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Complete complement deficiency in a large cohort of familial systemic lupus erythematosus

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Abstract

Genetic complete deficiency of the early complement components such as C1, C2 and C4 commonly results in a monogenetic form of systemic lupus erythematosus (SLE). However, previous studies have examined groups of complete complement deficient subjects for SLE, while a familial SLE cohort has not been studied for deficiencies of complement. Thus, we undertook the present study to determine the frequency of hereditary complete complement deficiencies among families with two or more SLE patients. All SLE patients from 544 such families had CH50 determined. Medical records were examined for past CH50 values. There were 66 individuals in whom all available CH50 values were zero. All but four of these had an SLE-affected relative with a non-zero CH50; thus, these families did not have monogenic complement deficient related SLE. The four remaining SLE-affected subjects were in fact two sets of siblings in which 3 of the 4 SLE patients had onset of disease at <18 years of age. Both patients in one of these families had been determined to have C4 deficiency, while the other family had no clinical diagnosis of complement deficiency. In this second family, one of the SLE patients had had normal C4 and C3 values, indicating that either C1q or C2 deficiency was possible. Thus, only 2 of 544 SLE families had definite or possible complement deficiency; however, 1 of 7 families in which all SLE patients had pediatric onset and 2 of 85 families with at least 1 pediatric-onset SLE patient had complete complement deficiency. SLE is found commonly among families with hereditary complement deficiency but the reverse is not true. Complete complement deficiency is rare among families with two or more SLE patients, but is concentrated among families with onset of SLE prior to age 18.

Introduction

Systemic lupus erythematosus (SLE) is an autoimmune disease of uncertain etiology that is complex in multiple aspects (1). At the clinical level, patients manifestation a wide array of disorders ranging from immediately life threatening disease of major organs such as heart or lungs or kidney to chronic but serious disease of organs such kidney or joints or skin. Manifestations also include mild abnormalities found only at laboratory examination such a lymphopenia. The disease is considered autoimmune largely because of the presence in the serum of antibodies binding self-structures (2). Virtually all patients have antinuclear antibodies, but many patients also have antoantibodies binding ribonucleoprotein particles such as the Ro (or SSA)/La (or SSB) complex or the spliceosome (RNP and Sm particles). Similar to the clinical disease, presence of these and other atuoantibodies is highly variable among SLE patients.

SLE is also complex in terms of its genetics (3–5). While the greater majority of patients do not have a family member with disease, evidence of the importance of genetics is shown by the risk of disease among first degree relative, which is 2%–5%. In addition, the concordance rate of identical twins is up to about 25% (6). Over the last few years, first using genetic linkage and now mostly with genetic association, the genes imparting susceptibility to SLE are being identified (3–5,7). Most are common alleles found in the general population and increase the risk of SLE less than 2-fold. There may be a few dozen genes that contribute to the complex population risk of the disease.

However, there are exceptions to SLE being a complex genetic illness with contributions from many genes that impart modest risk. In fact, there are patients with a monogenetic form of the disease in which genetic deficiency of early complement components results in a high likelihood of SLE (reviewed in 8). Among humans with complete deficiency of C2, about 75% of subjects ever described have SLE. Meanwhile, humans with complete C1q deficiency have had SLE in about 90% of reported subjects. Deficiency of C4, whose gene is found on chromosome 6 in the MHC III region, imparts a risk of SLE well above the population but lower than that found in either C2 or C1q deficiency with about 10% of those with complete C4 deficiency having SLE. Despite data that cohorts of complement deficient patients have SLE, study of cohorts of SLE patients for complement deficiencies have not been performed.

We have been identifying, characterizing and storing samples on families with 2 or more SLE patients for almost 20 years. We undertook the present study to determine the presence of genetic complement deficiencies among this large collection of familial SLE.

