The complement system in systemic lupus erythematosus: an update.

Leffler, Jonatan; Bengtsson, Anders; Blom, Anna

Published in:
Annals of the Rheumatic Diseases

DOI:
10.1136/annrheumdis-2014-205287

2014

The complement system in systemic lupus erythematosus – an update.

Jonatan Leffler[1,2], Anders A Bengtsson[3] and Anna M Blom[1,*]

[1]Lund University, Department of Laboratory Medicine Malmö, Division of Medical Protein Chemistry, Inga Marie Nilssons gata 53, 205 02 Malmö, Sweden. [2]Telethon Institute for Child Health Research, University of Western Australia, Division of Cell Biology and Immunology, 100 Roberts Road, Subiaco WA 6008, Australia. [3]Lund University, Department of Clinical Sciences, Section of Rheumatology, Skåne University Hospital Lund, 221 85 Lund, Sweden.

*corresponding author
Anna Blom
Lund University
Department of Laboratory Medicine Malmö
Division of Medical Protein Chemistry
Inga Marie Nilssons gata 53, level 4
205 02 Malmö
Sweden
E-mail: Anna.Blom@med.lu.se
Telephone: +46 40 33 82 33
Fax: +46 40 33 70 43

Key words: systemic lupus erythematosus, inflammation, autoantibodies, complement system, complement inhibitors, c1q

Word count (excl. title page, abstract, references and legends): 3208

Formatted: German (Germany)

ABSTRACT

The complement system plays a major role in the autoimmune disease, systemic lupus erythematosus (SLE). However, the role of complement in SLE is complex since it may both prevent and exacerbate the disease. In this review we explore the latest findings in complement-focused research in SLE. C1q deficiency is the strongest genetic risk factor for SLE although such deficiency is very rare. Various recently discovered genetic associations include mutations in the complement receptors 2 and 3 as well as complement inhibitors, the latter related to earlier onset of nephritis. Further, autoantibodies are a distinct feature of SLE that are produced as the result of an adaptive immune response and how complement can affect that response is also being reviewed. SLE generates numerous disease manifestations involving contributions from complement such as glomerulonephritis and the increased risk of thrombosis. Furthermore, since most of the complement system is present in plasma, complement is very accessible and may be suitable as biomarker for diagnosis or monitoring of disease activity. This review highlights the many roles of complement for SLE pathogenesis and how research has progressed during recent years.

INTRODUCTION

The autoimmune disease, systemic lupus erythematosus (SLE) includes a range of manifestations from skin rashes, chronic fatigue and arthritis to the more severe glomerulonephritis, serositis and neurological involvement. Although improved monitoring and earlier diagnosis have had great impact on prognosis, progress in treatment has not been as significant as for some other rheumatic diseases such as rheumatoid arthritis. SLE still causes disease flares in the patients and leads to irreversible organ damage over time (1). SLE affects approximately 70 per 100 000 but varies between countries, populations and genders with a 6-10 times increased frequency in women (2). Early during the disease, infections pose the largest threat whereas as the disease progresses, vascular diseases such as myocardial infarction or stroke can become up to 10 times more common compared to normal (3).

Autoantibodies directed against DNA and histones (4) are commonly found in SLE are thought to cause many of the manifestations. The autoantibodies induce complement activation but also a general Fc gamma receptor (FcγR) mediated

immune reaction (5). More importantly, the autoantibodies form immune complexes (ICs) that deposit in the kidney glomeruli. These ICs effectively activate the classical complement pathway and cause tissue damage, which leads to lupus nephritis (LN) (6). Interestingly, patients with SLE display elevated levels of autoantibodies several years before outbreak of the disease, which indicates an early loss of tolerance. Often, antibodies against SSA are detected first whereas antibodies against DNA are detected closer to disease outbreak (7, 8).

The complement cascade is a part of the innate immune system (9). Genetic deficiency of the initiator of the classical pathway, C1q predisposes strongly to SLE. Deficiencies or mutations in other complement proteins of the classical pathway, such as C1r, C1s, C4 and C2 also increase the risk although to a lower extent compared to C1q. It was initially suggested that SLE development in C1q deficient patients was due to a reduced ability to clear apoptotic cells since C1q is an important opsonin of such cells. However, new exciting roles for C1q and other complement proteins in SLE have since emerged. In this review we explore the latest developments in complement research, focusing on SLE and highlighting areas where research has progressed during the last couple of years (also summarized in figure 1). We hence aim to provide the reader with an updated view of this exciting field.

Genetic associations

The genetic predisposition is evident in SLE and close relatives to patients have an increased risk of developing the disease. However, the concordance for homozygous twins is only 24% (10) which indicates there is also a strong environmental impact on disease. Apart from genes encoding certain MHC variants (11), genetic deficiencies of many classical pathway components are strongly associated with development of SLE (12) see online supplementary (Table S1). This is paradoxical since complement is thought to contribute to the inflammatory tissue destruction observed in SLE (13). However, the classical pathway of complement is important for preventing SLE but once the disease is established, complement may amplify the disease (14, 15).

C1q associations

Although only less than 70 cases have been reported, almost all patients with C1q deficiency develop SLE. C1q deficiency not only overrides the sex predisposition but

also often causes early onset of the disease in childhood. Two novel mutations in C1qA and the C1qB gene were recently found to cause C1q deficiency (16)(17) adding to previously reported mutations (18). Another mutation in the C1q genes renders a functional defect. The C1q can still bind to apoptotic cells but fails to assemble with C1r and C1s and hence fails to activate complement (19). Interestingly, phagocytosis is also decreased, indicating that other complement proteins than C1q itself (C2, C4, C3) may be important for proper phagocytosis. Common variants of C1q may also be associated with certain SLE manifestations such as photosensitivity and nephritis (20). However, these associations may be population specific and do not associate with SLE diagnosis but only with specific manifestations (21). Restoring C1q levels by plasma transfusion in C1q deficient patient ameliorates the pathology (22) and recently a C1q deficient patient with SLE was cured with bone marrow transplantation reconstituting C1q, which is produced mainly in macrophages (23).

C2 and C4 associations

C2 deficiency has a frequency of 1 in 20 000 and increases the risk of infections and SLE with antibodies against C1q and cardiolipin (24). In the C2 gene, a 28 bp deletion is commonly associated with SLE in Caucasians (25), but not in a small Malaysian population (26). C4 is expressed in two isoforms C4A and C4B. Complete C4 deficiencies are rare but variations in gene copy number are common and affect plasma levels. As a physician, this is important to keep in mind when using C4 levels to monitor disease activity (24). Low gene copy numbers of the C4A gene increase the risk of SLE in a Korean population whereas increased gene copy numbers are protective (27). On the other hand, copy numbers of the C4B gene are not associated with SLE (28). By using multiple cohorts from northern and southern Europe a recent study could however not find any protective effects of C4A. Although they established that complete C4 deficiency was a risk factor, partial C4 deficiency was dependent on its genetic localization, which is close to the strongly associated HLA haplotypes (29).

