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CD11b agonists offer a novel approach for treating lupus nephritis

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Abstract

Lupus nephritis (LN) develops in more than a third of all systemic lupus erythematosus (SLE) patients and is the strongest predictor of morbidity and mortality. Increased circulating levels of type I interferon (IFN I) and anti-double stranded DNA (anti-dsDNA) and anti-RNA binding protein (anti-RNP) antibodies lead to increased glomerular injury via leukocyte activation and glomerular infiltration. Uncontrolled Toll-like receptor (TLR) signaling in leukocytes results in increased production of IFN I and anti-dsDNA antibodies. *ITGAM* gene codes for integrin CD11b, the α-chain of integrin heterodimer CD11b/CD18, that is highly expressed in leukocytes and modulates TLR-dependent pro-inflammatory signaling. Three nonsynonymous SNPs in the *ITGAM* gene strongly correlate with increased risk for SLE and LN and with IFN I levels. Here we review the literature on the role of CD11b on leukocytes in LN. We also incorporate conclusions from several recent studies that show that these *ITGAM* SNPs result in a CD11b protein that is less able to suppress TLR-dependent pro-inflammatory pathways in leukocytes, that activation of CD11b via novel small molecule agonists suppresses TLR-dependent pathways, including reductions in circulating levels of IFN I and anti-dsDNA antibodies, and that CD11b activation reduces LN in model systems. Recent data strongly suggests that integrin CD11b is an exciting new therapeutic target in SLE and LN and that allosteric activation of CD11b is a novel therapeutic paradigm for effectively treating such autoimmune diseases.

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Conflicts of Interest

The authors have no additional financial interests.

Overview

Lupus nephritis (LN) is a debilitating complication of systemic lupus erythematosus (SLE) with poorly defined etiology and ineffective treatment options. LN is characterized by infiltration of immune cells to the kidneys, leading to damaging inflammation and proteinuria. CD11b, the α-chain of the integrin CD11b/CD18 (αMβ2, CR3, Mac-1) is expressed on the surface of infiltrating macrophages and neutrophils. The *ITGAM* gene, which encodes for CD11b, has single nucleotide polymorphisms (SNPs) which reduce integrin activation and are strongly associated with SLE and LN susceptibility. CD11b modulates several biological functions including cell adhesion, migration, and signaling. Toll-like receptor (TLR) signaling in leukocytes mediates several pro-inflammatory cytokines and type 1 interferon (IFN I), a circulating biomarker for SLE and LN. Recent studies show that CD11b activation suppresses TLR-dependent pro-inflammatory signaling reducing inflammatory damage and LN in experimental systems. Here, we give an overview of CD11b, its associations in SLE and LN, and suggest that allosteric activation of CD11b is potential novel therapeutic strategy for SLE and LN.

Lupus nephritis and systemic lupus erythematosus

Systemic lupus erythematosus (SLE) is a debilitating autoimmune disease that affects multiple end organs. It is also highly heterogeneous in its clinical manifestations, with mild-to-moderate presentations in some patients, versus significantly debilitating symptoms in many others. The disease etiology is multifactorial, and the exact disease mechanism remains unclear (1). SLE affects >2 million Americans and >5 million people worldwide (2,3). More than 40% of all adult SLE patients and >80% of pediatric SLE patients develop glomerular injury and cardiovascular complications, resulting in kidney impairment and the development of lupus nephritis (LN), which remains the strongest predictor of morbidity and mortality (4–6). Young women of childbearing age are disproportionately more susceptible to SLE and LN, with a ratio of 9:1 (2,7,8). LN is a severe end-organ manifestation of lupus which often develops within the first 5 years of SLE diagnosis, though it is also often the presenting manifestation of underlying SLE (9). LN is characterized by immune complex deposition in the glomerular capillaries and glomerulonephritis, which can rapidly progress from chronic kidney disease (CKD) to end stage renal disease (ESRD) (5). LN is currently managed via glucocorticoids and immunosuppressive aggressive treatments, remission is achieved in only a fraction of patients, and a majority develop kidney complications resulting in end-stage renal disease (ESRD) within 5–10 years of disease diagnosis. Thus, targeted and improved LN treatments are urgently needed and an unmet medical need. A key to development of improved therapeutics is a better understanding of the underlying mechanisms at a molecular and cellular level.

Immune complex deposition in the glomerular capillaries, in conjunction with elevated levels of circulating pro-inflammatory factors, is often the initiating cause of LN and drives tissue damage (10). The immune complexes comprise of anti-nuclear or anti-double stranded DNA (dsDNA) antibodies, anti-RNP antibodies, anti-glomerular antibodies, activated complement fragments, neutrophil extracellular traps (NETs), DNA fragments and apoptotic cell debris. The complexes are deposited in the glomerular capillaries due the presence

of autoreactive antigens in the matrix, the inability of the glomerular filter to clear these complexes or both. The deposited complexes induce influx of innate immune cells from circulation, where the recruited cells initiate a pro-inflammatory program that results in recruitment and activation of leukocytes and lymphocytes, loss of glomerular integrity and a progressive glomerular damage. The recruited cells also secrete pro-inflammatory mediators that initiate such signaling in nearby resident cells in the glomeruli, including podocytes, mesangial cells and the resident macrophages. Furthermore, the deposited pro-inflammatory milieu contains damage-associated molecular patterns (DAMPs) that are recognized by various cellular receptors, such as the family of toll-like receptors (TLRs) (11), and initiate pro-inflammatory signaling, which produces a feed-forward loop that causes further cellular injury, apoptosis, glomerulosclerosis and kidney damage. Macrophages are increased in LN and are associated with more severe disease and poorer outcomes (12).

