Commentary

Links between complement deficiency and apoptosis

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Abstract

Deficiency in the classical pathway complement components displays a hierarchical association with the development of systemic lupus erythematosus (SLE). In addition, SLE causes consumption of complement. C1q- and C4-deficient mice develop a lupus-like disease and exhibit impaired clearance of apoptotic cells. The autoantigens targeted in SLE have been localised to the surface of apoptotic cells, which may be the source of these antigens. Although apoptosis was originally thought to be an immunologically inert process, dendritic cells can present epitopes derived from apoptotic cells, and immunization with apoptotic cells leads to the generation of autoantibodies. These findings taken together indicate that a defect in complement-dependent clearance of apoptotic cells may increase susceptibility to the development of autoimmunity.

Keywords: apoptosis, complement, deficiency, SLE

Introduction

Homozygous deficiency of each of the classical pathway complement components (C1q, C1r, C1s, C4, C2) is associated with an increased susceptibility to systemic lupus erythematosus (SLE). The severity and the strength of this association vary according to the position of the missing component within the classical pathway of activation (C1>C4>C2) [1,2]. Inherited deficiencies of C1q and C4 are invariably associated with the development of a severe, lupus-like disease early in life, while C2 deficiency is only weakly associated with a milder form of SLE, an association which has most likely been overestimated [2]. In contrast, whereas deficiency of C3 predisposes to recurrent pyogenic infections and membranoproliferative glomerulonephritis, it is rarely associated with SLE [2]. This is a quite surprising finding, as C3 is the point of convergence for all three complement-activating pathways and therefore has the most substantial effect on all the activities mediated by complement activation. The association between complement deficiency and SLE appears even more paradoxical: complement deficiency causes SLE, and yet SLE causes activation and consumption of complement. These clinical observations suggest that the early part of the classical pathway plays a key protective role against the development of SLE. A recently described link between the complement system and apoptosis may explain these apparently paradoxical findings.

Apoptosis and SLE

Apoptosis is an active process that leads to the programmed destruction of cells without the release of intracellular contents into the extracellular microenvironment, where they could cause an inflammatory reaction and tissue damage. Apoptotic cells undergo a series of distinct physical changes, including alteration of the surface lipid membrane, cytoskeletal disruption, cell shrinkage, and a characteristic pattern of DNA fragmentation. Although the apoptotic cell-death program is executed in hours, the removal of dying cells is normally so rapid that few cells are seen even in tissues such as the thymus,

SLE = systemic lupus erythematosus.

where up to 95% of cells undergo apoptosis. The mechanisms whereby apoptotic cells are efficiently identified, removed, and degraded by phagocytosis in mammalian cells are not well understood. Several receptors/ligands have been reported to play a role in the initial engulfment of apoptotic cells *in vitro*. These include the $\alpha_v\beta_3$ vitronectin receptor [3], the phosphatidylserine receptor [4,5], CD36 [6], CD14 [7], scavenger receptor A [8], receptors for low-density lipoprotein [9–11], and complement receptors 3 and 4 [12]. These different receptor/ligand systems may function in conjunction with one another, but individual types of phagocyte may show specific ligand/receptor preferences [4].

There is a mounting body of evidence that apoptotic cells are the source of the autoantigens of lupus. First, Rosen and his collaborators have demonstrated that apoptotic cells express many of the nuclear autoantigens of SLE in surface blebs and apoptotic bodies [13,14]. In addition, negatively charged phospholipids, such as phosphatidylserine, which are found predominantly in the inner lamella of the cell membrane in healthy cells, are translocated to the outer layer as part of the process of apoptosis, and the antiphospholipid autoantibodies, found in approximately one third of patients with lupus, bind phosphatidylserine. Secondly, lupus autoantigens undergo post-translational modification during the process of apoptosis and may be cleaved or phosphorylated. This process could generate neo-epitopes of autoantigens, which might appear as 'nonself' to the immune system [15]. Thirdly, recent experiments have shown that injection of apoptotic cells into mouse strains not normally susceptible to the development of SLE induces an autoantibody response [16].

The finding that apoptotic cells may be the source of the autoantigens in SLE raises two questions: 1) can a breakdown of the normal mechanisms of removal of apoptotic cells promote the development of SLE? and 2) does the complement system play a role in this clearance function?

Is SLE a disease of defective clearance of apoptotic cells?

