

Review

Genotyping in the MHC locus: potential for defining predictive markers in sarcoidosis

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Abstract

In sarcoidosis, host genetic factors are discussed as contributing to disease susceptibility and course. Since tumor necrosis factor (TNF)- α is a central mediator of granuloma formation and since elevated TNF- α levels are found during active phases of sarcoidosis, genetic polymorphisms correlating with influences on TNF- α levels are of special interest. The complete sequencing of the MHC region and the increase in the number of identified gene polymorphisms in this locus associated with TNF- α production offer the opportunity of detecting new genes associated with sarcoidosis and perhaps of defining disease-associated haplotypes that bear the potential of serving as predictive markers for this disease.

Keywords: disease haplotypes, gene polymorphisms, HLA-DR, tumor necrosis factor- α

Introduction

Sarcoidosis is a multisystemic granulomatous disease of unknown etiology in which environmental exposures are believed to interact with genetic factors in determining the pattern of sarcoidosis presentation, progression and prognosis [1–3]. The consensus is that the disease results from an exaggerated cellular immune response (acquired, inherited or both) to a limited class of antigens or self-antigens [1,3]. Disease activity is accompanied by mononuclear cell infiltrates and granuloma formation, the most common organs involved being the lung and lymph nodes, although almost any organ can be affected, including the eyes, heart, skin and the central nervous system. The course and prognosis of the disease are correlated with the mode of onset, where an acute onset accompanied by fever, arthralgia and erythema nodosum (sarcoid arthritis, Löfgren syndrome [4]) usually heralds a self-limited course with spontaneous resolution, whereas an insidious onset may be followed by chronic disease with relentless, progressive fibrosis [1,3].

For an individual patient, however, only time and close observation will provide a specific answer regarding the disease course, making an appropriate treatment with respect to disease manifestation at the time of disease diagnosis difficult. Although many attempts to find prognostic markers for a better assessment of sarcoidosis and to ameliorate treatment of patients have been made, a reliable marker is still lacking [1,3,5].

Granulomatous reactions have been shown to be regulated by numerous endogenous mediators, of which the immunoregulatory cytokine TNF- α is thought to play a pivotal role. In sarcoidosis, the presence of TNF- α mRNA transcripts and protein in granulomas has been reported [6] and, interestingly, TNF- α mRNA levels were shown to be increased in sarcoid granulomas in lymph nodes compared with control lymph nodes from patients with localized primary lung carcinoma [7]. On the cellular level, elevated spontaneous and induced *ex vivo* release of

HLA = human leukocyte antigen; MHC = major histocompatibility complex; TAP = transporter associated with antigen processing; TNF = tumor necrosis factor.

TNF- α is observed by alveolar macrophages of sarcoidosis patients [8–10]. Furthermore, the individual capacity of the patient to release TNF- α from alveolar macrophages is a phenomenon linked with active and progressive disease, implying that this cytokine plays a role in the pathogenesis of sarcoidosis [9] and may even represent a candidate marker for determining the course of the disease [11].

Because of the important role of TNF- α as a central immunomodulator and mediator of granuloma formation, and since elevated TNF- α levels are typically found during active phases of sarcoidosis, genetic polymorphisms correlating with influences on TNF- α levels appear to be of special interest in the context of disease pathology. A number of different polymorphisms in the MHC locus have been shown to be associated with variations in TNF- α production and to be associated with predisposition to and disease presentation of sarcoidosis. These investigations, and others examining genetic polymorphisms not located in the MHC locus, imply that it is unlikely one gene locus is responsible for predisposition to and clinical outcome of the disease. It rather seems that multiple genetic factors may be involved, perhaps constituting an extended disease-associated haplotype.

The complete sequencing of the MHC region and the increase in the number of identified gene polymorphisms in this locus associated with TNF- α production offer the opportunity of detecting new genes associated with sarcoidosis, and perhaps of defining disease-associated haplotypes. This is discussed in the context of the authors' own data and published data, with the focus on the TNF and human leukocyte antigen (HLA)-DR gene loci that indicate such a hypothetical disease-associated haplotype is a probability. A correlation of haplotypes with the clinical presentation and outcome of sarcoidosis bear the potential of serving as predictive markers for this disease and may even show to be of prognostic value, a prerequisite for improving treatment regimes of patients suffering from this elusive disease.

