stimulated conditions, the respective median difference in TNF levels after two hours differed between the groups ($p = 0.048$). In post hoc analysis these differences were greater in the B27+ReA+ group than in the B27-ReA- group ($p = 0.021$).

TNF production in relation to *CD14*(-159) genotype

TNF levels calculated per 10^5 *CD14* positive monocytes in whole blood culture increased in a T allele dose dependent manner in response to LPS in test tubes at four hours (fig 3); median (range) was 2317.0 (1500.0 to 3271.0) pg in the CC group, 2702.0 (1538.0 to 3734.0) pg in the CT group, and 3114.5 (1866.0 to 4107.0) pg in the TT group ($p = 0.030$, Jonckheere-Terpstra test).

TNF levels and kinetic ratios obtained with PMA plus A23187 were not associated with *CD14*(-159)C/T.

T allele prevalence was 36.7% in the B27+ReA+ group, 40.0% in the B27+ReA- group, and 56.7% in the B27-ReA- group, and did not differ significantly between the groups.

Monocyte *CD14* expression

There were no significant differences in median *CD14* fluorescence intensity of monocytes in whole blood samples between the B27+ReA+ group (median ABC 14 434, range 7629 to 17 342), the B27+ReA- group (14 295, 10 194 to 19 286), or the B27-ReA- group (13 232, 6926 to 20 046). Median proportion of *CD14*+*CD16*+ monocytes in whole blood was comparable in the B27+ReA+ (4.2%, 1.0% to 13.4%), B27+ReA- (4.3%, 0.6% to 6.8%), and B27-ReA- (2.5%, 0.5% to 8.8%) groups.

DISCUSSION

In adherent whole blood cultures, samples from HLA-B27 positive healthy subjects with previous yersinia arthritis released more TNF in response to direct activation of the protein kinase C pathway, stimulated by the combination of PMA and Ca^{2+} ionophore A23187, than samples of HLA-B27 positive reference subjects. In agreement with a previous study,³¹ the *CD14*(-159)T allele increased LPS induced TNF production in an allele dose dependent manner. However, the frequency of *CD14*(-159)T and the proportion of *CD14*+*CD16*+ monocytes in whole blood samples were comparable in the subject groups. Whereas these results do not explain the difference in TNF levels in PMA/A23187 wells between arthritis and reference subjects, adhesion may provide an explanation. When adherent and non-adherent

PMA/A23187 culture conditions were compared, the former increased the two hour TNF production more in the arthritis group than in the reference groups. Additionally, the kinetic ratio (TNF level at four hours/TNF level at two hours) of non-adherent PMA/A23187 cultures was higher in the arthritis group than in the reference groups. These results raise a question as to whether abnormal signalling upon adhesion plays a role in the enhanced TNF induction in the arthritis group. Of note, signalling through *CD11b*/*CD18*—the major molecule mediating monocyte adhesion to cell culture well surfaces^{11 12}—enhances TNF production¹³ and prolongs monocyte survival.¹⁴ Thus some aberrations in *CD11b*/*CD18* signalling may explain the increased TNF production.

Transferring the HLA-B27 gene to epithelial cells³² or monocytic cells³³ apparently increases cytokine generation in response to bacteria or bacterial LPS, suggesting a direct role of HLA-B27. In the present study, the enhanced TNF production capacity, revealed by PMA/A23187 stimulation as described above, was associated with the presence of previous arthritis not with HLA-B27. However, in LPS stimulated cultures, adherent conditions increased TNF levels more in the HLA-B27 positive arthritis group than in the HLA-B27 negative reference group but not the HLA-B27 positive reference group. This suggests that HLA-B27 positivity and enhanced LPS receptor mediated response may be independent but additive factors operating in the pathogenesis of reactive arthritis. It is of interest that low TNF secretion by mononuclear cells in response to phytohaemagglutinin may correlate with the chronicity of reactive arthritis.⁴ Thus it is possible that a vigorous TNF response of adhered phagocytes provokes clinical joint inflammation, but, on the other hand, helps to overcome the disease more rapidly.

A novel feature of our assay was that TNF production was measured concurrently in adherent and non-adherent cultures. After LPS induction, TNF production differed dramatically between these conditions. TNF levels remained stable in adherent cell culture wells after two hours, indicating only a rapid initial burst of TNF. In non-adherent conditions, TNF levels were lower than in wells at two hours but release continued thereafter. These findings suggest that adhesion mediated signals for TNF production are stimulatory in the early stage but not in the advanced stage of the response, or, in general, that the course of the response is more controlled in adhesive conditions. The more restrained TNF production of adhered phagocytes may be aimed at protecting endothelial cells from undue exposure to TNF in vivo. An intriguing possibility is that circulating monocytes of patients with systemic inflammation lack adhesion mediated inhibitory signals, and thus generate inappropriately large amounts of pro-inflammatory cytokines, which activate vascular endothelium. This would promote leucocyte adhesion and inflammation mediated end organ injury³⁴.

These preliminary results indicate that HLA-B27 positive healthy subjects with previous yersinia arthritis show enhanced TNF production, which may be regulated at the level of monocyte adhesion. Whether or not this innate immune aberration derives from abnormal *CD11b*/*CD18* signalling warrants further study.

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