Complement inhibitors

Mutations in complement inhibitors such as complement factor H (FH) are found in numerous kidney diseases. Mutations in FH and CD46 may also be involved in LN where these lead to an earlier onset of LN in SLE patients (30). Deficiencies of the

FH-related proteins 1-3 have previously been associated with age related macula degeneration and atypical hemolytic uremic syndrome (aHUS) and lead to lower plasma levels of FH-related protein 1 and 3. These deficiencies may also be important in SLE (31). Interestingly, other previously described exonic polymorphisms in FH such as the Y402H are not associated with the SLE (30, 31). The role of the membrane bound CD46 in SLE is not clear and observations range from increased transcripts level in peripheral immune cells (32) to decreased protein expression on neutrophils (33). Membrane bound complement inhibitors not only inhibit complement but may also be involved in lymphocyte proliferation, as observed in $CD59^{-/-}$ mice, which also displayed a more severe disease. This was complement independent and neither deletion of C3 nor blockade of C5 affected the phenotype (34). Similar to this, SLE patients show decreased expression of complement inhibitors on their peripheral immune cells and erythrocytes (33), which may ameliorate the disease (35). In general it appears that a decreased expression of complement inhibitors makes the patient more sensitive to complement activation and may also contribute to the deregulated immune cells observed in SLE.

Complement Receptor 3

Complement receptors bind activated complement fragments and ICs and are hence important to the pathogenesis of SLE. Polymorphisms in complement receptor 3 (CR3) have previously been shown to be associated with SLE (36) and this was recently confirmed (37). One recent publication explores the functional outcomes of the identified haplotype R77H (38, 39) in an *in vivo* setting observing reduced phagocytic abilities by macrophages, neutrophils and monocytes expressing the H-variant but with no apparent difference in CR3 expression, cell migration or release of inflammatory markers (40). Neighboring SNPs could however yield similar effects in neutrophils due to strong linkage disequilibrium that may have additional effects in SLE (41). A difference in cytokine release may be expected since activation of the CR3 H-variant less efficiently prevents TLR7/8 responses in macrophages (42).

C1q antibodies

Autoantibodies against C1q in SLE are commonly observed (43, 44). C1q antibodies could result in decreased C1q levels, mimicking a C1q deficient state with impaired clearance of dying cells (45). Whether the antibodies are directed at C1q or simply

cross-reacting due to antigen resemblance is a matter of debate (46). The level of C1q antibodies is a good predicative marker for LN (47, 48), since the antibodies amplify the effect of C1q-containing immune complexes in the kidneys (49). Recent work identified two linear peptides that are recognized by C1q antibodies and represent functional regions of the C1q-A and B chain (50). The A-chain peptide could inhibit activation of the classical pathway *in vitro*. Others have also identified regions in the globular part of the C1q-B chain as important antibody targets for a small subset of SLE patients (51).

Complement and clearance of dying cells

Apart from causing complement activation and formation of immune complexes, antibodies against complement proteins may also affect opsonization and phagocytosis of apoptotic cells. Impaired clearance of apoptotic cells is believed to be one of the main reasons behind SLE and is a process where complement is crucial. A recent study has found that antibodies against C3 may bind to deposited C3b on apoptotic cells and there prevent phagocytosis in mice (52). However, another study showed that both C4 and autoantibodies facilitated phagocytosis of necrotic cells (53), which is similar to the effect of antibodies directed against histones (4). The state of the dying cells may explain the discrepancy. The phagocytic pathways are key factors in SLE and are additionally dependent on differential expression of Fcγ receptors (54).

C1q opsonizes apoptotic cells (55), however the C1q receptor on the phagocyte is still a matter of controversy (56). A recent study described the scavenging receptor SCARF1 as a C1q dependent receptor for clearance of apoptotic cells (57). C1q is not only an important opsonin but further acts to ensure silent removal of the apoptotic cells by preventing inflammasome assembly (58).

To further prevent unwanted inflammation, complement inhibitors such as and C4b-binding protein are also present on apoptotic cells (59). Autoantibodies against FH however, do not seem to be a common feature in SLE (60) in contrast to in aHUS and rheumatoid arthritis where they affect binding and protection of cellular surfaces in sensitive organs such as kidneys. It has been proposed that binding of FH to the apoptotic cells may affect C1q binding by competing for similar ligands (61).

However such competition was not observed in a study that identified Annexins A2 and A5 as C1q ligands (62) in addition to DNA (63) and phosphatidylserine (64) on the apoptotic cells.

Cellular debris and microparticles also become opsonized with complement and antibodies and are more prominent in SLE (65). Further, the microparticles found in SLE serum contain proteins marking them for clearance (66) and indicates overloaded or less efficient clearance mechanisms in SLE.

Complement interactions with the adaptive immune system

Much of the pathogenesis in SLE is caused by pathogenic autoantibodies and therefore B cells have a central role in the disease. Complement can affect the activation of both B and T cells (67).

B cells

Proper B cell maturation is dependent on negative selection of autoreactive cells by exposure to self-antigens and complement delivers such antigens to B cells (68). Deficiencies in complement hence lead to less exposure of autoantigens to B cells and increase the risk for development of autoreactive B cells, which is observed in SLE (69). Locally produced C4 is also directly involved in the negative selection of autoreactive B cells by inducing anergy and $C4^{-/-}$ mice produce more autoreactive B cells (70). Much of the interaction between complement and B cells is through complement receptor 2 (CR2), which is expressed on B cells (71). In SLE, polymorphisms in CR2 are observed that may alter the expression patterns during B cell development (72). C3-independent binding of DNA to CR2 may be important for generation of DNA antibodies (73) although much remains to be clarified since CR2 expression on B cells is usually lower in SLE (74, 75).

T cells

Lately the importance of complement in T cell regulation has received attention (76). C3d can alter T cell function and C3d deposition was found to cause less intracellular Ca^{2+} release in T cells, which led to increased production of inflammatory cytokines (77). Complement activation products may also, in combination with ICs, bind to and

activate T cells in SLE and may explain why FcRγ activated T cells are often found in SLE patients (78).

IFN-α

Involvement of type I IFN system with elevated serum level of IFN-α is a key feature of SLE that has gained more and more attention. Interestingly, long-term IFN-α treatment may also induce SLE like symptoms. The IFN-α is thought to come from activated plasmacytoid dendritic cells (pDCs) upon stimulation with ICs and IFN-α levels correlates with autoantibody levels (79). Expression is reduced if the ICs contain C1q, which would provide an additional explanation as to why C1q deficiency predisposes to SLE (80). This phenomenon was further confirmed by analyzing the gene expression in pDCs upon IC stimulation with and without C1q (81). The C1q binding protein on the pDC has further been identified as LAIR-1 (82).

