

# The many faces of type I interferon in systemic lupus erythematosus

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**Systemic lupus erythematosus (SLE) is a multisystem autoimmune disease with a broad spectrum of clinical presentations involving multiple organ systems. An abnormal response to self-antigens is thought to drive the development of SLE; however, the factors that underlie this dysfunction are not clear. In this issue of the *JCI*, Li and colleagues present compelling evidence to show that type I interferons (IFNs) produced by plasmacytoid dendritic cells inhibit the clearance of apoptotic cells (ACs) by marginal zone macrophages. Specifically, type I IFNs increase the translocation of marginal zone (MZ) B cells to the follicular region of the spleen, thereby disrupting interactions between these B cells and MZ macrophages (MZMs), which in turn disrupts megakaryoblastic leukemia 1-mediated (MKL1-mediated) mechanosensing and inhibits AC phagocytosis by MZMs. The results of this study provide important insight into factors that inhibit AC clearance and promote the development of SLE.**

## Systemic lupus erythematosus: a heterogeneous disease

Systemic lupus erythematosus (SLE) is a systemic autoimmune disease with diverse clinical presentations that can affect multiple organ systems (1). The multiplicity of symptoms and pathologies are a result of the heterogeneous immunological abnormalities that contribute to disease pathogenesis. While the precise etiology of SLE is unknown, it is hypothesized to be a combination of genetic and environmental factors (1). The onset of clinical symptoms and subsequent diagnosis of SLE occur long after the initiation of disease, making it difficult to identify the causative factor(s).

## Defective apoptotic cell clearance by marginal zone macrophages

An abnormal response to autoantigens is considered an important driving force in the development of human SLE (2) and is thought to be due to a defect in clearance of

apoptotic cells (ACs), resulting in increased exposure of lymphocytes to internal cellular antigens (3). Lupus-associated autoantigens, including nucleosomal DNA and small nuclear ribonucleoproteins, have been identified on the blebs of ACs (3). The lack of AC clearance has been shown to be pivotal in the production of anti-nuclear antibodies (ANAs) against these antigens, and the presence of circulating ANAs is a diagnostic criterion for lupus. Immune complexes formed by autoantibodies and self-antigens accumulate in multiple organs, cause tissue damage, and promote a systemic inflammatory response (4). Although it is widely accepted that ACs contribute to SLE pathogenesis, the mechanisms responsible for the defective clearance of ACs remain poorly understood. Recently, it has become apparent that, in addition to B cells, several other cells of the immune system are defective in patients with SLE. These include cells of the innate immune system, such as macrophages and monocytes, which contribute

to impaired phagocytosis and defective AC clearance (5). In particular, marginal zone macrophages (MZMs) surrounding the splenic follicles have been reported to play a crucial role in the efficient clearance of ACs and in the induction of tolerance to AC autoantigens (AC-Ags) (6). MZM dysfunction results in a loss of tolerance to self-antigens and a decrease in AC clearance (7).

## Type I IFNs in SLE

Type I IFNs are produced in response to both viral and self-nucleic acids and are primarily secreted by plasmacytoid dendritic cells (pDCs) (8). Whereas many cell types produce IFN $\beta$  and some other subtypes of type I IFNs, IFN $\alpha$  is almost exclusively produced by pDCs (9, 10). Chronic activation of nucleic acid-sensing receptors expressed on the surface of pDCs stimulate IFN $\alpha$  overproduction and activation of signals downstream of IFN $\alpha/\beta$  receptors (IFNARs) on target cells. The overproduction of type I IFN has been implicated in the pathogenesis of SLE, as this phenotype has been observed in gene expression studies and serum analysis of patients (11). Moreover, excessive IFN $\alpha$  production contributes to SLE pathogenesis via increased induction of plasma cells, production of autoantibodies, and promotion of T cell-dependent inflammation (10). While the role of ACs in promoting type I IFN overproduction in SLE is well documented, the effect of excess IFN on the clearance of ACs has remained uninvestigated. In this issue, Li and colleagues use a combination of genetic mouse models to demonstrate a novel role for type I IFNs in defective MZM-mediated clearance of ACs in SLE (12).

