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Basophils and Autoreactive IgE in the Pathogenesis of Systemic Lupus Erythematosus.[*]

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Abstract

Systemic lupus erythematosus (SLE) is a heterogeneous disease that can affect multiple organs. A hallmark of this disease, like for other autoimmune diseases, is the presence of large amounts of autoantibodies. As such SLE is considered to be a B cell disease perpetuated by the expansion of autoreactive T and B cells. The T cells involved have long been considered to be Th1 and Th17 cells as these potent pro-inflammatory cells can be found in the tissues of SLE patients. Recent advances point to a role for the Th2 environment in contributing to SLE through promotion of autoantibody production. Here we describe the recent work focusing on autoreactive IgE and the activation of basophils as promoting the production of autoantibodies in SLE. The findings, both in a mouse model of SLE and in human SLE subjects, support the concept that the activation of the basophil by autoreactive IgE-containing immune complexes serves to amplify the production of autoantibodies and contributes to the pathogenesis of disease. We propose that therapeutic targeting of this amplification loop by reducing the levels of circulating autoreactive IgE may have benefit in SLE.

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Introduction

Systemic Lupus Erythematosus (SLE) is a complex, multifactorial, autoimmune disease that can affect multiple organs [1]. SLE is heterogeneous both in symptoms and in which target organs may be involved with damage occurring in the central nervous system (CNS), kidney, heart, skin, joints and vessels. It is well recognized that tissue damage is associated with immune complexes deposition and chronic inflammation [1]. The immune complexes formed are generally comprised of auto-reactive antibodies, auto-antigens and complement components [1]. In SLE, most of the auto-reactive antibodies are raised against nuclear components. This self-immunization has at its origin the loss of tolerance due to environmental and/or genetic factors that promote cell death and release of nucleosomal components that are a source of self-antigens. The loss in tolerance is exacerbated through increased numbers of self-reactive T cells and B cells, ultimately leading to the persistent and prolific production of autoantibodies against double stranded DNA (dsDNA), nucleosomal proteins (Ro, La, Sm), neurotransmitter receptors (N methyl D aspartate (NMDA) receptors), plasma membrane components (phospholipids), cytoskeleton associated proteins (α-actinin), or complement components (C1q) [1]. These auto-reactive antibodies (which can be of IgA, IgM, and IgG subclasses) form circulating immune complexes (CIC) in the periphery when they encounter their self-target [1]. They can deposit into organs, irrespective of the particular isotype of auto-reactive antibody. As a direct consequence, chronic inflammation (with inflammatory cells infiltrates and pro-inflammatory cytokine production) is established leading to symptoms of disease and tissue damage: i.e, cognitive impairment and hippocampal damage in the CNS, nephritis in the kidney, skin rashes, arthritis in the joints and fetal heart block in pregnant women [1].

As many autoimmune diseases, SLE has no specific treatment nor early diagnostic tools allowing disease prevention, disease control or definitive healing. Strong immunosuppressive therapy is still the preferred manner to temporarily silence the disease, with all of its accompanying side effects [1]. SLE affects about 1 person in 2,500 in northern Europe and over

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1 in 1,000 in the United States, thus lupus prevention and treatment is an important international challenge. Environmental and/or genetic factors as contributors to development or severity of disease are evident but their roles are poorly understood. For example, approximately 90% of SLE patients are child-bearing aged women and incidence of disease is 10 fold higher in African American women than in women of northern European. In some geographic areas within the US, the disease can affect 1 out of every 200 people [1]. Thus, how genetics and environment contribute remains an enigma whose resolution may well advance treatment of this disease.

The immunological basis of SLE has allowed considerable exploration on the factors and types of immune cells involved in its pathogenesis. Animal models (mainly mouse models with some features of human disease) have allowed the study of the contribution of particular T cell subsets, B cells, monocytes, and dendritic cells in the development of lupus-like disease ([2]). These models have been useful in defining that the pathogenesis of disease lies in the loss of tolerance in the T and B cell compartments [2,3]. B cells themselves were shown to be essential for manifestation of the disease [4]. Studies in human SLE subjects have also confirmed that dysregulation of tolerance in these cellular compartments is a hallmark of disease [5]. These advances in an immunological understanding of disease has led to a number of clinical trials aiming to disrupt the production of auto-antibodies by targeting B cells [6-8]. Interestingly, depletion of B cells with an anti-CD20 monoclonal antibody (Rituximab, Rituxan®) did not show increased efficacy in alleviating refractory disease in a phase III clinical trial [8]. In contrast, clinical trials aiming to disrupt B cell activation by B cell activating factor (BAFF) through the use of another monoclonal antibody (Belimumab, Benlysta®) showed more encouraging results [7,8]. However, it should be noted that the criterion used for determining efficacy in these two clinical trials were markedly different. BAFF (or Blys, for B lymphocyte stimulator) is a member of the TNF ligand superfamily. This cytokine is able to activate B cells to mature towards antibody-secreting plasma cells and to proliferate. This cytokine is found in higher titers in the periphery of SLE patients [9]. Thus, while some of these trials have shown some promise, it is clear that such strategies are not a panacea for all individuals with SLE and

