Perspective

Nomination of a Candidate Susceptibility Gene in Sarcoidosis

The Complement Receptor 1 Gene

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Sarcoidosis likely occurs in a genetically-susceptible host following exposure to some airborne antigen. Although epidemiologic studies seeking to identify the exposure responsible for sarcoidosis have been ongoing since the 1940s, the etiology remains unknown. Recently, the multicenter study ACCESS (A Case Control Etiologic Sarcoidosis Study) (1) has suggested a few potential environmental risk factors such as bioaerosols, but more importantly confirmed that sarcoidosis is unlikely to be due to a single predominant airborne antigen. Although finding the environmental cause has so far remained elusive, progress has been made in understanding genetic susceptibility in sarcoidosis.

Gene Search in Sarcoidosis

Racial differences in disease incidence (2) and familial clustering of cases (3) support that a genetic susceptibility to sarcoidosis exists. In fact, race and family history are the two strongest risk factors for sarcoidosis (4). The identification of disease genes that cause simple Mendelian disorders has spurred on gene searches in complex disorders such as sarcoidosis. Complex disorders do not follow a clear Mendelian mode of inheritance and generally involve both environmental factors and several interacting genes each with only weak to moderate effect. Two main approaches to identify disease genes in complex disorders are the positional candidate and the functional candidate gene approach.

For the positional candidate approach, a genome screen by linkage analysis is used to identify linked chromosomal regions and then potential candidate genes in the linked regions are identified by mining the human genome database. The identified candidate genes are further scrutinized by mutational or polymorphism analysis. A variation on this strategy is to evaluate a chromosomal region because it is rich in potential disease genes. Using affected sibling pair linkage analysis, we found that the inflammatory bowel disease (IBD)/Blau syndrome locus on chromosome 16q could be excluded from containing a susceptible gene conferring moderate risk for sarcoidosis (5). This chromosomal region was chosen because Blau syndrome, an autosomal dominant disorder, is quite similar to childhood sarcoidosis (6). The gene from this IBD/Blau syndrome region, CARD15, has been identified with CARD15 mutations found in both patients with Blau syndrome and in patients with Crohn's disease (7).

The major histocompatibility complex region (MHC) on chromsome 6p has long been thought to be important in sarcoidosis (8, 9). Schurmann and colleagues, in a sample of 122 siblings from 55 German nuclear families, found evidence for linkage to sarcoidosis (10). A follow-up genome scan by these same investigators using a slightly larger sample of families yielded several other chromosomal regions with suggestive evidence for linkage (11). At present, the MHC region remains the most intensively evaluated linked chromosomal region. The region of interest is $\sim$ 5 million megabases long (Figure 1), with several potential candidates present; however, the HLA-DRB1 and HLA-DQB1 genes show the most promise (12–17).

In the U.S., the sarcoidosis genetic linkage analysis consortium (SAGA) has planned a linkage study that focuses on African Americans. SAGA will perform a genome-wide scan using 360 African American sib pairs and their family members. The SAGA study has focused on African Americans because in the United States African Americans are more likely to report a family history of sarcoidosis (18), present at an earlier age, and have more severe disease (19). The 400-marker genome screen on the first half of this sample is now being analyzed. Those linked regions found in the German family study (11) that are confirmed by SAGA would be prime targets for candidate gene evaluation.

Rather than choosing candidates based on their chromosomal position, the other approach is to choose candidates based on a gene's function and how that gene might fit into disease pathophysiology. An attractive candidate should make biologic sense. Most often investigators rely on the case control study design to determine if a candidate gene is associated with disease. Table 1 lists recently reported candidate genes associated with sarcoidosis using a case control study design. Zorzetto and coworkers report the results of such an analytic approach in this issue of the *AJRCMB* (20).

How Appealing a Candidate Gene is Complement Receptor Type 1?

The removal of immune complexes from blood depends on complement receptor (CR)1 function. CR1 (alias CD35 antigen, C3-binding protein, complement component [3b/4b] receptor-1) is a widely distributed membrane glycoprotein present on polymorphonuclear leukocytes, macrophages, B

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Abbreviations: A Case Control Etiologic Sarcoidosis Study, ACCESS; complement receptor 1, CR1; inflammatory bowel disease, IBD; major histocompatibility complex, MHC; sarcoidosis genetic linkage analysis consortium, SAGA; systemic lupus erythematosus, SLE.