Systemic inflammation due to aberrant activation of the immune compartment, including leukocytes, B-cells and lymphocytes, drives the formation of immune complexes and increased production of pro-inflammatory mediator in SLE. Defective clearance of apoptotic materials and an unrestrained immune response towards it creates a feed-forward loop that results in systemic inflammation and autoimmunity (13). Overactive neutrophils release NETs during a unique form of cell death, called “NETosis,” which also serves as a source of autoantigen upon B cell activation (5,14,15). SLE patients display higher NET levels compared to healthy controls (14). These levels have been associated with disease severity and failure of NET removal is associated with LN development. Failure to remove apoptotic debris and NETing neutrophils leads to accumulation of these debris and immune complexes in the kidneys, promoting the damaging inflammation seen in LN (16).

Among the circulating pro-inflammatory mediators, levels of a major family of immunomodulatory cytokines, type I interferon (IFN I), are elevated in the sera of SLE patients, correlate with disease activity and are diagnostic (17–20). Increased IFN I activity is also a heritable risk factor and is pathogenic (21). Although type I interferons are key mediators of host response against viral and bacterial pathogens, their activation has also been recognized as being central in the development of SLE/LN (22). Increased IFN I levels are correlated with increased severity of LN (23) and are associated with accelerated glomerulonephritis in mice (24,25). Elevated IFN I levels result in increased expression of several downstream targets, that can be measured as an IFN I signature (26). Elevation in IFN I levels also activates T cells, dendritic cells and B cells, that lead to secretion of autoantibodies and IC generation, resulting kidney IC deposition and in tissue scarring in LN (21,27,28). The plasmacytoid DCs (pDCs) and myeloid cells are among the main cellular sources of IFN I production, via stimulation of their TLRs and other molecular sensors of pathogen-associated molecular patterns (PAMPs) (29,30). Given the central role of IFN I and its receptor (type I interferon receptor, *IFNAR*), an antibody blocking this interaction, anifrolumab, was recently approved for treatment in SLE (31,32).

CD11b is encoded by the *ITGAM* gene

Integrins are heterodimeric receptors with an α- and a β-subunit that are non-covalently associated with each other (for an excellent recent review, please see (33)). They have a

large extracellular domain that binds to its ligands, single-pass transmembrane helices and short cytoplasmic tails, that bind to a variety of intracellular proteins to link integrins to the cytoskeleton and induce signaling. The integrin CD11b/CD18 comprises of α-chain CD11b (also known as αM, encoded by the gene *ITGAM*) and β-chain CD18 (also known as β2, encoded by gene *ITGB2*) and is highly expressed on leukocytes, such as neutrophils, monocytes and macrophages (34–37). The dimeric integrin receptor CD11b/CD18 is also known as αMβ2, Mac-1 and CR3 and mediates cell adhesion, migration and signaling in leukocytes to modulate many biological functions of these cells, including phagocytosis, inflammatory damage and tissue repair (27,38–41).

The function of CD11b/CD18 is dynamically regulated on the cell membrane by large conformational changes. In circulating leukocytes, CD11b/CD18 is expressed in a low affinity, closed conformation. Structural studies with the recombinant ectodomain of homologous integrin CD11c/CD18 showed that its large extracellular domain adopts a bent "V" shaped structure in its low affinity conformation, with a "head" where ligands can bind and two "legs" which further span the plasma membrane connecting the ectodomain to the intracellular proteins and cytoskeleton (Figure 1A) (42,43). The various domains of the CD11b and CD18 are organized in a way to allow for conformational switching of the receptor (Figure 1B) and the short cytoplasmic tails in each of the two sub-units bind to cytoplasmic proteins, that help convey signaling changes from inside of the cell to the extracellular domains (inside-out signaling) or from extracellular ligands to the inside of the cells (outside-in signaling) by these receptors. The binding of cytoplasmic proteins to the integrin cytoplasmic tails or engagement of extracellular ligands by the integrin head domain induces large conformational changes in the integrin structure that drives the intracellular signaling pathways. In CD11b/CD18, the ligand binding is mediated by a compact domain in CD11b called the αA-domain (also called αI-domain) and is mediated via a *m*etal *i*on *d*ependent *a*dhesion *s*ite (MIDAS) (44–46). Previous structural studies showed that the αA-domain also exists in two major conformations, with its low affinity, closed state predominating in circulating cells that converts into a high-affinity, ligand competent open state upon activation (Figure 1C) (47,48).

Coding SNPs in *ITGAM* are a risk factor for LN

The increased incidence of SLE in individuals with specific genetic backgrounds, such as of African, Hispanic or Asian ancestry, as compared to those of European ancestry suggests a role for genetic predisposition for this autoimmune disease. Indeed, genome-wide association studies (GWAS) over the last two decades have identified over 100 genetic risk loci that are linked to the increased risk for SLE and LN (49). Fine mapping of these loci identified single-nucleotide polymorphisms (SNPs) in a number of immune response genes. Studies also showed that SNPs in *ITGAM*, including three coding region SNPs (rs1143678, rs1143679 and rs1143683, which result in mis-sense mutations P1146S (a C>T substitution), R77H (G>A) and A858V (C>T) respectively in the protein, significantly correlate with incidence of SLE and other complications of SLE such as LN, discoid rash and cardiovascular disease (1,9,14,40,50–53). This association of *ITGAM* SNPs with SLE and LN holds among diverse ancestral backgrounds, suggesting it to be a common disease linked pathway (7,52,54–56). Recent studies have also shown that two of the three coding

SNPs in *ITGAM* (rs1143678 and rs1143683) are in complete linkage disequilibrium (LD), forming a haplotype (27,51,54) and the SNPs rs1143678 and rs1143679 are often in LD (27,52,56). Finally, the prevalence of LN among SLE patients increases in patients with the *ITGAM* SNPs rs1143679 or rs1143683 as compared to SLE patients without renal nephritis (54,57).