continued efforts towards gaining a further understanding of the contributing factors in SLE are essential.

Herein, we present novel insights on the underlying mechanisms contributing to SLE pathogenesis. Whereas the involvement of Th1 and Th17 cells and cytokines in SLE are well documented [10,11], the contribution of the Th2 environment was poorly understood. Our recent work has demonstrated that basophils and auto-reactive IgE play a role in SLE pathogenesis [12]. This finding, and the demonstrated interruption of SLE pathogenesis through inhibiting production of IgE or activation of basophils in a mouse model of spontaneous SLE, provides a novel strategy with therapeutic promise in disease intervention.

SLE: a B cell disease that includes IgE autoantibody production

As mentioned in the introduction, most of the pathologic features of SLE are linked to the levels and types of auto-reactive antibodies present in the serum of SLE patients. Disease activity is associated with the levels of autoantibodies. These auto-reactive antibodies are produced by plasma cells derived from auto-reactive B cells. Minimal data is available on how tolerance is broken leading to the accumulation of auto-reactive lymphocytes. However, there is considerable evidence that the innate immune response is somehow involved [1]. The presence of an interferon alpha (IFNα) signature in a large proportion of SLE patients corroborates the activation of an innate immune response [13]. Studies in both mouse models and human subjects also suggest a defect in T regulatory cells [14,15]. Nonetheless, it is well accepted that SLE is primarily a B cell disease and that escape from negative selection of auto-reactive B cells is the key element leading to the production of auto-reactive antibodies that results in the presence of circulating immune complexes able to deposit in the targeted organ [1].

While autoantibody involvement in SLE pathogenesis is well documented, the relevance of antibody isotypes is not well understood. It is widely accepted that circulating immune complexes and immune complexes deposits contain IgM, IgG and IgA auto-reactive antibodies [1]. Several studies [16-18] have also shown the presence of auto-reactive IgE in such immune

complexes. While IgG autoantibodies have been intimately linked to tissue damage and the

pathology of SLE, there is also evidence for the involvement of IgM and IgA in lupus nephritis

[1,19,20]. In some SLE patients high levels of IgE have been observed [18,21], whereas in

others IgE levels were normal. Interestingly, whether allergy is associated with lupus is

controversial and while a few reports [22] have linked these diseases there is also considerable

evidence to the contrary [18,23,24]. Thus, little was known about the role of IgE in SLE. As we

describe in more detail below, a key findings is the presence of auto-reactive IgE in a mouse

model of spontaneous SLE and in a large proportion of SLE patients [12]. Importantly, the

levels of auto-reactive IgE was associated with disease severity. This unexpected finding

provided a strong impetus for deciphering the role of auto-reactive IgE in lupus.

The Basophil

Basophils are rare immune cells (<1% of circulating leukocytes) well known for their

involvement in allergic reactions and parasite infections [25] . Their ability to produce large

amounts of Th2 promoting cytokines such as interleukin 4 (IL-4) and thymic stromal

lymphopoietin (TSLP) provides them the ability to serve as immune regulators, able to influence

T and B cells to promote and produce antibodies, respectively [25]. Recent studies showed that

basophils are able to regulate humoral memory responses by producing IL-4 and IL-6 [26]. They

were also shown to induce Th2 differentiation of naïve CD4 T cells *in vivo* [27-30], present

antigen as professional antigen presenting cells (APCs) through expression of MHC class II [31-

33], to promote plasma cells survival [12,34], and to organize the recruitment of other immune

cells in an IgE-mediated chronic allergic inflammation model [35]. Whether basophils

exclusively play these roles is uncertain. For example, basophil-deficient mice were recently

shown to mount normal Th2 responses as well as normal humoral responses [36] suggesting that

basophils are dispensable for these responses. However, one must be careful in overinterpreting

experiments based on the use of a particular challenge or model. On the other hand, it is also

likely that basophils do not replace dendritic cells as initiators of Th2 responses and, in fact,

evidence has been presented for collaboration of these two cell types in initiating Th2 responses [37]. Thus, it is likely that the basophil's role as an immunoregulatory cell may well be context dependent, where in some settings it may play a dominant role and in others it may be less important.

