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The SLE review series: working for a better standard of care

Modelling clinical systemic lupus erythematosus: similarities, differences and success stories

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Abstract

Mouse models of SLE have been indispensable tools to study disease pathogenesis, to identify genetic susceptibility loci and targets for drug development, and for preclinical testing of novel therapeutics. Recent insights into immunological mechanisms of disease progression have boosted a revival in SLE drug development. Despite promising results in mouse studies, many novel drugs have failed to meet clinical end points. This is probably because of the complexity of the disease, which is driven by polygenic predisposition and diverse environmental factors, resulting in a heterogeneous clinical presentation. Each mouse model recapitulates limited aspects of lupus, especially in terms of the mechanism underlying disease progression. The main mouse models have been fairly successful for the evaluation of broad-acting immunosuppressants. However, the advent of targeted therapeutics calls for a selection of the most appropriate model(s) for testing and, ultimately, identification of patients who will be most likely to respond.

Key words: systemic lupus erythematosus, SLE, mouse models, SLE treatment, SLE modelling, NZB/W mouse, MRL/lpr mouse, TLR7, IFN

Rheumatology key messages

- Mouse models are indispensable tools to study human SLE.
- Mouse models recapitulate specific elements of SLE, particularly with regard to the mechanism of disease.
- Selection of the most appropriate model(s) for future testing of targeted SLE therapeutics is essential.

REVIEW

Introduction

SLE is a complex autoimmune disease with an extremely heterogeneous clinical presentation, which reflects the multiple roles of the environment, genetics and immune response in disease initiation and progression [1]. Variability in disease manifestation and severity makes it extremely challenging to study in clinical trials, especially in terms of selecting the patient population who will be most likely to respond to the treatment under investigation [2].

Murine models of disease represent genetically homogeneous populations to study the initiation and the progressive pathogenesis at the local, peripheral and end-organ stage [3]. They provide a much faster system for therapeutic screening, because mice reach 50% mortality due to GN at 5–9 months of age [4, 5]. Moreover, they allow for examination in the absence of any therapy, which is a major caveat of studying samples from SLE patients, who still take chronic doses of immunosuppressants even when in remission [6]. Additionally, they allow for easy assessment of drug combinations with the aim of reducing individual doses and side effects of high-dose monotherapy.

The limitations to use of murine models are the increasing costs, the longevity of projects and the scientific expertise to design, fulfil, analyse and interpret results to ascertain meaningful data applicable to human disease. In this review, we briefly discuss the commonalities and differences of the most commonly used mouse strains in lupus research and highlight how they have provided a meaningful path forward for therapeutic intervention.

Main mouse models

Defining whether a system is a good model of disease requires an analysis of its requirements. A hallmark feature of human SLE is the presence of ANAs, anti-dsDNA

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antibodies and anti-RNA or RNA-associated antibodies. Clinical manifestations include GN, arthritis, heart disease, cutaneous lesions and neurological symptoms [1]. Likewise, as each patient presents with a unique phenotype, mouse models can recapitulate only limited features of the disease (Table 1).

Spontaneous New Zealand black×white F_1 (NZB/W), Murphy Roths Large/lymphoproliferative (MRL/lpr) and BXSB mouse models have been extensively used for studying immunological mechanisms and therapeutic targets over the last 40 years [5]. All three models exhibit serum ANAs and develop GN according to a strain- and sex-specific time line [4, 5]. Based on their specificities, these models can be used to examine the role of different genes and pathways, cellular dependency in disease progression and therapeutic targeting. Additionally, IFNα-dependent models and Toll-like receptor 7 (TLR7)-associated strains are gaining in importance, based on the extensive data supporting these pathways in the pathogenesis of SLE [7–9].

