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Review

Recent findings on the role of natural killer cells in the pathogenesis of systemic lupus erythematosus

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ABSTRACT

Systemic lupus erythematosus is a chronic, multifactorial autoimmune disease of complex etiology, characterized by loss of tolerance to nuclear autoantigens, expansion of autoreactive T and B cell clones, polyclonal B cell activation that gives rise to hypergammaglobulinemia, and increased autoantibody production, as well as immune complex deposition and multiorgan tissue inflammation. As disease progresses, immune cells, mainly T cells and macrophages, infiltrate affected organs and amplify the local inflammatory response. Natural killer cells are large, granular lymphocytes that are an important link between the innate and adaptive immune systems; variations in their activity correlate with several autoimmune diseases. To date, the literature has disregarded natural killer cells as relevant modulators in systemic lupus erythematosus pathogenesis, as these cells are few in number and show a dysfunctional phenotype in patients with active systemic lupus erythematosus. This review focuses on research that could help define the role of natural killer cells in systemic lupus erythematosus and their function in regulating this autoimmune disorder in nonlymphoid organs. *J. Leukoc. Biol.* 98: 479–487; 2015.

Introduction

NK cells are large, granular lymphocytes that constitute an essential element of the innate immune system and are the third main lymphocyte lineage after T and B cells [1]. NK cells were first characterized for their capacity to kill tumor targets without prior sensitization; they are able to lyse susceptible target cells by exocytosis of perforin and granzyme granules, which can induce apoptosis in targeted cells [2]. NK cells are also rapid producers of various cytokines and chemokines, such as IFN-γ, TNF-α, CCL5, CCL3, and CCL4, which amplify and recruit an inflammatory response through various mechanisms [3, 4].

In contrast to T and B cells, NK cells do not use the RAG to rearrange their receptor genes and do not have a unique antigen recognition receptor [5, 6]. Instead, these cells express inhibitory and activating receptors to sense their environment and discriminate between healthy and diseased cells. The balance in signaling input between these receptors allows NK cells to mount an antiviral response or to distinguish altered self-cells, such as tumor cells [7].

NK cells can interact with virtually all nucleated cells via a family of inhibitory receptors, termed the Ly49 family in mice, or the KIR family in humans [8]. These inhibitory receptors allow the NK cell to determine whether a cell expresses the correct self-MHC profile. Lack of a correct MHC profile (usually as a result of the lack of MHC I molecules) does not allow binding of these inhibitory NK cell receptors, which block inhibitory signals and allow NK cell activation and lysis of the target cell.

NK cells also express numerous activating receptor subsets, including the CD49/NKG2C complex, NKp46 and NKG2D (in mouse and man), Ly49 subsets (in mice), and the KIR S family in humans [9]. These activating receptors can identify foreign and self proteins. Some, such as Ly49H, can recognize the cytomegaloviral MHC I-related molecule; others, such as NKp46, bind viral hemagglutinins, whereas receptors, including NKG2D, bind self-molecules that are up-regulated on the cell membranes of "stressed" or virally infected cells [10–12]. It is the integration and the balance of signals received from activating and inhibitory receptors that drive the NK cell activated/quiescent state [13]. NK cell function is modulated by a complex network of soluble factors (cytokines, chemokines, soluble receptor ligands) and cell-to-cell interactions. This allows for fine tuning of NK cell activity but also highlights the complexity of analyzing their role in diseases.

The generic definition of autoimmune disorders includes immune-mediated elimination or debilitation of endogenous cells and/or tissue. These diseases are typically characterized by inappropriate cell activation in the adaptive and the innate immune systems,

Abbreviations: Are-Del = adenylate-uridylate-rich element deleted, BxSB = C57BL6/J x SB/Le, DNAM-1 = DNAX accessory molecule-1, Eomes = eomesodermin, Gas6 = growth arrest-specific 6, KIR = killer cell Ig-like receptor, LAIR-1 = leukocyte-associated Ig-like receptor-1, MHC I/II = MHC class I/II, MICA = MHC class I polypeptide-related sequence A, MINECO = Spanish Ministry of Economy and Competitiveness, MRL = Murphy Roths Large, MS = multiple sclerosis, NKP = NK precursor, NZB = New Zealand Black, NZW = New Zealand White, pDC = plasmacytoid dendritic cell, RA = rheumatoid arthritis, SLE = systemic lupus erythematosus, T1D = Type 1 diabetes

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which leads to specific or nonspecific cell and/or organ damage. Alterations in NK cell activities are implicated in various autoimmune diseases, such as T1D, RA, MS, and Crohn's disease [14–19].

