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SLE-associated risk factors affect DC function

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Summary: Numerous risk alleles for systemic lupus erythematosus (SLE) have now been identified. Analysis of the expression of genes with risk alleles in cells of hematopoietic origin demonstrates them to be most abundantly expressed in B cells and dendritic cells (DCs), suggesting that these cell types may be the drivers of the inflammatory changes seen in SLE. DCs are of particular interest as they act to connect the innate and the adaptive immune response. Thus, DCs can transform inflammation into autoimmunity, and autoantibodies are the hallmark of SLE. In this review, we focus on mechanisms of tolerance that maintain DCs in a non-activated, non-immunogenic state. We demonstrate, using examples from our own studies, how alterations in DC function stemming from either DC-intrinsic abnormalities or DC-extrinsic regulators of function can predispose to autoimmunity.

Keywords: *dendritic cells, immune tolerance, risk alleles, systemic lupus*

Introduction

Research over the last decades has revealed a fundamental role for the innate immune system in initiating protective responses against different pathogens and tolerogenic responses to tissue antigens. Dendritic cells (DCs) sit at the interface of the innate and adaptive response and perform both immunogenic and tolerogenic functions. This paradigm strongly supports the idea that DCs are critical to immune homeostasis and that breaking the balance among DC subsets, either by changes in differentiation or disturbance of life span, might cause immunological defects including immunodeficiency, autoimmune disease, or cancer.

Understanding how systemic lupus erythematosus (SLE)-associated risk alleles affect DC subsets and function has a great potential to lead to new therapeutic targets. It should be possible, over the next few years, to use our current and expanding knowledge of inhibitory receptors on DCs and the tolerogenic function of DCs to devise therapeutic strategies that enhance their ability to modulate inflammation and autoreactivity.

DC subsets

DCs are a heterogeneous population with different abilities to respond to external stimuli and regulate distinct effector lymphocytes. The developmental pathway for both DCs and monocytes starts with multipotent self-renewing hematopoietic stem cells residing in the bone marrow (BM). In the steady state, common myeloid precursors give rise to monocyte/DC precursors (MDP). MDPs differentiate into either monocytes or common DC progenitors (CDPs). CDPs have lost their potential to differentiate into monocytes or macrophages and give rise to plasmacytoid DCs (pDCs) and preconventional (c)DCs (reviewed in 1). While MDP and CDPs are restricted to the BM, pre-cDCs egress from the BM and circulate in the blood. They migrate into peripheral tissues and lymphoid organs, where they further differentiate into DC subsets such as $CD11b^+$ cDCs, $CD103^+$ cDCs or $CD8\alpha^+$ cDCs and pDCs. Both cDCs and pDCs are dependent on Fms-related tyrosine kinase 3 signaling, which promotes terminal pDC differentiation and mature pDC survival. They also share features of their transcription profile (1).

Mouse DC subsets

Several subsets of DCs have been identified in lymphoid organs and non-lymphoid tissues in mice. DC subsets are identified by particular combinations of surface markers. Classical DCs (cDCs: $CD11c^{hi}$ MHC class II^{hi}) and pDCs ($CD11c^{int}$ Siglec-H^+) are currently considered to be the two major categories of DCs. CDCs are further grouped into lymphoid DCs ($CD11b^+$; termed $CD11b^+$ cDCs) and myeloid DCs (CD11b− $CD8\alpha^+$ and termed $CD8\alpha^+$ cDCs in spleen or $CD103^+$ cDCs in lymph nodes [LNs]). An increasing number of studies have identified an even greater variety of DCs that reside in non-lymphoid tissues. Tissue-resident DCs often express additional markers such as CX3CR1, CD103 and/or signal-regulatory protein alpha (SIRPα).

$CD11b^+$ cDCs are the major cDC subset (approximately 60–70% of total cDCs). They present exogenous antigens in the context of major histocompatibility class II (MHC II), activating cognate $CD4^+$ T-helper cells. $CD11b^+$ cDCs are migratory DCs and induce T-cell proliferation in LNs. They also produce high levels of IL-4, IL-5, IL-10, IL-12, and interferon γ (IFN-γ), thereby providing signals necessary for $CD4^+$ T-cell activation and differentiation. $CD8\alpha^+$ cDCs are a minor population (approximately 10% of total cDCs) and are functionally unique due to their ability to cross-present exogenous antigens on MHC I to activate $CD8^+$ T cells. Unstimulated $CD8\alpha^+$ cDCs do not produce IL-12 constitutively but when activated by engagement of Toll-like receptors (TLRs) and CD40, produce high levels of IL-12 (2, 3) and preferentially induce a Th1 immune response (4, 5). $CD8\alpha^+$ cDCs have a capacity to phagocytose apoptotic cells and to induce regulatory T cells (Tregs), suggesting that $CD8\alpha^+$ cDCs participate in immune tolerance. $CD103^+$ cDCs, a functional counterpart of $CD8\alpha^+$ cDCs in the mucosal immune system, have also been suggested to be a tolerogenic DC subset. They reside primarily in the intestinal lamina propria and are a critical DC subset for Treg induction and maintenance of differentiated Tregs (6). $CD103^+$ cDCs and Treg differentiation are critical for maintenance of intestinal homeostasis preventing aberrant inflammation (7–9). Treg induction by both of these DC subsets is transforming growth factor (TGF)-β and retinoic acid dependent.

pDCs are a functionally distinct DC subset and are major producers of type I IFN but are weak antigen-presenting cells (APCs). MHC II expression on pDCs can be induced by activation but is significantly lower than on cDCs at the basal level, consistent with their poor APC function (10). It is still controversial whether pDCs, even when activated, can present antigen and regulate an adaptive immune response. Tezuka *et al.* (11) identified that pDCs isolated from mesenteric lymph nodes (MLNs) or Peyer's patches can induce IgA production from naive B cells through secretion of April and Baff. Both April and Baff expression depends on type I IFN production in pDCs. A recent study demonstrated that pDCs present endogenous antigen with newly synthesized MHC II with slower kinetics than cDCs. Thus, pDCs and cDCs present antigen at different times throughout immune response (12). This finding might partially explain the previous controversy on APC function of pDCs.

pDCs express high levels of intracellular TLR 7 and 9, and as a consequence of TLR activation secrete 100- to 1000-fold more IFN-α than other immune cells (13, 14). PDCs induce T cells to become IFN-γ and IL-10-producing Tregs (15). PDCs have been also identified as a critical cell type for B-cell activation, inducing naive B cells to become antibody-secreting plasma cells in humans. This also depends on IFN-α as well as IL-6 produced from pDCs (16).

Human DC subsets

Compared to the information available about mouse DC subsets, knowledge of human DC subsets is in its infancy. The major obstacle to learning about human DC biology is their scarcity in blood [approximately 0.1% of peripheral

blood mononuclear cell (PBMC)] and their unique surface marker expression which is different from that of mouse. Although CD11c has been considered a DC-specific marker in most lymphoid organs and in blood, CD11c is not uniquely expressed in DCs and is broadly expressed in monocytes in human blood. Therefore, indirect methods are used to distinguish DCs from monocytes, including the absence of CD14 and CD16 expression. These lineage-negative DC populations have been further subgrouped into CD11c-high cDCs or CD11c-intermediate pDCs. Human $CD11c^+$ DCs have not been further subgrouped like mouse $CD11c^+$ DCs as human DCs do not express CD8α or CD103 which distinguish DC subsets in mice. Ito *et al.* (17) have identified blood dendritic cell antigen-1 (BDCA-1: $CD1c^+$ cDC) as a marker to help subset human DCs. Dzionek *et al.* (18) subsequently showed that BDCA-2 ($CD123^+$ pDCs), and BDCA-3 ($CD141^+$ cDC) are also expressed on blood DCs. Most blood DC subsets have also been found in tissues including $CD1c^+$ cDCs and $CD141^+$ cDCs (19).