How these *ITGAM* SNPs confer risk for SLE and LN is not completely clear, although recent studies have shed some new light. These SNPs in *ITGAM* are present in up to 20% of patients and only occur in 1–2% of the general population, suggesting a strong mechanistic link between these SNPs and SLE/LN (27,40,51–53). They appear to reduce function of the encoded protein CD11b, including integrin activation, ligand binding and cell adhesion, phagocytosis and catch-bond formation (42,58–60) without change in surface expression (51,61). Mapping of the SNPs on integrin structure shows that they are dispersed in three different domains, with rs1143679 (resulting in the R77H mutation) in the propeller domain, rs1143683 (resulting in the A858V mutation) in the calf-2 domain and rs1143678 (resulting in the P1146S mutation) in the cytoplasmic tail, suggesting their role in modulating CD11b-dependent signaling (Figure 2).

CD11b is a key modulator of pathogenic mechanisms

The integrin CD11b/CD18 plays important roles in leukocyte biology (62). It is expressed on monocytes, neutrophils, dendritic cells, natural killer cells, macrophages, and a subset of B and T cells (63–68). As a receptor, CD11b/CD18 dimer binds over 40 ligands, including intercellular adhesion molecule (ICAM) family members, complement protein iC3b and fibrinogen (69–73), and modulates multiple leukocyte functions, including cell adhesion, transmigration, phagocytosis, pro-inflammatory signaling, tissue recruitment, oxidative burst and apoptosis. Given these roles, CD11b/CD18 has long been considered to promote host defense pathways as a pro-inflammatory receptor (74), although recent studies have also shown it to have an anti-inflammatory role by modulating intracellular signaling of other pro-inflammatory receptors (21,27,53,75). This suggests that CD11b acts in a highly contextual manner to promote or regulate inflammation.

CD11b, the α-chain of CD11b/CD18, was initially identified in patients with leukocyte adhesion deficiency-1 (LAD1) (76–79). LAD1 is an autosomal recessive disorder that is characterized by recurrent bacterial infections, impaired wound healing and aberrant granulocyte and leukocyte functions. Leukocytes in LAD1 patients show a lack of β2 integrin surface expression, due to mutations in either the β- or the α-chains of CD11b/CD18. LAD1 patients present with recurrent, life-threatening infections and an inability to fight off and destroy invading pathogens. LAD1 neutrophils show markedly reduced adhesion, chemotaxis and extravasation, and an inability to kill bacteria. This suggested that a main role of CD11b was to promote inflammation to control infections. Mice deficient in CD11b ($CD11b^{-/-}$) mimic many, although not all, of the characteristics observed in LAD1 patients (80). $CD11b^{-/-}$ neutrophils display impaired adhesion, spreading, phagocytosis and oxidative burst and show defective transmigration and tissue recruitment (their extravasation is paradoxically increased) and delayed apoptosis. CD11b deficient mice also show increased susceptibility to inflammatory and autoimmune diseases (81–86),

although they also show increased tissue infiltration of leukocytes, elevated IC deposition and immune-mediated glomerular injury in lupus-prone mice (84), suggesting additional anti-inflammatory roles for CD11b. CD11b deficiency results in increased levels of pro-inflammatory cytokines in circulation in models of sepsis and SLE (21,27,87). Thus, this propensity of genetically or functionally CD11b deficient animals to display overly increased immune responses and tissue injury is not unexpected as CD11b has a key functional role in leukocyte migration and recruitment and in phagocytosis mediated clearing of opsonized particles, apoptotic debris and ICs, and the lack of CD11b would affect control of exuberant immune responses. Conversely, some studies have also shown that CD11b promotes inflammatory injury and that blocking CD11b (or CD18) or the deficiency of CD11b reduced inflammation and tissue injury in other animal models (88–91). Therefore, the published literature is not completely clear as to whether blocking CD11b with antagonists or its absence (via knockouts or knock downs) has therapeutic applicability in inflammatory and autoimmune diseases, and that the approach may be highly experimental model system and tissue context dependent.

Aberrant TLR-dependent pro-inflammatory signaling plays a key role in driving systemic inflammation in SLE and LN. Over-abundance or ineffective clearance of apoptotic cells and cellular debris, such as single-stranded RNA (ssRNA), double-stranded DNA (dsDNA) and non-methylated endogenous DNA fragments (CpG DNA) stimulate TLRs 3, 7/8 and 9. Stimulated TLRs recruit adaptor proteins MyD88, TRIF, TRAF3 or TRAM, which as subsequently sequentially detected by a set of kinases, such as TBK1, and ubiquitinases, such as Cbl-b, to activate transcription factors NFκB, AP-1 and IRF3/5/7. This results in activation of pro-inflammatory pathways generating IL-1β, IL-6, TNFα and IFN I (92,93) (Figure 3). The resulting signaling produces increased levels of pro-inflammatory mediators in circulation, including IFN I, and a feed-forward loop that results in activation of immune cells, further production of ICs and end-organ injury. CD11b cross-talks with a number of cellular receptors on immune cells, including TLRs, to regulate intracellular signaling. Recent data suggests that CD11b acts to negatively regulate TLR-signaling in leukocytes (21,27,94,95). Stimulation with TLR4 agonist LPS increases secretion of proinflammatory cytokines IL-6, TNFα, and IL-1β through activation of TLR-dependent canonical NFκB pathways, leading to end-organ damage over time, and significantly higher levels of these cytokines are produced in CD11b-deficient cells. Basally, circulating leukocytes express CD11b/CD18 on cell surface in a low ligand-competent (inactive) conformation, where it rapidly changes its conformation to ligand-competent (active) conformation, in response to various inside-out or outside-in signals, such as increased chemokine and cytokine gradients near sites of inflammation and stimulation of other receptors, such as TLRs (96–98). TLR stimulation, in addition to activating NFκB and IFN I pathways, also results in inside-out signaling mediated activation of CD11b that occurs via the activation of phosphatidylinositol-3-OH kinase (PI3K), the second messengers diacylglycerol (DAG) and Ca^{2+} activating protein kinase C and small GTPase Rap1, that subsequently engages interacting proteins RIAM and RapL that un-restrain cytoplasmic protein talin-1 to bind to the integrin tail and stabilize its active conformation (99,100). Subsequently, active CD11b induces phosphorylation of kinases Src and Syk, which further phosphorylate MyD88 and TRIF leading to their ubiquitin-mediated degradation

