Published in final edited form as:

Int J Clin Pract. 2008 June ; 62(6): 852–854. doi:10.1111/j.1742-1241.2008.01745.x.

Whole genome association study results shed light on elusive aetiopathogenesis of systemic lupus erythematosus

V. C. Kyttaris¹ and K. H. Costenbader²

¹Rheumatology, Beth Israel Deaconess Medical Center, Boston, MA, USA

²Brigham and Women's Hospital, Robert B. Brigham Arthritis and Musculoskeletal Diseases Center, Boston, MA, USA

The events that lead to the development of systemic lupus erythematosus (SLE) remain largely elusive. External factors, such as viruses, and endogenous molecules, such as hormones, are hypothesised to lead to the development of the disease in the genetically predisposed person [reviewed in (1)]. Nevertheless, the exact contributions of genetic, epigenetic and environmental factors and how they interact are not understood. On a pathophysiological level (2), clinical SLE develops as a two-step process. First, the tolerance mechanisms that under normal conditions prevent autoimmunity fail to prevent the generation of autoreactive cells or fail to suppress their function. These autoreactive cells, both B and T cells, may be present in the blood and tissues of patients with SLE years before the emergence of clinical symptoms, attested to by the detection of autoantibodies in the sera up to 9 years before disease onset (3). The second step in disease pathogenesis occurs when autoantibodies, immune complexes and abnormally activated immune cells infiltrate target organs, leading to the various clinical manifestations of SLE such as nephritis, arthritis and dermatitis.

Abnormally activated immune cells infiltrate target organs, leading to the various clinical manifestations of SLE

Given our lack of clear understanding of SLE aetiopathogenesis, results from recent whole genome association scans conducted in large populations of SLE patients are all the more exciting. In the past, family-based studies and linkage association studies have allowed the identification of a few regions of the genome with quite strong effects on disease risk, such as the HLA region. New technologies allowing for the simultaneous testing of over 500,000 single nucleotide polymorphisms in huge collections of patients and comparison with those in healthy controls is facilitating discovery of many more loci associated with disease susceptibility. Results of huge collaborative efforts in SLE are reported in the *New England Journal of Medicine* (4) and *Nature Genetics* (5). In the first study, two genetic regions, that surrounding *BLK/C8orf13* on chromosome 8p23.1 and that near the *ITGAM/ITGAX* gene region on chromosome 16p11.22, were identified as strongly associated with risk of SLE. This whole genome association study employed the using the Illumina HumanHap550 platforms on samples from 1311 cases and 1783 controls from the New York Cancer Registry. Results were replicated in a Swedish SLE cohort of 793 and 857 controls. Results reached genome-wide significance with p-values of 1×10^{-10} and 3×10^{-11} respectively.

Of the several newly identified genetic regions associated with SLE in recent genetic studies, these two, *BLK/C8orf13* and *ITGAM/ITGAX*, are especially enticing for their potential roles in SLE pathogenesis. Several variants in the 8p23.1 region were associated with risk of SLE, the strongest of which was from a single nucleotide polymorphism in the interval between the *BLK* gene and the *C8orf13* gene. While *C8orf13* is a ubiquitously expressed gene of unknown function, the *BLK* gene encodes a tyrosine kinase in the src family that signals downstream of the B-cell receptor. The identified polymorphism is upstream of *BLK*, possibly in a promoter region. In separate experiments reported in the same paper, the authors examined gene expression in over 600 EBV-transformed B-cell lines and demonstrated that the SLE-associated risk allele was associated with decreased mRNA transcription of the *BLK* gene. This implies that underexpression of BLK increases the risk of developing SLE. BLK protein levels were not directly measured in SLE patients or controls however. Murine data suggest that the BLK protein is expressed mainly during early stages of B-cell ontogeny, and is gradually downregulated in more mature B cell (6). Similarly, in humans BLK is expressed in immature B cells (pro- and pre-B cells), as well as mature B cells and to a lesser extent plasma cells (7). Interestingly, human BLK is also expressed in the thymus possibly by immature T cells (7). These data suggest a role of BLK in the maturation process of B cells and/or T cells. Indeed, constitutively activated *BLK* in mice mimicked pre-B cell receptor signalling and was able to support maturation of progenitor B cells (8).

