8

Antioxidant status associated with inflammation in sarcoidosis: a potential role for antioxidants

AW Boots, M Drent, ELR Swennen, HJJ Moonen, A Bast, GRMM Haenen

Abstract

Background

Enhanced production of reactive oxygen species (ROS) is suggested to play a pivotal role in sarcoidosis. The high production of ROS is expected to reduce antioxidant levels. Since ROS can enhance inflammation, antioxidant supplementation might, also mitigate elevated inflammation. The aim of the present study is to determine both the antioxidant and inflammatory status in sarcoidosis. Furthermore, the potential of the antioxidant quercetin to mitigate inflammation will be assessed.

Methods

Non-smoking patients with sarcoidosis were enrolled as well as matched controls. Measurements included assessment of total plasma antioxidant capacity, vitamin C, uric acid, glutathione, basal and lipopolysaccharide (LPS)-induced levels of tumour necrosis factor alpha (TNFα), interleukin (IL)-8 and IL-10 and the effect of quercetin on these levels.

Results

Sarcoidosis patients displayed a significantly lower total plasma antioxidant capacity and decreased levels of the antioxidants vitamin C, uric acid and glutathione compared to their controls. Basal TNFα and IL-8 levels were significantly increased in the sarcoidosis group, whereas basal IL-10 levels were unaffected. Quercetin significantly decreased *ex vivo* LPS-induced TNFα- and IL-8 production in a concentration-dependent manner, but did not affect LPS-induced IL-10 production, in both the patient and control group. Interestingly, this quercetin effect was more pronounced in sarcoidosis patients.

Conclusions

The endogenous antioxidant defence is significantly reduced in sarcoidosis, suggesting that increased production of ROS is associated with the pathology of this disease. Moreover, the levels of the pro-inflammatory cytokines TNFα and IL-8 are significantly enhanced in sarcoidosis. The antioxidant quercetin significantly reduces the *ex vivo* TNFα and IL-8 production and this effect is more pronounced in sarcoidosis patients. This implies that these patients might benefit from antioxidant supplementation not only by empowering the relatively low protection against ROS but also by reducing inflammation.

Introduction

Sarcoidosis is an interstitial lung disease which incidence varies among different countries over the world. In Scandinavian countries the incidence is higher compared to more southern countries (1). In the Netherlands and Germany it is estimated that the incidence is approximately 20-25 per 100.000 inhabitants (1) .

Sarcoidosis is an antigen-driven, multisystem, granulomatous disorder of which the exact aetiology is unknown (2,3). Current evidence supports the concept that the pathogenesis of sarcoidosis involves a highly polarized T-helper 1 Th1 immune response to pathogenic tissue antigens or specific environmental factors (1). Granuloma formation is regulated by a complex interaction between Th lymphocytes and macrophages, in which cytokines such as tumour necrosis factor alpha (TNFα) play an important role (2).

Enhanced formation of reactive oxygen species (ROS) is suggested to play a pivotal role in the aetiology of sarcoidosis (4,5). However, little is known about the endogenous defence levels present in sarcoidosis, i.e. the antioxidant levels that can offer protection against ROS-mediated damage. This prompted us to determine the endogenous antioxidant levels present in sarcoidosis patients. It is expected that the high production of ROS that occurs in sarcoidosis will consume antioxidants, thereby reducing their levels. As a result, antioxidant supplementation might be beneficial in sarcoidosis treatment.

ROS are also capable of initiating and mediating inflammation in the lung (6,7). Besides enhanced ROS formation, inflammation plays a key-role in the occurrence and progression of sarcoidosis too (8,9). Conventional treatment is focused on attenuating granuloma formation with drugs that inhibit antigen presentation or with nonspecific anti-inflammatory agents such as glucocorticosteroids, methotrexate, or azathioprine (2). However, all these therapies fail to be completely efficacious (10,11). Strengthening the endogenous antioxidant defence with antioxidant supplementation, and thereby decreasing the levels of ROS production and ROS-mediated damage, might mitigate the elevated inflammation present.

The past few years, much attention has been given to the potential health-beneficial properties of flavonoids, natural occurring polyphenolic compounds, and to quercetin, the most commonly occurring flavonoid, in particular. Quercetin is an extremely powerful antioxidant (12) and therefore it is tempting to speculate that quercetin can exert positive effects in sarcoidosis.

The aim of the present study is to determine both the anti-oxidant and inflammatory status in sarcoidosis. As markers of the antioxidant status, the endogenous antioxidants gluthathione (GSH), vitamin C and uric acid are

measured as well as the total antioxidant capacity. As markers of the inflammatory status, the pro-inflammatory cytokines TNFα and interleukin (IL)-8 and the anti-inflammatory cytokine IL-10 are measured. Furthermore, the potential anti-inflammatory effects of antioxidants, exemplified with the flavonoid quercetin, will be assessed in sarcoidosis. To this extent, cytokine production will be evoked *ex vivo* by lipopolysaccharide (LPS), a patho-physiological relevant stimulator of monocytes, neutrophils and B lymphocytes (13-16).