via Cbl-b (21). Consequently, there is reduction in activation of transcription factor NFκB, which correlates with reduced production of IL-6 and other pro-inflammatory proteins. Macrophages deficient in CD11b have reduced TLR-induced degradation of MyD88 and TRIF resulting in enhanced activation of NFκB and other TLR-dependent pathways and inflammatory cytokine production (21,27). This suggests that CD11b suppresses pro-inflammatory signaling pathways downstream of TLR stimulation, that this break on inflammatory signaling is missing in the $CD11b^{-/-}$ cells, and that defective regulation of this pathway underlies LN and SLE pathobiology. CD11b also helps maintain autoreactivity by negatively regulating BCR signaling (101), T-cell activation (34), Th17 cell development (83) and DC maturation. Collectively, functional CD11b is more than a pro-inflammatory receptor and likely maintains homeostasis and tolerance by modulating pro-inflammatory pathways. Moreover, recent studies also suggest that CD11b also engages specific cell surface expressed ligands on the same cells, *in cis*, thereby regulating biological functions of such ligands. These include ICAM-1, FcγRIIA, and SIRPα, suggesting the multiple additional mechanisms CD11b uses to limit pro-inflammatory signaling by other proteins and ligands (100,102,103).

Conversely, CD11b can also positively induce pro-inflammatory signaling (87) (Figure 4). CD11b/CD18 engagement with soluble or immobilized ligands, such as fibrinogen and ICAM-1, induces integrin clustering that activates pro-inflammatory NFκB signaling, delays neutrophil apoptosis, releases proteolytic granules and induces cytotoxic activity (104–110). Ligation of CD11b/CD18 with ligands, such as fibrinogen, and clustering also induces increased production of pro-inflammatory cytokines, superoxide and proteases in neutrophils that result in increased tissue damage (111–115), although studies have also shown that high avidity ligation of integrins produced rapid but transient expression of inflammatory cytokines IL6 and TNFα, and that longer term engagement resulted in a suppression of pro-inflammatory pathways (116). CD11b can similarly positively regulate TLR signaling in DCs (117). Studies have also shown that integrins cluster only upon engagement with multimeric or immobilized ligands and that integrin conformational change *per se*, such as with an activating mutation I316G in the αA-domain, that converts it into a ligand-competent conformation, does not lead to induction of the pro-inflammatory pathways or the reduced neutrophil apoptosis as is observed with ligand engaged, clustered integrins (118,119). Collectively, these studies suggest that CD11b has a regulatory role in controlling run-away TLR signaling, that absence of CD11b fails to suppress the TLR-signaling pathways and that although integrin engagement with high avidity ligands is synergistic with inflammatory pathways and is pro-inflammatory, integrin conformational change to a ligand-competent conformation alone does not induce pro-inflammatory signaling (Figure 4).

Recent studies with the LN associated three *ITGAM* coding region SNPs (rs1143679 (R77H), rs1143678 (P1146S), and rs1143683 (A858V)) describe the effects of mutation-induced functional deficiency in the integrin receptor. CD11bR77H mutant expressing cells display a reduced ability to bind to ligands, including ICAM1 and iC3b (61,120,121). Similarly, firm adhesion of CD11b expressing neutrophils was significantly reduced in cells from individuals carrying any of the three *ITGAM* SNPs (51). Neutrophils from donors carrying any of the three *ITGAM* variant alleles showed significantly reduced phagocytosis

(51,121,122) and that the effect for pronounced even in cells heterozygous for the mutant allele, suggesting the importance of functionally competent integrin (51,61,121,122). Furthermore, monocytes carrying the R77H substitution failed to suppress the expression of pro-inflammatory mediators IL-6, TNF-α, and IL-1β, suggesting that the mutation impaired the ability of expressed CD11b to restrain the TLR-mediated NFκB pathways (95,120,121). The regulatory effect of CD11b on BCR signaling was also significantly diminished in the cells carrying the R77H mutation (101). Finally, we recently showed that IFN-I serum activity is significantly increased in SLE patients carrying these three *ITGAM* SNPs (27). Given that IFN-I is transcriptionally induced via activation of transcription factors IRF3 and IRF7, downstream of TLR stimulation, where AKT phosphorylates repressor FOXO3 to mark it for degradation, freeing up IRF3/7 mediated transcription of IFN I (123,124) (Figure 3). Donor cells carrying the above three *ITGAM* SNPs showed increased IRF3/7 and decreased FOXO3 in the nucleus, and consequently, increased levels of IFN I, due to the mutant CD11b having reduced functionality that failed to suppress TLR-dependent pro-inflammatory signaling (27).

Together, these studies firmly establish that integrin CD11b plays a major role in modulating exuberant pro-inflammatory signaling pathways. The genetic deletion or functional deficits in CD11b, associated with the LN linked mutations, fail to suppress the overactive signaling pathways, indicating that restoration of CD11b function could be a therapeutic opportunity in LN.

