Chapter 8

Relationship of myeloperoxidase promotor polymorphism and disease severity in sarcoidosis?

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Abstract

Background

Previously, we demonstrated that the number of polymorphonuclear neutrophils (PMNs) in the bronchoalveolar lavage fluid (BALF) is useful in distinguishing sarcoidosis patients with a more favourable outcome from those having a more severe course of disease. Neutrophils contain the oxidant generating enzyme, myeloperoxidase (MPO). Cellular levels of MPO can be influenced by functional promotor polymorphisms, -463G/A and -129G/A, which might modulate disease severity.

Methods

In the present study, we investigated two MPO promotor polymorphisms in 110 sarcoidosis patients and in 191 ethnically matched controls. Pulmonary disease severity was evaluated by means of radiographic staging, HRCT scoring, lung function- and exercise capacity testing.

Results

No significant differences were found between sarcoidosis patients and healthy controls, with regard to either polymorphism. Furthermore, no association was observed between -463 G/A and -129 G/A polymorphism, and the severity of sarcoidosis.

Conclusion

Functional MPO promotor polymorphisms, -463G/A and -129G/A, did not explain for disease severity in the studied sarcoidosis population. Future studies are needed to identify predictive features useful in guiding therapeutic strategies and to determine difficult-to treat cases.

Introduction

Sarcoidosis, a disease predominantly affecting young adults, is the most common diffuse lung disease with a population prevalence of around 40:100.000[1,2]. Sarcoidosis is an immune mediated inflammatory disease with unknown etiology, although genetic predisposition is likely to be important[2,3]. The main pathologic feature of the disease is a chronic inflammation resulting in non-caseating granuloma formation. The prognosis of sarcoidosis is rather variable. A great deal of uncertainty exists on how to predict the natural course of the (untreated) disease, and identify those individual cases who should be treated immediately. More important, as it is still not possible to identify the cause of sarcoidosis, therapy is directed at controlling the inflammatory reaction[4].

Bronchoalveolar lavage fluid (BALF) analysis appears to reflect the severity of sarcoidosis[5-9]. Notably, the number of polymorphonuclear neutrophils (PMNs) is increased in advanced, chronic sarcoidosis[5-7,9]. In line with these findings, interleukin 8 (IL-8), a potent chemoattractant and activator of neutrophils, in BALF appears to be highly elevated in sarcoidosis patients who showed progressive disease[8,10].

Myeloperoxidase (MPO) is an abundant protein in PMNs and monocytes, stored in the azurophilic granules of neutrophils and released during phagocytosis[11,12]. MPO in BALF originates from lung PMNs and can be used to estimate the presence and/or activation of PMNs[13]. Indeed, a strong correlation between the MPO level and the PMNs count in BALF has been demonstrated in patients with Wegener's granulomatosis[14]. The MPO concentrations appear to be associated with two described functional promotor polymorphisms, namely the -463 G/A MPO promotor polymorphism[15,16] and the recently described -129 G/A promotor polymorphism[17]. Several diseases were reported to be associated with -463 G/A promotor polymorphism[16,18-22], whereas no association studies of -129 G/A have yet been reported.

MPO appears to be involved in tissue damage through production of potent oxidant HOCl, which is able to oxidize many cellular compounds such as thiols and NADPH and lead to chlorination of DNA bases and tyrosine residues in proteins[11,12.23-25]. Therefore, it can be assumed that decreased availability of MPO related to functional MPO promotor polymorphisms, has a protective effect by decreasing the chance of tissue damage.

It was hypothesized that MPO polymorphism might contribute, at least in part, to disease severity in sarcoidosis and make it possible to determine those cases who might benefit from agents controlling the inflammatory reaction at an early stage. Hence, the incidence of two MPO promotor polymorphisms (-463 G/A and -129 G/A) was analyzed in a Dutch Caucasian sarcoidosis population as well as a possible relationship with disease severity.

Materials and Methods

Study population

Caucasian sarcoidosis patients (n = 110), who visited the Sarcoidosis Management Center of the University Hospital Maastricht, a Dutch referral center for sarcoidosis between January 2000 and April 2001, were included in this study. Patients were diagnosed based on consistent clinical features and BALF fluid analysis, according to the ATS/ERS/WASOG guidelines[2,26]. The diagnosis was histologically confirmed in 85% of the cases. The control population consisted of 191 healthy individuals. Informed consent was obtained from all participating subjects. Patient characteristics are summarized in Table 8.1.