Material and Methods

Chemicals

Quercetin and lipopolysaccharide (LPS, *E. coli* 0.26:B6) were purchased from Sigma Chemical Co. (St. Louis, USA). RPMI 1640 medium containing L-glutamine was obtained from *Gibco (UK)*. Human TNFα (7300 pg/ml), human IL-10 (4000 pg/ml) and human IL-8 (10 ng/ml) were acquired from CLB/Sanquin (Amsterdam, The Netherlands). All other chemicals were of analytical grade.

Methods

Participants

General information

All participating patients were recruited from the patients visiting the out-patient clinic of the university hospital Maastricht, a tertiary referral for the Netherlands. The experiment took place at the day they had to come in for a regular check-up. Based on food questionnaires, all patients had comparable dietary habits with an average daily intake of quercetin of approximately 15 mg (data not shown). None of the participants used any vitamin or food supplementation.

The Medical Ethical Committee of Maastricht University and the University Hospital Maastricht had approved the protocol before the beginning of the study. All participants were fully informed about the aim and details of the study and have given their written informed consent.

Sarcoidosis patients

Twenty non-smoking patients with symptomatic sarcoidosis (age 44±1) were enrolled in this study. Patients had been diagnosed with sarcoidosis based on clinical features, together with bronchoalveolar lavage (BAL) fluid analysis results (data not shown) (17). Moreover, 17 out of the 20 sarcoidosis

patients had a biopsy confirmation of the disease. The clinical symptoms of all patients included respiratory symptoms, i.e. dyspnea, coughing and chest pain. The characteristics of the studies population are summarized in Table 8.1.

Table 8.1 Characteristics of the participants.

	Sarcoidosis	Controls
number (m/f)	20 (16/4)	11 (7/4)
age	31-60 (44 ± 1)	40-58 (48 ± 3)
length	158-194 (177 ± 2)	162-188 (175 ± 3)
weight	52-107 (80 ± 4)	59-95 (77 ± 3)
body mass index	19-31 (25 ± 1)	19-30 (26 ± 1)
time since diagnosis	0-30 (5 ± 2)	-
biopsy taken	yes: 17 no: 3	-
DLCO	39-107 (80 ± 3)	-
FEV$_1$	42-135 (84 ± 6)	-
FVC	49-152 (92 ± 6)	-
chest radiograph stage 0/I/II/III/IV (n)	1/4/9/2/4	-

Controls are matched on age and gender and do therefore not significantly differ from the patients regarding these parameters. Age is expressed in year, length in cm, weight in kg, and DLCO (diffusing capacity of the lung for carbon monoxide), FEV$_1$, FVC (forced vital capacity) (forced expiratory volume in 1 second) in % of the predicted value based on age and gender. Data are expressed as range (mean ± SEM).

According to their chest radiographic stage, patients were subdivided into two groups, i.e. mild (stage 0-1) and severe sarcoidosis (stage 2-4). In the sarcoidoisis patients, both the DLCO (80±3%) and the FEV$_1$ (84±6%) were reduced.. Both the FEV$_1$ and DLCO values of stage 4 sarcoidosis patients were significantly lower than those of the other sarcoidosis patients (52±10% (n=4) vs 91±5% (n=16) and 60±4% (n=4) vs 83±3% (n=16) respectively).

At the moment this study was performed, none of the sarcoidosis patients used any medication.

Controls

The control group consisted of 11 non-smoking healthy volunteers, all with dietary habits comparable to those of the patients and a quercetin

intake of approximately 15 mg per day. All controls did not use any medication or vitamin/ food supplementation.

Lung function measurement

Lung function measurements included FEV_1 and DLCO. FEV_1 was measured with a pneumotachograph, DLCO by the single-breath method (Masterlab, Jaeger, Würzburg, Germany). Values were expressed as a percentage of those predicted based on age and gender (18)

Preparation of blood samples

Blood was collected from all participants in EDTA-containing vacutainer tubes (Vacutainer, Becton-Dickinson) and kept on ice prior to processing which occurred within 1 hour after blood collection. Blood was ailquoted into eppendorfs for both the ascorbic acid and the GSH/GSSG analysis: for the former 10% TCA was added to the whole blood, whereas 1.3% SSA in 10 mM HCl was used to preserve the samples for the latter. Another aliquot of blood was used for the incubations required for the blood-based cytokine production assay as described in that section below. The remaining blood was centrifuged (3000 rpm, 5' at 4ºC) to obtain plasma. Deproteinization of an ailquot of this plasma, using 10% TCA (1:1) followed by centrifugation (13.000 rpm, 5' at 4ºC), was carried for the trolox equivalent antioxidant capacity measurement. All samples were stored at -80ºC prior to analysis.

Trolox antioxidant capacity

The trolox equivalent antioxidant capacity (TEAC value) is a measurement for the total antioxidant status, relating the free radical scavenging properties of a solution or a compound to that of the synthetic antioxidant trolox. The assay is performed as previously described (19).

Ascorbic acid measurement

Calibrators were prepared freshly, containing the same amount of TCA as the samples. Samples and calibrators were processed identically as described previously (20).

Uric acid measurement

Uric acid was measured in the plasma of all samples as described previously (21).