Allosteric activation of CD11b is a novel therapeutic paradigm in LN

Recent publications establish that CD11b plays a largely protective role in regulating systemic inflammation and autoimmunity in SLE and LN. Thus, agents that would promote the anti-inflammatory functions of CD11b without impacting its ligand binding and other biological functions, allosteric agonists of CD11b, have the potential to further strengthen the immune-protective activities of CD11b. Towards that, we and others utilized novel small molecule allosteric agonists of CD11b, such as molecule LA1, that we previously discovered (41) to determine their applicability in controlling pro-inflammatory signaling in cells and inflammation and tissue damage *in vivo* (41,125,126). LA1 does not block the ligand binding pocket of CD11b and promotes CD11b-dependent cell adhesion and reduces cell migration, although, like the activating I316G mutation, does not induce clustering (41,127). LA1 binding to CD11b in human monocytes and macrophages down-regulated TLR7/8-dependent pro-inflammatory signaling (125), including TLR-dependent NFκB signaling and generation of proinflammatory mediators IL-1β, and IL-6 (27). CD11b activation via LA1 also suppressed TLR dependent IRF3/7 pathway by repressing the phosphorylation mediated degradation of FOXO3 leading to significant reductions in IFN I production. In two different *in vivo* models of LN, CD11b activation with LA1 significantly reduced levels of pro-inflammatory mediators in circulation, renal IC deposition, kidney infiltration of activated leukocytes and significantly decreased glomerular injury and proteinuria (27,37). Importantly, *in vivo* effects of LA1 were dependent on CD11b and were not observed in $CD11b^{-/-}$ animals. Studies also showed that TLR-stimulated increased expression of pro-inflammatory IL1β, IL6 and IFN I in donor cells carrying the above three *ITGAM* SNPs was significantly reduced by treatment with the CD11b agonist. Similar studies with other

β2 integrin agonist antibodies showed that integrin activation can rescue functional deficits in cells carrying the LN ITGAM mutations (61).

To further confirm this mechanism using an orthogonal system, we recently generated a novel knock-in model of constitutive active CD11b in mice (39). Here, the knock-in mice constitutively express the I332G mutation in the activation-sensitive allosteric pocket of ligand-binding αA-domain of CD11b that promotes high-affinity conformation of the integrin, mimicking binding of the allosteric agonist LA1. CD11bI332G knock-in mice showed significant reduction in recruitment of neutrophils and macrophages upon induction of acute inflammation. The mutation also protected animals against development of hyperlipidemia induced atherosclerosis, suggesting that allosteric activation of CD11b is protective in the setting of inflammatory diseases. Future studies will test its role in the setting of SLE and LN.

Collectively, the studies with pharmacologic or genetic activation of CD11b show that allosteric activation of CD11b can suppress the dysfunctional TLR-dependent pro-inflammatory pathways in primary leukocytes and *in vivo*, reduce proteinuria and protect kidney from injury, suggesting it to be a highly promising novel therapeutic strategy for LN.d

CD11b agonism is therapeutic in other inflammatory diseases

Leukocytes extravasate from circulation and into infected, injured or inflamed tissues to better control the damage and help heal the tissues (128–130). However, excessive leukocyte infiltration results in increased tissue damage, reduced healing and fibrotic scarring. Similarly, tumors recruit leukocytes from circulation and an increased abundance of $CD11b^+$ leukocytes, such as tumor associated macrophages (TAMs), in the tumor microenvironment correlates with poorer outcomes in multiple types of cancer (131–133). As CD11b/CD18 dimer is a key adhesion receptor that mediates such leukocyte trafficking, it has been extensively targeted as a therapeutic strategy in experimental models of inflammation and cancer. Antibodies that block CD11b or its various ligands, thus interfering with leukocyte adhesion, blocked leukocyte influx in models of acute injury, chronic injury and cancer, and reduced disease (134–140).

Counterintuitively, although CD11b agonists transiently increase cell adhesion it also reduces leukocyte extravasation in a CD11b-dependent fashion (41). Administration of CD11b agonists into models of acute inflammation, chronic injury and transplantation showed significant reduction in recruitment of $CD11b^+$ leukocytes, resulting in significant disease amelioration (41,53,126,127,141–143)

Similarly, administration of CD11b agonists in experimental models of breast cancer, lung cancer, melanoma and pancreatic cancer showed a significant reduction in the infiltration of $CD11b^+$ cells into tumor microenvironment, and a significant suppression of tumor growth (38,144,145). Similarly, CD11b KI mice show significant reduction in tumor growth (38). Collectively, these data confirm the validity of CD11b as a therapeutic target in inflammatory diseases and cancer, and suggest CD11b agonism as a promising therapeutic

strategy. Indeed, a novel CD11b agonist, GB1275, is currently completing Phase I clinical trials in oncology (146– 148).

Conclusions

SNPs in *ITGAM* are associated with risk for SLE and LN in approx. 20% of SLE and LN patients and are also associated with increased IFN I levels in patients. Recent studies have shown that integrin CD11b plays a vital role in modulating pro-inflammatory TLR-signaling pathways in leukocytes. Studies have also shown that the coding *ITGAM* SNPs associated with SLE and LN produce a defective integrin that shows reduced biological functions in leukocytes, including reduced binding to known ligands, reduced cell adhesion, phagocytosis, and catch-bond formation, and inability to reduce pro-inflammatory cytokine production downstream of TLR-pathways, suggesting that exogenous activation of CD11b could be a potential therapeutic strategy. Indeed, allosteric activation of CD11b, via pharmacologic or genetic approaches, results in suppression of TLR-dependent inflammatory pathways in leukocytes, including production of IFN I, as well as rescues the functional deficits in human donor cells expressing the mutant integrin. These studies show that integrin CD11b is an important therapeutic target in SLE and LN and firmly establish allosteric activation of CD11b as a novel, key therapeutic paradigm for effectively treating such autoimmune diseases.

