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Do follicular dendritic cells regulate lupus-specific B cells?☆

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ABSTRACT

The factors that allow self-reactive B cells to escape negative selection and become activated remain poorly defined. In this review we describe recently published results in which a B cell receptor-knock-in mouse strain specific for nucleolar self-antigens was bred with mice deficient in complement C4 and discuss the implications for the lupus field. Absence of C4 leads to a breakdown in the elimination of autoreactive B cell clones at the transitional stage. This is characterized by a relative increase in their response to a range of stimuli, entrance into follicles and a greater propensity to form self-reactive germinal centers. In this review, a model is proposed in which, in the absence of complement C4, inappropriate clearance of apoptotic debris promotes chronic activation of myeloid cells and follicular dendritic cells, resulting in secretion of Type I interferon. This allows for the maturation and activation of self-reactive B cell clones leading to increased spontaneous formation of germinal centers and subsequent generation of autoantibodies.

1. Introduction

Systemic lupus erythematosus (SLE) represents a multigenic autoimmune disease for which there is no current cure (Walport, 2001). It is characterized by autoantibodies specific for nuclear antigens found in apoptotic blebs such as ribonuclear proteins (RNP), histones and dsDNA that form immune complexes (IC) with self-antigen and deposit in tissues. Given this phenotype, SLE is considered an immune complex disease in which failure to clear apoptotic blebs or IC containing nuclear material from apoptotic cells can lead to alteration in negative selection of autoreactive B cells and production of autoantibody.

One of the major pathways for clearance of apoptotic cells and IC is the complement system (C′) (Reid and Porter, 1981). In particular, the classical pathway becomes activated when the first component (C1q) binds to IC containing IgM or IgG. This leads to an activation of C1-associated serine proteases that cleave the fourth component (C4) exposing an internal thioester and results in covalent attachment to the IC (van den Elsen et al., 2002). Subsequently, the central component of complement, i.e. C3, is activated and forms a covalent bond with the IC complex (Law and Levine, 1977; Nagar et al., 1998). In normal individuals, IC and apoptotic debris are cleared efficiently through a combination of scavenger receptors and specific complement receptors that bind activated fragments of complement C3, such as CR1 (CD35) and the CR-Ig receptor (Helmy et al., 2006; Smith et al., 2002).

Deficiency in C1q or C4 but not C3 is a major risk factor for SLE (Walport, 2001; Walport et al., 1998; Yang et al., 2007). Although relatively rare, genetic or acquired deficiency in C1q leads to SLE in 90% of individuals identified. Total deficiency in complement C4 also results in SLE at a high frequency, i.e. 75%, among affected individuals (Walport, 2001). Interestingly, in contrast to the normal population where women have a much higher susceptibility to disease than men (9:1), men and women deficient in C4 are equally susceptible to lupus. Mice deficient in C1q or C4 are also predisposed to a lupus-like phenotype (Botto et al., 1998; Prodeus et al., 1998). Although spontaneous disease is mild, crossing either C1q or C4 deficient strains with mice bearing another susceptibility locus such as *lpr* (lymphproliferation and autoimmunity; CD95 or Fas) on the C57Bl/6 background lead to elevated anti-nuclear antibodies (ANA) and glomerulonephritis (Botto et al., 1998; Carroll, 2004; Pickering et al., 2000; Prodeus et al., 1998).

How activation and binding of C1q and C4 to IC bearing apoptotic debris is protective is not known. Earlier hypotheses suggested that direct opsonization of apoptotic IC with C1q or C4 enhanced

Abbreviations: RNP, ribonucleoprotein; SSB/La, Sjögren's syndrome antigen B; Id, idiotype; PNA, peanut agglutinin; Tfh, follicular T helper cells.