GSH,GSSG and haemoglobin measurement

Both GSH and GSSG calibrators were prepared freshly and contained the same concentrations of SSA as the samples. Samples and calibrators were treated identically as described previously (22).

Blood-based cytokine production assay

Within one hour after blood collection, the blood-based cytokine production assay was performed as described previously (14). Care was taken that handling of the blood prior to LPS-stimulation did not influence the cytokine release.

Enzyme linked immune sorbent assay (ELISA) measurement

All cytokines were quantified using PeliKine Compact human ELISA kits (CLB/Sanquin, The Netherlands) based on appropriate and validated sets of monoclonal antibodies. Assays were performed as described in the manufacturer's instructions. Cytokine production was related to that of the control incubation without quercetin. The ethanol (0.5%) used to dissolve quercetin did not show any influence on the *ex vivo* LPS-induced cytokine production (data not shown).

Statistics

The data of both the patient and control group were compared using a Mann Whitney U test. A two-tailed probability value (P-value) equal to or less than 0.05 was considered to be statistically significant.

Results

All antioxidant parameters measured were decreased in the blood from sarcoidosis patients (Figure 8.1) compared to the antioxidant-levels of their age-, gender- and dietary behaviour matched controls.

Reduced glutathione (GSH) was significantly declined in the blood of sarcoidosis patients compared to the GSH-level in matched controls (25% decline). Glutathione disulphide (GSSG) levels, that are relatively low because of the efficient reduction of GSSG by endogenous glutathione reductase, did not significantly differ between the patient and control group.

The total plasma antioxidant capacity, i.e. the total sum of all plasma antioxidants that is expressed as trolox equivalents, was in sarcoidosis patients approximately 75% of that of the matched controls.

Two endogenous antioxidants that are known to contribute substantially to the total plasma antioxidant status are uric acid and vitamin C. Uric acid levels as well as vitamin C levels were significantly declined in the plasma of sarcoidosis patients (to respectively 60% and 79% of the control levels) compared to their matched controls.
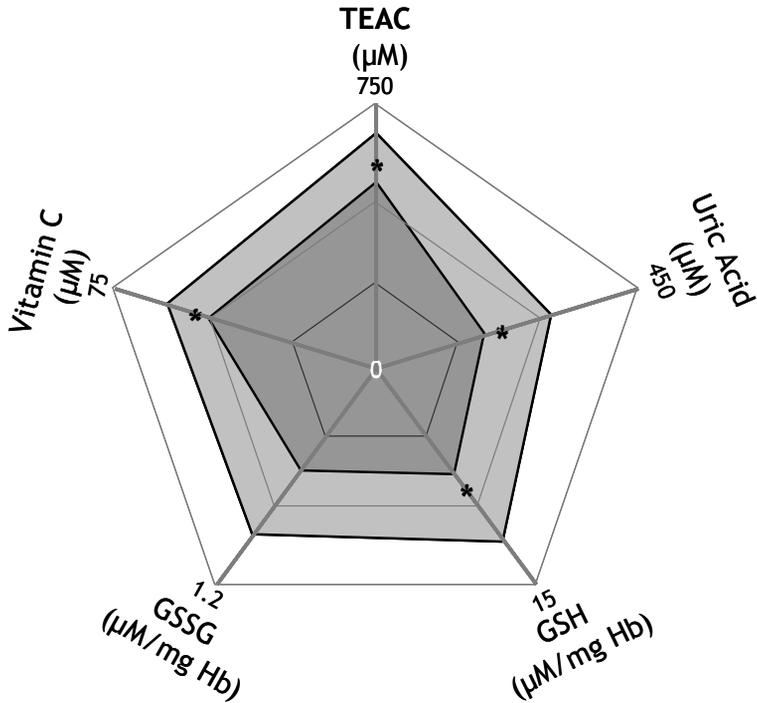
Figure 8.1. Antioxidant status in sarcoidosis patients compared to that in their matched controls. The values representing the levels of the various parameters in the patient group are connected to form the dark grey area whereas the light grey area reflects the same in the control group. Total plasma antioxidant status (TEAC) is expressed as Trolox equivalents. All axes have a linear scale. The origin is zero. The value at the end of the axes is indicated by the number depicted there. Data are expressed as mean $\pm$ SEM; *=P<0.03 vs the matched controls.

The TEAC value of sarcoidosis patients is significantly lower than that of their matched controls (535$\pm$21 μM versus 652$\pm$21 μM). The levels of individual antioxidants. i.e. uric acid, glutathione and vitamin C, are also significantly reduced in the patient group compared to the controls (respectively 185$\pm$16 μM vs 324$\pm$15 μM; 8.2$\pm$0.6 μM vs 13$\pm$1.9 μM; 52$\pm$4 μM vs 65$\pm$4 μM).

Basal levels of both TNFα and IL-8 (Figure 8.2A), two pro-inflammatory cytokines, were significantly increased in patients suffering from sarcoidosis compared to their controls. Basal levels of the anti-inflammatory cytokine IL-10 were not different between the sarcoidosis patients and the matched controls (Figure 8.2A). As a result, the ratios of the pro- versus the anti-inflammatory cytokines TNFα/IL-10 and IL-8/IL-10 were also significantly increased in the sarcoidosis patients.