Lyn Kinase: a molecular rheostat in the development of lupus

Lyn is a Src family tyrosine kinase (SFK) that functions to promote or inhibit molecular signals downstream of multiple receptors including cytokine receptors (such as IL-4R or IL-3R), growth factor tyrosine kinase receptors (such as Kit or CD117) or Fc receptors (such as the high affinity IgE receptor, FcεRI). This kinase is expressed in the hematopoietic compartment [38] but is not known to be expressed by T cells [39]. B cells express Lyn kinase and it serves as a key component of the B cell antigen receptor (BCR) through its ability to phosphorylate this receptor and initiate signal transduction upon BCR engagement [40]. Interestingly, however, mice deficient in Lyn kinase show a B cell hyperreactivity to IL-4 and CD40L stimulation [41,42], whereas BCR activation of Lyn-deficient B cells is impaired. Interestingly, mast cells [43-45] and monocytes [46](and more recently basophils [30]) from Lyn deficient mice have also been shown to be hyperreactive to various stimuli, thus demonstrating that Lyn kinase plays an important role in controlling cell homeostasis. It is now well recognized that this is mediated, to a large extent, through the requirement for Lyn kinase in the phosphorylation and activation of various phosphatases [38] whose activity is important in controlling immune cell homeostasis. Importantly, too much Lyn activity also has undesired immunological consequence and has been shown to promote uncontrolled monocytic proliferation and tumor development [47] as well as mast cell activation [44]. Thus, the amount of Lyn kinase activity must be well controlled and it appears that Lyn kinase serves as a rheostat in controlling immune cell homeostasis.

As they age (about 12-14 weeks of age), Lyn kinase deficient mice begin to produce auto-reactive antibodies against nuclear components, such as double stranded DNA and other anti-nuclear antigens. These mice develop an autoimmune phenotype with some similarities to

human lupus including the development of lupus nephritis. As early as 20 weeks of age, Lyn deficient mice show glomerular deposits of anti-dsDNA IgG antibodies and circulating immune complexes. They develop impaired renal function and ultimately die of renal failure [48,49]. Interestingly, it has been shown that Lyn expression is decreased in peripheral B cells of some SLE patients [50] and recently a single nucleotide polymorphism in the promoter region of the Lyn gene was linked with SLE patients of northern European heritage [51]. Of particular note, the described polymorphism was associated with autoantibody production. Thus, while yet undefined, the role of this Lyn polymorphism in the development of SLE is of considerable interest. Nonetheless, in animal models, the role of Lyn in controlling B cell responsiveness (and thus antibody production) is well appreciated; less is known about its contributory role in other cellular compartments that may also contribute in the pathogenesis of SLE.

Lyn kinase and basophils: Regulation of the Th2 environment

It has long been recognized that the high affinity IgE receptor (FcεRI) requires the activity of Lyn kinase to transmit signals that promote the effector responses of mast cells and basophils [52]. This kinase phosphorylates FcεRI and initiates signal transduction, but it also has both positive and negative regulatory roles downstream of FcεRI phosphorylation [44]. Multiple studies have demonstrated that Lyn kinase has a dominant role as a negative regulator of cellular homeostasis [38] and in mast cells its absence was shown to cause enhanced mast cell degranulation and cytokine production [44]. Strikingly, $Lyn^{-/-}$ mice developed an early life atopic-like allergic phenotype where high levels of IgE are produced and circulating histamine can be detected as well as an eosinophilia [43,44]. Additional studies demonstrated that these mice had an exacerbated response to challenge in an asthma model [53] and were generally hypersensitive to a Th2 challenge but also mounted a Th2 response to normally innocuous substances [30]. A significant proportion of CD4^{+} T cells from the spleen of $Lyn^{-/-}$ mice spontaneously produced IL-4. This Th2 bias was dependent on IL-4 and IgE since the deletion of these genes in the context of $Lyn^{-/-}$ mice completely ablated their Th2 skewing [30].