The role of NK cells differs considerably in these diseases. Perricone et al. [20] suggested great variability among autoimmune diseases, in which NK cells act as "shields" against autoimmunity or "swords" that enhance/promote it. Even within the same disease, data on NK cell activity can appear contradictory. In MS, NK cells appear to behave as shields, as patients in remission tend to have high percentages of NK cells that secrete Th2 cytokines, such as IL-5 and IL-13 [21, 22], although, NK cells might also have a pathogenic role in relapsing-remitting MS [23]. In some cases of T1D, enterovirus infection promotes NK cell infiltration of β-islets, which leads, in turn, to insulitis and β cell dysfunction [18]. In contrast, NK cells can abolish disease onset by interfering with β-islet-specific T cell activation in a murine model of diabetes [24, 25] and usually have an attenuated cytotoxic phenotype in the periphery in long-term T1D patients [26]. In RA, large numbers of NK cells are found in inflamed joints; recent studies show that synovium-infiltrating NK cells from patients produce more IFN-γ and are more active than peripheral blood NK cells from the same individuals [27, 28]. Likewise, NK cells could contribute locally to Crohn's disease, as they are abundant in inflamed mucosa and secrete IFN-γ [29]. These studies emphasize analysis of peripheral and local cells as essential in dissecting the NK cell contribution in autoimmunity.

SLE is a chronic autoimmune disease that can affect various organs, including the heart, lungs, blood, kidneys, and nervous system [30, 31]. Its etiology is not known completely, and various studies postulate roles for genetic and/or environmental factors in its development [30]. The hallmarks of SLE are autoantibody production to a broad range of self-antigens, immune complex formation, and self-reactive T and B cells able to produce these autoantibodies [32–34]. Autoantibody-mediated tissue damage in SLE patients is thought to arise from the deposition of immune complexes, which leads to leukocyte activation. As immune complexes can activate cells, such as macrophages or NK cells that express FcγRs, the deposits could contribute to macrophage infiltration of the kidney [35]. Studies often analyze kidney injury in SLE as a model for organ involvement, as it leads to lupus nephritis, a marker of poor prognosis [36]. Active human lupus nephritis is also associated with lymphopenia [37], probably as a result of anti-T cell autoantibody production in these cases [38]. Autoantibody production is nonetheless insufficient for pathogenesis. The production of high-affinity autoantibodies, a result of antigen-driven T cell help of B cells, is considered essential in SLE immunopathogenesis; this is particularly apparent in kidney injury studies [39, 40]. As a result, research interests in the past decades focused mainly on identifying autoantigens and studying the role of adaptive immunity in SLE, often neglecting the involvement of cells central in innate immunity, such as NK cells.

NK CELLS IN SLE—PERENNIAL UNDERACHIEVERS?

The role of NK cells in SLE pathogenesis remains unclear; to date, it has been relegated to the sidelines in understanding this complex, multifactorial disease. Reports from several groups show significantly lower proportions and total NK cell numbers in SLE patient blood compared with controls, especially in lupus nephritis patients [41–43]. This has been linked to elevated serum levels of IFN-α, a cytokine that promotes activation-induced apoptosis in SLE patients [42]. The reduction in total NK cell numbers in SLE patient peripheral blood appears to be common to various autoimmune diseases, such as MS, RA, T1D, and Sjögren syndrome, although its significance is not well understood [20, 44, 45]. It is not known whether this phenomenon is induced by the same environmental or genetic factors (i.e., anti-T cell antibodies) that cause the generalized lymphopenia, characteristic of SLE and other autoimmune disorders.