The LPS-induced TNFα and IL-8 levels (Figure 8.2B) found in the sarcoidosis group were significantly higher compared to the controls, whereas IL-10 production was similar in both groups after LPS stimulation (Figure 8.2B).
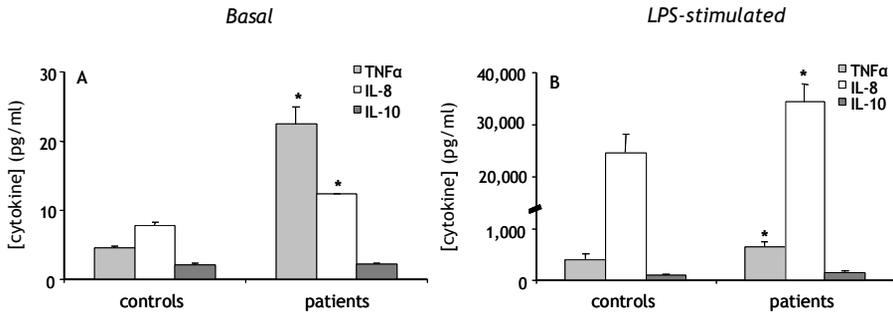

Figure 8.2. Basal (panel A) as well as LPS-induced levels (panel B) of TNFα, IL-8 and IL-10 in sarcoidosis patients compared to their matched controls. Basal TNFα- and IL-8 levels are both significantly higher in the patient group compared to their matched controls (TNFα: 22±2 pg/ml vs 5±0.3 pg/ml; IL-8: 12.3±1.7 pg/ml vs 7.9±0.4 pg/ml). LPS-induced TNFα- and IL-8 levels are both significantly higher in the patients than in the controls. LPS-induced IL-8 levels are approximately 8 times higher than the LPS-induced TNFα- and IL-10 levels.
Data are expressed as mean ± SEM; *P≤0.01 vs the matched controls for panel A; *P<0.04 vs the matched controls for panel B.

The flavonoid quercetin significantly reduced the TNFα (Figure 8.3A) and IL-8 production (Figure 8.3B), induced in blood by LPS, in the sarcoidosis group as well as in their matched controls. The extend of this reduction depended on the quercetin concentration. Both cytokines responded more sensitive (50% for IL-8 and 80% for TNFα) to quercetin in the sarcoidosis group compared to the matched controls (Figure 8.4A and 8.4B). The IL-10 production, induced in blood by LPS, of both patients and controls was not affected by quercetin (Figure 8.3C). As a result, the ratios of the pro- versus the ant-inflammatory cytokines TNFα/IL-10 and IL-8/IL-10 were also significantly reduced by the flavonoid.
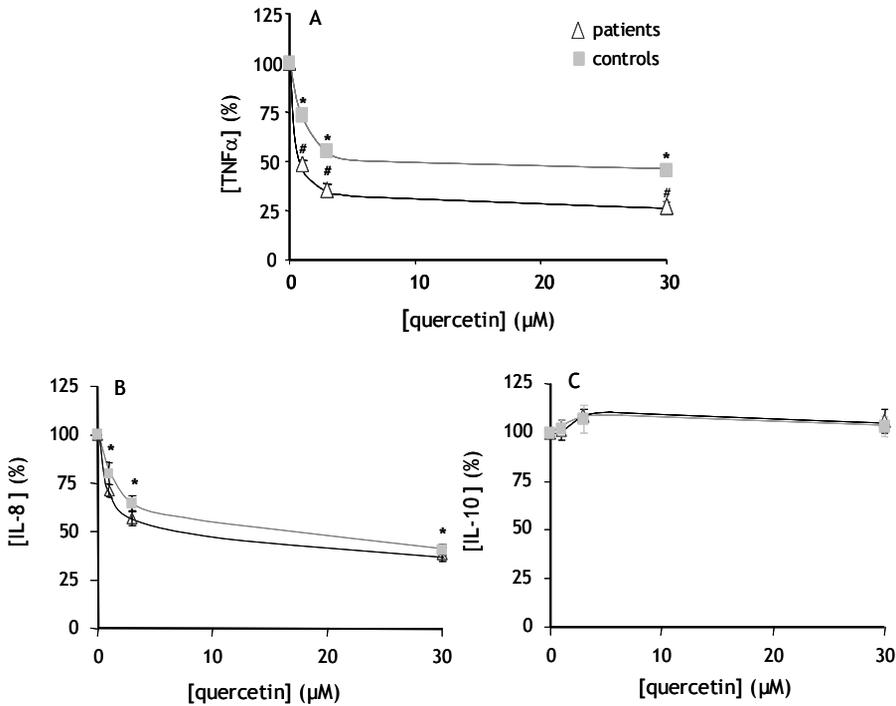
Figure 8.3 Modulating effect of quercetin on the LPS-induced TNFα, IL-8 and IL-10 production in sarcoidosis (panel A, B and C respectively) compared to their matched controls. Results are expressed in percentage, with 100% representing the cytokine-release under stimulation of LPS in the absence of quercetin. Data are expressed as mean ± SEM; *= P<0.01 vs the incubation containing no quercetin; #= P<0.05 vs controls and vs the incubation containing no quercetin.

