Impaired Apoptotic Clearance and Clonal Deletion: Onset or Manifestation of Systemic Lupus Erythematosus?

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Abstract
Systemic Lupus Erythematosus (SLE) is a prevalent widespread autoimmune disease where patients present numerous systemic manifestations like rashes, ulcers, hemolytic anemia, fever, thrombocytopenia, lymphadenopathy, and seizures. Although the disease is multifactorial in nature, this article aims to review the involvement of apoptotic pathways with SLE and to figure out if the pathways are instrumental to the onset or to the manifestation of the disease. It was found that impaired apoptotic pathways contributing to SLE included delayed apoptotic clearance and production of hyperactive B cells due to evasion of clonal deletion in the B cell development pathways. After thorough and detailed analysis, it was concluded that the contribution of the apoptotic pathways towards onset may be in the context of autoreactive B cell formation while incomplete apoptotic clearance being associated with the clinical manifestation of the disease.

Keywords: Systemic Lupus Erythematosus, Apoptosis, Hyperactive B cells

Introduction

Systemic Lupus Erythematosus (SLE) is an autoimmune disorder that causes widespread inflammation to multiple organ systems. The onset of the disease is multifactorial in nature. It is seen that SLE varies with gender, age, hormones, genetics, environment, and regions. Incidence and prevalence of SLE are observed in various parts of the world such as Europe specifically in Sweden, Iceland, and Spain, USA, Asia, and Australia. A greater prevalence of this disease is exhibited by women compared to men, and around 80-90% of the females are affected especially females who are in their child bearing age [1]. Due to greater incidence and prevalence rates of this disease internationally, it is essential to identify the factors that give rise to onset and pathophysiology of this disease so that earlier identification and personalized treatment options can be made available.

Systemic Lupus Erythematosus: Clinical Presentation and Pathophysiology

Systemic lupus erythematosus (SLE) is a chronic inflammatory autoimmune disease affecting a wide spectrum of organ systems. Patients with SLE present numerous systemic manifestations like rashes, ulcers, hemolytic anemia, fever, thrombocytopenia, lymphadenopathy, seizures with frequently reported renal involvement. These are often indicative of any autoimmune conditions, infections and hematologic diseases. SLE also extends to include musculoskeletal...
manifestations; arthralgia, arthritis, osteonecrosis, myopathy are a few to name [2]. Differential diagnosis of SLE is made with the antinuclear antibody (ANA) titre, antiphospholipid antibodies, C3, C4 or CH50 complement levels etc. A lot of other tests including detecting abnormal levels of rheumatoid factor, anti-cyclic citrullinated peptide antibodies, creatine kinase are performed in selected patients [3]. Because some of these markers show up negative in lupus patients the diagnosis of SLE remains a great challenge.

Human leucocyte antigen (HLA) class II gene polymorphism was studied to be related to the susceptibility of SLE. This has been shown to be associated with the occurrence of anti-Sm (small nuclear protein), anti-nRNP (nuclear ribonuclear protein) and anti-DNA antibodies. A lack or reduction in complement activity makes the elimination of self-antigens incomplete thereby contributing to the susceptibility. Hormones like estrogen, androgen, testosterone, and prolactin have been found to be immunostimulatory though the detailed molecular relevance of the same is yet to be understood [4]. Abnormal B and T cell activation precedes the development of SLE. SLE B cells are more sensitive to stimulatory cytokines and the SLE T cells induce susceptibility by their impaired interaction with the antigen presenting cells (APCs). Overall, the susceptibility, onset, clinical manifestations, and increasing severity of SLE are due to a multi-channeled repertoire of cellular, genetic and hormonal disturbances.

**Apoptosis and its Pathways**

Apoptosis, also known as Programmed Cell Death, is a multi-signaling pathway involving various cell signals which regulate cell survival or cell death (cell fate). The word apoptosis is derived from the Greek language which describes ‘dropping off’ or ‘falling off’ of leaves from the tree and is an essential pathway involved in the death of unwanted cells. This usually happens in a safe, controlled and in a well-coordinated manner ensuring that the contents of the cells are not released thereby protecting the neighboring cells from further attack. The cells that are marked for apoptosis go through a series of changes involving rounding up and retraction of cells from the neighboring cells, condensation of the nucleus and hydrolysis of the DNA to form fragments as low as 200bp, fragmentation of golgi, endoplasmic reticulum and mitochondrial networks, dynamic blebbing of the plasma membrane, pinching up of membranes leading to formation of small and intact apoptotic bodies (vesicles) which exclude vital dyes, alteration of surface signals promoting recognition and recruitment of phagocytes for recycling of cellular contents. The resulting cellular debris is then removed by the process of phagocytosis [5].