Methods

Patient collection methods

Families with two or more SLE patients were identified through the Lupus Family Registry and Repository at the Oklahoma Medical Research Foundation (9). These patients were collected from a multicenter effort expanding over the US, Canada, Puerto Rico, Virgin Islands and a few overseas collaborations. All subjects were enrolled and consented under protocols approved by the Institutional Review Boards of Oklahoma Medical Research Foundation and the University of Oklahoma Health Sciences Center. Diagnosis of SLE made by the primary physicians was confirmed by questionnaire, interview and review of available medical records by a physician, physician's assistant or specially trained nurse. Patients thus enrolled met at least four of the 11 American College of Rheumatology revised classification criteria for systemic lupus erythematosus (10,11). Putatively SLE-unaffected family members were screened for SLE-related manifestations by questionnaire, and further interview if indicated, in order to uncover undiagnosed SLE. Family trees were recorded. Typing at 308 microsatellites was performed as previously described (9). Relationships were confirmed by examination of Mendelian inheritance of markers using the statistics generated by RELTEST, a feature of the S.A.G.E. 4.0 package, version Beta 3 (12,13). Pediatric onset SLE was defined as clinical diagnosis prior to the age of 18.

Serology

All SLE patients underwent serologic testing in the Clinical Immunology Laboratory at Oklahoma Medical Research Foundation. This included measurement of total complement hemolytic activity, or CH50, by determination of the degree of hemolysis of sheep erythrocytes coated with anti-erythrocyte antibodies (14). Instructions supplied by the manufacturer were followed for this assay (EZ Complement, Diamedix Corporation, Miami, Florida, USA).

Complement measurement

C1q measurement: Serum C1q concentrations were quantified in triplicate using a sandwich ELISA (15).

Analysis of genomic DNA for type I C2D

The patients were screened for type I C2 deficiency in accordance with methods previously described. (16–18). Genomic DNA was isolated from the buffy coat fraction of blood samples collected in EDTA using standard procedures. The genomic DNA was then subjected to PCR using the oligonucleotide pair, 5'-GCCTGGGCCGTAAAATCCAAAT-3' and 5'-GCACAGGAAGGCCTCTGCTGCA-3', which was designed to amplify exon 6 of the C2 gene and its downstream boundary (16). Agarose gel electrophoresis of PCR products amplified from normal samples will yield a fragment of 174 bp while the type I C2D alleles should yield fragment of 146 bp. All oligonucleotides were synthesized and obtained from IDT technologies.

Further Record Review

Medical records of selected patients were further reviewed by one of us (RA) to determine previous complement studies.

Results

A total of 544 families with at least two SLE patients were identified, containing 1190 total SLE patients. In order to enter the study completely, each SLE-affected family member completed an extensive questionnaire and interview, and was shown to meet at least four of 11 ACR SLE criteria. In addition, blood was drawn. Among the 544 families, 251 (46.1%) consisted of affected siblings only. There were 32 families (5.8%) that had only an SLE-affected parent and offspring.

Our strategy to find families in our collection with complete hereditary complement deficiency is summarized in Figure 1. Essentially, we identified families where all the SLE patients had CH50 values of zero, either from testing in our laboratory or in the medical record. In families so identified, complement deficiency is a possibly the, or a, genetic susceptibility factor leading to familial SLE.

Thus, we first examined the CH50 values determined as part of the Lupus Family Registry and Repository. There were 70 SLE patients with a value of zero. Thus, these 70 potentially had a complete deficiency of one of the complement components. Of course, there are in fact several reasons to explain a zero value other than genetic deficiency. For example, SLE patients with active disease may have hypocomplementemia from consumption of complement (8). Also, almost all subjects had their blood samples collected elsewhere and the blood was sent to us by overnight courier. Thus, CH50 might be zero in samples not handled and sent properly because of complement consumption after collection during transportation. Therefore, we examined the available medical records of these 70 SLE patients for previous complement level determinations. We found that four of 70 had non-zero CH50 values. We then concluded that these four did not have complete genetic complement component deficiency.