Genetic determinants of TNF- α production and correlations to disease

TNF- α is an inducible cytokine with a broad range of pro-inflammatory and immunostimulatory actions, and it is thought to play a fundamental role in many diseases. It is classified as an early response mediator since it may be released in copious amounts by TNF- α -producing cells on stimulation, which in turn set into motion a cascade of events leading to the development of cytokine networks important in orchestrating an inflammatory response.

Variation in TNF- α production level is an innate host characteristic that was shown to vary between individuals and to be associated with certain HLA-DR alleles [12,13]. The TNF gene locus, comprising the TNF- α , lymphotoxin- α

(formerly TNF- β) and lymphotoxin- β genes, is located in the class III region of the MHC, telomeric to the class II region and centromeric to the class I region. Genetic analysis has revealed a number of polymorphisms in these genes, and new polymorphisms with potential functional consequences continue to be discovered [14].

The MHC III region contains other genes than TNF that are becoming increasingly interesting with respect to autoimmune and infectious diseases since they encode for proteins involved in immune and inflammatory responses [15]. These genes include, for instance, the allograft inflammatory factor 1, the leukocyte-specific transcript 1, IKBL (NFKBIL1), BAT1 and the immunoglobulin superfamily gene member 1C7. The most extensive data, however, have accumulated on two of the most studied polymorphisms in the TNF locus (the biallelic polymorphisms at position -308 in the promoter region of the TNF- α gene and in the first intron of the lymphotoxin- α gene), both of which have been associated with variation in TNF- α production and with susceptibility to infectious and autoimmune diseases [14]. Moreover, these two polymorphisms have been found to be strongly associated with the polymorphic MHC 8.1 (A1, B8, DR3) ancestral haplotype [16,17], which is associated not only with multiple immunopathological diseases [18], but also with variations in TNF- α production [16]. Given these findings, the genetic background of an individual may thus predispose for production levels of TNF- α and, given the importance of this immunomodulating cytokine, this may influence the immune response and thus, finally, the clinical course of disease.

Gene polymorphisms implicated in susceptibility to sarcoidosis

Evidence for genetic predisposition for sarcoidosis has long been assumed due to the prevalence of the disease in different ethnic groups and due to familial occurrence [2,3]. It is believed that a supposed genetic susceptibility is most probably conferred by more than one immune regulatory gene, which act synergistically on disease risk. Numerous studies have been performed to clarify this aspect [2] and, especially, the highly polymorphic HLA locus has been investigated intensely. The findings for HLA associations have not been consistent, however, because of differences in the HLA allele distribution in different ethnicities [19] and because of additional susceptibility genes adjacent to or within the MHC.

First reports examining other genes in the MHC have also not conclusively found an association with susceptibility to sarcoidosis, as shown for example for the transporter associated with antigen processing (TAP2) or the latent membrane protein 1 gene in the Japanese (no association) [20,21], and complement protein C4A, C4B and factor B in Caucasians (no association) [22]. A second

investigation on TAP1 and TAP2 in Caucasians based on two different ethnic patient/control groups from the UK and Poland found significant differences in TAP2 when comparing the respective ethnic case-control groups [23]. An interesting point of this study was, however, that on comparing UK and Polish control groups, significant differences were found in TAP1 and additional differences were found between the two patient groups concerning TAP2, indicating that the use of multiple defined ethnic populations may help with, and should be considered in, defining the role of genetic factors in sarcoidosis [24].