Recent studies have emphasised that apoptotic cells are not immunologically inert, but rather have either positive or negative effects, depending on the antigen-presenting cell with which they interact. Is autoimmunity simply caused by an increase in the load of autoantigens? For example, dendritic cells have been shown to be able to phagocytose apoptotic cells via the $\alpha_v\beta_5$ receptor [17] and afterwards efficiently present antigens derived from apoptotic cells to MHC class I- and II-restricted T cells [17,18,19] in a dose-dependent manner [20]. When macrophages are also present, the presentation of apoptotic cells is markedly inhibited. Two possible mechanisms might explain this inhibition. One is that phagocytosis of apoptotic cells by macrophages is so rapid and so efficient that the amount of apoptotic material ingested by dendritic cells is minimised. The other is that phagocytosis of apoptotic cells by macrophages induces the production of anti-inflammatory cytokines (e.g. transforming growth factor-β) and inhibits the production of pro-inflammatory cytokines (e.g. interleukin-1β, tumour necrosis factor-α) [21,22]. This has led to the hypothesis that an abnormality in any of these pathways that are involved with the rapid clearance of apoptotic material or with expression of its anti-inflammatory activities may initiate autoimmunity. In the context of this hypothesis, inherited defects in the mechanisms for clearing apoptotic cells would be strong candidates as susceptibility genes for SLE.

The role of complement in the clearance of apoptotic cells

Complement was implicated for the first time in the clearance of apoptotic cells by the observation that C1q could bind *in vitro* specifically and directly to the surface blebs of human keratinocytes that had been rendered apoptotic by exposure to UVB [23]. This observation suggested that C1q may promote the clearance of apoptotic cells, and hence of exposed autoantigen, preventing stimulation of the immune system. This hypothesis was tested in C1q-deficient mice. These mice, on the hybrid genetic background (129 × C57BL/6), developed significant titres of antinuclear antibodies and proliferative glomerulonephritis characterised by an increased number of uncleared apoptotic bodies [24], independently of C3 activation [25]. These findings, taken together, suggested that impairment in the clearance of apoptotic cells might be the primary defect. In addition, C4-deficient mice have recently been shown to develop a lupus-like disease, while mice deficient in complement receptors 1 and 2 (CR1 and CR2) did not share this susceptibility [26], supporting the hypothesis that complement deficiencies (C1q and C4) in mice cause autoimmunity through mechanisms that do not depend on CR1 and CR2.

However, studies by Mevorach and colleagues, using complement-depleted sera and human monocyte-derived macrophages *in vitro,* suggested a role for both the classical and alternative pathways of complement in the phagocytosis of apoptotic cells [12]. To address the relative contribution of different complement components to the phagocytic removal of apoptotic cells *in vivo,* we studied apoptotic cell clearance in complement-deficient mice. We used an *in vivo* peritoneal model of apoptotic cell clearance, in which the mice were initially injected intraperitoneally with thioglycollate, to induce recruitment of inflammatory macrophages, and four days later the same animals were injected with syngeneic apoptotic thymocytes. The phagocytic uptake of the apoptotic thymocytes by the elicited macrophages was significantly less in both C1q- and C4-deficient mice than in wild-type control animals. However, the phagocytic uptake was only partially

impaired in the C4-deficient mice compared with the C1q-deficient animals. Furthermore, the C1q-deficient mice, but not the C3- and C4-deficient animals, also exhibit a defect in phagocytic uptake of apoptotic cells by resident macrophages [27]. A similar phagocytic defect was also observed in macrophages derived from the monocytes of C1q-deficient humans and cultured in autologous serum [26]. This defect was corrected in a dose-dependent manner with purified human C1q. In this context, it is interesting that monocyte-derived macrophages from humans with SLE also exhibited impaired phagocytosis of apoptotic cells *in vitro* [28]. These observations, taken together, indicate that there is a hierarchy of importance within the classical complement pathway in the phagocytic clearance of apoptotic cells that mirrors the hierarchy of disease susceptibility in humans with complement deficiency. Although there are at present no data that directly address whether, and by what mechanism, impaired clearance of apoptotic cells may initiate or exacerbate the autoimmune state *in vivo*, one can hypothesise that the delayed removal might cause two major effects. One would be a change in the compartmentalisation of the autoantigen, allowing leakage of autoantigens during secondary necrosis and access of soluble molecules to efficient uptake by dendritic cells. Another effect would be that the delay provides a source of apoptotic cells to (pro-immune) populations of antigen-presenting cells from which they are normally excluded.

Conclusion

All of these findings are compatible with the hypothesis that complement deficiency causes SLE by impairment of the physiological clearance of apoptotic cells by macrophages. These uncleared apoptotic bodies in turn may provide the source of the autoantigens that drive the autoimmune response of SLE. A reduced ability of macrophages to remove apoptotic cells at sites of inflammation may promote the clearance of these cells by pro-immune antigen-presenting cells, and if the necessary pro-inflammatory cytokine milieu is present, this may drive dendritic cell maturation and initiate an autoimmune response followed by tissue damage and complement activation. Autoimmunity in individuals deficient in the early classical pathway components of complement may hence be the consequence of impaired waste disposal. Further experimentation is required to identify the receptors involved and further delineate the mechanism of the hierarchy within the early classical pathway.

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