MRL/*lpr*

MRL/*lpr* mice have a loss-of-function lymphoproliferation (*lpr*) mutation within the gene encoding Fas, a cell-surface protein that mediates apoptosis [10]. They are characterized by lymphoproliferation, enlarged lymph nodes (lymphadenopathy) and GN, and between 25 and 75% mice develop arthritis. Serologically, they display hyperimmunoglobulinaemia, high ANAs, high anti-dsDNA antibodies and anti-small nuclear ribonucleoprotein (sn-RNP) antibodies. Male and female MLR/*lpr* mice are equally affected [4, 5]. Lymphadenopathy and splenomegaly are attributable to expansion of an unusual double-negative $CD4^-CD8^-CD3^+B220^+$ T cell population [11]. Aside from examining the mechanisms of autoantibody production and renal failure, MRL/*lpr* mice are also used to examine cutaneous and neurological aspects of lupus, in contrast with other strains [12, 13].

Human relevance

MRL/*lpr* mice recapitulate many features of lupus; however, massive lymphadenopathy is not typical of human disease. Nonetheless, several recent studies reported an association of Fas and Fas ligand polymorphisms with the susceptibility to SLE, and increased double-negative T cells have been found in the periphery and in the kidneys of SLE patients [14–17].

IFN dependency

Pre-autoimmune MRL/*lpr* mice do not show evidence of elevated IFN-induced genes (i.e. IFN signature) [18]. IFN receptor (IFNAR) deficiency enhanced the disease, and anti-IFNAR antibody treatment did not mediate any long-term effects in this model [19, 20]. Consequently, MRL/*lpr* mice are not appropriate for studying the role of type I IFN in lupus.

NZB/W

The disease in New Zealand black×white F_1 hybrid (NZB/W) mice has a strong female bias, and it is characterized by lymphadenopathy, splenomegaly, increased concentrations of ANA and anti-dsDNA antibodies and IC-mediated GN [4, 5]. NZB/W mice are also used as a model of lupus-related cardiovascular disease [21].

Crossing and selective inbreeding generated several New Zealand mixed (NZM) strains, with diverse phenotypic traits and variability in penetrance, severity, onset and gender bias [22]. The NZM2410 strain rapidly develops severe GN in both female and male mice, whereas GN in NZM2328 mice is female biased [22, 23].

Human relevance

Arguably, the most important contribution of the genetic studies in NZB/W-congenic derivatives was the identification of the NZM2410-derived *Sle1* and NZB-derived *Nba2* locuses, which are responsible for the production of autoantibodies [24]. *Sle1* and *Nba2* overlap in the telomeric region of chromosome 1, which encodes members of the FcγR, SLAM and IFN-inducible (*Ifi*) receptor families [25–30]. This region has a human syntenic equivalent on chromosome 1, 1q21–44, which has been associated with SLE in human linkage studies [31–33]. Human gene associations include *Cr2* [34, 35], *FcγRIIA*, *FcγRIIIA* and *FcγRIIIb* [36–38], *PARP* [39] and *CRP/SAP* [40, 41]. SLAM family members *Ly108* and *CD84* have been identified as disease causative in mice, but they may be less significant in human SLE [29, 42]. NZM2328-derived susceptibility locuses associated with GN, *Cgnz1* and *Agnz1*, are also located on the distal region of chromosome 1, overlapping with *Sle1* [23, 43]. *Cgnz1* has a nearly identical homologous region in the human genome; however, further studies are needed to identify possible susceptibility genes [43].

NZM2410-derived *Sle3* on chromosome 7 is responsible for generalized T cell activation and development of nephritis [44, 45]. The kallikrein genes within this region were associated with nephritis in both mice and humans [46]. NZM2410-derived region *Sle2*, responsible for the expansion of autoreactive B cells, and NZM2328-derived region *Adnz1*, responsible for autoantibody production, are located on mouse chromosome 4 [47, 48]. These regions are under investigation to identify novel susceptibility genes.