Antioxidant as well as cytokine levels did not display significant differences between the two sexes. No difference was found between the mild and severe form of sarcoidosis regarding the residual plasma antioxidant capacity or the GSSG, uric acid, vitamin C, basal IL-8 and basal IL-10 levels. Compared to the mild form of sarcoidosis, the severe form tended to display slightly lower levels of GSH (6.8±1.1 μM (n=15) vs 7.6±1.3 μM (n=5); and total plasma antioxidant capacity (519±19 μM (n=13) vs 533±30 μM (n=5); and slightly higher levels of TNFα (24±3 pg/ml (n=15) vs 19±3 pg/ml (n=5). Stage 4 sarcoidosis patients displayed a significant lower total plasma antioxidant capacity compared to the other sarcoidosis patients (473±15 μM vs 543±23μM; P≤0.05).
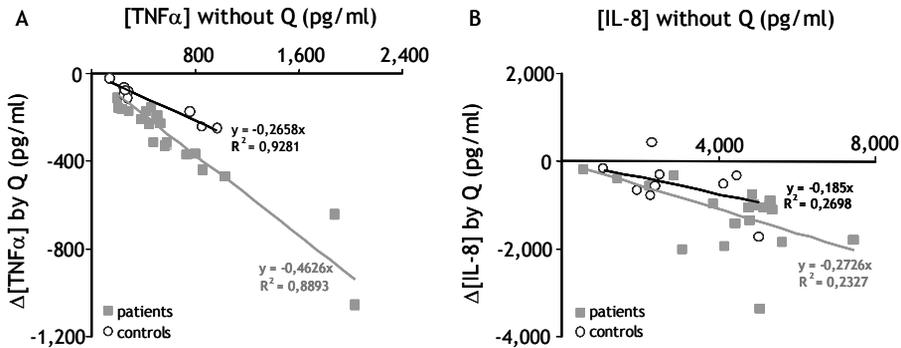
Figure 8.4 The effect of quercetin (1 μM) on LPS-induced TNFα (panel A) and IL-8 production (panel B) in both sarcoidosis patients and their matched controls. The decrease in LPS-induced cytokine production caused by quercetin (Q) (y-axis) is plotted against the LPS-induced cytokine production in the absence of the flavonoid (x-axis). The slopes of panel A indicate that on average, 1 μM quercetin reduces the TNFα production in controls with 27% and in patients with 46%. For IL-8, this reduction is on average 19% in controls and 27% in patients (panel B).

Data are expressed as individual values (n=20 for the patients; n=11 for the controls).

Discussion

The role of enhanced ROS production has been implicated in the pathology of sarcoidosis (4,5), but the influence of this increased ROS formation on the endogenous antioxidant levels in sarcoidosis have not yet been reported. To the best of our knowledge, this study is the first that demonstrates a significant decrease in the total plasma antioxidant capacity as well as in the blood levels of the important endogenous antioxidants GSH, vitamin C and uric acid in sarcoidosis patients compared to healthy controls matched for age, gender and dietary behaviour. The severe form of sarcoidosis tends to display a lower total plasma antioxidant capacity as well as a lower GSH level, indicating that the severity of the disease might be related to the level of oxidative stress occurring.

The patients and controls are matched for their dietary behavior, indicating that the observed decreased antioxidant levels are not the result of a different dietary intake. The lower endogenous antioxidant defence found, therefore, confirms the elevated production of ROS present in sarcoidosis. Low endogenous antioxidant levels combined with enhanced ROS formation is defined as oxidative stress. Oxidative stress can cause serious oxidative damage to biological macromolecules like DNA, lipids and proteins (23).

Several studies have shown that the levels of biomarkers of oxidative damage, i.e. exhaled ethane (4) and both 8-isoprostane (24) and oxidized proteins (25) in the bronchoalveolar lavage fluid (BALF), are indeed increased in sarcoidosis patients of different clinical stages. Furthermore, the transcription factor NF-κB, of which it is known that it is activated by radical damage, is increased in alveolar macrophages (26) and mononuclear blood cells (27) of active sarcoidosis patients compared to those of healthy controls.

To strengthen the endogenous antioxidant defence and thus offer more protection against ROS, antioxidant supplementation seems a logical strategy in the treatment of sarcoidosis. Moreover, antioxidant supplementation might not only protect against ROS-mediated damage, it might also mitigate elevated inflammation since ROS can enhance inflammation. ROS are capable of promoting inflammation in the lung by activating transcription factors like NF-κB and activator protein-1 that induce pro-inflammatory cytokines and chemokines (6,7). *In vitro* studies, using both macrophages and alveolar and bronchial epithelial cells, have demonstrated that oxidants can indeed initiate the production of inflammatory mediators like IL-8 and NO· (28). Because of this relation between ROS and inflammatory processes, the inflammatory status was also evaluated in sarcoidosis patients. To this end, basal levels of the pro-inflammatory cytokines TNFα and IL-8 and the anti-inflammatory cytokine IL-10 were measured.