Decreased BLK expression could lead to the development of autoreactive B cells producing pathogenic autoantibodies, inflammatory cytokines and might even trigger antigen presentation and co-stimulation to T cells. Contrary to previous beliefs that autoreactive B cells do not exist in normal individuals, we now know that healthy individuals without autoimmune disease have autoreactive B cells as part of their immune repertoire (9). In SLE, these autoreactive B cells become predominant and produce high titres of high affinity IgG autoantibodies. For example, Yurasov et al. (10) found that approximately 50% of B cells from SLE patients were capable of producing autoantibodies, in contrast to around 20% in normal individuals. It is therefore presumed that in SLE, during maturation in the bone marrow from pro-B to pre-B to mature B cells, B cells evade the classic mechanisms of tolerance such as receptor editing, clone deletion and anergy. This is where low levels of BLK may play a role (Figure 1). Decreased or abnormal BLK may prevent proper signal transduction from the B-cell receptor in immature B cells, a process critical for the deletion of autoreactive B cell clones called negative selection. Further studies are needed to elucidate the physiologic role of BLK in human B-cell ontogeny. Studies in SLE patients will need to address whether the levels of BLK are truly decreased and in which subsets of cells (pre-B cells, immature B cells, mature B cells or even T cells). These studies will be able to address whether molecules that coordinate proper selection of B cells influence the emergence of autoimmunity in humans.

The second polymorphism that was strongly associated with SLE risk in the whole genome association study by Hom et al. (4) lies in close proximity to the integrin alpha gene *ITGAM/ITGAX*. *ITGAM* was also found to be associated with SLE susceptibility in other large recent SLE genetic studies reported by Nath et al. (5). Under physiologic conditions, the integrin alpha chain μ (α_M , CD11b) and χ (α_X , CD11c) chains, encoded respectively by *ITGAM* and *ITGAX*, pair with the β_2 integrin protein and form the $\alpha_M\beta_2$ (CD11bCD18) and $\alpha_X\beta_2$ (CD11cCD18) molecules respectively. These heterodimeric proteins are involved in leucocyte adhesion to endothelial cells and the extracellular matrix and thus play a role in the translocation of leucocytes into the tissues. It has been shown that the expression of $\alpha_M\beta_2$ is increased in SLE neutrophils (11). This may signify that changes in the quantity or the structure of $\alpha_M\beta_2$ integrin alter the potential of neutrophils to ingress into areas of inflammation in SLE. The $\alpha_M\beta_2$ integrin, also called complement receptor 3, can also bind complement split products (iC3b) and may play a role in the clearance of complement-coated immune complexes (12, 13). It is therefore possible that changes in the structure or the number of $\alpha_M\beta_2$ molecules

prohibit the correct handling of immune complexes thus predisposing individuals to SLE. In a single case report, a patient with a defect in the iC3b-binding area of $\alpha_M\beta_2$, developed lupus-like cutaneous vasculitis and panniculitis (14). Studies that analyse the expression pattern and the presence of mutations in the $\alpha_M\beta_2$ integrin in SLE are therefore needed.

The findings of whole genome association studies in SLE are exciting for their potential in elucidating underlying mechanisms of disease. Two newly identified genetic regions, *BLK* and *ITGAM/ITGAX*, hold promise in this regard. Both regions are close to genes whose products are expressed in immune cells: *BLK* may be significant in the first step to the development of SLE by altering B-cell selection, while *ITGAM/ITGAX* may participate in the second step by facilitating cell and/or immune complexes accumulation in target organs. SLE is a complex autoimmune disease with multiple subphenotypes that preferentially strikes women of childbearing age and individuals of African, Hispanic and Asian heritage. The story of its aetiopathogenesis is likely not a simple one. These recent whole genome association studies open new avenues of research on intracellular signalling abnormalities in SLE B and T cells, as well as for further large collaborative genetic studies that can be stratified by racial and ethnic background and specific SLE disease subphenotypes.