IFN dependency

Pre-autoimmune NZB/W mice display elevated IFN-regulated gene expression in the spleen [18]. The IFN signature has also been observed in myeloid dendritic cells from the triple-congenic NZM2410-derived *Sle123* strain [49]. Additionally, IFNAR deficiency has been shown to reduce disease in NZM2328 and NZM2328-derived B6.*Nba2* mice [50, 51].

BXSB and associated strains with TLR7 upregulation

BXSB mice develop a rapid-onset severe disease in males [4, 5]. The male bias is attributable to the presence of the Y-autoimmune accelerator (*Yaa*) locus, which arose from an X to Y chromosome translocation [52, 53]. This doubled the genomic copy number and therefore the expression of a number of genes, including TLR7. TLR7 is

Table 1 Main mouse models of SLE and their characteristics

	Lupus models	Main genetic components	Related human genetic associations	Immunological characteristics	Model used to assess
Spontaneous	NZB/W related: NZM2410 NZM2328 B6.*Sle123*	Locuses: NZM2410 derived: *Sle1–3* NZM2328 derived: *Cgnz1*, *Agnz1*, *Adnz1*	*Ccr2*, *FcgRIIA*, *FcgRIIIA*, *FcgRIIIb*, *PARP*, *CRP/SAP*, *kallikrein* genes, possibly *SLAMF* genes	Splenomegaly GN (subacute to chronic) Moderate ANAs, high anti-dsDNA antibodies Persistence of long-lived plasma cells Weak IFN signature	Immune dysregulation Chronic kidney disease (acute and chronic in NZM2328) Endothelial and cardiac effects
	MRL/*lpr*	Fas mutation (*lpr*)	*Fas*/*FasL* polymorphisms	Lymphoproliferation Splenomegaly Extremely enlarged lymph nodes GN (subacute proliferative) High ANAs, high anti-dsDNA antibodies, high anti-snRNP antibodies Expansion of $CD4^{-}CD8^{-}CD3^{+}$ T cells No IFN signature	Immune dysregulation Kidney disease Cutaneous lupus Neurological manifestations Arthritis
	BXSB related: B6.TLR7.Tg B6.*Sle1*Tg7	Locuses: *Bxsb1–6* *Yaa* *TLR7* upregulation	*TLR7* copy number variations, *TLR7* polymorphisms, polymorphisms of TLR7-signalling pathways (i.e. *IRF5*)	Splenomegaly GN (acute proliferative) Monocytosis Moderate ANAs, moderate anti-dsDNA antibodies (high anti-snRNP antibodies in B6.*Sle1*Tg7) Weak IFN signature in the kidney	Immune dysregulation Kidney disease (acute)
Accelerated	IFNα accelerated (various strains, i.e. NZB/W, B6.*Sle123*, NZM2328)	Strain dependent	Strain dependent, IFN signature	Accelerated disease, with characteristics of the conventional strain Highly reproducible, rapid-onset GN without increased leucocyte infiltration T cell dependent Strong IFN signature	Immune dysregulation Acute severe kidney disease Drug resistance attributable to IFN signature
Induced	Pristane induced (BALB/c, C57BL/6, DBA/1, SJL)	Pristane induces lupus in mice lacking genetic predisposition	IFN signature, TLR7 dependent	Rapid-onset, severe disease High anti-RNP, anti-Sm, anti-dsDNA antibodies Strong IFN signature	Immune dysregulation Acute severe kidney disease

crucial for the severe aspects of pathogenesis in this model [54–56]. *Yaa* can also accelerate disease in MRL, NZW and NZB lupus-prone mice [57, 58]. Likewise, a 2-fold upregulation of TLR7 on a B6.*Sle1* mild autoimmune-prone background (*Sle1*.Tg7) or larger increases in expression (>4- to 8-fold; TLR7.Tg) on a non-autoimmune-prone background are sufficient to drive severe disease [54, 59]. BXSB susceptibility loci are required for the development of the disease, because *Yaa* is not sufficient to cause lupus in mice that lack an autoimmune genetic predisposition [57, 60]. BXSB-derived loci, designated *Bxsb1–6*, are present on various chromosomes and many overlap with known NZB/W-derived regions [61, 62].