Neutrophil extracellular traps

Neutrophil extracellular traps (NETs) are released by activated neutrophils as defense against microbes. NETs consist of chromatin covered with antimicrobial enzymes and constitute an antigen target in SLE. NETs are more easily formed in SLE (83) and additionally some patients, particularly those with LN (84), are not able to degrade NETs (85). NETs can further activate pDCs through TLR7/9, which leads to the IFN-α release that is observed in SLE (86). NETs also activate complement through C1q, which also prevents degradation of NETs both alone and in combination with autoantibodies (87). C1q facilitates clearance of NETs by macrophages and the decreased degradation may therefore be a trade-off for proper phagocytosis (88). The role of NETs in SLE has lately grown into an exciting area of research as reviewed (89).

Complement and lupus nephritis

Complement plays a major role in multiple kidney diseases (90) and so also in LN. An important function for complement is to remove IC from circulation. This is achieved by binding of ICs to complement receptor 1 (CR1) expressed on the erythrocytes (91). In LN, deposits of ICs in the glomeruli are regularly found and these activate complement in the kidneys. As such, this pathologic finding (C4d +

ICs) in kidney biopsy is a strong evidence for diagnosis of SLE. In LN, C4d deposition is common, however, little correlation of C4d-deposition in the kidneys and SLE activity appears to exist (92). This is maybe not surprising since ICs are found in glomeruli in patients even without manifestations indicating LN. C3b-deposition in the kidneys is however a good marker for LN and a MRI based study explored the possibility to non-invasively detect C3b-deposition in kidneys to monitor LN progression in mice (93). Antibodies against DNA and C1q are regularly found in glomerular IC deposits and these antibodies co-localize with antibodies to CRP in a small recent study on kidney biopsies (94).

Complement and thrombosis

A long-term consequence of SLE is an elevated risk for cardiovascular disease. Venous thrombosis may also occur early in SLE and is associated with the presence of antibodies against cardiolipin (95, 96). A recent study in SLE patients has pointed out that increased complement levels correlate with increased levels of HDL in plasma and early signs of atherosclerosis such as thickening of the heart vessel wall (97). This is puzzling since low complement levels are common in SLE patients although the risk for cardiovascular disease is increased. Complement activation products on platelets may instead explain the elevated risk of thrombosis as a consequence of platelet activation (98). Complement may also contribute to thrombosis in antiphospholipid syndrome, which is a common secondary syndrome to SLE. In antiphospholipid syndrome, complement may also be involved in the common complications observed during pregnancy (99).

Complement in diagnostics and therapeutics

Complement levels are easily measured in serum but caution should be taken due to easily occurring *in vitro* activation. Complement serum levels further do not differentiate between consumption and production, which may be important for diagnosis. Despite this they do provide a good and well-established tool to monitor SLE activity, and measurements of C3 and C4 are used worldwide and also included in some disease activity indexes such as SLEDAI. Levels of C1q are less widely used but can be of great value for monitoring LN activity. Disturbingly, there are rather large differences in detection methods as is apparent in a recent comparative study

including also detection of DNA antibodies (100). Instead, complement deposition on immune cells may be used as more robust method to monitor SLE and LN activity (101, 102). The usefulness of these for actual SLE diagnosis may however be questioned. Instead a panel of parameters including C4d-deposition on B-cells and erythrocytes has been suggested (103). Since activation of complement contributes to disease activity, therapeutics such as a monoclonal antibody against C5 (eculizumab) that blocks the pro-inflammatory activation of complement has been tested with promising results in a murine lupus model (104). However, when one injection of eculizumab was given to 24 SLE patients with low disease activity no clinical effect or side effects were observed within 2 months of observation (105). Based on the success of eculizumab in treatment of aHUS (106) and dense deposits disease (107) this and other drugs aiming to inhibit complement such as compstatin (108) may still prove efficient for patients suffering from in particular certain types of glomerulonephritis.

Concluding remarks

Complement plays an important and dual role in SLE. It is crucial to prevent disease but one of the villains once disease is initiated. SLE is most likely a result of a faulty biological network with the main task to clear cellular debris. A large number of molecules and biological pathways are involved in this process, which also includes tolerance induction. Most likely the classical complement pathway is central in this network. Recent C1q research focuses not only on its role as an opsonin but also on its role as a signaling molecule as well as antibody target. In the periphery of the network, amongst many other proteins involved in clearance, the complement receptors and inhibitors tailor the response of the adaptive immune system and the IFN-α signaling pathways. Recent research has focused on finding genetic alterations in these pathways to explain why SLE develops. Together the studies mentioned in this review provide important clues as to what roles complement play in SLE and highlights the diverse roles of complement in the disease. Since complement C5 targeted treatment is available and additional drugs with complement as a target are likely to appear in a near future, research should focus on the suitability of such therapeutics in SLE.

ACKNOWLEDGMENTS

The authors would like to acknowledge Dr. Naomi Scott (Telethon Institute for Child Health Research) for language revision of the manuscript and the following funding bodies: Swedish Research Council (K2012-66X-14928-09-5), Foundations of Kock, King Gustav V´s 80th Anniversary, the Royal Physiographic Society in Lund, Skåne University Hospital, the Swedish Rheumatism Association and Österlund's Foundation**.**

COMPETING INTEREST: None declared.

FIGURE LEGENDS

Figure 1

Schematic overview of some aspects of complement involvement in SLE. This ranges from participation in clearance of apoptotic cells, signaling in B and T cells to the role of complement in lupus nephritis and IFN-α signaling. The main areas covered in this review are indicated in the figure.