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binding and direct clearance through phagocytic receptors. One developing model is that opsonization of apoptotic debris with C1q or C4 acts to dampen activation of myeloid cells following phagocytosis of the debris. Thus, C1q or C4 may "mark" IC for clearance without inducing inflammation. Support for this novel role comes from several recent reports. The Elkon group reported that uptake of apoptotic debris by human peripheral blood monocytes (PBMC) or dendritic cells (DC) is relatively non-activating when pretreated with lupus sera of patients in the presence of C1q (Santer et al., 2010, 2012). In contrast, when the source of lupus sera is C1q deficient, uptake of the apoptotic IC leads to activation of human PBMCs and DCs. In their system, activation was assayed by cell expression of proinflammatory cytokines such as Type I interferon (IFNα). Thus, they proposed that C1q acted to suppress the activation of inflammation and secretion of IFNα. Further support for this hypothesis comes from the finding of Diamond and colleagues that the leukocyte-associated Ig-like receptor 1 (LAIR-1) binds the collagen stalk of C1q and mediates negative signaling through its ITIM (immunoreceptor tyrosine-based inhibitory motif) of plasmacytoid DC (pDC) (Son et al., 2012). In a more recent study, Means and colleagues report that the scavenger receptor SCARF-1 (scavenger receptor expressed by endothelial cell 1) is required for efficient uptake of dying cells; and mice deficient in the receptor develop an autoimmune phenotype similar to that of C1q deficient strains (Ramirez-Ortiz et al., 2013). In their study, SCARF-1 interacts on the cell surface of DC with C1q bound to apoptotic cells via exposure of phosphatidylserine. C1q cannot only bind Ig-coated IC or apoptotic cells through the Fc region of Ig but also via its affinity for phosphatidylserine. Thus, similar to calreticulin-CD91 (Gardai et al., 2005), MFG-E8 (Hanayama et al., 2004; Kranich et al., 2008), TIM 3-TIM-4 (Kobayashi et al., 2007), C1q recognizes dying cells through exposure of phosphatidylserine and promotes phagocytosis without triggering of inflammation.

Whether C4 interacts directly with scavenger receptors similar to C1q is not clear. One possible interaction is with the TAMs (Tyro-3, Axel, and c-Mer), which are a family of tyrosine kinases that act as negative regulators of myeloid cell activation following phagocytosis of apoptotic debris. A combined deficiency of all 3 family members results in severe lupus-like disease (Rothlin and Lemke, 2010). Alternatively, deficiency in c-Mer alone leads to a dysregulation of B cell tolerance and a mild lupus phenotype (Cohen et al., 2002). The primary ligands for TAMs are Gas 6 and Protein S, which recognize apoptotic cells through phosphatidylserine (Anderson et al., 2003). In the latter example, Protein S is known to interact with C4 binding protein (C4bp) in human sera. Interestingly, while Protein S promotes clearance of apoptotic cells, the complex of Protein S and C4bp is inhibitory (Kask et al., 2004). One explanation for a protective role for C4 is that it may compete with Protein S to bind C4bp. Thus, C4 may displace Protein S-bound apoptotic debris from C4bp and promote clearance via scavenger receptors and activation of TAMs.

2. Loss of B cell tolerance in absence of C4

Mice deficient in C1q or C4 not only have impaired clearance of IC but develop elevated ANA suggesting a loss of B cell tolerance to lupus antigens (Botto et al., 1998; Paul et al., 2002; Prodeus et al., 1998). A current paradigm to explain dysregulation of lupus-specific B cells is based on the observation that in general lupus antigens are ligands for TLRs such as TLR-7 and 9. Thus, uptake and internalization of nuclear debris containing ribonucleolar proteins, such as Ro and La, can activate cytoplasmic TLRs and result in activation of NFkB and induction of downstream pathways such as IFNα and IL-6 (Avalos et al., 2010; Lau et al., 2005; Leadbetter et al., 2002). TLR signaling can overcome intrinsic anergy leading to expansion and differentiation of the autoreactive B cells to plasma cells and possibly memory B cells. Whether this pathway allows for escape of tolerance at the immature stage has not been reported.