A unique feature of the BXSB strain is the TLR7-dependent expansion of circulating monocytes (monocytosis) [56]. An increased proportion of monocytes, especially non-classical $CD14^{+}CD16^{++}$, has also been observed in SLE patients [63, 64]. Additionally, strains with increased TLR7 expression have reduced splenic marginal zone cells and an expansion of T follicular helper cells and myeloid cells [52–54, 56, 59]. Aged mice develop GN and show increased leucocyte infiltration into the kidney, particularly $CD11b^{+}$ myeloid cells [53–56, 59]. TLR7-associated strains have rarely been used to assess drug targets, possibly because of their male gender bias, which is unlike human disease. However, they have provided important insight into the immune mechanisms driving end-organ pathology.

Human relevance

The *Bxsb3* locus overlaps with *Sle1* and *Nba2* and has been associated with autoantibody production and GN [61, 62]. The genes in this region that might be of relevance in human SLE are *FcγgRII* and *Ifi202* [65]. Human translocations from the X to Y chromosome have not been found; nonetheless, SLE is more prevalent in men who have an additional X chromosome [66]. Increased *TLR7* gene copies and two single nucleotide polymorphisms, rs179008 and rs3853839, have been associated with SLE in different ethnicities [67, 68]. Signalling pathways downstream of TLR7 can also be affected, as exemplified by *IRF5*, which is strongly associated with SLE susceptibility [69, 70]. Elevated *TLR7* expression appears to be a common feature of peripheral blood mononuclear cells from SLE patients; it can correlate with IFNα expression and can be induced by IFNα itself in several immune cells [71]. Immunological studies of human SLE have also shown a role of TLR7 in neutrophil extracellular trap cell death and generation of anti-snRNP antibodies [72, 73].

IFN dependency

The IFN signature has been observed in the kidneys of BXSB mice [74]. Treatment with an anti-IFNAR antibody was effective, particularly if started at early stages [20]. Thus, BXSB might represent a model for elucidating the role of type I IFN in the early stages of the disease.

IFNα-dependent/driven mouse models

A strong IFN signature in the peripheral blood can be induced by pristane and other hydrocarbons in non-autoimmune-prone mice, such as BALB/c, C57BL/6 and DBA/1 [75]. The disease in these mice is TLR7 and IFN dependent and characterized by GN, ANAs, anti-dsDNA antibodies, anti-snRNP antibodies and arthritis [75].

Additionally, IFNα can be used to induce rapid-onset, severe lupus in spontaneous models, such as NZB/W, B6.*Sle123* and NZM2328. These mice represent a reliable but stringent model to evaluate new drugs, because they do not respond well to standard therapies [76].

Evaluation of standard-of-care SLE therapeutics in mouse models

To date, only a handful of drugs have been approved for the treatment of SLE. In the 1950s, the US Food and Drug Administration approved aspirin, CSs and the antimalarial drug HCQ as non-specific treatments for SLE. These drugs were not approved following clinical trials, but based on clinical experience and eminence-based intuition. It took nearly 60 years of research before the approval of the next therapeutic and first targeted biologic, belimumab, in 2011 [77]. Additionally, several off-label agents were introduced to SLE therapy, predominantly systemic immunosuppressants such as CYC, MTX and MMF [77]. Despite being the pillars of SLE treatment [78], their mechanisms of action are not completely understood. NZB/W and MRL/*lpr* models have been extensively used to study these mechanisms and to evaluate side effects, dosage regimens and response to treatment, especially the ability to delay or prevent renal disease [5].