Table 8.1. Characteristics of the Dutch Caucasian sarcoidosis patients and controls genotyped for -463 G/A and -129 G/A myeloperoxidase (MPO) promotor polymorphism.

	Sarcoidosis patients (n = 110)	Control subjects (n = 191)
Male / female, n	55 / 55	100 / 91
Age[a], years	40.7 ± 11.0 (17 – 70)	36.4 ± 14.4 (18 – 82)
Time since diagnosis[a], years	4.25 ± 5.67 (0 – 37)	-
Smoking (no / yes), n	101 / 9	-
Prednison (no / yes), n	78 / 32	-

[a] Data are expressed as mean ± SD with range in parentheses.

Evaluating Sarcoidosis Pulmonary Disease Severity

Chest radiographs were graded according to the radiographic staging of DeRemee (0 to III), adding stage IV, the end stage of lung fibrosis[2,27]. With high-resolution computer tomography (HRCT), thin-section scans with 1-mm collimation were obtained at 10-mm intervals through the chest. The scanning parameters included 137 kVp, 255 mA, and 1-second scanning time.

Both mediastinal (width, 400 HU; level, 40 HU) and lung (width, 1600 HU; level, -800 HU) window images were obtained. The semiquantitive HRCT scoring system was used as described previously[28].

Lung function parameters, including the forced expiratory volume in 1 s (FEV1) and forced vital capacity (FVC), were measured with a pneumotachograph. The diffusing capacity for carbon monoxide (DLCO) was measured by the single-breath method (both Masterlab, Jaeger, Würzburg, Germany). Values were expressed as a percentage of those predicted[29].

Serum ACE (sACE) was measured by colorimetric method (Fujirebio Inc., Tokyo, Japan, cat. nr. FU 116). ACE acts on a substrate p-hydroxybenzoyl-glycyl-L-hystidyl-L-leucine and separates p-hydroxybenzoyl-glycine, which is converted in two consequent reactions in quinoneimine dye. The absorbance of quinoneimine dye is measured at 505 nm to evaluate the ACE activity.

Soluble Interleukin-2 receptor (sIL-2R) was measured on the IMMULITE Automated Analyzer, which is a two-site chemiluminescent enzyme immunometric assay with a detection limit of 50 kU/L and a measuring range of 50 – 7500 kU/L (Diagnostic Product Corporation, Los Angeles, CA, cat no LKIP1).

Molecular studies

The -463 G/A polymorphism was determined as previously described[16,18,19]. The -129 G/A polymorphism was determined as described by Hoy *et al*.[17]. The most important features are presented in Table 8.2. When screening for -129 G/A polymorphism, we identified a 129G to A specific Apa1 restriction site within the 278 bp PCR product. PCR products were digested with 20 units of Apa1 (Roche, Mijdrecht, The Netherlands) for two hours or overnight at 30°C, separated on a 2%-agarose gel and stained with ethidium-bromide.

Table 8.2. Sequences of primers and digestion fragments length used for myeloperoxidase (MPO) genotyping.

MPO Promotor	Primers	Digestion of PCR product	Genotype (characterized by fragments in bp)
-463 G/A	5'-CGGTATAGGCACACAATGGTGAG 5'R-GCAATGGTTCAAGCGATTCTTC	Aci I	GG (168, 121, 61) GA (289, 168, 121, 61) AA (289, 61)
-129 G/A	5'- CCTCCACAGCTCACCTGATAT 5'R- CGCTTGAACCATTGCACATCA	Apa I	GG (124) GA (154)

Statistics

All analysis were performed using the SPSS10.0 for Windows (SPSS, Chicago, IL, USA). Agreement with Hardy-Weinberg-equilibrium and differences in allele and genotype frequencies between groups were examined using chi-square tests. Because multiple comparisons were performed, a probability value divided by number of comparisons was considered statistically significant (Bonferroni's correction). Univariate logistic regression analysis was used to calculate odds ratios with 95% confidence intervals. Group comparisons were performed by means of Independent Samples t-test or Mann-Whitney U test.