Phenotypic analyses of NK cells in SLE patients have yielded divergent results (**Table 1**). Studies of various, typical NK-specific receptors in PBMCs, such as NKp30, NKp80, NKp44, and LAIR-1, showed no variations between healthy individuals and SLE patients or between SLE patients with active or inactive disease [46–49]. A decrease in the frequency of NK cells positive for the inhibitory receptor NKG2A, the KIR family receptors 2DL1/DS1, 2DL3, and 3DL1, and the activating receptor DNAM-1 (CD226) was found in SLE patients compared with healthy individuals [46–48, 50]. NK cells in patients with active disease also express higher levels of the activating receptor CD69 [46]. Discrepancies begin to appear among various groups when the frequencies of NK cell subsets for the activating receptors NKp46, 2B4, NKG2C, and NKG2D are analyzed. A study by Hervier et al. [46] reported an increase in the frequency of NKG2C-positive cells in SLE patients, whereas Ye et al. [48] found a decrease. The frequency of NKG2D-positive cells was lower in SLE patients in 2 studies (Puxeddu et al. [47] and Li et al. [50]), whereas Hervier et al. [46], Schepis et al. [49], and Ye et al. [48] found no substantial differences. The same can be said of the frequency of NK cells positive for the activating receptor 2B4 (increased in 1 study; unchanged in another) or the cytotoxicity receptor NKp46 (increased in SLE patients 2 two studies; unchanged in another 2) [46–49]. Some authors attributed these discrepancies to possible variations in patient populations, treatment type (e.g., Ye et al. [48] studied only newly diagnosed SLE patients, whereas Hervier et al. [46] studied treated patients), gating strategies, and difference in reagents. Analysis of these publications does not give a clear picture as to whether NK cells in SLE patients are actually more activated than controls. Whereas there is an apparent reduction in inhibitory receptors, the variable expression of activating receptors (Table 1) implies deregulated NK cell differentiation rather than a simple "more active" cell state. Analysis of CD122 and CD16 expression, 2 markers linked to NK cell differentiation, also supports this hypothesis.

CD122 is the 70–75 kDa IL-2R β chain, also known as IL-2Rβ, shared by IL-2R and IL-15R [1, 51]. Its expression allows NKPs to respond to IL-15 stimulation and further their development [52]. The FcγR type IIIa/CD16 marker is expressed when NK cells have reached their final differentiation state and is considered to act as an inhibitory receptor [53]. Patient data show decreased CD122 and CD16 expression in NK cells; 2 groups thus proposed that this phenotype might indicate a defect in NK cell differentiation, which is altered in patients with active SLE [41, 46, 48].

TABLE 1. Modulation of NK cell receptors in PBMC of patients with active SLE

	Activating receptors									Inhibitory receptors						
Reference	NK-p30	NK-p46	NK-p80	NK-p44	CD-244	NK-G2C	DNAM-1	NKG2D	CD69	NKG2A	LAIR-1	CD-16	2DL1/DS1	2DL2-3/DS2	3DL1/DS1	Treatment
[46]	=	=	=	=	=	↑	NA	NA	↑	↑	=	↓	↓	=	=	HC, PD, MM
[47]	=	=	NA	↓	↓	NA	↓	↓	NA	NA	NA	NA	NA	NA	NA	NA
[48]	=	↑	NA	NA	NA	↓	NA	=	NA	NA	NA	↓	NA	↓	↓	NT
[49]	=	↑	NA	=	NA	=	NA	=	=	NA	NA	↓	NA	NA	NA	PD, MM
[42]	NA	=	NA	NA	NA	↓	↓	=	=	=	NA	↓	NA	NA	NA	HC, PD, CP, MM
[50]	NA	NA	NA	NA	NA	NA	NA	↓	NA	↑	NA	↓	NA	NA	NA	NA

↑, Up-regulation compared with healthy controls; NA, not available; ↓, down-regulation compared with healthy controls; HC, hydroxychloroquine; PD, prednisolone; MM, mycophenolate mofetil; NT, untreated patients; CP, cyclophosphamide.

NK cells in the periphery usually undergo 3 stages of maturation that correlate with the gradual acquisition of effector functions. These stages are defined by the acquisition of CD11b and CD27 expression in the following manner: $CD11b^{Low}CD27^{High}$ (the most immature), $CD11b^{High}CD27^{High}$ (intermediate), and $CD11b^{High}CD27^{Low}$ (the most mature) [54–56]. In humans, these maturation markers correlate with CD56 levels [57]. NK cells in humans can be divided into a $CD56^{dim}$ population (more mature, highly cytotoxic, and generally found in periphery) and a $CD56^{bright}$ population (less mature, less cytotoxic, with high cytokine-secreting capacity). The work by Schepis et al. [49] shows an increase in the proportion of $CD56^{bright}$ NK cells in patients with active SLE, which could indicate that NK cells have reduced cytotoxic capacities but increased cytokine production during active stages of the disease.