TNFα was chosen since it is suggested to be the most prominent cytokine present in sarcoidosis (2,29). TNFα has been shown to play a pivotal role in the granula formation occurring in sarcoidosis (2). Furthermore, the individual capability of a patient to release TNFα is suggested to be linked to the progression of the disease, thereby linking this cytokine to the pathogenesis of sarcoidosis (30). TNFα is capable of activating transcription factors NF-κB and AP-1 that subsequently will further enhance other inflammatory mediators such as IL-8 (7,31) and TNFα itself, thereby amplifying the TNFα-mediated inflammatory effects.

Our study shows that basal TNFα-levels are significantly enhanced in sarcoidosis patients compared to healthy controls. This finding is in line with previous studies (32,33). The severe form of sarcoidosis tends to display higher TNFα levels, indicating that the severity of the disease might be related to the level of inflammation occurring. Conventional therapy with glucocorticoids fails to reduce the enhanced NF-κB activity found in sarcoidosis patients (27), whereas alternative strategies using anti-TNFα agents display a clinical beneficial effect (34).

Basal IL-8 levels are significantly increased in sarcoidosis, indicating that this cytokine is also involved in the enhanced inflammatory basal status in

this disease. This is again in line with previous studies, which show an increased expression of IL-8 in sarcoidosis (2,35,36). It should be noted that IL-8 can also be induced by TNFα and that the higher IL-8 level might reflect the higher TNFα level.

No changes were found in the basal levels of anti-inflammatory cytokine IL-10 between the sarcoidosis and control group. The results of previous studies on this cytokine are ambiguous. Some studies report enhanced IL-10 levels in the broncho-alveolar lavage fluid (BALF) of sarcoidosis patients as a compensatory mechanism (35,36), whereas other studies fail to demonstrate this increase (37,38).

To determine the anti-inflammatory potential of antioxidants, the effect of quercetin on LPS-induced production of TNFα, IL-8 and IL-10 was quantified in blood. The employed model, unlike models using isolated cells or cultured cells grown in medium, represents a more physiological and well reproducible model to measure cytokine production *ex vivo* (14,39). The natural cell-to-cell interactions are preserved and all blood components are present in *in vivo* ratios with non-cellular components, resulting in a system that reflects the *in vivo* condition well (14,39).

In both the sarcoidosis and the control group, the antioxidant quercetin significantly reduces the LPS-induced TNFα and IL-8 production, whereas the LPS-induced IL-10 production is not significantly altered by the flavonoid. The inhibitory effect of quercetin on both anti-inflammatory cytokines is dose-dependent and could already be achieved at an *in vivo* attainable concentration of 1 μM (40). Interestingly, the anti-inflammatory effects of quercetin are much more pronounced in the sarcoidosis patients than in their matched controls.

The anti-inflammatory actions of quercetin observed in the present study could very well be related to its anti-oxidative activity. This association between the anti-oxidative and anti-inflammatory capabilities of flavonoids has also been suggested in several *in vitro* (41,42) and *in vivo* studies (43,44). The reduced antioxidant status may also explain both (i) why *ex vivo* LPS-induced TNFα and IL-8 production are significantly higher in sarcoidosis patients than in their matched controls and (ii) why quercetin exerts a more pronounced effect on this TNFα and IL-8 production in sarcoidosis patients compared to the controls. Therefore, sarcoidosis patients are expected to profit from the use of antioxidant supplementation. The fact that quercetin is a dietary antioxidant indicates that the diet is an important factor in sarcoidosis. However, the toxicity of quercetin should be carefully evaluated especially when it is chronically administered (45). Clinical studies are needed to substantiate the efficacy as well as the safety of quercetin supplementation.

In conclusion, our study shows for the first time that the endogenous anti-oxidant levels as well as the total plasma antioxidant capacity are significantly reduced in sarcoidosis, emphasizing that increased production of ROS is associated the pathology of this disease. Moreover, inflammation is enhanced in sarcoidosis as shown by the increased basal levels of the pro-inflammatory cytokines TNFα and IL-8. The antioxidant quercetin shows ex vivo significant anti-inflammatory effects in both sarcoidosis patients and controls that are, interestingly, more pronounced in the first group. Probably, this larger anti-inflammatory effect of the flavonoid in sarcoidosis is associated with the compromised antioxidant status present in these patients. This suggests that particularly in sarcoidosis patients empowering the antioxidant defense system with alimentary antioxidants, such as quercetin, might be fruitful. This is especially important since conventional treatment with glucocorticoids fails to be efficacious.