Comparative analysis of the transcriptomes of human and mouse DC subsets confirmed phenotypic and functional similarities between mouse and human subsets in blood and intestine; for example, human blood $CD141^+$ cDCs are related to human intestinal $CD141^+$ DCs and mouse intestinal $CD103^+$ $CD11b^-$ DCs, and human blood $CD1c^+$ DCs are related to mouse intestinal $CD103^+$ $CD11b^+$ DCs. Like mouse $CD103^+$ cDCs, human $CD141^+$ DCs can cross-present antigen, confirming the functional similarities between human and mouse subsets (20). These data suggest that human blood DC subsets are functionally related to both human tissue-resident DCs and mouse DC subsets.

Although most DCs in the steady state derive from DC precursors, during inflammation an additional DC subset has been described, named inflammatory DCs (infDCs). InfDCs are found in inflamed tissues *in vivo*; for example, $CD1c^+$ DCs are found in synovial fluid from rheumatoid arthritis patients and inflammatory tumor ascites from cancer patients (21). InfDCs share phenotypic markers with mouse infDCs, such as expression of CD11b, CD206, CD172a, and FcεRI. Although it is not clear whether human infDCs are derived from monocytes or not, transcriptome profiles show that infDCs express transcription factors which are required for both monocyte and DC differentiation. Moreover, infDCs express transcriptional signatures similar to those of mouse monocyte-derived infDCs (22). These data suggest that infDCs have particular lineage development and function in immune system and may represent monocyte-derived DCs (MO-DCs). In addition, infDCs produce IL-12, IL-23, type I IFN, inflammatory chemokines, or the proinflammatory cytokines IL-1α and IL-1β in different inflammatory milieus (reviewed in 23). In contrast to mouse infDCs, human infDCs can induce differentiation of Th17 cells by production of IL-6 and IL-23. InfDCs can also activate $CD8^+$ T cells through antigen cross-presentation. Therefore, these DCs, whether in inflammatory tissue or a lymphoid organ, can activate an adaptive immune response. In order to maintain homeostasis, a careful regulation of infDC differentiation from monocytes is important.

Other DC subsets can express inducible indoleamine-2,3-dioxygenase (IDO), thereby representing a tolerogenic DC subset in humans (24) and mouse (25) as IDO has been shown to inhibit antigen-specific or allogenic T-cell proliferation and to induce a direct expansion of Tregs *in vitro*.

Transcription factors required for DC subset differentiation

Many molecules are known to regulate and influence DC subset differentiation. Several transcription factors are identified as key molecules for the development of specific DC subsets. IFN-regulatory factor 8 (IRF8) has been determined to play a critical role in myeloid cell differentiation in humans and mice. IRF8-deficient mice exhibit an increased production of granulocytes with a lack of pDCs, $CD8\alpha^+$ cDCs and $CD103^+$ cDCs in lymphoid organs and non-lymphoid tissues (26). A spontaneous point mutation of IRF8 in BHX2 mice also leads to a defect in $CD8\alpha^+$ cDC and $CD103^+$ cDC differentiation with normal pDC development (27). In contrast to IRF8-deficient mice, IRF4 deficiency results in a low number of $CD11b^+$ cDCs with normal numbers of $CD8\alpha^+$ cDCs in the spleen (28).

The basic leucine zipper transcription factor ATF-like 3 (Batf3) has a selective role in $CD8\alpha^+$ and $CD103^+$ DC differentiation. Although Batf3 is expressed in all cDC subsets, Batf3-deficient mice show a selective defect in $CD8\alpha^+$ and $CD103^+$ cDCs (29, 30). Notch 2 signaling and Notch RBP-J, a transcription factor activated following Notch receptor engagement, are critical to $CD11b^+$ cDC development. Defects in the Notch signaling pathway result in a selective reduction in $CD11b^+$ cDCs in the spleen and $CD11b^+$ $CD103^+$ cDCs in the small intestine (31). Inhibitor of DNA binding 2 (ID2) is also required for the development of cDCs. Mice lacking ID2 show a selective reduction in $CD8\alpha^+$ and $CD103^+$ cDCs (32, 33). A member of the family of helix loop helix transcription factors, E-protein E2-2/Tcf4, is preferentially expressed in pDCs; E2-2 directly regulates gene expression in pDCs and is required for pDC

development and function (34). Either constitutive or inducible deletion of E2-2 leads to a selective blockade of pDC differentiation (35), confirming its key function in pDC development. The Ets transcription family member Spi-B is highly expressed in pDC, but not in cDCs. Although Spi-B expression is required selectively for pDC differentiation from human $CD34^+$ progenitor cells *in vitro* (36), mice with Spi-B deficiency show only a mild reduction of pDCs only in BM and not in other lymphoid organs (37).

B cell lymphoma 6 protein (Bcl-6) and B lymphocyte-induced maturation protein-1 (Blimp-1) have been demonstrated to be involved in DC subset development in humans and mice (20). Watchmaker *et al.* demonstrated that these functionally antagonizing factors are selectively enriched in specific subsets of DCs, Bcl-6 in $CD103^+$ $CD11b^-$ cDCs and Blimp-1 in $CD103^+$ $CD11b^+$ cDCs. Interestingly, these selective expression patterns have been observed in both human and mouse DCs. Mice with a selective deficiency of either Bcl-6 or Blimp-1 in DCs show reductions in $CD103^+$ $CD11b^-$ cDC or $CD103^+$ $CD11b^+$ cDC development, respectively. These reductions are greater in tissue-resident DCs than DCs in lymphoid organs, implicating a predominant requirement in tissue-resident DC development or survival.

DC maturation and activation

DCs have dual functions depending on their maturation status. Mature DCs (mDCs) have been identified as potent immune activators of the adaptive immune response, while immature DCs (iDCs) play a role in maintenance of immune homeostasis in the steady state (reviewed in 38). IDCs can actively induce immune tolerance by modulating T-cell responses, leading to an anergy or deletion of self-reactive T cells or a differentiation of Tregs. Tissue-resident DCs, for example, continuously obtain antigens from the environment. However, they have poor immunogenic APC function as they express low levels of MHC II, co-stimulatory molecules and proinflammatory cytokines (39). Tissue-resident iDCs participate in the removal of self-reactive T cells during T-cell development in the thymus, shaping the naive T-cell pool in the periphery. This regulatory mechanism critically depends on a tolerizing APC function, as autoreactive T cells develop in mice with a cathepsin deficiency and impaired antigen presentation by MHC II (40). A regulatory function for iDCs has been demonstrated in peripheral tissues, including intestine, skin, lung and liver (41, 42). IDCs can present antigens that are obtained from dying cells and this mechanism is critical to the prevention of self-reactive T cells development (43).

DC activation

IDCs detect the presence of pathogens through pathogen-associated molecular patterns (PAMPs) or sterile injury through damage-associated molecular patterns (DAMPs) which are released from damaged tissues. These danger signals trigger signaling cascades in iDCs and change iDCs to mDCs. This maturation process includes profound phenotypic and functional change driven by changes in gene expression (reviewed in 44). These include an increased expression of surface molecules and proinflammatory cytokines which are required for efficient antigen presentation and T-cell activation, loss of phagocytic ability, increased migratory capacity by upregulation of the chemokine receptor, CCR7, enabling transit from tissues to local lymphoid organs (reviewed in 45). These data suggest that the combination of stimuli during antigen sampling determines whether iDCs induce immune tolerance or mature and induce immune activation. Thus, environmental factors are critical in determining whether iDCs remain tolerogenic or become activated and capable of cognate T-cell activation.