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Since Lyn is not expressed in T cells, our initial bias was that mast cells might be driving this unexpected Th2 skewing in $Lyn^{-/-}$ mice. To test this possibility, mast cell deficient mice ($Kit^{w-sh/w-sh}$) were crossed to $Lyn^{-/-}$ mice. However, the resulting mice ($Kit^{w-sh/w-sh} Lyn^{-/-}$) showed no alteration of the Th2 bias [30]. This meant that the Th2 bias was initiated by another cell type able to produce substantial amounts of IL-4 to induce naïve CD4^{+} T cells to differentiate to Th2 cells. Analysis of other cell types demonstrated that $Lyn^{-/-}$ mice had a peripheral basophilia (up to 4 times the amount of circulating basophils when compared to wild type (WT) mice). In addition, $Lyn^{-/-}$ basophils could produce high levels of IL-4 being hyperresponsive to FcεRI stimulation. To assess the role of these basophils in causing Th2 skewing in the absence of Lyn kinase, we first activated basophils *in vivo* in the $Kit^{w-sh/w-sh} Lyn^{-/-}$ double deficient mice by FcεRI stimulation. In the absence of mast cells or immune challenges, basophils are the principal cells that express FcεRI in the mouse. FcεRI stimulation of $Kit^{w-sh/w-sh} Lyn^{-/-}$ double deficient mice revealed a marked increase in the Th2 skewing of these mice with as much as 25% of the CD4^{+} T cells in the spleen producing IL-4. The inverse experiment where basophils were depleted from $Lyn^{-/-}$ mice with a monoclonal antibody (clone MAR-1, anti-FcεRIα [29]) showed a complete rescue of the Th2 skewing, clearly demonstrating that basophil was promoting the Th2 bias seen in these mice. Of particular note, the CD4^{+} T cells from spleens of $Lyn^{-/-}$ mice constitutively produced only Th2 cytokines, no IFN-γ was detected in these cells. Moreover, $Lyn^{-/-}$ mice failed to effectively fight an infection with the prototypic Th1 parasite Toxoplasma Gondii, which requires IFN-γ production for survival from infection. Measurement of IFN-γ levels demonstrated that $Lyn^{-/-}$ mice did not mount a normal Th1 response. However, it should be noted that *in vitro* restimulation of CD4^{+} T cells from $Lyn^{-/-}$ mice with PMA and ionomycin caused increased IFN-γ production (as well as IL-4 and IL-13) when compared to WT cells [30]. This suggests that T cells from these mice are primed by the $Lyn^{-/-}$ environment in a manner that can result in potent Th1 or Th2 responses.

The mechanism by which basophils can induce the IL-4 production necessary for the potent Th2 skewing of $Lyn^{-/-}$ mice was explored in some detail. In mast cells, the

hyperresponsiveness observed upon Lyn deficiency is, at least in part, through increased Fyn kinase activity [43,45,54] and the loss of SHIP activity [54]. As these molecules regulate the activity or the amount of product, respectively, of phosphatidylinositide 3-OH kinase (PI3K), a kinase that generates phosphatidylinositide 3,4,5-trisphosphate (PIP_3), loss of regulatory control on PIP_3 production in these cells causes their hyperresponsiveness [55].   However, whether this mechanism also provided the underpinnings of the hyperresponsiveness of basophils was not known. Studies to address the role of PI3K in basophils revealed that its activity was necessary for the increased expression of GATA3 (a transcription factor known to regulate IL-4 production in T cells) in $Lyn^{-/-}$ basophils. $Lyn^{-/-}$ basophils showed a marked increase in GATA3 expression relative to their WT counterparts and this (as well as IL-4 production) was inhibited by pharmacological inhibition of PI3K activity [30]. In addition, basophils from $Fyn^{-/-}$ mice failed to upregulate GATA3 and were impaired in the production of IL-4. Thus, the Th2 skewing was linked to a Fyn/PI3K-dependent increased induction of GATA3 in $Lyn^{-/-}$ basophils.

The clear dominance of a Th2 environment in $Lyn^{-/-}$ mice and the late life development of a spontaneous lupus-like disease provided a good model system to explore the relationship between the Th2 environment and the development of a lupus-like phenotype.