REFERENCES

1. Urowitz MB, Gladman DD, Ibanez D, et al. Evolution of disease burden over five years in a multicenter inception systemic lupus erythematosus cohort. Arthrit Care Res. 2012;**64**:132-7.
2. Danchenko N, Satia JA, Anthony MS. Epidemiology of systemic lupus erythematosus: a comparison of worldwide disease burden. Lupus. 2006;**15**:308-18.
3. Manzi S, Selzer F, Sutton-Tyrrell K, et al. Prevalence and risk factors of carotid plaque in women with systemic lupus erythematosus. Arthritis and Rheumatism. 1999;**42**:51-60.
4. Gullstrand B, Lefort MH, Tyden H, et al. Combination of autoantibodies against different histone proteins influences complement-dependent phagocytosis of

necrotic cell material by polymorphonuclear leukocytes in systemic lupus erythematosus. J Rheumatol. 2012;**39**:1619-27.
5. Jovanovic V, Dai X, Lim YT, et al. Fc gamma receptor biology and systemic lupus erythematosus. Int J Rheum Dis. 2009;**12**:293-8.
6. Berden JH, Licht R, van Bruggen MC, et al. Role of nucleosomes for induction and glomerular binding of autoantibodies in lupus nephritis. Curr Opin Nephrol Hypertens. 1999;**8**:299-306.
7. Arbuckle MR, McClain MT, Rubertone MV, et al. Development of autoantibodies before the clinical onset of systemic lupus erythematosus. N Engl J Med. 2003;**349**:1526-33.
8. Eriksson C, Kokkonen H, Johansson M, et al. Autoantibodies predate the onset of systemic lupus erythematosus in northern Sweden. Arthritis Res Ther. 2011;**13**:R30.
9. Ricklin D, Hajishengallis G, Yang K, et al. Complement: a key system for immune surveillance and homeostasis. Nat Immunol. 2010;**11**:785-97.
10. Deapen D, Escalante A, Weinrib L, et al. A revised estimate of twin concordance in systemic lupus erythematosus. Arthritis Rheum. 1992;**35**:311-8.
11. Fernando MM, Stevens CR, Walsh EC, et al. Defining the role of the MHC in autoimmunity: a review and pooled analysis. PLoS Genet. 2008;**4**:e1000024.
12. Truedsson L, Bengtsson AA, Sturfelt G. Complement deficiencies and systemic lupus erythematosus. Autoimmunity. 2007;**40**:560-6.
13. Reid KB, Porter RR. The proteolytic activation systems of complement. Annu Rev Biochem. 1981;**50**:433-64.
14. Sjoberg AP, Trouw LA, Blom AM. Complement activation and inhibition: a delicate balance. Trends Immunol. 2009;**30**:83-90.
15. Cook HT, Botto M. Mechanisms of Disease: the complement system and the pathogenesis of systemic lupus erythematosus. Nat Clin Pract Rheumatol. 2006;**2**:330-7.
16. Namjou B, Keddache M, Fletcher D, et al. Identification of novel coding mutation in C1qA gene in an African-American pedigree with lupus and C1q deficiency. Lupus. 2012;**21**:1113-8.
17. Higuchi Y, Shimizu J, Hatanaka M, et al. The identification of a novel splicing mutation in C1qB in a Japanese family with C1q deficiency: a case report. Pediatric rheumatology online journal. 2013;**11**:41.
18. Schejbel L, Skattum L, Hagelberg S, et al. Molecular basis of hereditary C1q deficiency--revisited: identification of several novel disease-causing mutations. Genes Immun. 2011;**12**:626-34.
19. Roumenina LT, Sene D, Radanova M, et al. Functional complement C1q abnormality leads to impaired immune complexes and apoptotic cell clearance. Journal of Immunology. 2011;**187**:4369-73.
20. Namjou B, Gray-McGuire C, Sestak AL, et al. Evaluation of C1q genomic region in minority racial groups of lupus. Genes Immun. 2009;**10**:517-24.
21. Cao CW, Li P, Luan HX, et al. Association study of C1qA polymorphisms with systemic lupus erythematosus in a Han population. Lupus. 2012;**21**:502-7.
22. Mehta P, Norsworthy PJ, Hall AE, et al. SLE with C1q deficiency treated with fresh frozen plasma: a 10-year experience. Rheumatology (Oxford). 2010;**49**:823-4.
23. Arkwright PD, Riley P, Hughes SM, et al. Successful cure of C1q deficiency in human subjects treated with hematopoietic stem cell transplantation. J Allergy Clin Immunol. 2014;**133**:265-7.

24. Jonsson G, Sjoholm AG, Truedsson L, et al. Rheumatological manifestations, organ damage and autoimmunity in hereditary C2 deficiency. Rheumatology (Oxford). 2007;**46**:1133-9.
25. Sullivan KE, Petri MA, Schmeckpeper BJ, et al. Prevalence of a mutation causing C2 deficiency in systemic lupus erythematosus. J Rheumatol. 1994;**21**:1128-33.
26. Lian LH, Ching AS, Chong ZY, et al. Complement components 2 and 7 (C2 and C7) gene polymorphisms are not major risk factors for SLE susceptibility in the Malaysian population. Rheumatol Int. 2012;**32**:3665-8.
27. Kim JH, Jung SH, Bae JS, et al. Deletion variants of RABGAP1L, 10q21.3, and C4 are associated with the risk of systemic lupus erythematosus in Korean women. Arthritis Rheum. 2013;**65**:1055-63.
28. Yang Y, Chung EK, Wu YL, et al. Gene copy-number variation and associated polymorphisms of complement component C4 in human systemic lupus erythematosus (SLE): low copy number is a risk factor for and high copy number is a protective factor against SLE susceptibility in European Americans. Am J Hum Genet. 2007;**80**:1037-54.
29. Boteva L, Morris DL, Cortes-Hernandez J, et al. Genetically determined partial complement C4 deficiency states are not independent risk factors for SLE in UK and Spanish populations. Am J Hum Genet. 2012;**90**:445-56.
30. Jonsen A, Nilsson SC, Ahlqvist E, et al. Mutations in genes encoding complement inhibitors CD46 and CFH affect the age at nephritis onset in patients with systemic lupus erythematosus. Arthritis Res Ther. 2011;**13**:R206.
31. Zhao J, Wu H, Khosravi M, et al. Association of genetic variants in complement factor H and factor H-related genes with systemic lupus erythematosus susceptibility. PLoS Genet. 2011;**7**:e1002079.
32. Biswas B, Kumar U, Das N. Expression and significance of leukocyte membrane cofactor protein transcript in systemic lupus erythematosus. Lupus. 2012;**21**:517-25.
33. Alegretti AP, Schneider L, Piccoli AK, et al. Diminished expression of complement regulatory proteins on peripheral blood cells from systemic lupus erythematosus patients. Clinical & developmental immunology. 2012;**2012**:725684.
34. Miwa T, Zhou L, Maldonado MA, et al. Absence of CD59 exacerbates systemic autoimmunity in MRL/lpr mice. J Immunol. 2012;**189**:5434-41.
35. Miwa T, Maldonado MA, Zhou L, et al. Deletion of decay-accelerating factor (CD55) exacerbates autoimmune disease development in MRL/lpr mice. Am J Pathol. 2002;**161**:1077-86.
36. Nath SK, Han S, Kim-Howard X, et al. A nonsynonymous functional variant in integrin-alpha(M) (encoded by ITGAM) is associated with systemic lupus erythematosus. Nat Genet. 2008;**40**:152-4.
37. Toller-Kawahisa JE, Vigato-Ferreira IC, Pancoto JA, et al. The variant of CD11b, rs1143679 within ITGAM, is associated with systemic lupus erythematosus and clinical manifestations in Brazilian patients. Hum Immunol. 2013.
38. MacPherson M, Lek HS, Prescott A, et al. A systemic lupus erythematosus-associated R77H substitution in the CD11b chain of the Mac-1 integrin compromises leukocyte adhesion and phagocytosis. J Biol Chem. 2011;**286**:17303-10.
39. Rhodes B, Furnrohr BG, Roberts AL, et al. The rs1143679 (R77H) lupus associated variant of ITGAM (CD11b) impairs complement receptor 3 mediated functions in human monocytes. Ann Rheum Dis. 2012;**71**:2028-34.