All of these events are primarily orchestrated by a protein belonging to a family of cysteine proteases called Caspases. Caspases are protease that have an essential Cys residue in its active site and requires an Asp residue in the substrate cleavage site. They are classically divided into two types: initiator caspases that are auto-activated in response to stimuli and effector caspases that take part in further signaling processes via further proteolysis. In normal, healthy cells, caspases are present in the form ofzymogens. Thesezymogens, in turn, get activated when apoptotic pathways are triggered. Caspase-8 and caspase-9 are initiator caspases and caspase-3, caspase-6, and caspase-7 are effector caspases. There are two major caspase activation pathways: the extrinsic pathway and the intrinsic pathway. The extrinsic pathway involves binding of extracellular death ligands such as FasL or TNF-α to transmembrane death receptors. Binding of the ligand to the receptor promotes the recruitment of adaptor proteins such as Fas-associated Death Domain (FADD) which in turn activates caspase-8. Activated caspase-8 then activates the rest of the effector caspases through proteolytic cleavage. The intrinsic pathway involves the release of cytochrome c from the inner mitochondrial membranes in response to stress which thereby activates initiator caspase-9 by apoptotic protease-activating factor-1 (APAF-1) apoptosome formation. An apoptosome is a large protein complex consisting of cytochrome c and APAF-1 in the presence of ATP. The activated caspase-9 then activates the effector caspases proteolytically. BH3 group of proteins including BAD, BID, HRK, BMF, BIK, BIM, PUMA, and NOXA act as pro-apoptotic factors and BCL-2 subfamily of proteins act as anti-apoptotic factors [5].

In general, the clearing of apoptotic debris is the final event of apoptosis also called as the demolition phase. Recognition by phagocytes is due to membrane alteration wherein phosphatidylserine confined to the inner leaflet of the plasma membrane gets translocated to the outer leaflet. Binding sites for phagocytes are generated which result in the production of chemo attractants that recruit other immune molecules for further clearance [5].

**Role of Apoptosis in Systemic Lupus Erythematosus**

Although the causes of SLE appear to be multifactorial, one of the major factors contributing to the adverse pathophysiology is impaired apoptotic pathway leading to autoimmunity [6]. Impaired apoptotic pathways include:
1. Delayed apoptotic clearance
2. Hyperactive B cells due to aberrant B cell selection by evasion of checkpoints of clonal deletion

**Defect in Apoptotic Clearance Onsets and Manifests SLE**

The major phagocytic cells involved in the elimination of apoptotic cell debris include the tissue-resident macrophages and the immature dendritic cells. These are thought to further functionally differentiate themselves into several types like kuppfer cells, alveolar macrophages, microglia etc [7]. In addition to these functional cells certain other cell types, by default, perform the clearance in situ; like the epithelial cells of the airway, which are thought to clear off the airway apoptotic cells, the mammary gland, sertoli cells of the testes, etc. An incomplete or unregulated phagocytic clearance of the damaged or dying apoptotic cell is studied to be a pathophysiological route inducing several autoimmune cellular conditions. This phagocytic process is initiated when a cell destined for apoptosis decides to express (on the cell surface or intracellularly) or release (extracellularly) the signal compounds like phosphatidylserine, lysophosphatidylcholine, CX3CL1, pro-apoptotic factors like Smac/Diablo, HtrA2 and AIF [6]. BAI1, a transmembrane protein of the phagocyte binds with the Phosphatidylserine of the apoptotic cell. Tyro-3, Axl and Mer receptor tyrosine kinases also get expressed on the phagocytic cell surfaces which work similarly. These recognitions and bindings are mediated by a number of soluble proteins like plasma-protein β2-glycoprotein I, growth arrest specific gene 6 (Gas6) and protein S [8]. The binding triggers a series of activation episodes resulting in cellular changes, the significance of which is the mobilization of the actin cytoskeleton for engulfing action of the cells.

Any defect in the expression or function of their receptors or the soluble binding proteins result in accumulation of the apoptotic cells in the peripheral tissue areas. The concentrations of membrane Axl were found to be decreased in SLE conditions and this level was found to decrease with increasing severity of the autoimmune manifestation, suggesting incomplete apoptotic clearance. However, the soluble levels of the protein receptors Tyro-3, Mer, Alxetc were found to be elevated [9]. Also the levels of T-cell Immunoglobulin Mucin (TIM) proteins in the SLE cases correlated with the clinical implications of the tested animal models. TIM4 mRNA expression in PBMNCs of patients with SLE was recorded to be more than in the healthy individuals. A pro-inflammatory mediator, HMGB1 protein is expressed in elevated levels in sera of SLE patients. This protein blocks the phosphatidylserine molecules on the cell surface, affecting apoptotic clearance and also serve as an auto-antigen thus amplifying the autoimmune reactions in such patients. SLE patients also show a large number of microparticles in circulation. These are known to compete with the original apoptotic cells by expressing more of the phosphatidylserine on their cell surface. All these evidences cumulatively suggest the contribution of the membrane and soluble proteins of the ‘engulfment and cell motility signaling pathway’, to the onset, severity and clinical manifestations of SLE through the possibly studied mechanism of inadequate apoptotic demolition.