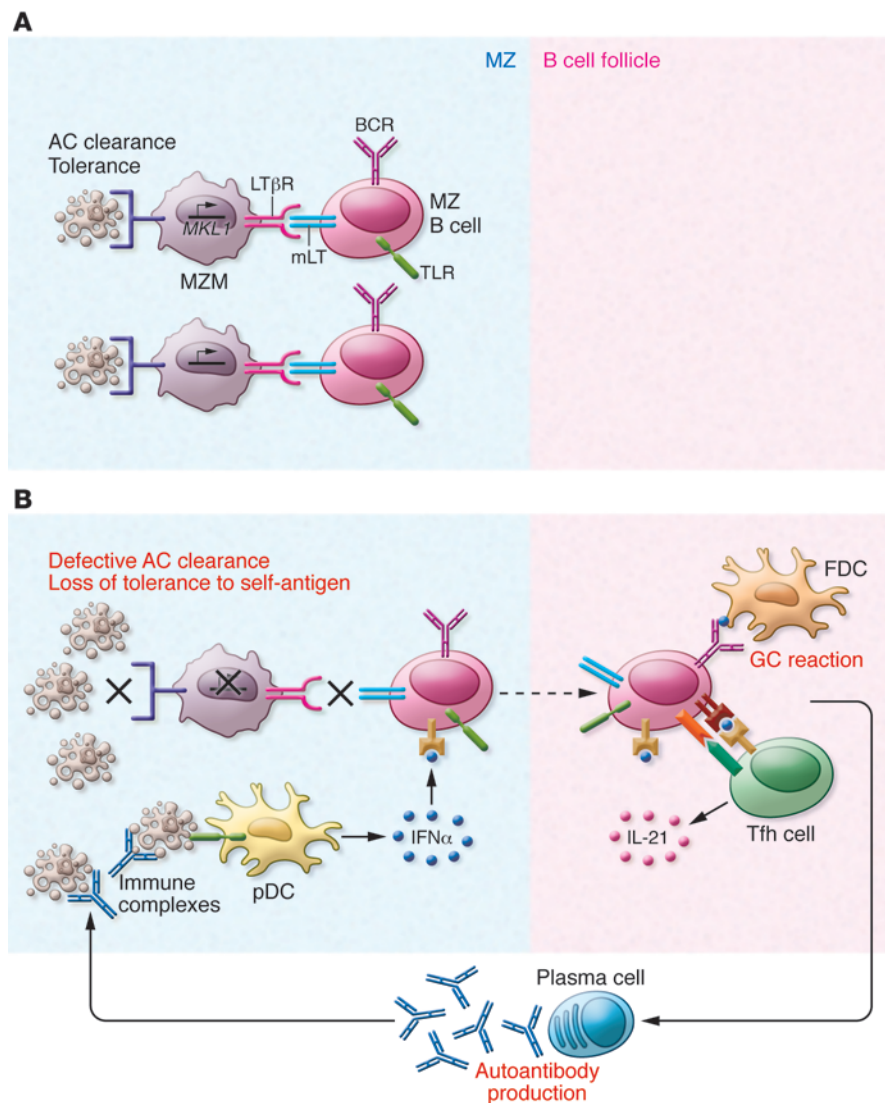
## Type I IFNs: dictators of defective AC clearance

Increased production of type I IFNs by pDCs and decreased clearance of circulating ACs is a common feature of both human patients and mouse models of SLE (2, 11). Using two lupus-prone mouse models (BXD2 and B6.Sle1.Sle2.Sle3 [B6.TC] mice), Li et al.

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**Figure 1. AC clearance in healthy individuals and patients with SLE. (A)** mLT-expressing MZ B cells interact with LT $\beta$ R-expressing MZMs in the perifollicular region of the spleen, which promotes MKL1 expression by MZMs and maintains their numbers and phagocytic function. **(B)** In lupus spleens, pDCs accumulate at the perifollicular region and produce IFN $\alpha$ , which induces the migration of mLT $^{+}$  MZ B cells to the follicle. This results in the loss of MZM numbers and function, leading to defective AC clearance and loss of tolerance to self-antigen. Furthermore, MZ B cells in the follicle interact with FDCs and T follicular helper (Tfh) cells and stimulate germinal center reactions that result in autoantibody production. The antinuclear autoantibodies form immune complexes to further stimulate pDCs to produce type I IFNs. Amplification of these defective interactions contributes to and perhaps initiates the pathogenesis of SLE.

found that an increase in pDCs is paralleled by a decrease in MZMs in the perifollicular region of the spleen (12). Remarkably, analysis of the distribution of MZMs and pDCs in the spleens of patients with SLE showed a similar pattern. Specifically, compared with healthy controls, patients with SLE exhibited a decrease in MZMs and an accumulation of pDCs in the spleen. Interestingly, IFNAR deficiency in BXD2 mice restored MZM numbers and prevented pDC aggregation in the spleen, suggesting

that IFNAR signaling may promote lupus by dissipating phagocytic MZMs in the spleen (12). However, at this stage, the possibility that reduced MZM numbers in the spleens of patients with SLE are a consequence of different therapies cannot be excluded.

To further investigate the dynamics of the interaction between MZ B cells, MZMs, and type I IFN during the development of lupus, BXD2-*Rag* $^{-/-}$  mice were reconstituted with bone marrow from either GFP $^{+}$  BXD2-*Ifnar* $^{+/+}$  or GFP $^{+}$  BXD2-*Ifnar* $^{-/-}$  mice.