Results

The two functional MPO polymorphisms fulfilled Hardy-Weinberg expectations in both sarcoidosis and control subjects. Genotype distributions associated with the -463 G/A or -129 G/A polymorphism did not differ significantly between patients with sarcoidosis and healthy controls (Table 8.3). Furthermore, for both polymorphisms sex-specific analysis showed no differences of allele or genotype frequencies (data not shown).
Base-line clinical data of the patients with sarcoidosis are presented in Table 8.4.

Additionally, it was tested whether any association could be found with MPO polymorphisms. For this purpose, for -463 G/A polymorphism, the GA and AA genotypes were grouped together and compared to the GG genotype. No relation with age at diagnosis was found. Furthermore, severity of the disease was evaluated in relation to genotype. The results of this analysis are presented in Table 8.5. As can be seen no significant differences in the severity as presented by clinical data could be found, the finding which remained also after correction for prednison use. The same analysis as shown in Table 8.5 was repeated with respect to -129 G/A polymorphism and no relation to clinical data was found (data not shown).

Table 8.3. Genotype frequencies of the myeloperoxidase (MPO) promotor polymorphisms in the Dutch Caucasian sarcoidosis and control population[a].

Polymorphism		Sarcoidosis Patients (n = 110)	Control Subjects (n = 191)	OR (95% CI)	p-value [b]
-463 G/A					
Genotype	GG	67 (60.9%)	113 (59.2%)	1.0	
	GA[c]	37 (33.6%)	68 (35.6%)	1.01 (0.35 – 2.91)	
	AA[c]	6 (5.5%)	10 (5.2%)	0.91 (0.56 – 1.52)	0.942
Allele	G	171 (77.7%)	294 (77.0%)	1.0	
	A[c]	49 (22.3%)	88 (23%)	0.95 (0.64 – 1.42)	0.829
-129 G/A[d]					
Genotype	GG[c]	97 (88.2%)	167 (87.4%)	1.0	
	GA[c]	13 (11.8%)	24 (12.6%)	0.93 (0.45 – 1.91)	0.849
Allele	G	207 (94.1%)	358 (93.7%)	1.0	
	A[c]	13 (5.9%)	24 (6.3%)	0.93 (0.46 – 1.88)	0.854

[a] Data are expressed as absolute numbers with percentages in parentheses.
[b] p-values are for overall comparison between cases and control subjects (Chi-square analysis). Odds ratios (OR) for genotypes/alleles were calculated by logistic-regression analysis.
[c] GG genotype or G allele is the reference group.
[d] AA genotype was absent from both sarcoidosis and control group.

Table 8.4. Evaluation of severity in Dutch Caucasian sarcoidosis patient population[a]

	Female (n = 55)	Male (n = 55)	Total (n = 110)
ACE (9 – 25 U/L)	20 (17 – 26)	20 (15 – 28)	20 (16 – 27)
sIL-2R (241 – 846 kU/L)	550 (404 – 937)	767 (409 – 1180)	667 (408 – 1085)
FEV1,% pred[b]	97.0 (84 – 106)	87.0 (63 – 99)	92.0 (69.8 – 103)
FVC, % pred[b]	105 (94.2 – 115)	91.0 (77.5 – 102)	98.5 (84.5 – 110)
DLCO, % pred	89 (78 – 96)	85.5 (72.5 – 97.5)	87.0 (76.5 – 96.5)
Radiographic stages 0/I/II/III/IV[b]	17 / 8 / 14 / 14 / 2	4 / 8 / 14 / 20 / 9	21 / 16 / 28 / 34 / 11
HRCTtot (0 – 18)[b]	4.0 (1 – 6)	6.0 (3 – 9)	5.0 (3 – 8)

[a] Data are median with interquartile range (25th – 75th percentile) in parenthesis, except for radiographic staging.
[b] $p < 0.01$ comparing males and females.