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Abbreviations

SLE	Systemic Lupus Eryethmatosus
LN	Lupus Nephritis
ITGAM	Integrin Alpha
IFN	Interferon
TLR	Toll-like Receptor

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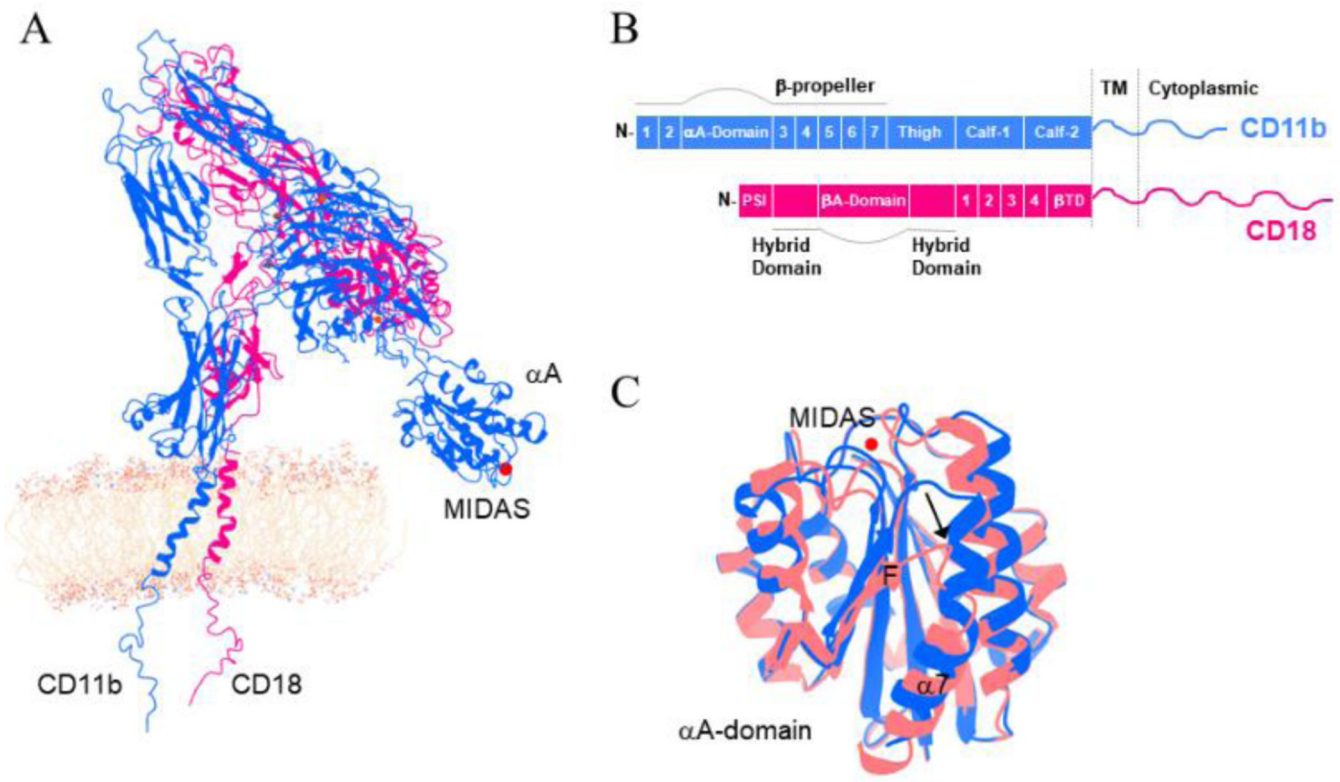

Figure 1. Structure of integrin CD11b/CD18.

A. A structural model of full-length integrin CD11b/CD18 in its low-affinity conformation based on published structure of the ectodomain of a homologous β2 integrin CD11c/CD18 (also known as αXβ2) (149). The model also depicts the relative location of the ligand binding αA-domain and the ligand binding site (metal ion dependent adhesion site, MIDAS). **B.** Domain organization in the individual chains of the integrin CD11b/CD18. **C.** Overlay model of the αA-domain of CD11b in two conformational states – low-affinity, inactive conformation (blue, from 1jlm.pdb (61)) and high-affinity active conformation (pink, from 1m1u.pdb (150)). The activation-induced changes in an allosteric pocket near the F-strand and the α7 helix, which shows the largest change between the two structures (arrow) are also shown. A metal ion at the MIDAS site is shown as a red sphere.