DCs express an array of pattern-recognition receptors including TLRs, c-type lectins, complement receptors, and Fc receptors (FcRs) on the surface that can specifically interact with PAMPs or DAMPs alone or adorned with antibody or complement (reviewed in 46). Specific molecules derived from dead tissue cells or pathogens represent DAMPs or PAMPs. PAMPs include double-stranded RNA, unmethylated CpG DNA, lipopolysaccharide (LPS), flagellin, and zymosan (47). DCs phagocytose necrotic cells, but in contrast to apoptotic cells, recognition of necrotic cells leads to an immune activation through exposure to DAMP. DAMPs include intracellular proteins such as heat shock proteins, high mobility group box 1 (HMGB1) and self-derived nucleic acids (reviewed in 48). Different molecules expressed by DCs detect distinct types of PAMPs or DAMPs.

TLRs are type I transmembrane proteins characterized by an extracellular domain containing leucine-rich repeats and a cytoplasmic tail that contains the Toll/IL-1 receptor (TIR) domain. To date, 10 human and 12 murine TLRs have been identified (49). They are located on the plasma membrane with the exception of TLR3, TLR7, TLR8, TLR9, and TLR13 which are localized in the endosomal compartment (50). The expression profiles of TLRs vary among cell types and tissues. Different subsets of DCs express different TLRs to

direct a response tailored to the threat. Binding to TLRs leads to the production of specific cytokines and the generation of specific T effector helper cell subsets and cytotoxic T cells (51).

TLR2 is essential for the recognition of PAMPs from Gram-positive bacteria, including bacterial lipoproteins, lipomannans, and lipoteichoic acids (52). TLR3 binds to virus-derived double-stranded RNA. TLR4 is predominantly activated by LPS. TLR5 detects bacterial flagellin. TLR7 and TLR8 recognize single-stranded RNA and TLR9 is required for a response to unmethylated CpG DNA. TLR13, which has been identified in mice, recognizes bacterial 23S ribosomal RNA (53, 54). The activation of TLRs is influenced by complex formation with accessory molecules; for example, MD-2 and CD14 form a complex with TLR4 to enable the response to LPS (55, 56). HMGB1 forms a complex with nucleic acids which binds to receptor for advanced glycan endo products (RAGE) and transports the nucleic acids to endosomal TLRs. Stimulation of TLRs initiates signaling cascades leading to the activation of transcription factors such as nuclear factor (NF)-κB and IRFs, a family of transcription factors known to play a critical role in antiviral defense, cell growth, and immune regulation (48, 57). This results in the production of pro-inflammatory cytokines and type I IFNs that direct the adaptive immune response. Three IRFs, IRF3, IRF5, and IRF7, function as direct transducers of TLR signaling. Activated IRFs are phosphorylated and translocate into the nucleus (58–60). Not surprisingly, genetic polymorphisms of IRF3, IRF5, and IRF7 have been associated with susceptibility to SLE (61–63), and elevated levels of nuclear IRF5 have been demonstrated in monocytes of SLE patients (64).

DCs in SLE

Numerous studies indicate that SLE has characteristic abnormalities in humoral immunity in conjunction with abnormal T-cell function (65). Abnormalities in T-cell function might be due to T-cell intrinsic defects, or to abnormal regulation of T cells by APCs such as DCs. Thus, there have been many attempts to identify whether DCs in SLE patients are different from DCs in healthy controls.

In early studies, investigators showed that the frequency of DCs is often decreased in blood of SLE patients compared to healthy controls. In one study, both $CD11c^+$ DCs and pDCs were significantly decreased in SLE patients regardless of therapeutic regimen. Moreover, the frequency of DCs in blood was inversely correlated with disease activity. A decreased ability to activate antigen-specific T cells was observed in studies of *ex vivo* derived DCs [granulocyte macrophage colony-stimulating factor (GM-CSF) stimulation], while there was no difference observed in monocyte differentiation to MO-DCs and induction of co-stimulatory molecules by GM-CSF and IL-4 (66). In other studies, however, induction of MHC II and TLR4 expression following maturation stimuli was compromised in MO-DCs from SLE patients and they showed a significantly decreased ability to induce T-cell activation in either autologous or allogeneic mixed lymphocyte reactions (MLRs) (67). It has been reported that chronically activated lymphocytes become hyporesponsive to external stimuli (68, 69). Thus, the decreased T-cell activation in autologous MLRs might reflect altered T-cell function as well as altered DC function. In contrast, other studies suggested that MO-DCs derived from SLE patients express higher levels of activation markers, CD80, CD86, and HLA-DR prior to exposure to maturation stimuli and increased allogenic T-cell stimulation. This positively correlated with clinical and serological features of SLE. These studies suggest that there are inflammatory factors which might precondition DCs in the blood of SLE patients, for example, nucleic acid-containing immune complexes or HMGB1. If these are present in the cultures of MO-DCs, the resulting cells might appear more activated than MO-DCs cultured in less pro-inflammatory conditions. Ding *et al.* (70) identified additional changes in MO-DCs from SLE patients by using additional markers, CD1a and CD83, to distinguish subsets of MO-DCs. There was an increase in CD1a-positive MO-DCs and decrease in CD83-positive MO-DCs from SLE patients compared to MO-DCs from healthy controls or patients with rheumatoid arthritis. The functional consequence of this difference is not clear.

Patients with SLE have reduced numbers of pDCs in the blood and an accumulation of pDCs in lesional tissue (71). The pathogenic role of pDCs in SLE is often considered to be a consequence of their production of type I IFNs. Patients treated with IFN-α for hepatitis C infection develop anti-nuclear antibodies or overt SLE (72). Moreover, long-term IFN-α treatment also induces SLE-like symptoms (73, 74). High expression of IFN-inducible genes in PBMCs (the IFN signature) correlates with disease activity in SLE. Notably, the IFN signature is seen in virtually all pediatric SLE patients and a substantial percentage of adult SLE patients. High type I IFN levels are a feature of some unaffected first-degree relations as well. Thus, pDCs and type I IFNs have been suggested to be central components of SLE pathogenesis. Genome-wide association study (GWAS) have identified SLE susceptibility alleles for genes which are associated with

type 1 IFN production such as IRF5, STAT4, and PTPN22 (75–77). It is worth noting, however, that the pathways that activate type 1 IFN production also activate production of several inflammatory cytokines which are also known to be involved in SLE pathogenesis such as IL-6 and TNF. Moreover, mouse models demonstrate that type 1 IFN can either exacerbate or ameliorate disease severity (78–80) and the PTPN22 SLE risk allele leads to decreased IFN production (81). Thus, it is not fully established whether the IFN signature is a marker of disease or a contributor to disease in all SLE patients.

Functional defects or decreased frequency of circulating DCs were repeatedly observed in early studies of DCs in SLE patients. However, these phenotypes were observed after disease onset, and might be a consequence of an inflammatory milieu in SLE patients. For example, it is difficult to understand whether low frequency of DCs in blood is due to DC-intrinsic defects or to a migration of DCs from blood to tissues in an inflammatory environment. In rheumatoid arthritis patients, activated DCs were shown to be selectively recruited to the inflamed synovium (82). In lupus nephritis patients, increased numbers of both cDCs ($CD1c^+$ cDCs and $CD141^+$ cDCs) and pDCs in kidney have been reported (83, 84).

Clearly, more data are needed to understand DC function in SLE. We chose to study DC function in SLE by investigating: (i) whether genetic DC-intrinsic alterations might occur in SLE or (ii) whether genetic alterations that are not DC-intrinsic affect DC development and maturation. Our approach has been to ask whether SLE risk alleles might affect DC function in healthy individuals in whom observed alterations in DC phenotype or function would not be secondary to the inflammation of established disease or to medication.