A recent investigation on 122 affected siblings from 55 families resulted in data showing highly significant evidence for the involvement of genes of the MHC region for genetic predisposition to sarcoidosis. The analysis suggested that no single gene may account for the significant results but, rather, that multiple additive MHC gene effects, possibly some of them in linkage disequilibrium, may be considered in conferring susceptibility to sarcoidosis [24]. Moreover, a genome wide scan performed by the same group confirmed and significantly underlined the major involvement of the MHC locus in which this major association was found besides six minor associations on five different chromosomes [25], substantiating the hypothesis that multiple genes may be involved in susceptibility to the disease.

Apart from genes of the MHC locus, other genes with a putative influence on susceptibility to sarcoidosis have been investigated. For example, the insertion/deletion polymorphism of the angiotensin-converting enzyme gene (I/D ACE), which is interesting because it correlates with variations in serum angiotensin-converting enzyme levels, in turn used as a disease activity marker in sarcoidosis, has been shown to correlate with disease progression in African Americans [26], to be significantly increased in Scandinavian patients with autoimmune manifestations [27], and to be associated with poor prognosis in Finns [28]. On the contrary, Arbustini *et al.* found no association in Caucasians [29], and no association was found in other Japanese cohorts [30,31]. A further study in Caucasians raises the question whether this I/D ACE polymorphism may rather be involved in the clinical manifestation of the disease, since it was not found to be an inherited main cause of sarcoidosis [32].

Overall, these various investigations with disparate results demonstrate that genetic susceptibility to sarcoidosis is multigenetic and that ethnicity should be considered. In addition, an important aspect to keep in mind with respect to genetic studies is environmental effects such as could be demonstrated by epidemiological studies in Japan and Iceland, where a homogeneous genetic background of the indigenous population is assumed [33,34]. For instance, the subpolar climate in contrast to the moderate or sub-

tropical climate on the Japanese islands increases sarcoidosis incidence in a Japanese population [33]; also, in Iceland, exposure to cristobalite leads to an increase in sarcoidosis incidence [34]. These findings give rise to the hypothesis that sarcoidosis is multigenetic and multifactorial in nature.

A further factor to be considered is the heterogeneity of patient groups used in the different studies with respect to the relative proportions of the different clinical manifestations of the disease in different cohorts, which could influence the results of case-control studies. The fact that environmental phenocopies of sarcoidosis exist complicates its genetic analysis. Berylliosis, an occupational disorder seen in up to 5% of beryllium-exposed workers, is a complete phenocopy of sarcoidosis with a distinct susceptibility locus in the MHC II, the HLA-DPB Glu69+ allele [35]. Moreover, other anorganic compounds (zirconium, aluminum, cristobalite [36]) or even bioaerosols [37] might cause the manifestation of granulomatous disorders presenting as phenocopies of sarcoidosis [38]. These considerations support the notion that sarcoid-like granuloma formation represents a final immunopathological pathway induced by different biological agents [1].

Genetic factors as potential predictive markers in sarcoidosis

Apart from contributing to susceptibility to sarcoidosis, it is also probable that genetic factors may be important in defining the pattern of disease presentation and progression as well as its overall prognosis.

Early indications for the involvement of inherited host factors influencing the clinical expression of sarcoidosis were reported by Smith *et al.* [39], who showed that HLA-B8 significantly correlates with resolution of the disease. In fact, the association of the B8-DR3 genotype has since been associated with good prognosis in a number of studies [40,41]. An association of HLA-DR3 was also found with acute onset of disease (sarcoid arthritis, Löfgren syndrome) in a series of investigations [40,42,43]. This is remarkable in conjunction with the aforementioned studies since acute onset of sarcoidosis usually leads to resolution of disease and is thus considered a sign for good prognosis. In this setting, an interesting finding was that the TNFA2 allele, which is in strong linkage disequilibrium with HLA-DR3 [17], was also shown to significantly correlate with Löfgren syndrome [44]. HLA-DR3 also showed additional correlations with resistance to severe, long-standing disease and predisposition to spontaneous remission [45] and good outcome [46]. A 10-year prospective study substantiated these previous observations by showing a correlation of DR3 with good prognosis and DR2 with chronic disease, thus propagating HLA-DR typing as a prognostic tool in predicting the outcome of disease [5].