40. Fossati-Jimack L, Ling GS, Cortini A, et al. Phagocytosis is the main CR3-mediated function affected by the lupus-associated variant of CD11b in human myeloid cells. PLoS One. 2013;**8**:e57082.
41. Zhou Y, Wu J, Kucik DF, et al. Multiple lupus-associated ITGAM variants alter Mac-1 functions on neutrophils. Arthritis Rheum. 2013;**65**:2907-16.
42. Reed JH, Jain M, Lee K, et al. Complement receptor 3 influences toll-like receptor 7/8-dependent inflammation: implications for autoimmune diseases characterized by antibody reactivity to ribonucleoproteins. J Biol Chem. 2013;**288**:9077-83.
43. Trouw LA, Roos A, Daha MR. Autoantibodies to complement components. Molecular Immunology. 2001;**38**:199-206.
44. Trendelenburg M. Antibodies against C1q in patients with systemic lupus erythematosus. Springer Semin Immunopathol. 2005;**27**:276-85.
45. Gullstrand B, Martensson U, Sturfelt G, et al. Complement classical pathway components are all important in clearance of apoptotic and secondary necrotic cells. Clin Exp Immunol. 2009;**156**:303-11.
46. Franchin G, Son M, Kim SJ, et al. Anti-DNA antibodies cross-react with C1q. J Autoimmun. 2013;**44**:34-9.
47. Yin Y, Wu X, Shan G, et al. Diagnostic value of serum anti-C1q antibodies in patients with lupus nephritis: a meta-analysis. Lupus. 2012;**21**:1088-97.
48. Chen Z, Wang GS, Wang GH, et al. Anti-C1q antibody is a valuable biological marker for prediction of renal pathological characteristics in lupus nephritis. Clin Rheumatol. 2012;**31**:1323-9.
49. Mahler M, van Schaarenburg RA, Trouw LA. Anti-C1q autoantibodies, novel tests, and clinical consequences. Front Immunol. 2013;**4**:117.
50. Vanhecke D, Roumenina LT, Wan H, et al. Identification of a major linear C1q epitope allows detection of systemic lupus erythematosus anti-C1q antibodies by a specific peptide-based enzyme-linked immunosorbent assay. Arthritis Rheum. 2012;**64**:3706-14.
51. Radanova M, Vasilev V, Deliyska B, et al. Anti-C1q autoantibodies specific against the globular domain of the C1qB-chain from patient with lupus nephritis inhibit C1q binding to IgG and CRP. Immunobiology. 2012;**217**:684-91.
52. Kenyon KD, Cole C, Crawford F, et al. IgG autoantibodies against deposited C3 inhibit macrophage-mediated apoptotic cell engulfment in systemic autoimmunity. J Immunol. 2011;**187**:2101-11.
53. Grossmayer GE, Munoz LE, Weber CK, et al. IgG autoantibodies bound to surfaces of necrotic cells and complement C4 comprise the phagocytosis promoting activity for necrotic cells of systemic lupus erythaematosus sera. Ann Rheum Dis. 2008;**67**:1626-32.
54. Orme J, Mohan C. Macrophages and neutrophils in SLE-An online molecular catalog. Autoimmun Rev. 2012;**11**:365-72.
55. Korb LC, Ahearn JM. C1q binds directly and specifically to surface blebs of apoptotic human keratinocytes: complement deficiency and systemic lupus erythematosus revisited. Journal of Immunology. 1997;**158**:4525-8.
56. Nayak A, Pednekar L, Reid KB, et al. Complement and non-complement activating functions of C1q: a prototypical innate immune molecule. Innate Immun. 2012;**18**:350-63.
57. Ramirez-Ortiz ZG, Pendergraft WF, 3rd, Prasad A, et al. The scavenger receptor SCARF1 mediates the clearance of apoptotic cells and prevents autoimmunity. Nat Immunol. 2013;**14**:917-26.

58. Benoit ME, Clarke EV, Morgado P, et al. Complement protein C1q directs macrophage polarization and limits inflammasome activity during the uptake of apoptotic cells. J Immunol. 2012;**188**:5682-93.
59. Trouw LA, Bengtsson AA, Gelderman KA, et al. C4b-binding protein and factor H compensate for the loss of membrane-bound complement inhibitors to protect apoptotic cells against excessive complement attack. J Biol Chem. 2007;**282**:28540-8.
60. Foltyn Zadura A, Zipfel PF, Bokarewa MI, et al. Factor H autoantibodies and deletion of Complement Factor H-Related protein-1 in rheumatic diseases in comparison to atypical hemolytic uremic syndrome. Arthritis Res Ther. 2012;**14**:R185.
61. Kang YH, Urban BC, Sim RB, et al. Human complement Factor H modulates C1q-mediated phagocytosis of apoptotic cells. Immunobiology. 2012;**217**:455-64.
62. Martin M, Leffler J, Blom AM. Annexin A2 and A5 serve as new ligands for C1q on apoptotic cells. J Biol Chem. 2012;**287**:33733-44.
63. Jiang H, Cooper B, Robey FA, et al. DNA binds and activates complement via residues 14-26 of the human C1q A chain. J Biol Chem. 1992;**267**:25597-601.
64. Paidassi H, Tacnet-Delorme P, Garlatti V, et al. C1q binds phosphatidylserine and likely acts as a multiligand-bridging molecule in apoptotic cell recognition. Journal of Immunology. 2008;**180**:2329-38.
65. Nielsen CT, Ostergaard O, Stener L, et al. Increased IgG on cell-derived plasma microparticles in systemic lupus erythematosus is associated with autoantibodies and complement activation. Arthritis Rheum. 2012;**64**:1227-36.
66. Ostergaard O, Nielsen CT, Iversen LV, et al. Unique protein signature of circulating microparticles in systemic lupus erythematosus. Arthritis Rheum. 2013;**65**:2680-90.
67. Fearon DT, Carroll MC. Regulation of B lymphocyte responses to foreign and self-antigens by the CD19/CD21 complex. Annu Rev Immunol. 2000;**18**:393-422.
68. Prodeus AP, Goerg S, Shen LM, et al. A critical role for complement in maintenance of self-tolerance. Immunity. 1998;**9**:721-31.
69. Carroll MC. A protective role for innate immunity in systemic lupus erythematosus. Nat Rev Immunol. 2004;**4**:825-31.
70. Chatterjee P, Agyemang AF, Alimzhanov MB, et al. Complement C4 maintains peripheral B-cell tolerance in a myeloid cell dependent manner. Eur J Immunol. 2013;**43**:2441-50.
71. Holers VM, Kulik L. Complement receptor 2, natural antibodies and innate immunity: Inter-relationships in B cell selection and activation. Mol Immunol. 2007;**44**:64-72.
72. Cruickshank MN, Karimi M, Mason RL, et al. Transcriptional effects of a lupus-associated polymorphism in the 5' untranslated region (UTR) of human complement receptor 2 (CR2/CD21). Mol Immunol. 2012;**52**:165-73.
73. Asokan R, Banda NK, Szakonyi G, et al. Human complement receptor 2 (CR2/CD21) as a receptor for DNA: implications for its roles in the immune response and the pathogenesis of systemic lupus erythematosus (SLE). Mol Immunol. 2013;**53**:99-110.
74. Wilson JG, Ratnoff WD, Schur PH, et al. Decreased expression of the C3b/C4b receptor (CR1) and the C3d receptor (CR2) on B lymphocytes and of CR1 on neutrophils of patients with systemic lupus erythematosus. Arthritis Rheum. 1986;**29**:739-47.