Among the remaining 66 SLE patients who had a CH50 value of zero in our laboratory and for whom we found no non-zero value in medical records, 62 of these had an SLE-affected relative with a non-zero CH50 value in our laboratory, or in their medical records. Therefore, these families contained at least one SLE patient with measurable CH50; and thus, complete hereditary complement deficiency did not contribute to SLE in these families.

The remaining four SLE patients with a zero CH50 in our laboratory and no non-zero value in medical records were, in fact, contained in two families and, were sets of SLE affected siblings (Figure 1). Thus, these two and only these two families met our criteria for the possibility of hereditary complete complement component deficiency. That is, all the SLE patients in the family had CH50 values of zero.

We further examined the medical records of these four subjects. The manifestations of SLE are shown in Table 1. We found that both SLE patients in Family A had been proven to have complete deficiency of C4 by their treating physicians. In Family B, Patient 1 in Table 1 had normal C4 and normal C3. Patient 2 in Family B had no record of complement component measurement. So, either C2 or C1 deficiency is possible in Family B, but there was no history of angioedema in this family suggesting C1q deficiency. In fact, we evaluated C1q serum levels in both patients from Family B and values were in the normal range. Furthermore, we examined both these subjects for the most common genetic cause of C2 deficiency and found PCR products in both were 176 bps in length (data not shown). Thus, this 28 bp deletion mutation was not present.

Thus, among the 544 families we have collected only one had previously diagnosed hereditary complement deficiency, while one other family has possible deficiency that is not C1q, C3, C4 or the most common C2 deficiency. Even when both are considered, only 0.37% of the SLE families in the LFRF have genetic complement deficiency. Of interest, however, was the age of onset of SLE in these 2 families (see Table 1). In family A, which had confirmed C4 deficiency, both patients had onset before they reached majority, and in Family B one of the two SLE patients had pediatric onset. In only 7 of the 544 families did all the SLE patients have pediatric onset. Further, 1 of these 7 had complete complement deficiency, while only 1 of the remaining 547 did so ($p=0.02$ by Fisher's exact test). Considering families in which at least 1 SLE patient had pediatric onset, 2 of 85 had complete complement deficiency with none ($n=459$) of the remaining had evidence of complete complement deficiency ($p=0.02$ by Fisher's exact test). Finally, we examined individual SLE patients parsed according to pediatric versus adult onset. Three of 103 with onset below age 18 had complement deficiency, while 1 of 1089 adult onset patients had complete deficiency as we have defined it ($p=0.0002$ by Fisher's exact test).

Discussion

In general, SLE falls among a large number of common human illnesses that have a complex genetic susceptibility with many genes increasing risk (19). However, there are patients with a monogenetic form of the illness in that hereditary complete deficiency of early complement components, namely, C1, C2 and C4, causes SLE.(8,20). For example, 93% of subjects with complete C1q deficiency have had SLE while 75% of those with complete C4 have SLE. A smaller percentage, 57%, of persons with C1r and C1s deficiency (combined deficiency is inherited together almost always) have SLE, while only 10% of individuals with complete C2 deficiency have SLE (reviewed in 20 and 21). Of course, even for the latter, the risk of SLE is much higher than the population risk. Nonetheless, the prevalence of hereditary complement deficiency among cohorts of SLE patients, especially familial SLE, has not been well described.

We find that among 544 families in which at least two members have SLE there was only one family with proven hereditary complement deficiency and one other family with possible hereditary complement deficiency. This latter family (Family B) did not have C3, C4 or C1q deficiency. Two distinct molecular mechanisms have recently been shown to cause C2 deficiency (17). In type I C2D, a 28 bp deletion removes 9 bp of exon 6 and 19 bp of the 5' end of the adjoining intron of the C2 gene. This deletion causes skipping of exon 6 during RNA splicing, resulting in a shift of the reading frame and a premature termination codon. Type I

C2 deficiency is in strong linkage disequilibrium with the MHC haplotype HLA-A25, B18, C2Q0, BfS, C4A4, C4B2, Drw2 (18). This extended haplotype occurs in >90% of C2D individuals. We demonstrated by PCR that the SLE patients from Family B do not have type II C2D. Individuals with type II C2D are rare, representing about 7% of all cases of C2 deficiency, and are characterized by a selective block of C2 secretion, leading to the retention of a full-length C2 polypeptide in the intracellular compartment (17). The patients in Family B may have type II C2 deficiency, although this is not proven.