DC intrinsic alterations

Blimp-1

Blimp-1, a transcriptional repressor of the IFNβ gene, was discovered by Keller and Maniatis (85) in humans and by Turner *et al.* (86) in mice. Blimp-1 binds to the positive regulatory domain I of the IFNβ promoter region. A consensus sequence for Blimp-1 was identified and shown to be similar to the binding sequence for IRF1 and IRF2, implying a regulatory mechanism of Blimp-1 through inhibition of IRF binding (87, 88). The mechanism by which Blimp-1 negatively regulates target genes was also studied in mouse germ cells. The positive regulatory (PR) domain in Blimp-1 is similar to the SET domain in histone methyl transferase (HMT) (89). Although Blimp-1 does not have HMT activity, Blimp-1 can recruit the G9a HMT, to the IFNβ promoter. Overexpression of G9a methylates lysine 9 on a repressive histone, histone 3, which leads to repression of IFNβ expression (90). Blimp-1 also forms a complex with prmt5, an arginine HMT, in primordial germ cells (91). However, further studies need to be performed to understand all the mechanisms by which Blimp-1 represses its target genes in each immune cell subset.

The expression and function of Blimp-1 in the adaptive immune system has been well studied (reviewed in 92). Blimp-1 expression is tightly regulated throughout B- and T-cell differentiation. In B cells, Blimp-1 is mainly expressed in antibody-producing plasma cells, in both short-lived plasmablasts and long-lived plasma cells (93). Blimp-1-deficient B cells show a selective defect in plasma cell differentiation with a normal germinal center (GC) reaction and memory B-cell formation (94). Blimp-1 expression in B cells is suppressed by Pax5, Bach2, and Bcl-6. Stress signals which activate NF-κB during the GC response induce Blimp-1 expression (95–98) which begins at a late stage of the GC B-cell response and enables the expression of genes that are required for antibody secretion (99).

Blimp-1 expression is also required for proper T-cell development, regulating T-cell survival during negative selection of double positive thymocytes (100). The level of Blimp-1 expression in T cells is increased by activation (101). In $CD4^+$ T cells, Blimp-1 can directly suppress the expression of IL-2, fos, IFN-γ, T-bet and Bcl-6, and orchestrate Th1 and Th2 differentiation (102). A Blimp-1 deficiency in $CD4^+$ T cells mediated by Lck-CRE or CD4-CRE in a mouse with floxed Blimp-1 leads to the spontaneous development of an inflammatory bowel syndrome, mediated by defective Treg generation (103). In $CD8^+$ T cells, Blimp-1 is required for the differentiation of effector $CD8^+$ T cells and a $CD8^+$ T-cell memory response, especially in chronic viral infection in mice (104–106).

Blimp-1 in DCs

An early study performed by Chang *et al.* suggested that Blimp-1 might play a role as a differentiation factor for the myeloid lineage. Our group also identified a significant level of Blimp-1 expression in LPS stimulated BM-derived DCs (BM-DCs) differentiated by GM-CSF, and the level of Blimp-1 was increased in BM-DCs of Fc γ-chain knockout mice (S. J. Kim and B. Diamond, personal observation). Iwakoshi *et al.* (107) found that X-box-binding protein 1 (Xbp-1) is

required for DC survival from endoplasmic reticulum-stress mediated cell death; Xbp-1-deficient mice show a significant reduction in both cDCs and pDCs in the spleen. Xbp-1 has been shown to be upregulated in association with Blimp-1 expression in B cells (99, 108), implicating a role of Blimp-1 in DC survival.

To identify whether Blimp-1 expression regulates DC development, we deleted Blimp-1 in DCs by mating Blimp-$1^{flox/flox}$ mice with CD11c-CRE mice (CKO) generating a significant reduction of Blimp-1 in $CD11c^+$ cDC subsets with a partial reduction in pDCs. In contrast to our initial speculation, all the DC subsets developed normally in both spleen and LNs. We found, however, a significant reduction in $CD11b^+$ $CD103^+$ cDCs in MLNs and in the intestinal lamina propria (20). Although DC development was generally normal in the peripheral lymphoid organs, DCBlimp-1 CKO mice develop a spontaneous lupus-like syndrome at approximately 6 months of age (109). Blimp-1-deficient DCs exhibit an activated phenotype with increased MHC II expression in cDCs. CDCs secrete increased levels of the proinflammatory cytokines IL-6, IL-2, IFN-γ, and TNF-α following LPS stimulation which is not due to increased levels of TLR4 or TLR4 downstream molecules in Blimp-1-deficient DCs. There is a broad range of alterations following TLR signaling in Blimp-1-deficient DCs, presumably secondary to a decreased expression of cytokine signaling-1 (SOCS-1) through the microRNA Let-7c (110).

Another striking phenotype in DCBlimp-1 CKO mice is constitutively increased GC formation. A stronger GC response in DCBlimp-1 CKO mice was confirmed by NP-immunization. DCBlimp-1 CKO mice immunized with NP-CGG developed an increased titer of high-affinity anti-NP antibodies but not low-affinity anti-NP antibodies (109). This increased GC formation is driven by an increased number of T follicular helper (Tfh) cells in DCBlimp-1 CKO mice. IL-6 produced by Blimp-1-deficient DCs is one of the mechanisms responsible for the enhanced Tfh cell differentiation; a reduction in IL-6 in IL-6 haploid DCBlimp-1 CKO mice reversed the phenotype, with a decrease in Tfh cells and GC formation and anti-dsDNA antibodies. Interestingly, the lupus-like phenotype observed in female DCBlimp-1 CKO mice is not recapitulated in male DCBlimp-1 CKO mice, suggesting an effect of either estradiol or an X-chromosome dose effect. Ovariectomy in DCBlimp-1 CKO female mice prevents the development of the lupus-like phenotype and reconstitution of estradiol in ovariectomized DCBlimp-1 CKO mice leads to disease development, clearly demonstrating a key role for estradiol in the development of SLE in DC Blimp-1 CKO mice (S. J. Kim and B. Diamond, unpublished data).

Blimp-1 gained attention in human SLE after GWAS showed that the gene encoding Blimp-1, PRDM1, has a SLE risk variant (111–113). The risk allele which is associated with SLE is located 3′ of the PRDM1 coding area in an intergenic region between PRDM1 and ATG5. To address whether the Blimp-1 SLE risk allele causes any functional change in leukocytes, Blimp-1 expression in various leukocytes was compared between individuals with a non-risk allele (controls) and individuals with the risk allele. Interestingly, Blimp-1 expression was decreased in MO-DCs of risk allele individuals but was not affected in B cells. Expression was increased in Tregs from risk allele individuals (S. J. Kim and B. Diamond, unpublished data). MO-DCs derived from Blimp-1 SLE risk allele individuals also expressed an increased level of HLA-DR and exhibited greater IL-6 expression following LPS stimulation (110). These data suggest that a proper expression of Blimp-1 is required for the regulation of the inflammatory function of DCs, and that Blimp-1-regulated pathways are similar in humans and mice.

A key function of DCs is their APC activity in the thymus and the periphery. A regulatory role of Blimp-1 in MHC II expression was first identified in studies of plasma cells where it was shown to suppress Class II transactivator (CIITA) which is a co-activator for MHC II expression (114). In DCs, Blimp-1 was also shown to regulate MHC II expression through suppression of CIITA (115). However, a recent study suggested that Blimp-1 regulates MHC II expression in $CD11b^+$ DCs jointly with IRF4 by regulating Cathepsin S (Ctss) (116). Ctss is a thiol protease which is exclusively expressed in APCs and regulates antigen presentation by removing the invariant chain facilitating surface expression of MHC II (117). Ctss also participates in antigen degradation, thereby shaping the pool of peptides that are loaded into MHC II (118). Manipulation of Ctss expression has been shown to affect the deletion of autoreactive T cells in the thymus, underscoring its key function in antigen presentation and in autoimmunity (119). The administration of a pharmacological inhibitor for Ctss can prevent disease development in animal models of Sjögren's syndrome (120) and SLE (121). In fact, there is a Blimp-1 binding sequence in the promoter of Ctss, and Ctss expression is higher in Blimp-1-deficient DCs than in Blimp-1-sufficient DCs (S. J. Kim and B. Diamond, unpublished data). This observation suggests another immunoregulatory function of Blimp-1,

through the regulation of Ctss and antigen presentation in DCs.