Analysis by confocal microscopy revealed that, whereas IgM $^{hi}$ CD1d $^{hi}$  MZ B cells derived from the bone marrow of IFNAR $^{+}$  mice had translocated to the follicle, those from IFNAR-deficient mice were retained in the MZ. These results demonstrate that the translocation of MZ B cells to the follicle is dependent on type I IFN signaling. There was no difference in the percentages of MZMs in the spleens of mice that received IFNAR-deficient bone marrow compared with the percentages in recipients of IFNAR-sufficient bone marrow, indicating that type I IFN does not directly affect MZM numbers or function. Additionally, Li et al. depleted MZ B cells to assess whether MZ B cell translocation affected MZM numbers and function. Indeed, administration of antibodies against the NOTCH2 ligand delta-like 1 (DLL1), which is crucial for MZ B cell development (13), to SLE mice resulted in depletion of MZ B cells as well as a marked reduction in the number of MZMs (12). As NOTCH2 is not expressed by MZMs, these results imply that MZ B cells are required for the maintenance of homeostatic levels of splenic MZMs in lupus-prone mice. These results raised the question of whether and how type I IFN regulates the numerical distribution of MZ B cells, which directly or indirectly affects the frequency of MZMs during lupus progression. Previous studies have implicated membrane lymphotoxin (mLT) expression by B cells in the maintenance of MZMs (14), as mLT is highly expressed on MZ and MZ-precursor B cells. Li and colleagues took advantage of a combination of IFNAR-deficient, lupus-prone mice and conditional knockout mice lacking mLT expression exclusively on B cells to demonstrate that the translocation of mLT $^{+}$  B cells out of the MZ into the follicle is driven by type I IFNs, resulting in a decrease in MZMs (12). These results highlight the importance of mLT-expressing MZ B cells in the maintenance of MZM numbers and phagocytic function.

### mLT/LT $\beta$ R signaling, loss of MZMs, and the development of lupus

Li and colleagues also detailed a molecular mechanism for the defective mechanosensing of signals by MZMs and the reduced MZM numbers in lupus. Specifically, the authors demonstrated that the interaction between lymphotoxin  $\beta$  recep-

tor (LT $\beta$ R) expressed on MZMs and mLT on the surface of MZ B cells is pivotal for the maintenance of MZMs. Loss of LT $\beta$ R signaling on macrophages resulted in alterations in the number and function of MZMs and led to the development of lupus-like disease (12). These results are in agreement with those of previous studies showing that disruption of the interaction between MZMs and mLT $^{+}$  B cells occurs in lupus-prone BXD2 and B6.TC mice (15, 16). Li et al. demonstrated that LT $\beta$ R signaling maintains expression of a mechanosensing transcriptional coactivator, megakaryoblastic leukemia 1 (MKL1) (17, 18), and that the activation of this signaling cascade is important for MZM homeostasis in the MZ as well as for clearance of ACs by MZMs (12). Moreover, MKL1-deficient mice displayed a decrease in MZMs and developed a spontaneous lupus-like disease, elegantly confirming the importance of the mLT/LT $\beta$ R pathway and associated MKL1 expression in preventing lupus.

Type I IFN has been shown to promote the translocation of mLT $^{+}$  B cells from the MZ to the follicles, where they can stimulate a spontaneous germinal center response by interacting with follicular dendritic cells (FDCs) (19). This result, coupled with previous reports that follicular translocation of AC-Ag-bearing MZ B cells results in the induction of autoreactive T cells (20, 21), highlights a shift in mLT $^{+}$  MZ B cells from a tolerogenic response in the MZ to an immunogenic response in the follicles that is driven by type I IFNs. Taken together, Li and colleagues highlight an important role of type I IFNs in the initiation of lupus, one that promotes defective AC clearance, loss of tolerance to self-antigens, and autoimmune germinal center reactions (Figure 1).

## Conclusions

The study by Li et al. (12) attributes additional pathogenic functions to type I IFNs in SLE. Type I IFNs not only disrupt the interactions between MZMs and MZ B cells, but also induce the translocation of MZ B cells into the follicles, leading to a consequential misplacement of MZMs. The similar loss of MZMs in the spleens of both mice with lupus as well as patients with SLE suggests that this disruption may be an important disease-promoting mechanism in humans. More work will need to

be done to address whether neutralization of type I IFN signaling can restore the defects and promote AC clearance. While such an approach may partly restore MZM function, it is unlikely to completely ameliorate disease, as the interaction of MZ B cells with FDCs would continue to promote autoimmune germinal center responses. One possibility to overcome this limitation would be the administration of combination therapy that includes rituximab (anti-CD20), followed by sifalimumab (anti-IFN $\alpha$ ). While rituximab depletes pathogenic B cells (22), sifalimumab would block IFN $\alpha$ -mediated signaling (23). Importantly, repopulated mLT $^{+}$  MZ B cells would be able to interact with MZMs and thereby promote AC clearance and stop the progression of lupus. The use of reagents targeting the SRF/MKL1 axis in MZMs, as suggested by Li et al. (12), may also be beneficial. In conclusion, this study highlights a novel pathogenic role of type I IFNs in defective AC clearance by MZMs in systemic autoimmunity.

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