Functional analysis of NK cells confirmed reduced cytotoxicity in SLE patients [41, 46] and in the MRL/MpJ^{lpr} mouse SLE model [58]. These deficiencies did not correlate with substantial degranulation defects based on CD107a labeling, a marker that correlates with NK cell cytotoxicity [46, 48, 59]. This alteration in natural NK cell cytotoxicity, which defines NK cell function, does not appear to affect production of IFN-γ, one of the most prominent proinflammatory cytokines generated by NK cells [60]. Indeed, NK cells from patients with active SLE produce higher IFN-γ levels than healthy controls after stimulation by IL-12 + IL-18 (2 cytokines that induce IFN-γ expression [61]) or by PMA + ionomycin [46, 48]. High IFN-γ levels are linked to SLE onset [62], as exemplified recently in a murine model, where chronic circulating levels of this cytokine (ARE-Del mice) trigger a SLE-like syndrome [63]. In this model, NK cells produced more IFN-γ compared with controls, both in basal conditions and after stimulation by IL-12 [63]. Little is known about their secretion of other cytokines in SLE, although NK cells from SLE patients produce lower levels of CCL4, which induces IFN-α production [64].

The discrepancy between dysfunctional cytotoxic ability and cytokine production might reflect the presence of immature or distinct NK cell subsets with different effector capacities. $CD27^{High}$, $CD11b^{Low}$, $CD56^{bright}$ immature NK cells have reduced cytotoxic function but increased IFN-γ secretion; these cells might be the predominant phenotype in SLE patient PBMCs [49, 57, 65].

All functional and phenotypic data collected from PBMCs of SLE patients must nonetheless be analyzed with extreme caution; prednisone, hydroxychloroquine, and mycophenolic acid are among the most common treatments for this disease and can down-modulate NK cell function, cytotoxicity, and proliferation [66–72]. Although limited to a small patient cohort, the study by Ma et al. [73] shows how classic SLE treatments can modulate expression of various receptors, such as NKG2C, KIR2DL3, NKp46, and NKG2D.

ORGAN-SPECIFIC DIFFERENCES IN SLE—LOOKING IN THE RIGHT PLACE?

Findings in recent years show that NK cell phenotype, function, and role can differ greatly between organs [74, 75]. The wealth of information, compiled to date, on PBMCs alone from SLE patients (with the exception of the study by Park et al. [41], who used bone marrow-derived NK cells) might show only part of NK cell effects in SLE. SLE mouse models could have considerable value in the evaluation of these aspects of the disease.

Whereas no mouse model perfectly mimics a complex disease like SLE, models, such as (NZBxNZW)F1, MRL/MpJ, MRL/MpJ^{lpr}, and BXSB mice, widely used as experimental, spontaneous models of human SLE (**Table 2**), can provide insights into disease pathogenesis [76] (**Fig. 1**). Studies that use MRL/MpJ^{lpr} mice showed reduced percentages of NK cells in blood and spleen and deficient cytotoxic cell activity that quite efficiently reproduces the phenotype of NK cells in SLE patient PBMCs [42, 58]. These data were corroborated in a recent article that highlights the presence of a spleen-resident, immature NK cell lineage in various murine SLE models, which could indeed explain the reduced NK cell cytotoxicity [78].

Indications of a potentially larger role of NK cells in SLE were derived from their analysis in SLE-affected organs. NK cells from liver and lungs of diseased MRL/MpJ^{lpr} mice have enhanced natural cytotoxicity compared with healthy controls [79, 80]. Long-term NK cell depletion delays glomerulonephritis onset in NZBxNZW(F1) mice [81], which suggests a local role for these cells. NK cells from diseased MRL/MpJ^{lpr} were also phenotypically more active than those from healthy mice, with increased