75. Erdei A, Isaak A, Torok K, et al. Expression and role of CR1 and CR2 on B and T lymphocytes under physiological and autoimmune conditions. Mol Immunol. 2009;**46**:2767-73.
76. Kemper C, Verbsky JW, Price JD, et al. T-cell stimulation and regulation: with complements from CD46. Immunol Res. 2005;**32**:31-43.
77. Borschukova O, Paz Z, Ghiran IC, et al. Complement fragment C3d is colocalized within the lipid rafts of T cells and promotes cytokine production. Lupus. 2012;**21**:1294-304.
78. Chauhan AK, Moore TL. Immune complexes and late complement proteins trigger activation of Syk tyrosine kinase in human CD4(+) T cells. Clin Exp Immunol. 2012;**167**:235-45.
79. Weckerle CE, Franek BS, Kelly JA, et al. Network analysis of associations between serum interferon-alpha activity, autoantibodies, and clinical features in systemic lupus erythematosus. Arthritis Rheum. 2011;**63**:1044-53.
80. Lood C, Gullstrand B, Truedsson L, et al. C1q inhibits immune complex-induced interferon-alpha production in plasmacytoid dendritic cells: a novel link between C1q deficiency and systemic lupus erythematosus pathogenesis. Arthritis and Rheumatism. 2009;**60**:3081-90.
81. Santer DM, Wiedeman AE, Teal TH, et al. Plasmacytoid dendritic cells and C1q differentially regulate inflammatory gene induction by lupus immune complexes. J Immunol. 2012;**188**:902-15.
82. Son M, Santiago-Schwarz F, Al-Abed Y, et al. C1q limits dendritic cell differentiation and activation by engaging LAIR-1. Proc Natl Acad Sci U S A. 2012;**109**:E3160-7.
83. Garcia-Romo GS, Caielli S, Vega B, et al. Netting neutrophils are major inducers of type I IFN production in pediatric systemic lupus erythematosus. Sci Transl Med. 2011;**3**:73ra20.
84. Leffler J, Gullstrand B, Jonsen A, et al. Degradation of neutrophil extracellular traps co-varies with disease activity in patients with systemic lupus erythematosus. Arthritis Res Ther. 2013;**15**:R84.
85. Hakkim A, Furnrohr BG, Amann K, et al. Impairment of neutrophil extracellular trap degradation is associated with lupus nephritis. Proc Natl Acad Sci U S A. 2010;**107**:9813-8.
86. Lande R, Ganguly D, Facchinetti V, et al. Neutrophils Activate Plasmacytoid Dendritic Cells by Releasing Self-DNA-Peptide Complexes in Systemic Lupus Erythematosus. Sci Transl Med. 2011;**3**:73ra19.
87. Leffler J, Martin M, Gullstrand B, et al. Neutrophil extracellular traps that are not degraded in systemic lupus erythematosus activate complement exacerbating the disease. Journal of Immunology. 2012;**188**:3522-31.
88. Farrera C, Fadeel B. Macrophage clearance of neutrophil extracellular traps is a silent process. J Immunol. 2013;**191**:2647-56.
89. Knight JS, Kaplan MJ. Lupus neutrophils: 'NET' gain in understanding lupus pathogenesis. Curr Opin Rheumatol. 2012;**24**:441-50.
90. Java A, Atkinson J, Salmon J. Defective complement inhibitory function predisposes to renal disease. Annual review of medicine. 2013;**64**:307-24.
91. Davies KA, Peters AM, Beynon HL, et al. Immune complex processing in patients with systemic lupus erythematosus. In vivo imaging and clearance studies. The Journal of clinical investigation. 1992;**90**:2075-83.
92. Kim MK, Maeng YI, Lee SJ, et al. Pathogenesis and significance of glomerular C4d deposition in lupus nephritis: activation of classical and lectin