**Aberrant B Cell Selection Contributing to SLE**

Aberrant B cell selection leads to the development of self-reactive B cells; cells that produce antibodies for self-antigens. These types of B cells are also known as hyperactive B cells. The pathway of B cell development is focused in order to understand the implications of the aberrant pathway in the manifestation of SLE.

B cell development consists of various sequential stages that lead to the assembly of a B Cell Receptor (BCR) which on differentiation to plasma cells produces unique antibodies. Unique antibodies are produced due to the production of a highly diverse range of BCR that is capable of producing a specific antibody on encountering a foreign antigen. The generation of highly diverse BCRs is done via the VDJ recombination which is the recombination of Variable, Diversity and Joining gene segments that encode the BCR heavy and light chains. Consequently, the BCR produced due to VDJ recombination can also lead to the formation of antibodies that react to self-antigens. The occurrence of these polyreactive B cells in immature B cells is about 50-75%. Due to this, B cell development has multiple stages housed with checkpoints at certain stages. These are checkpoints that differentiate normal B cells from autoreactive B cells. The checkpoints comprise of clonal deletion of autoreactive B cells (apoptosis), BCR editing, and the induction of anergy. The initial checkpoint lies in the pre-B cell stage where positive selection of normal B cells takes place. In case, any abnormalities leading to autoreactivity are observed, either receptor editing or clonal deletion takes place. Transitional B cells are cells wherein the immature B cells progress towards peripheral blood or other secondary lymphoid organs. Similar checkpoints are also observed in this stage. At this stage, any autoreactive B cells encountered are silenced via clonal deletion or induction of antigen unresponsiveness also known as anergy. B cells that have successfully passed through all of these checkpoints have proper immune functions [10].
A vast majority of abnormal B cells produced are killed via apoptosis unless they can edit their receptor. Receptor edition involves further VDJ recombination thereby altering the BCR specificity. Sometimes, the abnormal cells can also successfully pass through all the checkpoints thereby maturing into B cells which can differentiate into plasma cells secreting antibodies specific to self-antigens. These cells have reduced self-tolerance and hence immediately produce antibodies complementary to the self-antigen. This can lead to various autoimmune disorders, one of which is SLE. The pathophysiology of SLE also includes excessive exhaustion of mature B cells due to its continuous differentiation to plasma cells leading to decreased mature B cell levels and increased plasma cell levels in the peripheral blood [10].

Single gene defects in the BCR signaling pathway have proven to be sufficient to cause SLE in mouse models. It was found out that in these models, B cell restriction or mere increase in BCR signaling lead to the development of SLE-like disease. Enhanced BCR signaling is sufficient to decrease tolerance levels in autoreactive B cells. Overexpression, deletion or mutation of CD19, CD22, FcγRIIB, BTK (Bruton's tyrosine kinase), SHP-1 (Src homology domain 2-containing phosphatase-1), SHIP-1 (phosphatidylinositol-3,4,5-triphosphate 5-phosphatase-1) contributes to SLE in murine models. Mutations in molecules such as BLK (B lymphocyte kinase) and BANK-1 (B-cell scaffold protein with ankyrin repeats) have been known to be associated with an elevated risk of SLE in humans. Increased TLR signaling has also been observed with increased BCR signaling. Polymorphisms in TLR7 and TLR9 have been linked with SLE in Asian patients [10].

Understanding these pathways becomes essential as this knowledge can be used towards the field of translational and personalized medicine in order to develop drugs that can selectively inhibit BCR or TLR signaling pathways to cure SLE.

**Conclusion**

It is now well-known that both impaired apoptotic clearance and clonal deletion can develop autoimmune diseases such as systemic lupus erythematosus suggested by in vitro and in vivo evidence. The formation of autoreactive B cells in circulation due to failed checkpoints and clonal deletion could onset SLE. However, the clinical manifestations and extended pathophysiology of the condition is contributed by the incomplete apoptotic clearance facilitated by the pro-inflammatory mediators. With this conclusion, better comprehension in the immunobiology of this disease can help in further advancing detection and treatment options.

**References**