TABLE 2. Characteristics of NK cells in various murine SLE models

Organ	Major findings	Murine SLE model	Reference
Spleen	Reduced NK cell numbers	MRL/MpJ^{lpr}	[42]
Spleen	Reduced cytotoxicity	MRL/MpJ^{lpr}	[58]
Spleen	Increase in immature NK cell subsets	MRL/MpJ^{lpr} MRL/MpJ	[77]
Spleen	Increase in immature NK cell subset (NK1.1$^+$CD11c$^+$CD122$^+$ MHC II$^+$)	FcγRIIB2/2 FcγRIIB2/2.Yaa TLR7tg	[78][a]
Spleen	Increased IFN-γ production	Are-Del	[63][a]
Lung	Increased cytotoxicity	MRL/MpJ^{lpr}	[79]
Liver	Increased cytotoxicity	MRL/MpJ^{lpr}	[80]
Kidney	Increased NK cell numbers in prediseased and diseased models More active NK cells (CD69, CD226) Increased IFN-γ, perforin, and granzyme B production	MRL/MpJ^{lpr}	[42]
Kidney	Increased IFN-γ production Increased percentage of mature NK cells More active NK cells (CD11b, CD43, T-bet)	MRL/MpJ^{lpr}	[77]

[a]The SLE-like pathologies of the FcγRIIB2/2, FcγRIIB2/2.Yaa, TLR7tg, and Are-Del are detailed in Voynova et al. [78] and Hodge et al. [63].

CD69 expression, granzyme B and perforin production, and IFN-γ secretion [42].

As stated above, the kidney is one of the main organs targeted in SLE. Immune complex formation in kidney glomeruli is presumed to be an initiator of lupus nephritis, one of the main causes of morbidity and mortality in human SLE and in murine SLE models [82]. Once these complexes appear, interstitial infiltrates of macrophages and T and B cells form, in turn, amplifying the local inflammatory response [83, 84]. The cell infiltrates correlate with severity of glomerular lesions, leading to glomerulonephritis and eventual renal failure [85, 86].

Information on the phenotypical and functional properties of kidney NK cells is limited. Studies by Jevnikar's groups [87, 88] showed that NK cells contribute directly to renal injury by killing tubular epithelial cells during renal ischemia-reperfusion injury and that NK cells are involved in mediating damage in long-term kidney transplants. Our recent research shows that kidney NK cells from diseased SLE-like MRL/MpJ^{lpr} and MRL/MpJ mice are more active than in controls, indicated by increased CD11b and CD43 expression (mature NK cell markers), CD11b^{High}CD27^{Low} cell percentage (mature NK), STAT5 phosphorylation (a key NK cell-signaling modulator), IFN-γ production, and the percentage of T-bet$^+$Eomes$^-$ NK cells [77]. T-bet and Eomes are transcription factors that drive NK cell maturation and development locally and in the periphery and imprint the activated lymphoid program into these cells [89, 90]. These differences are kidney specific, as neither bone marrow nor spleen NK cells from diseased mice show increased activity [77]. These data support the findings of Huang et al. [42], who showed that kidney NK cells in mice with active disease produce more cytotoxic granules (perforin and granzyme B) and IFN-γ than controls when stimulated with PMA + ionomycin; this increase in IFN-γ production contributed, to some extent, to kidney damage in SLE [42, 77]. These activated NK cells could be among the main IFN-γ producers, responsible for promoting and sustaining inflammation in nephritic lupus kidney.

Huang et al. [42] also reported that the majority of NK cells that infiltrate kidney in mice with active disease expresses CD226, another important NK activating receptor. Our studies suggest that circulating NK cells are recruited early in disease progression, as mice in a predisease state had higher levels of CX3CL1 (a chemoattractant chemokine) in kidney compared with healthy and diseased controls [77]; the finding that NK cells in the prediseased MRL/MpJ^{lpr} kidney expressed Eomes supports this hypothesis. In mice, Eomes expression is linked to immature NK cell differentiation in the periphery [90]; in humans, Eomes is also expressed preferentially in the immature CD56^{bright} NK cell population [91]. These Eomes$^+$ NK cells in the prediseased kidney might thus indicate early-stage recruitment from the periphery to affected organs.