Disclosures

As Bone and Joint Diseases Associate Editor for the Journal, Karen Costenbader withdrew from the review process and deferred all editorial decisions to Graham Jackson. Dr Costenbader is supported by Arthritis Investigator Award from the American College of Rheumatology and the Arthritis Foundation, and a Building Interdisciplinary Research Collaborations in Women's Health (BIRCWH) award jointly funded by NIMH, NIAID, NICHD, and OD, and the Katherine Swan Ginsburg Memorial Award.

References

1. Krishnan, S.; Chowdhuri, B.; Juang, Y-T.; George, T. Overview of the pathogenesis of systemic lupus erythematosus.. In: George, C.; Tsokos, CG.; Josef, S., editors. Systemic Lupus Erythematosus: a Companion to Rheumatology. Elsevier; Philadelphia, PA: 2007. p. 55-63.
2. Kytтары VC, Juang YT, Tsokos GC. Immune cells and cytokines in systemic lupus erythematosus: an update. *Curr Opin Rheumatol* 2005;17:518–22. [PubMed: 16093827]
3. 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. [PubMed: 14561795]
4. Hom G, Graham RR, Modrek B, et al. Association of systemic lupus erythematosus with C8orf13-BLK and ITGAM-ITGAX. *N Engl J Med* 2008;358:900–9. [PubMed: 18204098]
5. Nath SK, Han S, Kim-Howard X, et al. A nonsynonymous functional variant in integrin- α (M) (encoded by ITGAM) is associated with systemic lupus erythematosus. *Nat Genet* 2008;40:152–4. [PubMed: 18204448]
6. Dymecki SM, Niederhuber JE, Desiderio SV. Specific expression of a tyrosine kinase gene, *blk*, in B lymphoid cells. *Science (New York, NY)* 1990;247:332–6.
7. Islam KB, Rabbani H, Larsson C, et al. Molecular cloning, characterization, and chromosomal localization of a human lymphoid tyrosine kinase related to murine *Blk*. *J Immunol* 1995;154:1265–72. [PubMed: 7822795]
8. Tretter T, Ross AE, Dordai DI, Desiderio S. Mimicry of pre-B cell receptor signaling by activation of the tyrosine kinase *Blk*. *J Exp Med* 2003;195:198.
9. Wardemann H, Yurasov S, Schaefer A, et al. Predominant autoantibody production by early human B cell precursors. *Science (New York, NY)* 2003;301:1374–7.
10. Yurasov S, Wardemann H, Hammersen J, et al. Defective B cell tolerance checkpoints in systemic lupus erythematosus. *J Exp Med* 2005;7:201.

11. Buyon JP, Shadick N, Berkman R, et al. Surface expression of Gp 165/95, the complement receptor CR3, as a marker of disease activity in systemic Lupus erythematosus. *Clin Immunol Immunopathol* 1988;46:141–9. [PubMed: 2961492]
12. Voice JK, Lachmann PJ. Neutrophil Fc gamma and complement receptors involved in binding soluble IgG immune complexes and in specific granule release induced by soluble IgG immune complexes. *Eur J Immunol* 1997;27:2514–23. [PubMed: 9368604]
13. Ohkuro M, Ogura-Masaki M, Kobayashi K, et al. Effect of iC3b binding to immune complexes upon the phagocytic response of human neutrophils: synergistic functions between Fc gamma R and CR3. *FEBS Lett* 1995;373:189–92. [PubMed: 7589463]
14. Witte T, Dumoulin FL, Gessner JE, et al. Defect of a complement receptor 3 epitope in a patient with systemic lupus erythematosus. *J Clin Invest* 1993;92:1181–7. [PubMed: 7690773]

Figure 1.

Potential role of BLK in the survival of autoreactive B cells. The encounter of an immature autoreactive B cell through its receptor with an autoantigen will lead to activation of the cell and triggering of either receptor editing (thus changing the cell into a non-autoreactive B cell) or cell death via apoptosis (negative selection). Thus, the body ensures the elimination of autoreactive B cells at this stage of their development. This process depends heavily on tyrosine kinases (such as BLK and others) that are activated once the B-cell receptor binds to the autoantigen and help propagate the signal to the nucleus. Decreased activity of these kinases (including BLK) may lead to survival of autoreactive B cells and their exit to peripheral lymphoid organs