A second risk allele of Blimp-1 is also highly associated with inflammatory bowel disease (IBD) (122). Blimp-1 expression in T cells has been known to contribute to intestinal homeostasis (100, 123). Although the initial studies suggested that T cells were responsible for Blimp-1-regulated intestinal homeostasis, DCs have been identified as a cell type showing the strongest expression of genes with IBD risk variants (124). We, therefore, studied the impact of DC-specific Blimp-1 deletion in IBD and showed that in DCBlimp-1, CKO mice are more susceptible to a chemically induced (dextran sodium sulfate) IBD model (125). Although Blimp-1-deficient DCs showed an increased expression of IL-6 which is required for Th17-cell differentiation, there was no difference in the frequency of Th17 cells in colonic lamina propria between DCBlimp-1 CKO and wildtype mice. However, stimulation of nucleotide-binding oligomerization domain-containing protein 2 by its ligand, muramyl dipeptide (MDP), induced an increased expression of IL-1β and IL-6 in Blimp-1 deficient intestinal DCs, and these cytokines were shown to induce matrix metalloproteinases (MMPs) in macrophages. The excessive secretion of MMPs from macrophages and an increased number of infiltrating neutrophils in the inflamed intestine is a plausible explanation for the excessive tissue damage and severe colitis which is seen in this model (see *Fig. 1*).

Blimp-1 in other innate immune cells

Blimp-1 expression in cells of the innate immune system was first identified in human monocytes and granulocytes (126). Blimp-1 expression is increased during differentiation of monocytes to macrophages and is sufficient to induce macrophage differentiation. c-myc was shown to be a target of Blimp-1 in monocytes, and suppression of c-myc leads to cessation of proliferation in monocytes. In mice, Blimp-1 expression was also observed in F4/80-positive macrophages and the expression of Blimp-1 was strongly increased by bacterial exposure. Macrophage-specific Blimp-1 CKO (LysoM-CRE) mice have an increased level of the chemokine CCL8 in blood and an increased infiltration of neutrophils into tissue following *Listeria monocytogenes* infection. Therefore, Blimp-1 suppresses the neutrophil-attracting chemokine, CCL8, thereby preventing the deleterious effects associated with excessive inflammation in target tissues (127). Blimp-1 is also expressed in natural killer (NK) cells in mouse, and IL-15 exposure is required for its expression. Blimp-1 is required for NK cell maturation and homeostasis. Moreover, Blimp-1 is critical to the cytotoxic effect of NK cells as it modulates granzyme B expression. Blimp-1 expression depends on T-bet, but not on IRF4, expression in NK cells, which further supports that cell type-specific regulatory mechanisms exist for Blimp-1 (128).

Fc γ receptor

FcRs are a group of surface molecules with binding specificity for the Fc region of antibodies (reviewed in 129). There are two functionally distinct groups of FcRs, activating and inhibitory FcRs. Some activating FcRs – FcRIIA, FcRIIC in humans – possess an immunoreceptor tyrosine-based activation motif (ITAM) in their cytoplasic domains while other activating FcRs (FcRI, FcRIII and FcRIV in mice and FcRI and FcRIIIA in humans) associate with the FcR common γ-chain which signals through an ITAM. Cross-linking of activating FcRs with immune complexes (IC) activates signaling cascades beginning with the activation of SRC family kinases (SFK) and spleen tyrosine kinase. Inhibitory FcRs (FcRIIB in mice and humans) possess an immunoreceptor tyrosine-based inhibition motif (ITIM) in their cytoplasmic domains, and the activation of inhibitory FcRs recruits SH2 domain-containing inositol 5′-phosphatase 1, counteracting activating receptor-mediated signaling cascades.

Various combinations of FcRs are expressed in DCs. The Immunological Genome Consortium generated a comprehensive data set on FcR expression patterns in DCs in blood and in tissue (skin) as well as *in vitro* cultured human MO-DCs, mouse BM-DCs, and in monocytes, which has been confirmed in other studies (130–132). Monocytes and macrophages exhibit the highest expression of activating and inhibitory FcRs. *In vitro* cultured MO-DCs also express high levels of both activating and inhibitory FcRs. However, human blood $CD141^+$ cDCs and mouse $CD8\alpha^+$ DCs express a limited array of FcRs and lower level expression. Interestingly, FcRI and FCRIII expression is particularly low in human and mouse cDCs. Human blood $CD1c^+$ cDC express activating FcRIIA and inhibitory FcRIIB. The level of FcRIIB in mouse cDCs is higher in tissue-resident cDCs compared to cDCs in spleen or LNs, suggesting a tolerogenic function of tissue-resident DCs. PAMPs and inflammatory cytokines have been shown to induce FcRllB expression in DCs; therefore, FcR-mediated immune modulation might occur following immune activation to prevent an excessive inflammatory response.