Owing to its strong linkage to A1, B8, DR3, the uncommon TNFA2 allele may contribute to associations of this haplotype. We therefore investigated both the HLA-DR and TNF loci in patients with sarcoidosis [47]. The results demonstrated that this genetic analysis clearly discriminated patients exhibiting Löfgren syndrome from sarcoidosis patients not presenting with this syndrome and thus being more prone to developing chronic disease. It was found that, in the Löfgren syndrome patient group, the TNFA2 and the HLA-DR3 alleles were represented significantly more, with a highly significant relative risk for developing Löfgren syndrome associated with the possession of the TNFA2 or HLA-DR3 allele, or of both alleles. In the non-Löfgren syndrome patient group, the phenotype expressing HLA-DR2 and lacking TNFA2 was represented significantly higher than in the Löfgren syndrome patient group.

Only the genotyping of both the TNFA and the HLA-DR locus (combined genotypes) allowed the discrimination between the two sarcoidosis patient subgroups of Löfgren syndrome and non-Löfgren syndrome [47]. Interestingly, both DR3 and TNFA2 are correlated with increased TNF- α levels, and DR2 and TNFA1 are correlated with lower TNF- α production levels [13,48]. The results of this combined genetic analysis thus imply that genetic predisposition for TNF- α production may play a role in the pathogenesis of sarcoidosis.

Supplementary statistical analysis of these published patient data [47] was recently performed to resolve which allele confers the greater risk (TNFA or HLA-DR) for expression of either disease phenotype and to determine whether disease haplotypes in correlation to the different disease courses can be defined [49]. The results essentially demonstrated that it seems very probable, with respect to this patient group and the discrimination between presentation with or without Löfgren syndrome, that the risk for developing or not developing Löfgren syndrome is conferred not by these two genetic traits alone, but that other elements possibly in linkage disequilibrium might contribute to the development of these different disease phenotypes. In addition, the construction of virtual haplotypes by means of a statistical calculation [49] showed that the DR3.TNFA2 haplotype was significantly associated with Löfgren syndrome and that the DR2.TNFA1 haplotype was associated with the non-Löfgren syndrome patients, giving substance to the hypothesis that haplotypes, rather than single genes interacting with each other, are a highly probable explanation for these correlations.

As a consequence, genetic analyses may have the potential of discriminating sarcoidosis patient subsets according to the clinical course of disease. The use of applying these genetic traits as predictive and potential prognostic markers in sarcoidosis must of course be substantiated

and investigated in prospective studies since, for example, although Löfgren syndrome usually heralds a self-limiting course of sarcoidosis with spontaneous resolution, recurrences may occasionally occur and may even develop into chronic sarcoidosis of the lung [3,4].

Questions addressed in this context were whether genotypes shown in the literature to be related to higher TNF- α production levels [14] lead to higher TNF- α levels in a disease setting, and whether assessment of TNF- α levels is of prognostic value. For the latter aspect, a study was performed in which patients were divided into a group with and a group without indications for therapy, with evaluation of disease course and measurement of release of TNF- α from cultured alveolar macrophages approximately 6 months after diagnosis [11]. Interestingly, despite both patient groups having significantly higher TNF- α levels compared with controls, patients without indications for therapy with a high level of TNF- α release had a significantly greater risk of disease progression than did those with normal TNF- α release. This finding thus sets a case for TNF- α release being a suitable parameter for predicting disease course, but with high levels correlating with disease progression, which seems to stand in contradiction to the genotyping findings. Since genotyping data for these patient groups were not obtained and since the patient cohorts differ, however, no direct comparison can be made to resolve this apparent contradiction.