CYC

CYC is widely used as a chemotherapeutic and immunosuppressant with remarkable immunodepletive properties [79]. The first murine lupus studies involving CYC began in the late 1960s using the NZB/W mouse model. In these studies, CYC decreased autoantibody production and repressed the progression of LN without reversing the existing abnormalities [80, 81]. Protection from severe GN was achieved with long-term high-dose CYC and correlated with decreased anti-DNA antibody levels [82]. Short courses of intermittent pulse CYC did not achieve sustained immunosuppression [83, 84]. The efficacy of CYC has been confirmed in the MRL/*lpr* model; it prolonged survival, decreased arthritis and nephritis, reduced adenopathy and splenomegaly, reduced antibody levels and normalized T and B cells [85, 86].

Immunological studies on NZB/W mice have shown that CYC therapy depletes dividing, short-lived plasmablasts, but does not delete long-lived plasma cells efficiently [87]. These persist in survival niches, which are provided by the bone marrow and inflamed tissues, and might explain the resistance to immunosuppressive therapy observed in both mice and humans [88–90]. Fifty per cent of SLE patients show persistent active nephritis despite the therapy showing apparent clinical response [91].

Long-term CYC therapy is more efficient; however, it is associated with more side effects. Prolonged administration of CYC, especially high doses, increases the

incidence of neoplasms in NZB/W mice [92, 93]. Long-term CYC (>1 year) in humans is also carcinogenic, causing most frequently bladder cancer, secondary acute leukaemia and skin cancer [79]. Owing to its toxicity, CYC is commonly used at a lower dose in combination with other drugs. In the 1970s, Steinberg *et al.* went on to study the combination of CYC, AZA and methylprednisolone (Mp) in NZB/W mice [94]. Prevention of GN was achieved either by intermittent high doses of CYC or by daily low doses of a combination of CYC, AZA and Mp. These treatments were beneficial only when started early or in older mice with mild renal disease [94]. These studies provided the basis for human trials, which concluded that the combination of an immunosuppressant and low-dose prednisone was superior to treatment with a high dose of CSs alone at preserving renal function [95, 96]. However, for most of the treatment regimens, the effect was evident only after 5–7 years from the initiation of the trial [95].

Methylprednisolone, prednisolone and prednisone

CSs have been extensively used to suppress inflammation in a variety of immune-mediated diseases [97]. CS therapy, especially high dose and long term, is associated with many complications, including weight gain, hypertension, atherosclerosis, diabetes, peptic ulcer, skin atrophy, acne vulgaris and increased risk for infections [97]. Despite their many side effects and unclear mechanism of action, CSs (in combination with HCQ, CYC, MTX or MMF) remain the preferred induction therapy for almost all clinical presentations of lupus [78]. CSs in combined regimens have been extensively studied in murine lupus; however, very few studies have addressed the effects and mechanism of action of steroids alone. Mp administered to NZB/W mice at the onset of nephritis preserved the glomerular structure and function by decreasing the amount of IgG, IgM and C3 deposits. This was associated with lower plasma concentration of IgG, but not of anti-DNA antibodies, C3 and C1q-reactive materials [98]. Mp also decreased proteinuria by preserving glomerular permeability and improved survival [99]. CSs might preserve renal function through the inhibition of nuclear factor-κB (NF-κB) activity, which has been implied in LN pathogenesis [100, 101]. Mp has been shown to inhibit the lipopolysaccharide-induced activation of NF-κB in the kidneys of MRL/*lpr* mice [102]. Additionally, prednisolone inhibited the expression of many NF-κB-inducible genes in the glomeruli of MRL/*lpr* mice, such as adhesion molecules, chemokines and their receptors and proteins involved in antigen presentation [103, 104]. Both prednisolone and methylprednisolone attenuated the expression of extracellular matrix components in the kidneys of MRL/*lpr* and NZB/W mice, respectively [103, 105].