These data could explain the conundrum of reduced NK cell numbers in SLE. This phenomenon, characteristic of SLE and other autoimmune diseases, remains unexplained, and it has not been determined whether this cell depletion is a result of disease or a possible cause of autoimmune progression. The interpretation that NK cells in PBMCs are depleted as a result of death, therefore, might be partially flawed, as it could also be a result of NK cell migration to target organs in SLE. NK cell trafficking to SLE target sites could explain the reduction in the percentage of CXCR3-positive NK cells in PBMCs of patients with active disease [65], in line with our data from murine models. As CXCR3 has a role in NK cell trafficking to inflamed organs, this reduction in mature CD56^{dim} CXCR3-positive cells could, in fact, reflect a shift of these cells from the periphery to target organs. The percentage of granzyme B- and perforin-expressing cells in this mature CD56^{dim} NK cell subset increases in patients with active

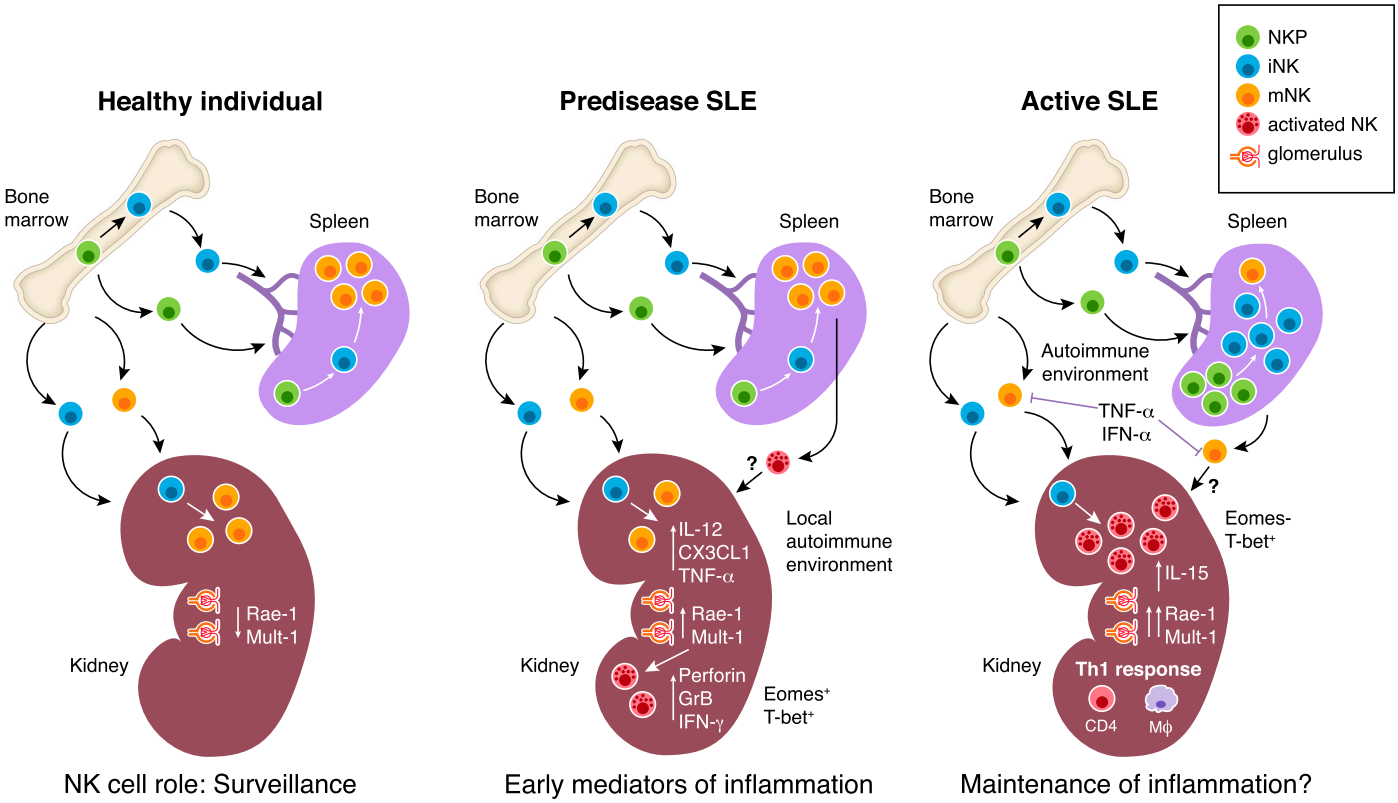

Figure 1. Model of altered NK cell phenotype in active SLE. In healthy individuals, NK cells have a role in infection surveillance. NK cell trafficking is normal, with some organ-specific differentiation. In predisease SLE, NK cell production remains unchanged in bone marrow and spleen; nonetheless, in the autoimmune kidney environment, in which inflammation occurs, NKG2D ligand expression [retinoic acid early transcript 1 (Rae-1) and murine UL16-binding-protein-like transcript 1 (Mult-1)] and production of proinflammatory cytokines (IL-12 and TNF-α) and the chemoattractant chemokine CX3CL1 increase. Peripheral NK cells that express the transcription factor Eomes are probably recruited to the kidney, where the autoimmune environment promotes NK activation and production of IFN-γ and cytotoxic granules [perforin and granzyme B (GrB)]. In this situation, NK cells could act as early mediators of inflammation. As a result of this kidney recruitment, NK cell numbers appear to be reduced in peripheral blood. In active SLE, NK cell trafficking and differentiation are altered in the spleen, where still-unknown genetic or environmental factors lead to accumulation of functionally immature NK (iNK) cells. As a result of active disease, high serum TNF-α and IFN-α levels probably contribute to the reduced NK cell activity often observed in SLE patients. The decreased NK cell numbers in the periphery of individuals with active SLE are not a result of a defect in NK cell production from NKP cells but rather, from a maturation pathway malfunction that seems to be organ specific (spleen). In kidney, several factors (such as increased NKG2D ligand expression and IL-15/IL-15R complex production) promote a locally, more active NK cell phenotype in mice with active disease. T-bet expression in these mature NK cells indicates local differentiation and activation as a result of the inflammatory environment. The increase in mature NK (mNK) cell levels and their increased IFN-γ secretion suggest that NK cells help maintain inflammation in the diseased kidney (↓, down-regulation; ↑, up-regulation; Mϕ, macrophage).