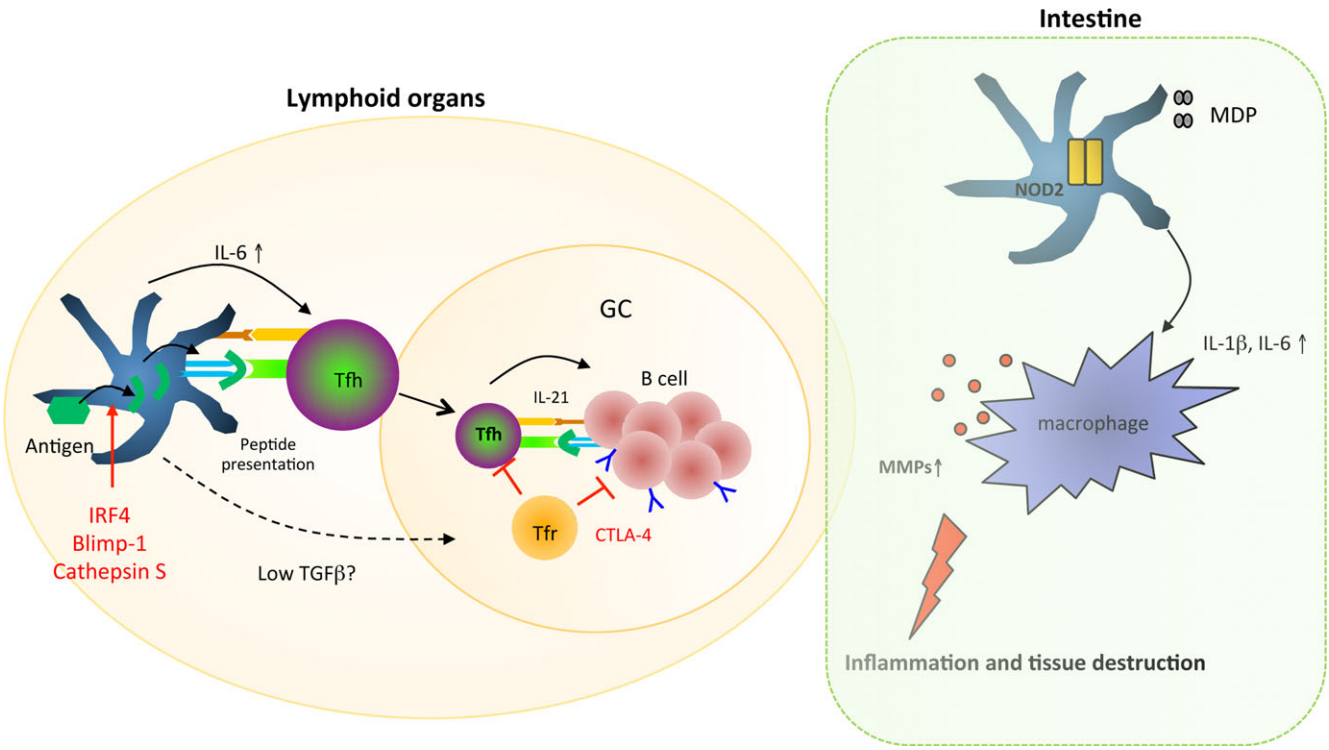

***Fig. 1.* Tissue-specific function of Blimp-1 expression in dendritic cells.** In peripheral lymphoid organs, Blimp-1 expression in cDCs regulates follicular helper T (Tfh) cell expansion and antigenic specificity. Together with Irf4, Blimp-1 regulates peptides which are presented on major histocompatibility class II (MHC II) through the expression of Cathepsin S. Blimp-1 expression also negatively regulates IL-6 which is required for Tfh cell differentiation. Therefore, low levels of Blimp-1 in DCs lead to an increase in Tfh cell differentiation, and autoreactive B cell activation in germinal centers. Blimp-1 expression is also necessary in tissue-resident DCs. Absence of Blimp-1 in intestinal DCs leads to a hyperactivation of the NOD2 signal pathway. This abnormal response generates increased IL-1β and IL-6 production, and subsequently induces increased matrix metalloproteinases (MMPs) from neighboring tissue macrophages. DC, dendritic cell; IL-6, interleukin-6.

FcR-mediated signaling has been shown to enhance APC function in DCs. Several studies demonstrated that particulate antigens, antibody-bound antigens (ICs), or apoptotic cells induce more effective antigen-specific T-cell activation than soluble antigens (133–135). Enhanced antigen presentation by ICs is mediated through activating FcRs. The engagement of activating FcRs induces DC maturation and production of proinflammatory cytokines. FcR engagement can also modulate the effects of engagement of other cell surface receptors. While TLR4 engagement induces IL-12, simultaneous stimulation of activating FcRs and TLR4 suppresses IL-12 production in monocytes, macrophages, and MO-DCs (136–138). IC-mediated FcR activation in DCs can also regulate B-cell differentiation. FcR γ-chain-deficient mice (deficient in the surface expression of both FcRI and FcRIII) develop an increased titer of anti-dsDNA antibodies in response to immunization with a peptide mimetope of dsDNA (139). In these mice, antigen-experienced B cells preferentially develop into short-lived plasma cells rather long-lived plasma cells derived from GC-matured B cells. DCs from the FcR γ-chain-deficient mice displayed an activated phenotype with a highly increased production of IL-12, consistent with data showing that ICs downregulate IL-12 production. Increased IL-12 production by splenic DCs from FcRγ-chain-deficient mice did not alter the T-cell response to immunization; however, IL-12 skewed B-cell differentiation to short-lived plasma cells, leading to increased dsDNA-reactive antibody secretion. Interestingly, while the titers were higher, the average affinity of the antibodies was lower, consistent with the diminished GC response.

FcR signaling also regulates *in vitro* differentiation of DCs in humans and mice. $CD14^+$ monocytes spontaneously differentiate to $CD1a^+$ $CD11b^+$ $CD206^+$ iDCs in the presence of plate-bound IgG. DC differentiation depends on the presence of FcRI on monocytes; FcRI engagement induces GM-CSF transcription which is a DC differentiation factor. MO-DCs that are generated by FcRI activation show a potent T-cell activation capacity (140). In contrast, another study showed that IC exposure during the differentiation of monocytes to DCs inhibits DC differentiation (141). In this study, IC-exposed MO-DCs expressed high levels of CD14 and CD68 with low levels of CD1a. Both FcRI and FcRIII

engagement were required for this inhibitory effect of ICs. IC-exposed MO-DCs are weak T-cell activators, but secrete high levels of the chemokines CXCL8 and CCL2. These phenotypic and functional changes driven by IC are similar to MO-DCs from SLE patients, implicating IC-FcR engagement as a potential factor which regulates differentiation from monocyte to DCs in SLE. Soluble immunoglobulin also regulates DC differentiation and maturation during BM-DC differentiation. Under the classical DC differentiation regimen with GM-CSF, DC precursor cells differentiate to $CD11c^+$ $CD11b^+$ cells with modest levels of MHC II and costimulatory molecules. However, when DC precursor cells are differentiated with GM-CSF in immunoglobulin-depleted fetal bovine serum (hypo IgG) or in serum-free media, immature BM-DCs express increased levels of MHC II, CD86, and CD80 constitutively and also following TLR4 stimulation. They also express increased levels of the proinflammatory cytokines IL-12 and IL-6 following TLR stimulation. Interestingly, DCs cultured with the hypo IgG serum showed a pDC-like phenotype with B220 and Ly6C/G expression together with CD11c expression (142). Most studies on FcR-mediated DC modulation and differentiation were performed *in vitro*. Therefore, the *in vivo* function of FcR in DCs needs to be investigated.

Importantly, polymorphisms, both single nucleotide polymorphisms (SNPs) and copy number variation, in the activating FcRs, FcRIIA and FcRIIIA, have been associated with SLE (143, 144). The SLE risk alleles all lead to lower affinity for IC binding and, therefore, reduced clearance of IC. Whether they also alter the cytokine profile of DCs and thereby the differentiation pathway of antigen-exposed B cells is not known. There are also several polymorphisms of the inhibitory receptor, FcRIIB that associate with SLE. These are uniformly loss-of-function. How these polymorphisms alter overall DC function has not been well studied.

DC extrinsic regulators

Vitamin D3 (VD3)

VD3 is the most physiologically relevant form of VD, and is synthesized in the skin from 7- hydrocholesterol by UV irradiation (reviewed in 145). VD3 is then converted in the liver to 25-dihydroxyvitamin D3 [25(OH)VD3], which is the main form of VD3 in blood. This compound is then metabolized in the kidneys to 1,25(OH)2VD3 (VD3), the active VD3 metabolite. VD3 is concentrated locally in lymphoid microenvironments. DCs, monocytes, pDCs, and macrophages express two types of VD3 convertease, CYP27A1 and CYP27B1 to hydroxylate 25(OH)VD3 to VD3. This locally produced VD3 can act on immune cells in an autocrine or paracrine manner (146, 147).

The effect of VD3 is mediated through binding to the vitamin D receptor (VDR). The VDR is a nuclear receptor and ligand-activated transcription factor that binds specific DNA sequence elements (VDRE) (reviewed in 148). The VD3-bound VDR activates transcription by heterodimerization with the Retinoid X receptor (RXR), resulting in dissociation of the nuclear receptor co-repressor, NCoR, and facilitating interactions with coactivators. In some conditions, the ligand-bound VDR can also repress transcription as the VDR–RXR complex interacts with a VDR-interacting repressor to cause ligand-induced repression.

VD3 has been shown to be a regulator of immune responses (148, 149). Although the influence of VD3 has been well studied in adaptive immune cells in the context of inhibiting T-cell proliferation or CD8 T cell-mediated cytotoxicity (150, 151), VD3 has important effects on innate immune cells as well. VD3 can inhibit MO-DC differentiation from monocytes *in vitro* and proliferation of monocytes (151). VD3 is known to inhibit the differentiation, maturation, and immunomodulatory capacity of DCs by decreasing the expression of molecules such as MHC class II, CD40, CD80 and CD86, the maturation proteins CD1a and CD83, and to decrease production of IL-6, IL-12, and IL-23 (152). In addition, VD3 increases levels of inhibitory receptors and anti-inflammatory cytokines. VD3-treated DCs are less potent inducers of effector T-cell responses but rather induce the generation of Tregs. Among the human DC subsets, blood $CD1c^+$ DCs produce retinoic acid in response to VD3, contributing to their ability to induce Tregs. VD3 induces $BDCA1^+$ DCs to express a high level of RALDH2, which converts retinal to retinoic acid in the presence of GM-CSF (153). Additionally, VD3 can inhibit mouse and human pDC maturation in a VDR-dependent fashion (146). Finally, VD3 downregulates the expression of TLRs and upregulates programmed death ligand-1 which acts as an inducer of IL-10 in DCs, thereby driving Treg differentiation (154, 155).