Table 8.5. Relationship of the -463 G/A myloperoxidase (MPO) promotor polymorphism and disease severity in a Dutch Caucasian sarcoidosis population (n = 110) [a]

Diagnostic parameter	Female		Male	
	GG (n = 32)	GA/AA (n = 23)	GG (n = 35)	GA/AA (n = 20)
sACE (9-25 U/L)	20 (18 – 26)	20 (16 – 27)	23 (15 – 27)	18.5 (15.3 – 32.8)
sIL-2R (241-846 kU/L)	511 (397 – 806)	732 (414 – 1581)	801 (409 – 1180)	664 (409 – 1181)
FEV1,% pred	99 (88 – 107)	92 (71 – 106)	79 (61 – 100)	90 (73 – 96)
FVC, % pred	105 (96 – 115)	105 (88 – 114)	89 (75 – 105)	95 (86 – 100)
DLCO, % pred	89 (81 – 95)	87 (72 – 99)	83 (69 – 96)	92 (80 – 102)
Radiographic stages 0/I/II/III/IV	11 / 8 / 6 / 7 / 0	6 / 0 / 8 / 7 / 2	3 / 5 / 7 / 13 / 7	1 / 3 / 7 / 7 / 2
HRCTtot (0-18)	3 (1 – 5)	6 (2 – 7)	7 (4 – 9)	6 (3 – 9)

[a] Data are median with interquartile range (25th – 75th percentile) in parenthesis, except for radiographic staging

Discussion

This study showed that distribution of two functional myeloperoxidase (MPO) promotor polymorphisms (-463 G/A and -129 G/A) was similar in sarcoidosis patients as compared to controls. Furthermore, no relation was found between these polymorphisms and disease severity. To exclude a possible bias due to corticosteroid use, treated and untreated sarcoidosis patients were also considered separately. The results in both subgroups appeared to be similar to those in the total population, *i.e.* no relation with MPO genotype (also after stratification for sex and age) was found.

Among the cells present in bronchoalveolar lavage fluid (BALF), polymorphonuclear neutrophils (PMNs) are considered to be a key mediator indicative of severity of the disease. In cases with more extensive long term radiographic features, impaired lung function parameters, having a worse prognosis and sometimes corticosteroid resistant chronic disease, PMNs were found to be increased in BALF[5-7,9]. Moreover, a strong correlation exists between the myeloperoxidase level and the PMN count in BALF[14].

Some could argue that the neutrophils might just be an epiphenomenon, coming in when disease is extensive and fibrotic. However, it was recently demonstrated that the number of neutrophils in BALF even at first presentation is indicative of prognosis and disease outcome[5,9].

GG genotype of -463 G/A polymorphism was previously found to correlate with higher levels of MPO mRNA and higher protein levels, as shown by Western blotting, than the GA or AA genotype[15]. In contrast, when circulating levels of MPO were measured in serum by means of enzyme immunoassay, no such correlation was found with -463 G/A polymorphism, but only with -129 G/A polymorphism[17].

It was recently demonstrated that -463 G/A MPO promotor polymorphism influences diseases severity and expression in chronic granulomatous disease[22] and Wegener's granulomatosis[20], both granulomatous diseases different from sarcoidosis. In the present study, however, no association of MPO promotor polymorphisms with sarcoidosis were found. One possible explanation could be, that disease expression in chronic granulomatous disease and Wegener's granulomatosis is modulated by infections with

extensive PMN involvement. Indeed, co-trimoxazol (antibacterial drug) positively influences the course of disease in both chronic granulomatous disease[30] and Wegener's granulomatosis[31,32], whereas this has never been shown in sarcoidosis.
Granuloma formation in sarcoidosis appears to be a strictly T cell dependent process[33]. Since MPO was shown to inhibit T cell proliferation[34,35], one can postulate that MPO promotor polymorphisms might also influence the T cell dependent disease process in sarcoidosis. Therefore, the role of MPO and its polymorphisms seem very complex and not completely defined in the different diseases that are characterized by granulomatous inflammation.
In conclusion, the PMN count in BALF appears to be useful in determining chronic and difficult to treat sarcoidosis patients. MPO plays a key role in the immunopathogenesis of several granulomatous disorders and MPO release appears to be related to MPO polymorphism(s). However, in the Dutch Caucasian sarcoidosis population studied, similar frequencies of functional MPO promotor polymorphisms (-463 G/A and -129 G/A) were found as compared to control subjects. Furthermore, no association between -463 G/A and -129 G/A MPO promotor polymorphisms, and the severity of sarcoidosis, including radiographic features, impaired lung function, time since diagnosis and corticosteroid use was found in our study population. Given the inability to identify the cause of sarcoidosis, therapy is directed to control the inflammatory reaction. Future studies are needed to determine those cases with a more advanced chronic course of the disease who might benefit from early treatment of the inflammatory reaction.

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