SLE, and their IFN-γ production capacity is unchanged compared with controls. This would imply intact NK cell activity in these "migrating," mature $CD56^{dim}$ NK cells, which therefore, would have the potential to exacerbate/promote local tissue damage. Park et al. [41] also showed a direct correlation between the onset of lupus nephritis and the reduction of circulating NK cells, supporting this hypothesis. One might speculate that this correlation is a result of recruitment of these NK cells to the kidneys of patients with active disease.

Data from murine models show that immature NK cells accumulate in the spleen but not bone marrow or kidneys of diseased mice [77]. The alterations in cell number and activity in SLE patient PBMCs imply that NK cell development and function are greatly influenced by organ-specific environmental changes during SLE progression (Fig. 1).

POSSIBLE FACTORS IN THE DYS(?) REGULATION OF NK CELLS IN SLE

The factors implicated in modulating NK cell phenotype and function in SLE have not been characterized extensively. Most studies focus on identification of factors that explain the lower proportion and total numbers of NK cells in SLE patient peripheral blood.

Several cytokines have been linked to this deregulation of NK cell activity. Levels of IL-2, a crucial cytokine in NK cell development [92], are decreased in SLE patients and in murine SLE models [93–95], which might limit NK cell differentiation in the periphery. Other cytokines, including TNF-α and IFN−α, are also linked to the immature NK cell phenotype in SLE patients. High TNF-α levels can block NK cell development and impair NK cell functions [96, 97]; the increased TNF-α in SLE patient serum

and in MRL/MpJ^{lpr} mouse kidney and serum might inhibit NK cell function [97–100]. IFN-α is also elevated in the serum of SLE patients with active disease, and modulations in IFN-α levels could have a role in NK cell development [42, 49, 101, 102].

Other mechanisms could also contribute to the presence of immature NK cells in the periphery of SLE patients. Recent work by Voynova et al. [78] showed a correlation between chronic TLR7 activation (which contributes to SLE pathogenesis [103]) and the appearance of a highly proliferative, immature NK cell population that correlates inversely with mature NK cell number. Aberrant T cell selection inhibits NK cell development; as double-negative T cell populations are expanded in SLE patients, these cells might also have a regulatory role in NK cell development during SLE [97, 104]. The Tyro3 receptors (Tyro3, Mer, Axl) on NK cells, and especially their ligands (Gas6 and protein S) are also critical for functional differentiation of NK cells [105]. Free protein S is decreased in SLE patient serum, whereas the opposite was observed for Gas6, which correlated with disease activity [106, 107]. Nonetheless, more detailed studies are needed to determine whether these ligands have direct or indirect influence on NK cell maturation and function in SLE.