MMF

MMF is an immunosuppressive drug used to reduce acute and chronic transplant rejection. In SLE, it is most commonly used together with CSs in the induction therapy of LN [78]. When administered to NZB/W mice, MMF (60 and 200 mg/kg/day) reduced proteinuria, albuminuria and blood urea nitrogen concentrations, decreased autoantibody production and prolonged survival [106, 107]. Low doses of MMF (30 mg/kg/day) were effective in diminishing glomerular lesions and promoted qualitative changes in autoantibody production, lowering specifically IgG2a antibodies, but did not affect serum IgG or anti-dsDNA antibody levels [108]. Likewise, in MRL/*lpr* mice, 90 mg/kg/day MMF efficiently reduced albuminuria and GN and caused less immunoglobulin and C3 deposition in the glomeruli, but did not diminish antibody formation [109]. However, when MMF was given at 100 mg/kg/day, it reduced serum levels of antibodies in both NZB/W and MRL/*lpr* mice [108, 110]. MMF at 300 mg/kg/day also effectively abrogated LN development in the IFNα-accelerated NZB/W model and led to a decrease in the number of antibody-secreting cells in the spleen, but did not affect serum levels of anti-dsDNA antibody [111].

In the MRL/*lpr* renal cortex, MMF inhibits expression of inducible nitric oxide synthase, at least in the initial stages of disease [112, 113]. In contrast with CSs, it does not affect the NF-κB pathway [112, 114]. In the kidneys of NZB/W mice, MMF has been found to reduce the activation of protein kinase C, reduce fibronectin expression and reduce the expression of urokinase receptor in podocytes [115, 116].

Antimalarial agents

Use of the antimalarial agents quinacrine, chloroquine (CQ) and HCQ in SLE has recently been extensively reviewed [117, 118]. Their principal modes of action are thought to be interference with lysosomal acidification and inhibition of TLR7/9 through binding of nucleic acids [118].

Two studies have assessed HCQ for the treatment of lupus-associated endothelial dysfunction in NZB/W mice. Long-term treatment with 10 mg/kg/day HCQ by oral gavage resulted in reduced hypertension, reduced endothelial dysfunction and less damage of the heart and kidneys, without altering anti-dsDNA antibody levels [119]. Early HCQ treatment with a lower dose (3 mg/kg/day) also reduced endothelial dysfunction, but did not decrease the degree of nephritis [21]. These effects have been attributed to anti-oxidative properties of HCQ, but further studies are needed to elucidate the exact mechanism. The potential for HCQ to treat lupus-related skin lesions has been evaluated in the MRL/*lpr* model, showing efficacy and safety [120].

Mouse models for the screening of targeted therapeutics

B cell-targeted therapies

B cells produce antibodies (including autoantibodies), cytokines and chemokines and are crucial for normal humoral immune function. They represent an obvious and valid drug target in autoimmune diseases, as recently proved by the approval of belimumab for SLE treatment [121].

Belimumab

Belimumab is a human monoclonal antibody that binds and neutralizes the B cell-activating factor, B-lymphocyte stimulator (BLyS, commonly known as BAFF). BAFF and its homologue APRIL (a proliferation-inducing ligand) bind to receptors that are expressed on B cells at different stages of maturation [122].

Mouse experiments played an indispensable part in elucidating the role of BAFF and establishing it as an effective target for SLE treatment [121, 122]. Administration of recombinant BAFF to non-lupus mice results in elevated immunoglobulin production [123]. BAFF-transgenic (BAFF-Tg) mice develop SLE-like manifestations, and introduction of a BAFF transgene into autoimmune-prone B6.*Sle1* or B6.*Nba2* mice accelerates the development of GN [124, 125]. BAFF inhibition reduces nephritis and prolongs survival in multiple mouse models: NZM2410, NZB/W and BXSB [126–128]. Additionally, genetic ablation of BAFF prevents IFNα-dependent acceleration of GN, suggesting that therapeutic targeting of BAFF could be successful in patients with an IFNα signature [129].