Is there a link between the Th2 environment and development of a lupus-like phenotype?
Removing IgE or IL-4 genes in the context of the $Lyn^{-/-}$ background led to the reversion of the Th2 bias seen in $Lyn^{-/-}$ mice. These double deficient mice provided a model to ask the question of whether the Th2 bias was linked to the development of a lupus-like disease. Aging of such mice for greater than 35 weeks along with $Lyn^{-/-}$ mice revealed that the penetrance of disease was almost 100% in the latter mice whereas IgE/Lyn or IL-4/Lyn double deficient mice showed little signs of kidney disease. Indeed, the double deficient mice had a greater than 50% reduction in autoantibody titers and immune complex deposition in the glomeruli was markedly reduced with normal albumin/creatinine levels in the urine. Analysis of the extent of glomerular nephritis by scoring immune infiltrates, mesangial proliferation, morphology of the glomeruli, and other

parameters revealed little evidence of kidney disease and damage. Thus, it was evident that inhibiting the Th2 skewing of $Lyn^{-/-}$ mice by depletion of IL-4 or IgE greatly reduced autoantibody production and ablated the development of overt kidney disease and damage [12]. This unexpected result led us to question if basophils were contributing to the development of the lupus-like phenotype seen in $Lyn^{-/-}$ mice.

Basophils and autoreactive IgE in the development of lupus.

Evidence from the Lyn$^{-/-}$ mouse model

At the time of this work basophil deficient mice were not available. Thus, once again, we depleted basophils with the anti-FcεRIα (MAR-1 antibody) and found that basophils were needed to maintain high autoantibody (IgM, IgG, IgA) titers in the circulation of $Lyn^{-/-}$ mice. Depletion of basophils caused a marked decrease in anti-ANA IgG and anti-dsDNA IgG antibodies within 6 days post-treatment. This was somewhat unexpected given the long half-life of antibodies in the blood, although immune complexes are cleared more rapidly than normal immunoglobulins by binding to cells. Nonetheless, we hypothesized that the role of basophils might extend to the support of plasma cell survival and function and in this manner could more rapidly influence control on antibody levels. This view was supported by the recent finding of others [26] showing that basophils contribute to humoral memory responses through their production of IL-4 and IL-6. Within 6 days of basophil depletion, the numbers of plasma cells (likely plasmablasts) in the spleen of $Lyn^{-/-}$ mice decreased by greater than 50%. These findings showed the direct involvement of basophils in supporting plasma cells in the spleen of $Lyn^{-/-}$ mice that can produce autoantibodies [12]. Our findings that basophil support plasma cell survival and antibody production has recently been confirmed by others [34].

Given that $Lyn^{-/-}$ mice had a peripheral basophilia and produced high levels of IL-4, IL-6 and TSLP, we asked: How do these basophils become activated? Our previous findings demonstrated that $Lyn^{-/-}$ basophils were hyper-reactive to stimulation via FcεRI. Moreover, given that IL-4/Lyn and IgE/Lyn double deficient mice (which are not Th2 skewed) did not

produce high levels of auto-reactive antibodies or CIC's to induce kidney disease, we reasoned that in this model, IgE and the FcεRI were involved in the activation of the basophils and pathogenesis of disease. In part our reasoning was based on the prior description of the presence of autoreactive IgE in the circulation of SLE patients [18,20]. Analysis of *Lyn*^{-/-} mice for autoreactive IgE in the circulation revealed the presence of anti-dsDNA and anti-ANA autoantibodies of the IgE isotype. Compared to WT mice, large amounts of anti-ANA and anti-dsDNA IgE were found in the sera of *Lyn*^{-/-} mice. Moreover, the presence of large amounts of CIC containing IgE was also detected by western blot. We then tested if IgE-containing immune complexes could induce IL-4 production from WT and *Lyn*^{-/-} basophils and found that indeed these immune complexes could effectively activate both WT and *Lyn*^{-/-} basophils, with the latter producing higher amounts of IL-4. In contrast, immune complexes that contained only IgG failed to induce IL-4 production from basophils of both genotypes. These findings provided a mechanistic explanation on how basophils might be continuously activated in the periphery of *Lyn*^{-/-} mice.

The support of autoantibody production would require that activated basophils be in the appropriate environment to support T and/or B cell function. This would require their migration to the secondary lymphoid organs. To assess their ability to migrate to the secondary lymphoid organs (such as the lymph nodes and spleen), we measured the levels of CD62L (L-selectin) on the surface of basophils from WT and *Lyn*^{-/-} mice. CD62L is recognized as a necessary molecule for leukocyte rolling and recruitment from blood stream to tissues. Measurement of CD62L expression on *Lyn*^{-/-} basophils revealed a marked increase in its expression relative to WT basophils. Under normal conditions (in aged WT mice), the numbers of basophils in the lymph nodes are very small and difficult to detect. In contrast, aged *Lyn*^{-/-} mice had very high numbers of basophils in peripheral lymph nodes as well as in the spleen. This placed the basophil in the appropriate environment where it could influence T and B cell function. However, the question as to whether the *Lyn*^{-/-} basophil expressed the appropriate molecules to interact with T and B cells remained to be answered. Recently, in both mouse and human basophils, it has been