References

1. Thomeer M, Demedts M and Wuyts W. Epidemiology of sarcoidosis. *Eur Respir Mon* 2005;32:1-10.
2. Baughman RP, Lower EE and du Bois RM. Sarcoidosis. *Lancet* 2003;361:1111-1118.
3. Newman LS, Rose CS and Maier LA. Sarcoidosis. *N Engl J Med* 1997;336:1224-1234.
4. Kanoh S, Kobayashi H and Motoyoshi K. Exhaled ethane: an in vivo biomarker of lipid peroxidation in interstitial lung diseases. *Chest* 2005;128:2387-2392.
5. Halliwell B, Gutteridge JM and Cross CE. Free radicals, antioxidants, and human disease: where are we now? *J Lab Clin Med* 1992;119:598-620.
6. MacNee W. Oxidative stress and lung inflammation in airways disease. *Eur J Pharmacol* 2001;429:195-207.
7. Rahman I. Oxidative stress, transcription factors and chromatin remodelling in lung inflammation. *Biochem Pharmacol* 2002;64:935-942.
8. Papakosta D, Kyriazis G, Gioulekas D, Kontakiotis T, Polyzoni T, Bouros D and Patakas D. Variations in alveolar cell populations, lymphocyte subsets and NK-cells in different stages of active pulmonary sarcoidosis. *Sarcoidosis Vasc Diffuse Lung Dis* 2005;22:21-26.
9. Katchar K, Soderstrom K, Wahlstrom J, Eklund A and Grunewald J. Characterisation of natural killer cells and CD56+ T-cells in sarcoidosis patients. *Eur Respir J* 2005;26:77-85.
10. Baughman RP and Lower EE. Therapy for sarcoidosis. Wouters EFM. *Sarcoidosis*, Wakefield: The Charlesworth Group, 2005.
11. Raghu G, Brown KK, Bradford WZ, Starko K, Noble PW, Schwartz DA and King TE, Jr. A placebo-controlled trial of interferon gamma-1b in patients with idiopathic pulmonary fibrosis. *N Engl J Med* 2004;350:125-133.
12. Boots AW, Kubben N, Haenen GR and Bast A. Oxidized quercetin reacts with thiols rather than with ascorbate: implication for quercetin supplementation. *Biochem Biophys Res Commun* 2003;308:560-565.
13. Thorn J. The inflammatory response in humans after inhalation of bacterial endotoxin: a review. *Inflamm Res* 2001;50:254-261.
14. Swennen EL, Bast A and Dagnelie PC. Immunoregulatory effects of adenosine 5'-triphosphate on cytokine release from stimulated whole blood. *Eur J Immunol* 2005;35: 852-858.
15. Fischer KD, Tedford K and Wirth T. New roles for Bcl10 in B-cell development and LPS response. *Trends Immunol* 2004;25:113-116.
16. De Groote D, Zangerle PF, Gevaert Y, Fassotte MF, Beguin Y, Noizat-Pirenne F, Pirenne J, Gathy R, Lopez M, Dehart I et al. Direct stimulation of cytokines (IL-1 beta, TNF-alpha, IL-6, IL-2, IFN-gamma and GM-CSF) in whole blood. I. Comparison with isolated PBMC stimulation. *Cytokine* 1992;4:239-248.
17. Drent M, Nierop MA, van Gerritsen FA, Wouters EF and Mulder PG. Computer program using BALF analysis results as diagnostic tool in interstitial lung diseases. *Am. J. Respir. Crit. Care Med.* 1996;153:736-741.
18. Quanjer PH, Tammeling GJ, Cotes JE, Pedersom OF, Peslin R and Yernaut JC. Lung volumes and forced ventilatory flows. Report working party. Standardization of lung functions tests. European Commission for Steel and Coal. Official statement of the European Respiratory Society. *Eur Respir J* 1993;16:5-40.
19. Fischer MA, Gransier TJ, Beckers LM, Bekers O, Bast A and Haenen GR. Determination of the antioxidant capacity in blood. *Clin Chem Lab Med* 2005;43:735-740.
20. Speek AJ, Schrijver J and Schreurs WH. Fluorometric determination of total vitamin C in whole blood by high performance liquid chromatography with pre-column derivatization. *J Chrom Biomed Appl* 1984;305:53-60.
21. Benzie IF, Chung W and Tomlinson B. Simultaneous measurement of allantoin and urate in plasma: analytical evaluation and potential clinical application in oxidant:antioxidant balance studies. *Clin Chem* 1999;45:901-904.

22. Tietze F. Enzymic method for quantitative determination of nanogram amounts of total and oxidized glutathione: applications to mammalian blood and other tissues. *Anal Biochem* 1969;27:502-522.

23. Boots AW, Haenen GR, den Hartog GJ and Bast A. Oxidative damage shifts from lipid peroxidation to thiol arylation by catechol-containing antioxidants. *Biochim Biophys Acta* 2002;1583:279-284.

24. Montuschi P, Ciabattoni G, Paredi P, Pantelidis P, du Bois RM, Kharitonov SA and Barnes PJ. 8-Isoprostane as a biomarker of oxidative stress in interstitial lung diseases. *Am J Respir Crit Care Med* 1998;158:1524-1527.

25. Lenz AG, Costabel U and Maier KL. Oxidized BAL fluid proteins in patients with interstitial lung diseases. *Eur Respir J* 1996;9:307-312.