Mouse models are also being used to elucidate the exact mechanism of action of belimumab. Older studies suggested that the blockade of BAFF would delete strongly self-reactive B cells and thus limit the autoimmune response [130]. However, new data suggest that BAFF blockade prevents signalling through its receptor transmembrane activator, calcium modulator, and cyclophilin ligand interactor, resulting in complete protection from autoantibody production without an extensive reduction in the number of B cells [131]. This could partly explain the controversial efficacy of B cell-depleting treatments, such as rituximab (anti-CD20 monoclonal antibody) [132].

Anti-CD20 B cell-depleting therapies

In humanized MRL/*lpr* mice, which express hCD20, anti-CD20 therapy effectively reduces B cell numbers and leads to amelioration of the disease, albeit only when used at high doses and for a prolonged time [133]. Unusually high doses (1–10 mg per mouse) have been used because of resistance to depletion that was later attributed to impaired IgG-mediated phagocytosis [134]. NZB/W mice also display less effective B cell depletion compared with C57BL/6 mice that increases with age and disease severity [135]. Nonetheless, a short course (4 weeks) of anti-CD20 therapy (10 mg/kg) in young NZB/W mice delayed the onset of the disease and delayed the development of nephritis in mice with advanced disease without decreasing anti-dsDNA antibodies [135]. Reduction of autoantibodies and prevention of renal injury and hypertension were achieved only after long-term B cell depletion [136].

Anti-CD20 B cell depletion can be further enhanced by concomitant BAFF blockade, leading to reduction of autoantibodies, reduced kidney disease and prolonged survival [135, 137]. Dual anti-CD20 and anti-BAFF therapy has also been effective in the IFNα- or pristane-accelerated NZB/W mice, particularly in attenuating renal injury [137].

Despite the promising results of preclinical trials, especially in NZB/W mice, clinical trials with the anti-CD20 mAb rituximab did not meet the chosen end points [132]. However, the cumulative data from randomized control trials, open clinical trials and cohort studies have shown that rituximab is a safe and effective treatment for non-renal manifestations of SLE, such as arthritis and thrombocytopaenia, and is currently being used off-label [132, 138]. Based on mouse data, the dual anti-BAFF/anti-CD20 therapy might be useful to treat renal manifestations of SLE, especially in the context of IFN signature.

T cell CTLA-4 co-stimulation blockade

Cytotoxic T-lymphocyte antigen 4 (CTLA-4 or CD152) is an inhibitory protein on the surface of T cells that binds CD80/CD86 on antigen-presenting cells. By inhibiting CD28-mediated T cell activation, CTLA-4 plays a role in regulation of central tolerance and prevention of self-reactivity [139]. The same effect is mediated by the recombinant fusion protein CTLA-4-Ig (abatacept). Abatacept has been approved for the treatment of RA and has shown promising results in murine LN; however, it failed in human SLE trials [139, 140].

Sustained CTLA-4-Ig treatment increases survival, dampens autoantibody production and reduces kidney disease in BXSB and NZB/W mice, whereas a short-course therapy only delays disease [141–143]. Long-term treatment regimens are not likely to be used in clinical practice, thus short-term CTLA-4-Ig in combination with other immunosupressive reagents (anti-CD40L, CYC) has been evaluated in NZB/W mice, showing promising results [142, 144]. Importantly, the combination of CTLA-4-Ig with CYC reversed proteinuria in the majority of mice with advanced renal disease and precluded the need for continuous administration of CYC [144, 145].

Based on positive outcomes in mouse studies, the addition of abatacept to the CYC/AZA regimen has been evaluated in the ACCESS trial for the treatment of proliferative LN. Disappointingly, the randomized double-blinded study did not show any improvement [146]. As in the case of rituximab, the failure of abatacept trials has been partly attributed to clinical trial design, and *post hoc* analyses have shown potential benefit in patients with active arthritis and LN [140, 147]. The IFN signature in patients might also be considered, because IFNα renders NZB/W F_1 mice relatively resistant to CYC/CTLA-4-Ig therapy [148]. Currently, the ALLURE trial is assessing the potential of abatacept for the treatment of advanced LN with CSs and MMF as background therapy [146].