described [31-33] that these cells can express molecules of the major histocompatibility complex II (MHC-II). Whereas circulating basophils from $Lyn^{-/-}$ mice expressed low levels of MHC-II molecules on their surface, the basophils found in the lymph nodes of these mice were expressing high levels of MHC-II. The antigen being presented by these basophils remains an enigma, however, one might speculate that autoantigenic epitopes may well reside on the surface of these cells. Thus, these findings provide evidence that the basophil is capable of antigen presentation, since the appropriate expression of MHC-II requires it's assembly in the context of the antigenic peptide epitope [56]. In addition, however, a membrane-bound form of BAFF (B cell activating factor) was also detected on the surface of $Lyn^{-/-}$ basophils found in the lymph nodes, as has been described for human basophils in other chronic inflammatory diseases [57]. Interestingly, this was independent of increased BAFF receptor expression suggesting that the expressed BAFF was not bound to the receptor. Thus, these findings show that $Lyn^{-/-}$ basophils home to the secondary lymphoid organs and are fully equipped to support T and B cells at these sites. This is consistent with our finding that basophils support plasma cells in the spleen of $Lyn^{-/-}$ mice [12]. Given that such plasmablasts require T cells for their expansion, our data suggests that basophils are also providing help to T cells in secondary lymphoid tissues.

Given the marked impairment of autoantibody production by the depletion of basophils, we asked whether the absence of basophils had any bearing on the kidney inflammation of $Lyn^{-/-}$ mice during the manifestation of lupus nephritis. Within 6 days of basophil depletion a marked reduction was observed in the pro-inflammatory cytokines (IL-4, IFN-γ, IL-1β, MCP-1, TNF-α and IL-6) in the kidneys of these mice [12]. This showed that beyond their role as contributors to autoantibody production, the basophil also contributes to the pathogenesis of lupus nephritis by promoting inflammation.

The data obtained through the study of $Lyn^{-/-}$ mice demonstrates that in this model of SLE, the Th2 bias is contributing to the pathogenesis of disease. Perhaps, the best evidence is the requirement for IL-4 and IgE for disease manifestation in $Lyn^{-/-}$ mice. Our findings suggest that the basophil and autoreactive IgE's serve to amplify the role of autoreactive T and B cells in

establishing the disease (Figure 1). This amplification seems to be key in determining the threshold of autoantibodies that are necessary to establish disease, presumably because the body can no longer effectively remove the high levels of autoantibodies and CIC's produced once this basophil-dependent amplification loop is fully established (Figure 1).

Basophils and autoreactive IgE in human SLE

While our findings in *Lyn*^{-/-} mice clearly provide evidence of a Th2 influence in the pathogenesis of SLE, the relevance of the findings in this model to human disease must be explored. Evidence for the role of Th1 and Th17 cell subsets in human SLE is considerable [1]. In contrast, evidence for Th2 involvement is sparse and is primarily restricted to certain populations of SLE patients with more circulating IgE than healthy individuals [18,21]. Interestingly, the increased levels of circulating IgE were not associated with an increased incidence of atopic disease in this population of patients [18]. This suggested that a Th2 skewing might be seen in certain SLE patients. A more informative finding was the description of autoreactive IgE and IgE containing immune complexes in some SLE patients [20]. While described, the role of these autoreactive or IgE containing immune complexes in disease was not known. Thus, we set out to explore whether the presence of autoreactive IgE's (anti-dsDNA and anti-ANA) might be associated with different measures of disease activity in SLE patients. We detected large amounts of autoreactive IgE in patient samples relative to healthy controls. Interestingly, the levels of both anti-dsDNA and anti-ANA IgE correlated with disease activity as determined by the SLEDAI score of the patients. Remarkably, patients with active lupus nephritis seemed to show the highest levels of anti-dsDNA IgE when compared to patients without kidney involvement [12]. These findings suggested that like shown in our mouse model, autoreactive IgE's might play a role in the pathogenesis of lupus in humans.