To test if deficiency in C4 results in loss of tolerance to a known lupus antigen, $C4^{-/-}$ mice were crossed with the B cell receptor (BCR) knock-in line 564 Igi. These mice bear an insertion of the rearranged Ig heavy ($V_HD_HJ_H$) and light (V_kJ_k) chain genes. The line was developed originally from a hybridoma (mAb) isolated from an autoimmune strain of mice that spontaneously develop a lupus-like phenotype (Berland et al., 2006). Initial characterization of the knock-in mice identified normal negative selection of the autoreactive B cells at checkpoint I and II (clonal deletion and clonal anergy in the periphery respectively). However, a fraction of the autoreactive B cells escape tolerance and expand in a TLR7-dependent pathway and secrete ANA. The 564 Igi strain is one of the first in vivo models that confirm the paradigm of TLR 7 signaling leading to loss of anergy and secretion of pathogenic autoantibody.

Characterization of the 564 mAb identified a nucleolar staining pattern as expected. Likewise, sera isolated from the $C4^{+/+}$ and $C4^{-/-}$ 564 Igi mice gave a similar staining pattern as the mAb (Fig. 1). The nucleolus is a major site for RNA splicing and accumulation of ribonucleoproteins (RNP). Biochemical analysis of immune precipitates prepared from 564 Ig serum, identified multiple RNP including the known lupus antigen SSB/LA. 564 Ig binding is dependent on both the RNA and protein components suggesting that the antibody recognizes an RNA binding domain (Chatterjee et al., 2013).

To characterize B cell tolerance, splenic B cells isolated from $C4^{+/+}$ and $C4^{-/-}$ 564 Igi mice heterozygous for Ig H and L chains were analyzed by flow cytometry. Autoreactive B cells were identified using an antibody that was specific for the 564 Igi BCR (idiotype or Id). Comparison of Id^+ B cells isolated from the $C4^{+/+}$ and $C4^{-/-}$ 564 Igi mice identified a relatively low frequency of autoreactive B cells in the former strain as expected (Berland et al., 2006; Chatterjee et al., 2013). By contrast, a significantly higher frequency of mature Id^+ B cells was observed in the spleen in the $C4^{-/-}$ 564 Igi mice suggesting that in the absence of C4 negative selection was less efficient (Fig. 2). Further analysis of Id^+ splenic B cells using cell surface markers such as AA4.1 to distinguish transitional from mature

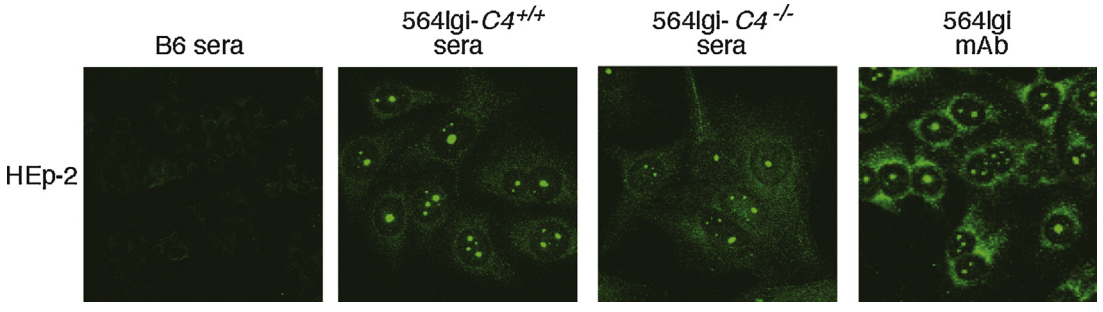

Fig. 1. Sera from B6, $C4^{+/+}$ 564 Igi and $C4^{-/-}$ 564 Igi-mice (1/100) were incubated on Hep2 slides. 564 Igi mAb was used as control. Images were acquired at 20× magnification.