VDR-deficient mice have increased numbers of mDCs in skin-draining LNs (156). In an experimental allergic encephalitis model (EAE), VD3 induces IDO^+ tolerogenic DCs, leading to an enhanced Treg number and reducing the severity of EAE (157). It has been shown that BMDCs cultured with VD3 produce high levels of IL-10 and have decreased expression of MHC II and CD80. Therefore, VD3

can act as a natural inhibitor of antigen presentation and T-cell activation.

VD3 deficiency is prevalent in patients with autoimmune diseases including SLE. VD3 has been demonstrated to inversely correlated with clinical SLE activity and with anti-C1q and anti-dsDNA titers. We and other groups have shown that VD modulates DC activation and blocks induction of an IFN signature by SLE serum (158, 159). Thus, the association of VD deficiency with increased disease activity in SLE may reflect the effects of VD on DC differentiation and maturation (158). Some studies suggest VD3 supplementation helps correct immune abnormalities of SLE patients, while other studies do not confirm this observation.

C1q

C1q, which initiates the classical complement pathway, also exhibits a variety of complement-independent activities (160–162). The interaction of C1q with immune cells induces numerous biological responses including stimulation of phagocytosis, suppression of T- and B-cell proliferation, cell adhesion and migration, suppression of cytokine production, and regulation of DC maturation (reviewed in 163). It plays a fundamental suppressive role in immune homeostasis.

C1q, present at high concentration in serum, is composed of 18 polypeptide chains; six triple helix units composed of a 6a, 6b, and 6c chain form the C1q molecule (460 kDa). Unlike most of the complement proteins which are primarily synthesized in the liver, C1q is synthesized by myeloid cells including monocytes, macrophages, microglia, and iDCs, but not by mDCs (163). C1q is actively produced and accumulates at inflammatory sites (164). While this may function to downregulate ongoing inflammation, it has also been shown that C1q can directly attract DCs from the blood to peripheral inflammatory tissues, and promote the migration of mDCs to secondary lymph organs (165).

The diversity of immunological functions mediated by C1q relates in part to its structure; it possesses a globular head domain (gC1q) and a collagen-like domain (C1q tail), both of which have distinct binding partners. Thus, the diverse functions of C1q are mediated by its interaction with distinct cell surface receptors for its gC1q or C1q tail domain. C1q is a major sensor of DAMPs and PAMPs (reviewed in 166). The gC1q domain recognizes a wide range of molecular patterns including self and non-self antigens. Cellular receptors for C1q include gC1qR, LAIR-1, calreticulin, CD93, CD91, CD44, DC-SIGN, RAGE, and β-integrin (167–171). Human MO-DCs express high levels of gC1qR and DC-SIGN which decrease in a maturation-dependent manner (170). In immature MO-DCs, C1q, DC-SIGN, and gC1qR form a trimolecular complex on the plasma membrane which inhibits DC maturation.

Our findings demonstrate that C1q can interact with cell-associated Leukocyte-associated Ig-like receptor 1 (LAIR-1, CD305) to control DC activation and maturation (169). LAIR-1 is the functional receptor for collagen, C1q collagen tail, and collagenous surfactant protein D (169, 172, 173). It has been proposed that during a normal response to tissue injury, extracellular matrix collagens engage LAIR-1-expressing cells to suppress inappropriate immune activity (reviewed in 174). The intracellular domain of LAIR-1 contains two ITIMs; when these are phosphorylated, they recruit the SH2-containing tyrosine phosphatases SHP-1 (but not in mice) and SHP-2, which negatively regulate intracellular signaling associated with immune cell maturation, differentiation, and activation (175). Phosphorylation of LAIR-1 is inhibited by PP2, an SFK inhibitor, suggesting that the kinase responsible for LAIR-1 phosphorylation is an SFK such as Lck, Hck, or Lyn (176) and our own data show Hck constitutively associates with LAIR-1, suggesting it may be the SFK that phosphorylates LAIR-1 (177). LAIR-1 is also associated with Csk, which is known to phosphorylate the inhibitory tyrosine of SFKs (178). Two soluble forms of LAIR-1, the splice variant LAIR-2 (CD306) which is not expressed in mouse or rat (179) and a shed form of LAIR-1 (sLAIR-1), have been implicated in antagonizing LAIR-1-meditated inhibition. LAIR-1 is highly expressed in $CD34^+$ progenitor cells, monocytes, pDCs, T and B cells (not expressed on mouse B cells). High expression of LAIR-1 is associated with a less differentiated or naive phenotype suggesting that LAIR-1 engagement will result in suppression of the activation immature cells. C1q and LAIR-1 activity have been separately ascribed important roles in maintaining immune homeostasis and defects in either molecule have been linked with flawed immunoregulation in distinct disease settings. When C1q binds to LAIR-1, nuclear translocation of IRF3 and 5 and NF-κB, mediated by CpG stimulation, is inhibited, thereby diminishing TLR9-mediated signaling and cytokine production (177). This constitutes one mechanism to downregulate the TLR-mediated transcription of multiple cytokines. While LAIR-1 is an important receptor for C1q, C57BL/6 mice with LAIR-1 deficiency fail to exhibit a lupus-like phenotype (180) suggesting that there

are additional inhibitory receptors to prevent autoimmunity.

C1q potentiates the anti-inflammatory effects of IgM natural autoantibodies. IgM autoantibodies are present in healthy individuals and have been shown to be tolerogenic (reviewed in 181). IgM may form immune responses with C1q to mediate immune suppression through engaging the inhibitory LAIR-1 receptor on monocytes and DCs. Indeed, IgM antibodies need not be autoreactive to mediate immunosuppression [(182), M. Son and B. Diamond, unpublished data]. In contrast, IgG ICs can bind activating FcRs to mediate cellular activation and inflammation. As mentioned above, gC1q also binds to several cell surface receptors. Our hypothesis is that the response to C1q may be a consequence of an interaction of C1q tail with LAIR-1 and an interaction of gC1q with other membrane receptor-specific combinations of LAIRs with gC1q receptors may have distinct effects on monocyte and DC function.

C1q also plays an important role in the clearance of dead and dying cells (183–185). Apoptotic material taken up by DCs through inhibitory receptors is normally non-immunogenic. C-reactive protein, natural IgM antibodies, and C1q can serve as opsonins, facilitating an efficient recognition of apoptotic cells by inhibitory receptors on phagocytes (186–188). Accordingly, apoptotic cells preincubated with C1q deficient serum lost their inhibitory effect on DCs (S. J. Kim and K. Elkon, unpublished data), implicating C1q as a critical molecule for anti-inflammatory effects in DCs. Moreover, DCs which phagocytose apoptotic cells secrete reduced levels of the proinflammatory cytokines IL-12 and TNF-α, and increased levels of the anti-inflammatory mediators IL-10, PGE2, and TGF-β (189). Some receptors that phagocytose C1q-decorated apoptotic cells have been identified. The scavenger receptor Scarf1 also has been described as C1q-dependent receptors for apoptotic cell clearance (190). C1q can enhance the uptake of apoptotic cells in a Scarf-1-dependent fashion as it increases uptake of apoptotic cells by wildtype $CD8\alpha^+$ DCs but not by $Scarf1^{-/-}CD8\alpha^+$ DCs. Moreover, Scarf1 deficiency led to spontaneous autoimmune disease and global activation of T cells, follicular helper T cells, and B cells. C1q elicited macrophage expression of the Mer tyrosine kinase (Mer) which resulted in enhanced clearance of apoptotic debris (191). Whether LAIR-1 also mediates phagocytose is not known. Olde Nordkamp *et al.* (172) recently showed that the collagenous C-type lectin, surfactant protein D (SP-D), engages LAIR-1 and inhibits FCαR-mediated reactive oxygen species production by a human myeloid leukemia cell line.