Whether basophils might be activated in human SLE was subsequently explored. Blood basophils from the same cohort of 42 patients and 40 healthy volunteers were analyzed for their activation status. Unlike mouse basophils, human basophils have known surface markers that

are expressed upon their activation, such as CD63, CD203c or CCR3. Each of these markers reflect slightly different activation conditions. For instance, CD63 expression reflects the degranulation of the basophil, since CD63 is a granule membrane protein that becomes expressed on the cell surface only after the intracellular granule fuses with the plasma membrane. In contrast, CD203c is expressed when basophils are activated under conditions where they may or may not degranulate. Thus, we used this marker to assay for basophil activation. Analysis of CD203c expression showed that basophils from SLE patients were highly activated. In addition, peripheral basophils from SLE patients expressed higher levels of MHC-II molecules (HLA-DR) than the healthy controls, suggesting that their activation could result in basophil communication with T and B cells.

When analyzed for whether basophils could home to the secondary lymphoid organs in SLE patients, CD62L levels were found to be increased as compared to healthy controls, confirming the ability of these cells to home to these organs and their activated phenotype. To confirm that these cells were present in the lymph nodes and spleen from SLE patients, we analyzed several biopsy specimens of these tissues from SLE patients. In contrast to non-SLE biopsies (controls), basophils were found in the lymph nodes and spleens of SLE patients with their primary localization in germinal centers, in the middle of the B cell zone and in the surrounding T cell zone. No basophils were detected in control samples. Thus, basophils were shown to be in secondary lymphoid tissues, alike to our observation in $Lyn^{-/-}$ mice. Given that basophils were recruited to secondary lymphoid organs, one might expect that the numbers of basophils in the circulation would decrease. Both the percentage and absolute numbers of circulating basophils were reduced in SLE patients. These findings once again demonstrated that the activation of basophils in SLE caused increased expression of cell surface molecules (CD203c, CD62L, MHC-II…) that promote the recruitment of basophils to the secondary lymphoid organs and may allow their interaction with T and B cells. Collectively, the studies on human SLE patients are consistent with the conclusions from the analysis of $Lyn^{-/-}$ mice. They

support that concept that autoreactive IgE and activated basophils are likely contributors to the pathogenesis of SLE (Figure 1).

Basophils and autoreactive IgE as therapeutic target in human SLE

The aforementioned findings suggest two new putative therapeutic targets in the treatment or prevention of lupus nephritis; namely autoreactive IgE and basophils. The involvement of these two factors, in an amplification loop that increases the levels of autoantibodies and CIC, suggests that disruption of this loop could have therapeutic benefit. Nonetheless, there are many poorly understood factors for the heterogeneity of disease. It is still not known what makes the difference between an SLE patient that develops active nephritis and one that does not. When looking at SLE patients with lupus nephritis, it is still not understood why some will develop class I or class II nephritis (with low severity, with mainly mesangial deposits) and others will develop class III or class IV nephritis (with high severity, mesangial deposits, extramembranous deposits and vasculitis). Our findings suggest that it is possible that the presence of level of activation of such amplification loops (whether basophil driven or not) might explain some of these features; based on the fact that class III and class IV states are correlated with the titers of autoantibodies and CIC. Thus targeting of basophils as one possible cell type for amplification of autoantibody production could lead to a beneficial outcome for some patients. This recent clinical trials targeting B cell activation [7] suggests that such approaches may have some clinical benefit in SLE. However, one must be cautious in developing such an approach for the basophil as the release of pro-inflammatory mediators (histamine, TNF-α, platelet activating factor…) contained in this cell could be an unwanted consequence of its depletion. In addition, one must consider the consequences of depleting a potential immune regulator and a potent effector against parasitic infections. Consideration of such therapeutic strategies requires further evaluation of short term and long term depletion of basophils in health and disease.

Thus, a more tempting therapeutic target might be to inhibit the activation of the basophil through depletion of autoreactive IgE. Depletion of IgE-containing CIC would disrupt the

persistent activation of basophils in SLE, blocking the amplification loop, decreasing the amounts of circulating autoantibodies. Depletion of IgE from the circulation is a strategy already in use in other diseases, such as allergic asthma. Omalizumab (Xolair®, Novartis) has shown efficacy in treatment of asthma [58]and allergic rhinitis [59] through blocking the binding of IgE to FcεRI. This monoclonal antibody was raised against the Fc portion of the IgE and binds the epitope on the IgE molecule that interacts with the FcεRIα. Once IgE is bound by this anti-IgE antibody, the IgE can no longer bind to the FcεRI. Thus, such an approach should stop basophil activation by IgE-containing CIC in SLE and may lead to interruption of the amplification loop described in Figure 1. Such studies are currently underway and should provide novel insights on the role of autoreactive IgE in basophil activation in SLE patients and beyond.