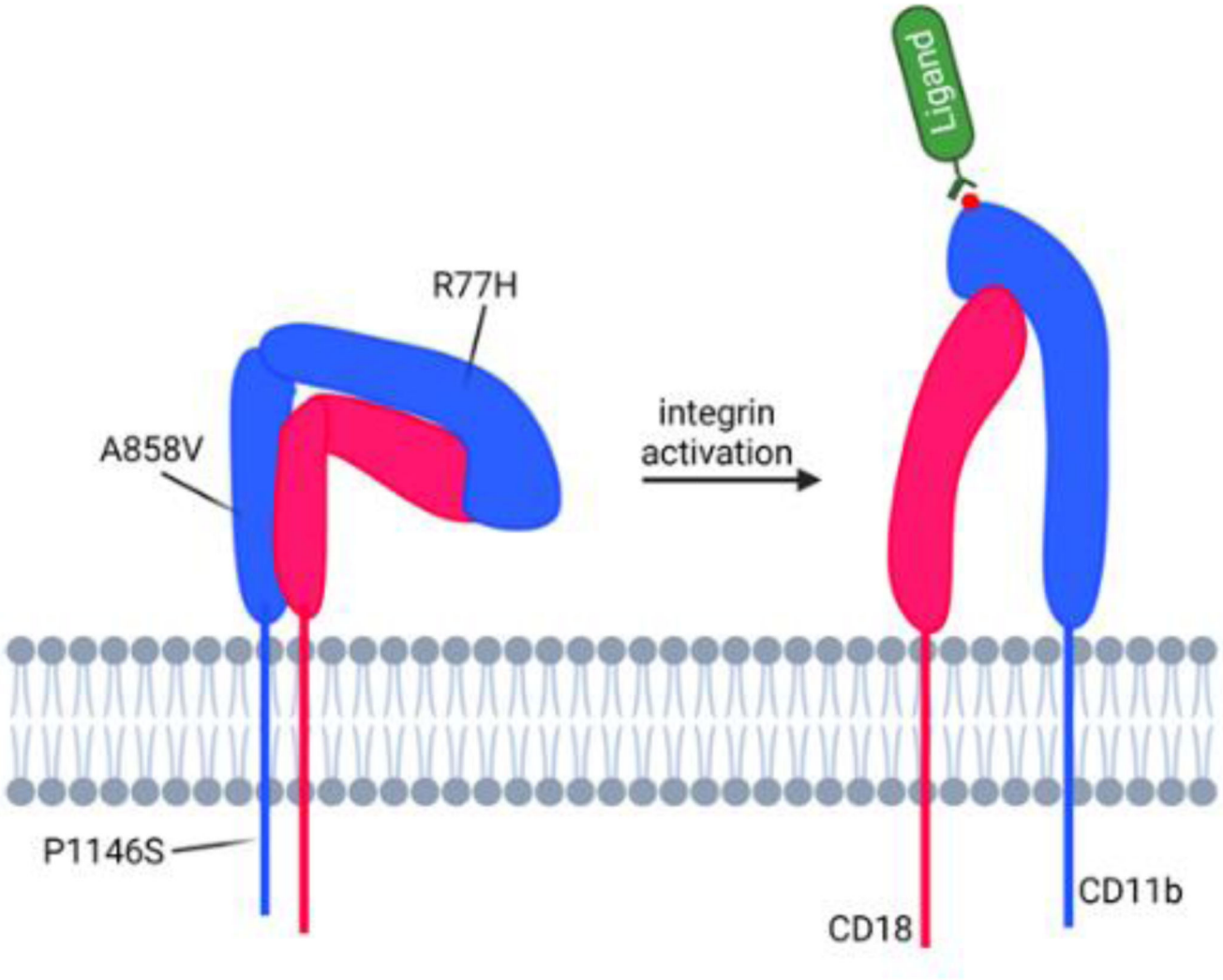

Figure 2. Integrin activation and LN associated mutations.

A diagram showing large conformational change in the integrin CD11b/CD18 that is expected upon integrin activation. The integrin exists primarily in a bent, low-affinity conformation on the surface of circulating leukocytes under basal conditions. Upon activation, the dimer changes conformation and adopts a more upright, active, high-affinity form that is bound to large, extracellular ligands with high affinity at the MIDAS site (shown with a red sphere representing a metal ion at the MIDAS site). Relative locations of each of the three CD11b mutations encoded by the comment *ITGAM* variants are also shown on the diagram.

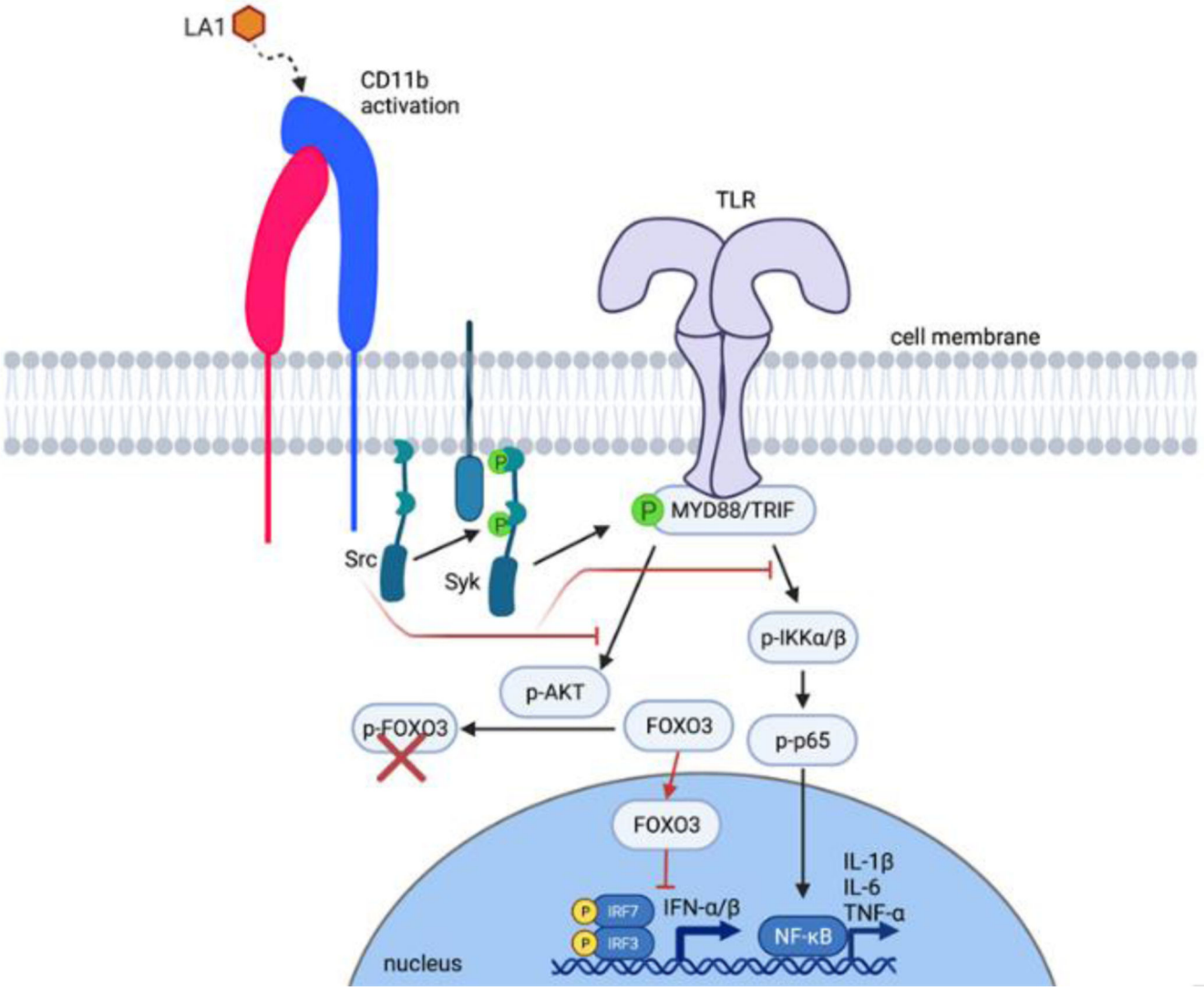

Figure 3. TLR-dependent signaling pathways are modulated by allosteric activation of CD11b.