pathways. International journal of clinical and experimental pathology. 2013;**6**:2157-67.
93. Sargsyan SA, Serkova NJ, Renner B, et al. Detection of glomerular complement C3 fragments by magnetic resonance imaging in murine lupus nephritis. Kidney Int. 2012;**81**:152-9.
94. Sjowall C, Olin AI, Skogh T, et al. C-reactive protein, immunoglobulin G and complement co-localize in renal immune deposits of proliferative lupus nephritis. Autoimmunity. 2013;**46**:205-14.
95. Palatinus A, Adams M. Thrombosis in systemic lupus erythematosus. Seminars in thrombosis and hemostasis. 2009;**35**:621-9.
96. Zoller B, Li X, Sundquist J, et al. Risk of pulmonary embolism in patients with autoimmune disorders: a nationwide follow-up study from Sweden. Lancet. 2012;**379**:244-9.
97. Parra S, Vives G, Ferre R, et al. Complement system and small HDL particles are associated with subclinical atherosclerosis in SLE patients. Atherosclerosis. 2012;**225**:224-30.
98. Lood C, Eriksson S, Gullstrand B, et al. Increased C1q, C4 and C3 deposition on platelets in patients with systemic lupus erythematosus--a possible link to venous thrombosis? Lupus. 2012;**21**:1423-32.
99. Samarkos M, Mylona E, Kapsimali V. The role of complement in the antiphospholipid syndrome: a novel mechanism for pregnancy morbidity. Semin Arthritis Rheum. 2012;**42**:66-9.
100. Julkunen H, Ekblom-Kullberg S, Miettinen A. Nonrenal and renal activity of systemic lupus erythematosus: a comparison of two anti-C1q and five anti-dsDNA assays and complement C3 and C4. Rheumatol Int. 2012;**32**:2445-51.
101. Li J, An L, Zhang Z. Usefulness of complement activation products in Chinese patients with systemic lupus erythematosus. Clin Exp Rheumatol. 2013.
102. Batal I, Liang K, Bastacky S, et al. Prospective assessment of C4d deposits on circulating cells and renal tissues in lupus nephritis: a pilot study. Lupus. 2012;**21**:13-26.
103. Kalunian KC, Chatham WW, Massarotti EM, et al. Measurement of cell-bound complement activation products enhances diagnostic performance in systemic lupus erythematosus. Arthritis Rheum. 2012;**64**:4040-7.
104. Wang Y, Hu Q, Madri JA, et al. Amelioration of lupus-like autoimmune disease in NZB/WF1 mice after treatment with a blocking monoclonal antibody specific for complement component C5. Proc Natl Acad Sci U S A. 1996;**93**:8563-8.
105. Barilla-Labarca ML, Toder K, Furie R. Targeting the complement system in systemic lupus erythematosus and other diseases. Clinical immunology. 2013;**148**:313-21.
106. Kavanagh D, Goodship TH, Richards A. Atypical hemolytic uremic syndrome. Seminars in nephrology. 2013;**33**:508-30.
107. Vivarelli M, Pasini A, Emma F. Eculizumab for the treatment of dense-deposit disease. N Engl J Med. 2012;**366**:1163-5.
108. Ricklin D, Lambris JD. Compstatin: a complement inhibitor on its way to clinical application. Advances in experimental medicine and biology. 2008;**632**:273-92.

Figure 1

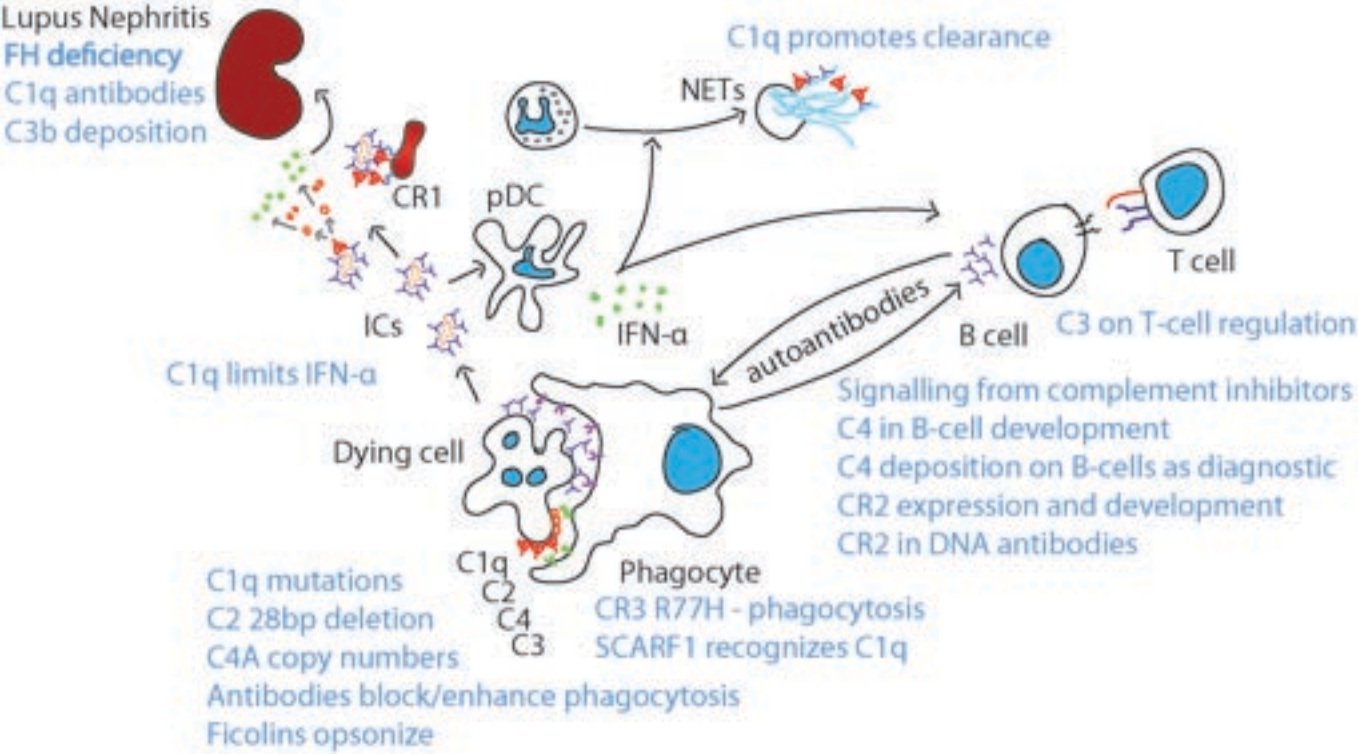
Lupus Nephritis
FH deficiency
C1q antibodies
C3b deposition
C1q promotes clearance
NETs
CR1
pDC
T cell
ICs
IFN-α
B cell
C3 on T-cell regulation
autoantibodies
C1q limits IFN-α
Signalling from complement inhibitors
C4 in B-cell development
C4 deposition on B-cells as diagnostic
CR2 expression and development
CR2 in DNA antibodies
Dying cell
C1q
C2
C4
C3
Phagocyte
C1q mutations
C2 28bp deletion
C4A copy numbers
CR3 R77H - phagocytosis
SCARF1 recognizes C1q
Antibodies block/enhance phagocytosis
Ficolins opsonize

Table S1

Complement protein	Number of cases/ Incidence in general population	SLE penetrance / Odds ratio (OR)	Population	SLE association	Ref.
C1q homozygous deficiency	~70 cases	93%	Varied	Glomerulonephritis, severe SLE	(1)
C1q polymorphisms including rs172378 and rs631090	1/3-1/20	OR 0.7-2.4	Hispanic, African-American	Photosensitivity and nephritis, possible SLE association	(2, 3)
C1r/C1s homozygous deficiency	~20 cases	65%	Varied	Glomerulonephritis, severe SLE	(4)
C4 homozygous deficiency	~30 cases	78%	Varied	Glomerulonephritis, cutaneous, severe SLE	(5, 6)
C4 gene copy number	1/3	None	Europeans	Unclear linkage to MHC	(6)
C2 homozygous deficiency	1/20 000	10-25%	Caucasian, Malaysian	Cutaneous, SLE, unclear in some populations	(7-9)
C3 homozygous deficiency	~30 cases	None	Varied	Glomerulonephritis	(10)
C3 polymorphisms including rs7951 and rs2230201	1/2-1/10	OR 1.4-2	Japanese	Glomerulonephritis, SLE	(11)
MBL, B allele (rs1800450)	1/4	OR 1.4	African, Asian, Caucasian	SLE, infections, cardiovascular manifestations	(12, 13)
MBL promotor polymorphisms including rs11003125 and rs7096206	1/2-1/20	OR ~1-2	North American	a-smith antibody, no SLE association	(12)
FH polymorphism including rs6677604	1/20	OR 1.2	Caucasian, African-American	SLE	(14)
FH and CD46 mutations	rare	Not determined	Caucasian	Glomerulonephritis onset	(15)
CFHR1-3 deletion	1/3	OR 1.2	Varied	SLE	(14)
CR1, Structural variant	1/5-1/10	OR 1.5	Caucasian	SLE	(16, 17)