IFNα- and TLR-targeted therapies

A prevalent IFN signature in the peripheral blood is present in > 80% lupus patients [8]. In BXSB and NZB/W models with a weak IFN signature, the blockade of the IFNα pathway either by mAb or by immunization with an IFNα kinoid ameliorated disease [20, 149]. A human IFNα kinoid has successfully reduced the IFNα signature in SLE patients [150]. The efficacy of anti-IFNα (rontalizumab,

sifalimumab) or anti-IFNAR1 (anifrolumab) antibodies showed variable results depending on the patient IFN signature [151–153]. It has to be noted that the assessment of the IFN signature varies greatly between these studies, and a standardized panel of genes should be used, ideally involving IFNα-, IFNβ- and IFNγ-related modules, as recently described [8]. It also remains to be determined whether a high IFN signature in patients makes them more resistant to treatment as has been observed in NZB/W mice following IFNα exposure [148].

In one study, the IFN signature could be normalized transiently only with aggressive high-dose Mp pulse therapy, but not by oral CSs [154]. In NZB/W and TLR7.Tg7.6 mice, this unresponsiveness was attributable to TLR7/9-mediated chronic activation. A TLR7/9 antagonist (IRS954) enhanced the sensitivity to CSs and could be potentially useful as a CS-sparing drug [154]. Additional mouse studies support TLR7/8/9 targeting as an important therapeutic venue in SLE [7, 71]. Single TLR7 antagonist (IRS661), dual TLR7/9 (IRS954) or triple TLR7/8/9 antagonists (IMO-8400, IMO-9200 and CPG 52364) have all shown efficacy in MRL/*lpr* and NZB/W models [155–159]. Despite the promising results in murine models, TLR antagonist development for treating SLE patients has somewhat halted at preclinical or early clinical phases [160]. DV1179, a dual TLR7/9 antagonist developed by Dynavax, did not reduce IFN-regulated genes in SLE patients in a Phase 1b/2a study [161]. There might be several reasons for these failures, including TLR antagonists being useful only for a specific subpopulation of SLE patients [71].

Summary statement

Mouse models of lupus are an indispensable tool for the study of lupus pathogenesis, especially the pathways involved in loss of tolerance, autoantibody production and progression to end-organ disease, such as GN. In the last two decades, these studies have provided many new target molecules and triggered a revival in lupus drug development. The same models are also used in preclinical studies of new drugs to assess safety, dosage and efficacy. Despite the promising results obtained in mouse studies, the majority of the novel drugs, with the exception of belimumab and IFNα-blocking agents, failed in clinical trials. This has prompted the US Food and Drug Administration to release special guidelines for SLE drug development [2, 77]. Selection of the patient population that is most likely to respond to treatment, the number of recruited patients, defining the outcomes of treatment, establishing the appropriate duration of the trial and the contribution of existing treatment are the main challenges of SLE trial design [2, 77, 146]. Better study design with a larger number of patients and less stringent end-point measures has probably contributed to a successful outcome of belimumab and anti-IFNα trials [147]. The failure of certain therapeutics in clinical trials could also be attributed to lack of efficiency, simply because the biology is not completely understood. There are also obvious differences between the human and mouse immune system, with lymphocyte frequencies being the most obvious one [162]. Lastly, laboratory mice are housed in relatively germ-free conditions, whereas humans are constantly being exposed to pathogens that activate the immune system in various ways, including by TLR engagement and IFN signalling. The involvement of the microbiome in the development of autoimmunity is currently a hot topic of investigation [163].

To summarize, mouse models of lupus will continue to be indispensable in multiple aspects of SLE research. Novel drug targets should be assessed across multiple spontaneous SLE models and, ideally, also in an IFNα-dependent or accelerated model if the impact of the IFN signature is expected. Improved understanding of the biology and better clinical trial design will be likely to generate more success stories similar to belimumab and anti-IFN treatment.

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