26. Culver DA, Barna BP, Raychaudhuri B, Bonfield TL, Abraham S, Malur A, Farver CF, Kavuru MS and Thomassen MJ. Peroxisome proliferator-activated receptor gamma activity is deficient in alveolar macrophages in pulmonary sarcoidosis. *Am J Respir Cell Mol Biol* 2004;30:1-5.

27. Drent M, van den Berg R, Haenen GR, van den Berg H, Wouters EF and Bast A. NF-kappaB activation in sarcoidosis. *Sarcoidosis Vasc Diffuse Lung Dis* 2001;18:50-56.

28. Antonicelli F, Parmentier M, Hirani N, Drost E, Rahman I, Donaldson K and MacNee W. LPS stimulation of IL-8 release is inhibited by thiol antioxidant at the transcriptional level in THP-1 macrophage cells. *Am J Respir Crit Care Med* 2000;161:A738.

29. Moller DR. Cells and cytokines involved in the pathogenesis of sarcoidosis. *Sarcoidosis Vasc Diffuse Lung Dis* 1999;16:24-31.

30. Seitzer U, Swider C, Stuber F, Suchnicki K, Lange A, Richter E, Zabel P, Muller Quernheim J, Flad HD and Gerdes J. Tumour necrosis factor alpha promoter gene polymorphism in sarcoidosis. *Cytokine* 1997;9:787-790.

31. Rahman I and MacNee W. Role of transcription factors in inflammatory lung diseases. *Thorax* 1998;53:601-612.

32. Baughman RP, Strohofer SA, Buchsbaum J and Lower EE. Release of tumor necrosis factor by alveolar macrophages of patients with sarcoidosis. *J Lab Clin Med* 1990;115:36-42.

33. Ziegenhagen MW, Schrum S, Zissel G, Zipfel PF, Schlaak M and Muller-Quernheim J. Increased expression of proinflammatory chemokines in bronchoalveolar lavage cells of patients with progressing idiopathic pulmonary fibrosis and sarcoidosis. *J Investig Med* 1998;46:223-231.

34. Yee AM and Pochapin MB. Treatment of complicated sarcoidosis with infliximab anti-tumor necrosis factor-alpha therapy. *Ann Intern Med* 2001;135:27-31.

35. Meloni F, Caporali R, Marone Bianco A, Paschetto E, Morosini M, Fietta AM, Patrizio V, Bobbio-Pallavicini F, Pozzi E and Montecucco C. BAL cytokine profile in different interstitial lung diseases: a focus on systemic sclerosis. *Sarcoidosis Vasc Diffuse Lung Dis* 2004;21: 111-118.

36. Rutherford RM, Staedtler F, Kehren J, Chibout SD, Joos L, Tamm M, Gilmartin JJ and Brutsche MH. Functional genomics and prognosis in sarcoidosis--the critical role of antigen presentation. *Sarcoidosis Vasc Diffuse Lung Dis* 2004;21:10-18.

37. Hauber HP, Gholami D, Meyer A and Pforte A. Increased interleukin-13 expression in patients with sarcoidosis. *Thorax* 2003;58:519-424.

38. Martinez JA, King TE, Jr., Brown K, Jennings CA, Borish L, Mortenson RL, Khan TZ, Bost TW and Riches DW. Increased expression of the interleukin-10 gene by alveolar macrophages in interstitial lung disease. *Am J Physiol* 1997;273:L676-683.

39. Yaqoob P, Newsholme EA and Calder PC. Comparison of cytokine production in cultures of whole human blood and purified mononuclear cells. *Cytokine* 1999;11:600-605.

40. Conquer JA, Maiani G, Azzini E, Raguzzini A and Holub BJ. Supplementation with quercetin markedly increases plasma quercetin concentration without effect on selected risk factors for heart disease in healthy subjects. *J Nutr* 1998;128:593-597.

41. Zern TL, Wood RJ, Greene C, West KL, Liu Y, Aggarwal D, Shachter NS and Fernandez ML. Grape polyphenols exert a cardioprotective effect in pre- and postmenopausal women by lowering plasma lipids and reducing oxidative stress. *J Nutr* 2005;135:1911-1917.

42. Kostyuk VA, Potapovich AI, Vladykovskaya EN, Korkina LG and Afanas'ev IB. Influence of metal ions on flavonoid protection against asbestos-induced cell injury. *Arch Biochem Biophys* 2001;385:129-137.
43. Donnelly LE, Newton R, Kennedy GE, Fenwick PS, Leung RH, Ito K, Russell RE and Barnes PJ. Anti-inflammatory effects of resveratrol in lung epithelial cells: molecular mechanisms. *Am J Physiol Lung Cell Mol Physiol* 2004;287:L774-783.
44. Comalada M, Camuesco D, Sierra S, Ballester I, Xaus J, Galvez J and Zarzuelo A. In vivo quercitrin anti-inflammatory effect involves release of quercetin, which inhibits inflammation through down-regulation of the NF-kappaB pathway. *Eur J Immunol* 2005;35:584-592.
45. Boots AW, Balk JM, Bast A and Haenen GR. The reversibility of the glutathionyl-quercetin adduct spreads oxidized quercetin-induced toxicity. *Biochem Biophys Res Commun* 2005;338:923-929.