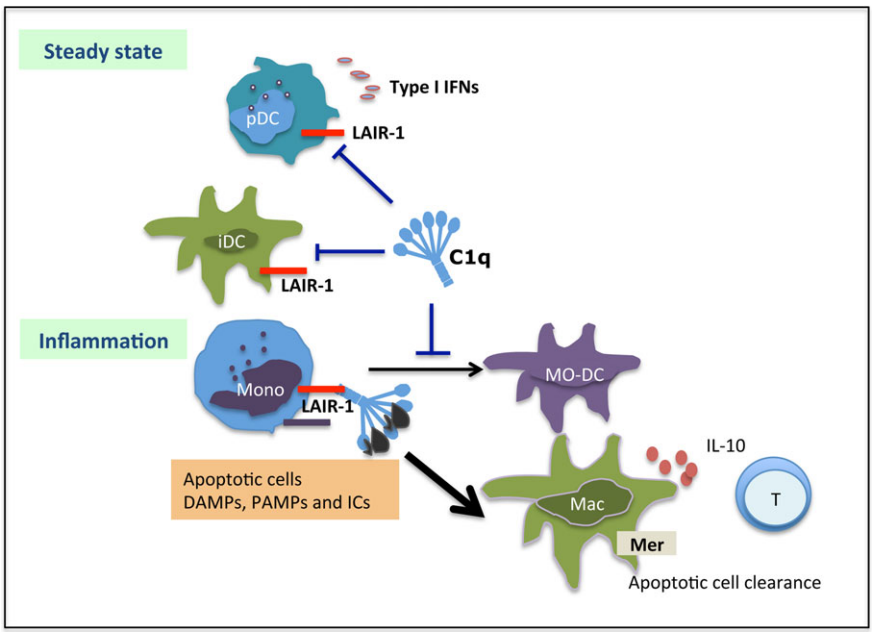

***Fig. 2.* C1q has a fundamental suppressive role in immune homeostasis.** Interaction between C1q and its receptor LAIR-1 inhibits DC differentiation and activation either during steady state or during inflammation. C1q prevents the production of interferon and inflammatory cytokines by both human and mouse DCs and monocytes. In inflammation or lupus progression, C1q enhances the clearance of apoptotic debris through regulating LAIR-1 and perhaps other C1q receptors. PDC, plasmacytoid DC; Mono, monocyte; MO-DC, monocyte-derived dendritic cell; Mac, macrophage; DAMPs, damage-associated molecular patterns; PAMPs, pathogen-associated molecular patterns; Mer, Mer tyrosine kinase.

SP-D is a member of collections and, like C1q, functions in lung clearance of apoptotic cells (192). In general, apoptotic debris decorated by a number of molecules including C1q activates inhibitory pathways in DCs and maintains their immature phenotype and tolerogenic function. It is possible that additional C1q receptors for apoptotic debris remain to be identified.

Genetic deficiency of C1q predisposes strongly to SLE. SLE occurs in approximately 90% of C1q-deficient patients in many studies (reviewed in 162). A less dramatic association suggested that half of C1q-deficient patients (55%) presented with definite SLE with another 25% presenting with a SLE-like syndrome (193). C1q-deficient patients have severe central nervous system and renal autoimmune disease. Studies in mice suggest that C1q deficiency acts as a disease-accelerating factor on genetic backgrounds that predispose to the development of SLE (194).

Associations between SNPs in C1q and specific clinical phenotypes have reported. The presence of SNPs in the C1q genes has been associated with differences in response to rituximab treatment in patients with lymphoma (195). Martens *et al.* (196) showed that C1q polymorphisms are associated with more severe SLE, low serum C1q, and low levels of total hemolytic complement. Trouw *et al.* (197) reported that SNP rs292001 is strongly associated with low serum levels of C1q and significantly associated with RA in GWAS from the North American Rheumatoid Arthritis Consortium study. The same rs292001 SNP and the G allele in rs172378 have been identified as risk factors for lupus nephritis in a Bulgarian population (198). While no LAIR-1 alleles have yet been associated with autoimmunity, transmembrane LAIR-1 expression is lower in pDCs, B cells and T cells in SLE patients than in healthy donors [(199), M. Son and B. Diamond, unpublished data].

In SLE, C1q deficiency may also be mediated by autoantibodies. Antibody to C1q has also been implicated in lupus nephritis, and is found in 30–50% of lupus patients (200) and increased serum levels of anti-C1q antibodies correlate with renal flares in SLE (201). We identified a subset of anti-DNA antibodies that binds C1q and so can bind to IC in glomeruli to enhance the activation of FcR-bearing cells in the kidney (202). Antibodies with this cross-reactivity are present in the serum of 40% of SLE patients. We postulate that anti-C1q antibodies participate in lupus pathogenesis through direct targeting of IC in glomeruli and also through removal of soluble C1q thereby limiting the ability of C1q to mediate immune homeostasis. Anti-C1q antibodies might also contribute to SLE by blocking the interaction of C1q with its inhibitory receptors and by preventing its contribution to immune quiescence.

One likely explanation for the importance of C1q in protecting against SLE is that C1q prevents the production of IFN and inflammatory cytokines by both human and murine DCs (203, 204) as C1q inhibits activation of cDCs and pDCs by SLE serum *in vitro*. Moreover, C1q limits the monocyte to DC transition and arrests DCs in an immature tolerogenic state. Human peripheral blood monocytes cultured with C1q and DC growth factors (GM-CSF plus IL-4) fail to downregulate the expression of the monocyte marker CD14 and upregulate expression of the DC markers CD83 and CD86 (169). In mice, soluble C1q caused BM-DC to produce less IL-12 (160) and DCs cultured on immobilized C1q exhibit elevated IL-10 but reduced IL-12 and IL-23 production (205). C1q also prevents antigen presentation to T cells by macrophages and DCs (206). These studies demonstrate that DCs developing in the presence of C1q are more tolerogenic. Another explanation for the association of C1q deficiency with SLE is the defective clearance of apoptotic debris. An increased presence of apoptotic cells in LNs and tissues is seen in many SLE patients (207, 208). The inability to properly remove apoptotic cells can facilitate a loss of immune tolerance (see *Fig.* 2).

In summary, C1q/LAIR-1 signaling mediates a major inhibitory pathway for the innate immune response both in the steady state and during inflammation. C1q and C1q-like molecules could be potent therapeutics to prevent activation of monocytes, cDCs, and pDCs, reducing the proinflammatory milieu in autoimmune patients.

Conclusions

The data presented above demonstrate that abnormal DC function can predispose to autoimmunity. As both DC intrinsic alterations and extrinsic regulators of DC function are able to skew DCs toward inflammation and immunogenicity, DCs become an important therapeutic target in SLE. There is much speculation that the lack of success of clinical trials for SLE is a consequence of the heterogeneity of the disease. As the expression of genes with SLE risk alleles is greatest in B cells and DCs, it is interesting to speculate that either cell lineage may be a driver of disease and that some individuals develop SLE due to aberrant DC maturation and activation and some due to aberrant B-cell tolerance and activation. If this speculation is confirmed, early SLE would be characterized first by either cytokine abnormalities or by autoantibodies. This is a testable hypothesis.

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