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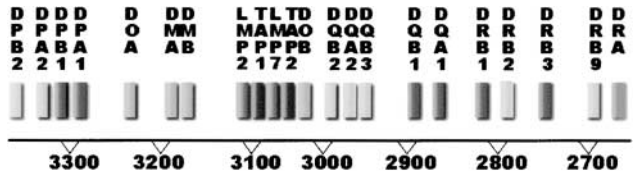

Figure 1. Map of MHC region linked to sarcoidosis. Distance given in kilobases.

lymphocytes, some T lymphocytes, dendritic cells, and erythrocytes (21–25). Immune complexes bound to CR1 are transferred to phagocytes as erythrocytes traverse the liver and spleen (26, 27). Immune complex clearance rates correlate to CR1 density. Low expression of erythrocyte CR1 is associated with impaired immune complex clearance and complex deposition outside the reticuloendothelial system (28–30). It is these extrareticuloendothelial immune complex deposits that incite local inflammatory responses and presumably granuloma formation (Figure 2). Examples of diseases where CR1 density have been evaluated include systemic lupus erythematosus (SLE) and HIV infection. Erythrocytes from patients with SLE (31) and patients with HIV(32) share an acquired reduction of CR1 expression.

CR1 density on erythrocytes is controlled by a codominant autosomal gene. Two alleles, associated with either high (H allele) or low (L allele) CR1 expression, are detected by a *Hin*dIII polymorphism located in intron 27 (33). Individuals homozygous for the H allele can have more than 1,000 CR1 molecules per erythrocyte; those with the L allele have fewer than 200. Heterozygous individuals have intermediate CR1 expression. Correlation between the *Hin*dIII RFLP and CR1 expression on erythrocytes has been confirmed in multiple populations (34) including African Americans (35). Differences in H and L allele frequencies have not been associated with susceptibility to immune diseases such as SLE (36–38) or rheumatoid arthritis (39), or to severe malaria (40).

What Is the Evidence that Immune Complexes Play a Role in Sarcoidosis?

All models of immune granuloma formation so far support that the first step begins with the processing of an antigenic agent by antigen-presenting cells. The antigen is then presented to antigen-specific T lymphocytes in the context of class II MHC molecules. The T cell then orchestrates the accumulation and differentiation of mononuclear phagocytes. Early fibrotic changes, fibroblast accumulation, and collagen deposition occur at the periphery of the granuloma. The nature of the initiating antigenic agent and whether or not it is complexed to immunoglobulin are simply not known.

That immune complexes may be involved in sarcoidosis was suggested in the early 1970s. In a series involving 3,676 patients from 11 cities around the world, James and coworkers (41) reported that an elevation in serum levels of γ-globulin, above 3.5 g/100 ml, was found in 23–96% of patients. There was an increase in all immunoglobulin classes, but IgG was the most consistently and persistently elevated (41, 42). Following these initial studies, several groups reported that immune complexes were present in 23–100% of patients. The wide variation in results was explained in part by the different sensitivities of the techniques used among these studies to identify immune complexes. These techniques included tests such as the platelet aggregation test, the C1q assay, rheumatoid factor radioimmunoassay, and the use of Raji cells. Selroos and colleagues (43), using five techniques to detect immune complexes in 33 sarcoid patients, found positive results in all patients during some stage of their disease. Furthermore, positive correlations to immune complex levels have been described with disease activity, extrathoracic manifestations, and disease duration (44–46). So it appears that immune complexes are always present in sarcoidosis depending on when and how you look.

A central question remains whether immune complexes play any direct causative role in granuloma formation. Immune complexes are not consistently found in granulomas (47–49), but the immune complex could be cleared by the time granulomas are sampled. Evidence does support that immunoglobulin production is locally enhanced at sites of disease activity. This was demonstrated by an increased number of plasma cells at sites of granuloma formation (50). *In vitro*, IgG immune complexes are a potent stimulus for alveolar macrophages to secrete cytokines (51, 52) and angiotensin-converting enzyme (ACE) (53).

TABLE 1

Candidate genes associated with sarcoidosis in case control studies

	Candidate gene	Reference
ACE	Angiotensin-converting enzyme	(62, 63)
CCR2	C-C chemokine receptor 2	(64)
CFTR	Cystic fibrosis transmembrane regulator	(65)
HLA-B		(66)
HLA-C		(66)
HLA-DPB1		(14,Lympany, 1996 #110)
IL-1	Interleukin-1	(67)
NRAMP	Natural resistance–associated macrophage protein	(68)
TAP2	Transporter associated with antigen processing 2	(69)
TNF-α	Tumor necrosis factor-α	(70)
TNF-β	Tumor necrosis factor-β	(71)
VDR	Vitamin D receptor	(72)