Concluding remarks

The recent advances in our understanding of the role of basophils in health and disease demonstrate that the longed ignored basophil granulocyte has an important immunomodulatory role in the immune system. The findings described herein show that, in some settings, the basophil, through its ability to communicate with T and B cells, links the Th2 environment as a contributor to the development of an autoimmune disease, like SLE. It will be of considerable interest to explore if this will translate to therapeutic benefit in disease.

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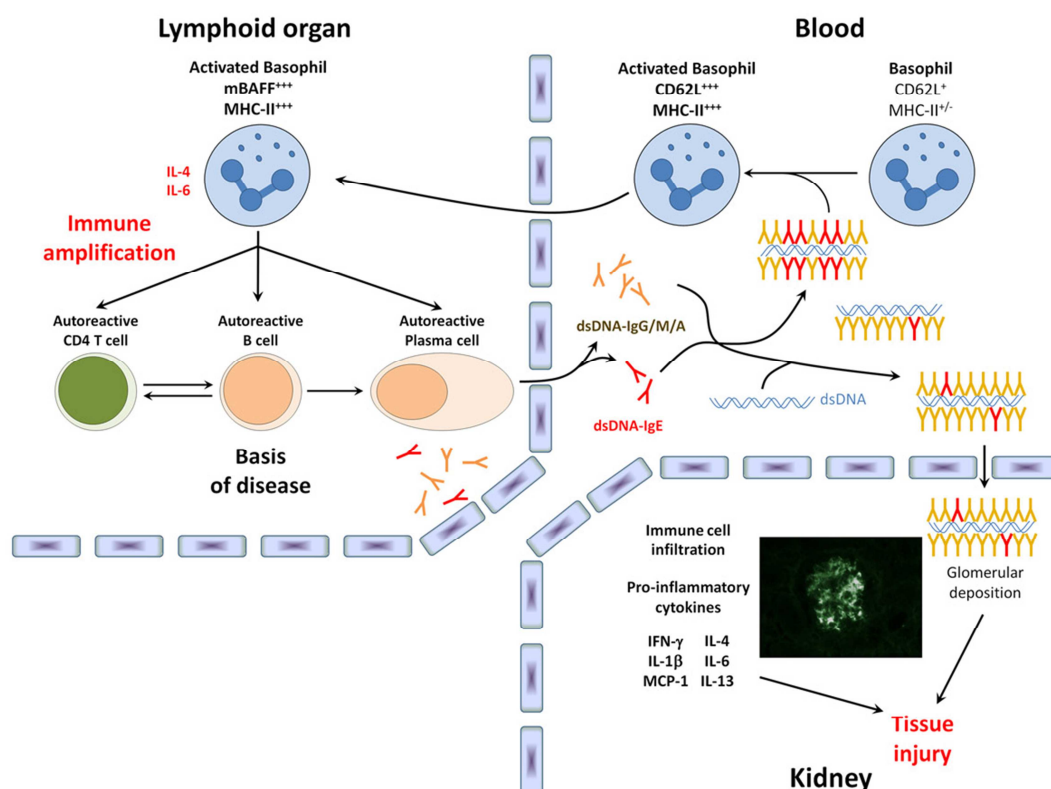
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Figure 1: Basophil and autoreactive IgE amplify autoantbody production in SLE.

SLE is a consequence of the loss of tolerance promoting autoreactive T and B cells that escape from negative selection. These autoreactive lymphocytes produce large amounts of circulating immune complexes (CICs) comprised of aggregated autoreactive antibodies, complement factors and autoantigens. Autoreactive IgGs, IgMs and IgAs comprise the majority of autoantibodies, but autoreactive IgE's also accumulate as CICs reaching a threshold that activates circulating blood basophils recruiting these cells to lymphoid organs via upregulation of CD62L on their cell surface. Once recruited to the secondary lymphoid organs, basophils can interact directly with autoreactive lymphocytes through upregulation of molecules like MHC-II and membrane bound BAFF. Together with the basophils ability to secrete large amounts of IL-4 (and IL-6), these cells can promote plasma cell survival and amplify autoantibody production, leading to the disease amplification. The presence of large amounts of autoantibody promotes the levels of pro-inflammatory cytokines found in the target organs, like the kidneys, resulting in uncontrolled inflammation and organ damage.