CR2 polymorphisms including rs3813946, rs1048971, rs17615 and rs4308977	1/2-1/20	OR 0.9-1.5	Caucasian, Chinese	SLE	(18, 19)
CR3, R77H (rs1143679)	1/10	OR 1.8	European, Brasilian	SLE	(20, 21)

Adopted from (22, 23)

Legend

Table indicating how alterations in common complement proteins associate with the risk of developing SLE or particular manifestations. The table shows frequency of the alteration in the general population and SLE penetrance/odds ratio for SLE or clinical manifestation of the genetic alteration. In case of reports of multiple polymorphisms this is indicated and the frequency and odds ratio ranges are shown. For some proteins, only the most common polymorphism is shown. This table does not aim to present all known genetic alterations but aims to provide an idea of the relative importance of the indicated complement proteins for the development of SLE.

References

1. Jlajla H, Sellami MK, Sfar I, et al. New C1q mutation in a Tunisian family. Immunobiology. 2014;**219**:241-6.
2. Namjou B, Gray-McGuire C, Sestak AL, et al. Evaluation of C1q genomic region in minority racial groups of lupus. Genes Immun. 2009;**10**:517-24.
3. Martens HA, Zuurman MW, de Lange AH, et al. Analysis of C1q polymorphisms suggests association with systemic lupus erythematosus, serum C1q and CH50 levels and disease severity. Ann Rheum Dis. 2009;**68**:715-20.
4. Amano MT, Ferriani VP, Florido MP, et al. Genetic analysis of complement C1s deficiency associated with systemic lupus erythematosus highlights alternative splicing of normal C1s gene. Mol Immunol. 2008;**45**:1693-702.
5. Yang Y, Lhotta K, Chung EK, et al. Complete complement components C4A and C4B deficiencies in human kidney diseases and systemic lupus erythematosus. J Immunol. 2004;**173**:2803-14.
6. Boteva L, Morris DL, Cortes-Hernandez J, et al. Genetically determined partial complement C4 deficiency states are not independent risk factors for SLE in UK and Spanish populations. Am J Hum Genet. 2012;**90**:445-56.
7. Jonsson G, Truedsson L, Sturfelt G, et al. Hereditary C2 deficiency in Sweden: frequent occurrence of invasive infection, atherosclerosis, and rheumatic disease. Medicine. 2005;**84**:23-34.
8. Sullivan KE, Petri MA, Schmeckpeper BJ, et al. Prevalence of a mutation causing C2 deficiency in systemic lupus erythematosus. J Rheumatol. 1994;**21**:1128-33.
9. Lian LH, Ching AS, Chong ZY, et al. Complement components 2 and 7 (C2 and C7) gene polymorphisms are not major risk factors for SLE susceptibility in the Malaysian population. Rheumatol Int. 2012;**32**:3665-8.

10. E SR, Falcao DA, Isaac L. Clinical aspects and molecular basis of primary deficiencies of complement component C3 and its regulatory proteins factor I and factor H. Scand J Immunol. 2006;**63**:155-68.
11. Miyagawa H, Yamai M, Sakaguchi D, et al. Association of polymorphisms in complement component C3 gene with susceptibility to systemic lupus erythematosus. Rheumatology (Oxford). 2008;**47**:158-64.
12. Lee YH, Witte T, Momot T, et al. The mannose-binding lectin gene polymorphisms and systemic lupus erythematosus: two case-control studies and a meta-analysis. Arthritis Rheum. 2005;**52**:3966-74.
13. Monticielo OA, Mucenic T, Xavier RM, et al. The role of mannose-binding lectin in systemic lupus erythematosus. Clin Rheumatol. 2008;**27**:413-9.
14. Zhao J, Wu H, Khosravi M, et al. Association of genetic variants in complement factor H and factor H-related genes with systemic lupus erythematosus susceptibility. PLoS Genet. 2011;**7**:e1002079.
15. Jonsen A, Nilsson SC, Ahlqvist E, et al. Mutations in genes encoding complement inhibitors CD46 and CFH affect the age at nephritis onset in patients with systemic lupus erythematosus. Arthritis Res Ther. 2011;**13**:R206.
16. Alegretti AP, Schneider L, Piccoli AK, et al. The role of complement regulatory proteins in peripheral blood cells of patients with systemic lupus erythematosus: review. Cell Immunol. 2012;**277**:1-7.
17. Nath SK, Harley JB, Lee YH. Polymorphisms of complement receptor 1 and interleukin-10 genes and systemic lupus erythematosus: a meta-analysis. Human genetics. 2005;**118**:225-34.
18. Wu H, Boackle SA, Hanvivadhanakul P, et al. Association of a common complement receptor 2 haplotype with increased risk of systemic lupus erythematosus. Proc Natl Acad Sci U S A. 2007;**104**:3961-6.
19. Douglas KB, Windels DC, Zhao J, et al. Complement receptor 2 polymorphisms associated with systemic lupus erythematosus modulate alternative splicing. Genes Immun. 2009;**10**:457-69.
20. Nath SK, Han S, Kim-Howard X, et al. A nonsynonymous functional variant in integrin-alpha(M) (encoded by ITGAM) is associated with systemic lupus erythematosus. Nat Genet. 2008;**40**:152-4.
21. Toller-Kawahisa JE, Vigato-Ferreira IC, Pancoto JA, et al. The variant of CD11b, rs1143679 within ITGAM, is associated with systemic lupus erythematosus and clinical manifestations in Brazilian patients. Hum Immunol. 2013.
22. Carneiro-Sampaio M, Liphaus BL, Jesus AA, et al. Understanding systemic lupus erythematosus physiopathology in the light of primary immunodeficiencies. Journal of clinical immunology. 2008;**28 Suppl 1**:S34-41.
23. Truedsson L, Bengtsson AA, Sturfelt G. Complement deficiencies and systemic lupus erythematosus. Autoimmunity. 2007;**40**:560-6.