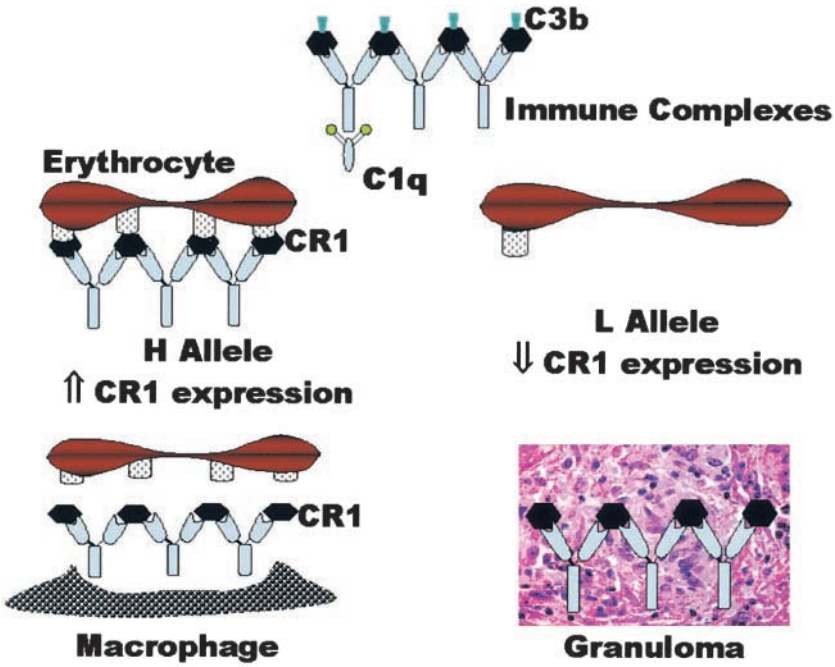

Figure 2. CR1 and immune complex clearance.

Case-Control Association Studies

The sarcoidosis literature is replete with case-control association studies that often conflict with one another. One potential explanation for these inconsistent results is that different genetic mechanisms lead to susceptibility in different populations. This could explain the African-American predominance of disease in the United States, but for genetic variation that predates human history, such as most of the variation found in the MHC gene region, one would not expect drastically different genetic associations between ethnic groups. A more likely explanation for the discrepancy among studies is the differences in sampling methodology. Although setting a more stringent level of statistical significance can protect against type I errors, there are limited statistical corrections for a poorly sampled control group. Population stratification arising from case and control groups with different genetic backgrounds can result in both false positive and false negative genetic associations. Potential statistical methodologies involving the typing panels of unlinked polymorphic markers have been proposed, but have not yet been widely been applied (54–56). The alternative approach to guard against population stratification is family-based association studies (57–59). Sampling families is generally more difficult than cases and controls, but this methodology has been applied successfully in a number of early-adult age onset disorders, including sarcoidosis (10, 60, 61). Ideally, in the functional candidate gene approach, case-control associations should be confirmed in family-based studies. However, the relative simplicity of case control studies compared with family studies has made this a rare occurrence. In lieu of family-based studies of CR1, the limitations of the case-control approach needs to be taken into consideration in the findings reported by Zorzetto and coworkers.

CR1 as a Candidate Gene in Sarcoidosis

A final consideration regarding the putative association between CR1 and sarcoidosis is that from a pathophysiologic view, a CR1–sarcoidosis association makes sense, but based on available linkage data, CR1 would not have been selected as a potential candidate. CR1 is located on chromosome 1q32. Schurmann and colleagues reported one minor peak suggesting linkage on chromosome 1 at marker D1S166, 100 megabases away from CR1 (essentially unlinked to 1q32). The failure of linkage studies to corroborate case–control associations of candidate genes does occur. If the relatively modest 3-fold increase in sarcoidosis risk for the GG genotype of CR1 gene that Zorzetto and collegues have estimated is accurate, it would likely not be detectable in a genome scan linkage analysis of moderate sample size. The results of Zorzetto and coworkers and the recent linkage studies of sarcoidosis in a German population exemplify the need for a comprehensive approach in the genetic dissection of complex disorders.

Although CR1 is now nominated as a candidate gene in sarcoidosis, it should be retested accounting for potential population stratification using population-specific alleles, or retested in family-based association studies. Chromosome 1q32 should be scrutinized in the larger linkage study planned in African Americans. Using both linkage and association study designs to search out all possible genetic susceptibilities that may exist in sarcoidosis may eventually give us a more complete picture of this puzzling disorder.

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