Even when considered both these families, only 0.37% of our SLE families have a monogenetic form of the disease related to complement deficiency. These data are similar to the findings of Ramos-Casals and colleagues (22). These investigators studied complement levels in 597 consecutive SLE patients and 70 primary antiphospholipid syndrome patients. There were two SLE patients with CH50 values consistently zero and normal C3 and C4. Thus, similar to Family B in the present study, at least prior to our further evaluation, these patients may have had either C1q or C2 deficiency. Meanwhile, one APS patient had CH50 values of zero and undetectable C4. We conclude that hereditary complement deficiency is uncommon in familial SLE and no more common than among SLE patients without an affected family member.

However, complete complement deficiency was concentrated in families with pediatric onset. One of 7 (14.7%) families in which all patients had onset before age 18 had complement deficiency. Eight-five of the 544 families had at least SLE patient with onset before age 18, and 2 of these had complete complement deficiency. Thus, among families with pediatric onset SLE, complete complement deficiency is significantly more common than among families where SLE had exclusively adult onset.

The situation in SLE concerning monogenetic versus polygenetic inheritance may be analogous to that seen in type 2 diabetes mellitus and maturity onset diabetes in youth (MODY). There are 5 known genes in which mutations cause MODY. These include glucokinase, which functions as an intracellular glucose sensor, and 4 transcription factors critical to molecular regulation of pancreatic beta cells (23). This knowledge has led to insights into the pathogenesis of type 2 diabetes (24,25). Similarly, the association of complement deficiency to SLE has led to insight regarding the role of defective apoptosis in SLE (see 21). However, again similar to complement deficiency in SLE, only a very small percentage of patients with type 2 diabetes mellitus have a loss of function mutation in one of these genes. Nonetheless, there is mounting evidence that common population alleles of the MODY genes may be risk factors to the vastly more common, routine type 2 diabetes mellitus (25–27). Recently, common population alleles of genes whose protein products are part of the complement system have been implicated in SLE susceptibility (28).

In conclusion, even among families with multiple SLE patients, monogenetic forms of the disease caused by loss of function mutations in complement components are highly unusual; and, thus, almost all of our SLE families have a polygenic form of the disease in which common alleles of several, if not many, genes impart risk. While these monogenetic forms of SLE may prove critical to understanding pathogenesis, these syndromes do not contribute substantially to the burden of disease in the population even among familial SLE, although among families with pediatric onset SLE complete complement deficiency is relatively much more common.

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Figure 1.

Summary of the strategy to find hereditary complement deficiency among families with two or more SLE patients.

Table 1

Clinical manifestation of SLE in the four patients from two different families with either proven complement deficiency (Family A), or putative complement deficiency (Family B)

	Family A		Family B	
	Patient 1	Patient 2	Patient 1	Patient 2
Age at Onset	8	12	14	34
Malar Rash	+	+	+	–
Discoid rash	–	–	–	–
Photosensitivity	+	+	+	–
Oral Ulcers	–	–	–	–
Arthritis	+	+	+	+
Serositis	–	–	–	–
Renal	–	–	–	–
Neurological	–	–	–	+
Hematological	–	–	–	+
Immunological	–	+	+	+
ANA	+	+	+	+
complement				
CH50	0	0	0	0
C3	normal	normal	normal	ND
C4	0	0	normal	ND
C1q	ND	ND	normal	normal
C2 type 1	ND	ND	normal	normal

SLE criteria were defined as in the ACR classification criteria (10,11) Antibodies to extractable nuclear antigens (anti-ENA) were measured by double immunodiffusion in our lab or positive results found in the medical record) ND = not done