A further study correlated TNF- α levels with TNF genotypes in a sarcoidosis patient cohort [10]. Although no significant differences in TNF- α release were found between carriers and non-carriers of alleles correlated with higher TNF- α production, there was nonetheless a trend to higher production levels in carriers of the TNFA2 allele. This patient cohort again differed from that in which the correlation with Löfgren syndrome and TNFA2/HLA-DR3 was established. In addition, the cohort did not include patients with Löfgren syndrome and only one of 44 patients was TNFA2 homozygous (in contrast to eight of 101 in the former study). A major question that thus remains to be resolved is whether the correlations on the genetic level of alleles associated with TNF- α production with disease course in sarcoidosis are in any way reflected by correlations with actual TNF- α production levels. Since investigations performed to find correlations of TNF-relevant gene polymorphisms with TNF- α production levels have also not produced clear cut results [14], it may well be that in this case multiple genetic factors acting in concert must also be considered.

Studies performed in Japanese patients with sarcoidosis further complicate the matter. Two recent studies have also shown correlations with sarcoidosis disease phenotypes and gene polymorphisms implicated in TNF- α production levels. In one study, the TNFA2 allele, associated

with higher TNF- α secretion levels, was found to be significantly associated with cardiac sarcoidosis, implicating a role for this allele in developing this more severe form of the disease [50].

Yamaguchi *et al.* [51] performed a first prognostic study on 110 patients with a mean follow-up period of 67 months to evaluate the potential prognostic value of the TNFA and TNFB polymorphisms. Interestingly, in this cohort, which consisted of patients with a relatively benign course not requiring corticosteroid therapy, a significant correlation with prolonged disease course was found with the TNFB1 allele. Since TNFB1 is in strong linkage disequilibrium to TNFA2 and correlates with high TNF- α production levels, these results stand somewhat in contradiction to the analyses correlating TNFA2 with a benign disease course.

Despite the fact that the correlations found with TNFA2 were not performed in a prognostic study, however, one should consider the different patient groups both with respect to the ethnic background and the disease phenotypes involved. It is striking, for instance, that in the Japanese control group the TNFA allele frequency (TNFA1/TNFA2, 0.99/0.01 [51]) differs markedly from that of the Caucasian control group (TNFA1/TNFA2, 0.81/0.19 [44]), and that HLA-DR3 specificities (in strong linkage with TNFA2) in Caucasian and Asian populations also markedly differ (Asian, 0.05; Caucasian, 0.10 [19]), indicating that a direct comparison of these differing results is not possible. In conclusion, however, all these studies provide evidence for the assumption that gene polymorphisms influencing TNF- α production levels contribute to disease course in addition to determining susceptibility to sarcoidosis.

Concluding remarks

In summary, data from the literature indicate that genetic polymorphisms play a role in regulating TNF- α levels, which in turn may influence the immune response of an individual and, finally, the course and outcome of diseases. It appears very probable that different polymorphisms shown to correlate with TNF- α production, and possibly new genetic traits not yet defined, act synergistically on TNF- α production levels. This statement is also underlined by the fact that, for instance, investigations on variations in TNF- α production in correlation with the -308 TNF gene polymorphism have given disparate results, therefore implying that more than one genetic factor may be involved in potentiating effects.

A first point to consider in searching for additional genes influencing TNF- α production is to look for genes in strong linkage disequilibrium, as for instance HLA-DR3, but other genes have also been described and should be taken into consideration [14]. In addition, the availability of sequence

data of the MHC [52], in which the important genes pertinent to TNF- α production are localized, holds the promise of discovering new polymorphisms influencing TNF- α production and thus potentially playing a role in disease susceptibility and disease course. The application of new technologies (DNA arrays) combined with new insights into gene linkages, single nucleotide polymorphisms and extended haplotypes of the MHC may aid in closer defining genetic influences on TNF- α production.

With respect to defining disease course in sarcoidosis in correlation with genetic traits, the summary presented implies that it may be worthwhile to investigate TNF- α -relevant polymorphisms for two reasons. First, since the MHC locus in which the TNF genes are located has recently been shown to be the most significant locus associated with sarcoidosis [24,25] and, second, since two MHC loci alone (HLA-DR and TNFA) already allow a significant discrimination between two patient subgroups to be made [47,49], thus holding the promise of functioning as potential predictive markers and perhaps in defining clinical subsets for this disease.

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