The conventional TLR-dependent signaling is mediated via membrane recruited MyD88/TRIF proteins, that result in phosphorylation and nuclear import for NFκB complex, thereby transcriptionally upregulating pro-inflammatory molecules, such as IL-6, IL-1β and TNFα. A second arm of TLR-dependent signaling results in AKT-dependent phosphorylation and degradation of FOXO3, that results in de-repression of IFN I pathway, resulting in transcriptional upregulation of IFN I. Novel small molecule allosteric agonists, such as LA1, activate CD11b that results in recruitment of kinases Src and Syk, leading to phosphorylation and subsequent degradation of MyD88/TRIF downstream of TLRs. This leads to suppression of p65 phosphorylation and reduced nuclear translocation of NFκB, suppressing generation of pro-inflammatory cytokines IL-6, IL-1α and TNFα. Allosteric activation of CD11b also suppresses AKT phosphorylation, decreasing pFOXO3 levels, that allows import of FOXO3 to repress IRF3/IRF7 mediated expression of IFNα/β.

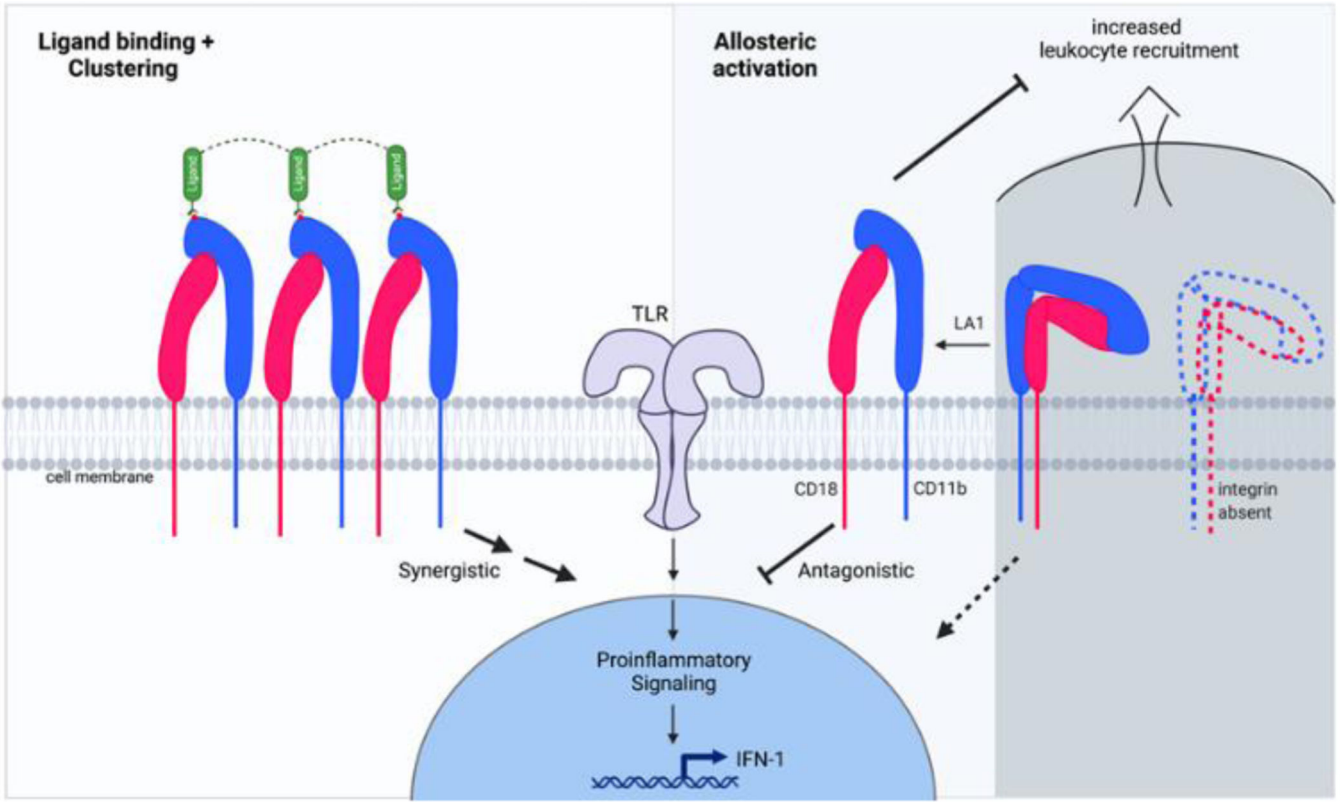

Figure 4. The many roles of CD11b in promoting or suppressing inflammatory pathways.

Ligand binding and clustering of CD11b/CD18 integrins on cell surface typically result in enhancing TLR-dependent pro-inflammatory signals and are involved in pathogen clearance, oxidative burst and other immune surveillance functions of the innate immune cells (left half). Absence of CD11b results in loss of this integrin on cell surface, that also promotes pro-inflammatory pathways, increased tissue recruitment of leukocytes, reduced ability to clear pathogens, yet increased expression of pro-inflammatory mediators, such as IL-6, IL-1βand TNFα, due to over-active TLR-dependent signaling pathways in $CD11b^{-/-}$ cells. Conversely, the presence of CD11b limits TLR-pathways and its allosteric activation, either pharmacologically or genetically, significantly dampens the exuberant TLR-dependent pro-inflammatory pathways in leukocytes, reduces leukocyte infiltration and tissue damage. Such activation is also able to rescue some of the functional deficits in cells carrying LN associated *ITGAM* SNPs, suggesting allosteric agonism of integrins as a novel therapeutic strategy.