NKG2D ligand expression is another well-described pathway that could modulate NK cell activity in SLE, both peripherally and locally. In tumors, cell-bound NKG2D ligand expression improves tumor cell immunogenicity, whereas soluble NKG2D ligands suppress tumor immunity [108]. Soluble MICA (an NKG2D ligand) is reported in SLE patient plasma and could contribute to the impaired NK cell activity often observed in PBMC samples [15]. When it is membrane bound, MICA polymorphism is associated with SLE [109], with some alleles able to increase NK cell activity [110]. In kidney biopsies, we detected MICA in lupus nephritis patients. In 3 murine models of SLE, we found increased NKG2D ligand levels in glomeruli, even at the predisease stage, which indicates that local NKG2D ligand expression might promote NK cell activity and drive kidney injury [77]. As with other factors that control NK cell activity, the context in which NKG2D ligands are expressed is likely to influence their effect.

Other factors could also contribute to NK cell activity and enhance local autoimmune injury. IL-15 is considered central to NK cell development in bone marrow, as IL-15 knockout mice are almost completely devoid of NK cells [111–113]. IL-15 levels are increased in SLE patient serum and in diseased BXSB mice [114–116]. At first glance, this would contradict the observation that NK cell activity is low in the periphery in SLE; nonetheless, PBMCs (including NK cells) from SLE patients respond poorly to IL-15 stimulation [115]; high serum IL-15 levels might thus be insufficient to enhance NK cell activity in PBMCs. In diseased target organs, however, IL-15 effects probably differ. Our studies show elevated IL-15/IL-15R complex levels in kidneys of diseased MRL/MpJ^{lpr} mice. This and the association of high transmembrane IL-15 expression by immune cells with murine lupus development suggest that IL-15 is able to maintain NK cell activity in diseased kidney [77, 116].

IL-12, a cytokine that promotes proinflammatory NK cell activation, is triggered in SLE patients before disease flares and might influence NK cell activity immediately before active disease [117]. These data are reinforced by the observed increase in IL-12 in (NZBxNZW)F1 mice after pristane-induced lupus [118] and by our report [77] of high IL-12 levels in MRL/MpJ^{lpr} mouse kidneys before disease onset. The ability of predisease kidney-infiltrating NK cells to produce IFN-γ in response to IL-12 + IL-15 stimulation remained intact [77]. These findings indicate that IL-12-promoted NK cell production of IFN-γ could have a role in the early stages of kidney damage in SLE.

Increased expression of CD319, a member of the signaling lymphocyte-activated molecule receptor family, was reported recently in NK cells from SLE patients [119]. PBMC stimulation with nucleic acid-containing immune complexes (typical of SLE) up-regulated CD319 expression in CD56^{dim} NK cells, linking this ligand functionally to the disease. Increased CD319 expression is also documented in pDCs and B cells in SLE [119, 120]. Thus, it could be conjectured that interactions among NK cells, pDCs, and B cells via CD319 could contribute to SLE pathogenesis, although further studies are needed to untangle the complicated network of cell interactions at play in this disease.

CONCLUDING REMARKS

The innate complexity of NK cell function and development, coupled with the multifactorial components that characterize SLE, have made it difficult for researchers to understand the role of NK cells in the pathogenesis of this disease. Whereas the importance of NK cells as factors in SLE has almost been disregarded, a closer look into their function and phenotype during active disease stages shows that they could have a larger role than originally thought. Mouse models could aid considerably in providing technical tools, which will allow better understanding of NK cell function in SLE, particularly in relation to NK cell activity in various organs. It will be of great interest to determine whether the variations in NK cell deficiencies are the cause or the effect of autoimmune manifestations and/or whether these deficiencies are the result of alterations that are "central" (in bone marrow) or "peripheral" (blood or target organs).

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DISCLOSURES

The authors declare no competing financial interests.

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KEY WORDS:
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