Chapter

A Spontaneous Mouse Model of Lupus: Physiology and Therapy

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Abstract

Spontaneous models of lupus were recognized four decades ago beginning in the early 1960s with the NZB/NZW F1 (NZB/W F1) mouse, an F1 hybrid between the New Zealand Black (NZB) and New Zealand White (NZW) mice. Although the parental strains display limited autoimmunity, the NZB/W F1 develops severe lupus-like features similar to that of human lupus patients. Here, we will address the genetic characteristics of the model and discuss its main characteristics such as the presence of serum antinuclear autoantibodies (ANA) including anti-dsDNA, mild vasculitis, and the development of immune complex-mediated glomerulonephritis. Similar to human lupus, the disease develops primarily in female mice after six months of age, with a lesser percentage and severity in male mice. The relation of this phenomenon will be examined in the context of estrogen levels. The participation of both innate and adaptive immunity will be addressed as well as the contribution of both T and B cells in the development of the clinical aspects of the disease. We will focus on the use of the model as a valuable tool for elucidating the pathogenic mechanisms of the disease, as well as its use as preclinical testing of therapeutic for human use.

Keywords: lupus, mouse model, histopathology, autoreactive cells and antibodies, genetics, sex

1. Introduction

Autoimmune diseases are generally defined by the existence of autoantibodies and the presence of autoreactive T and B lymphocytes. More than 80 different autoimmune disorders have been described, including systemic lupus erythematosus (SLE). Animal models of human diseases are an invaluable tool for defining pathogenic mechanisms, finding novel therapeutic targets, and testing new therapies. These models have the advantage of having a shorter lifetime, a characteristic that allows to study the full cycle of the disease and to test for the possible therapies in much shorter period. Although using animal models may have some disadvantages due to the obvious genetic and physiological differences with humans, they have been an invaluable tool to study human diseases, especially in autoimmunity. Although the exact etiology of SLE has not yet been identified, there is a consensus that numerous factors such as genetics, environment, and hormonal aspects are involved in the development of this disease. Several mouse models resemble specific elements of the human disease and have been employed to understand the cellular and genetic treats linked to SLE susceptibility. Most of them, share in common, the development of glomerulonephritis and

Spontaneous models of lupus					
	Generation	**Autoantibodies**	**Sex Bias**	**Main clinical manifestation**	**Age for 50% Mortality (months)**
NZB/NZWF1	New Zealand Black crossed with the New Zealand White mouse	Anti-dsDNA , anticardiolipin, rheumatoid factors, cryoglobulin, gp70, RNA polymerase I, RNA, ubiquitin, helicase.	Develops primarily in females with lesser percentage and severity in male mice.	Splenomegaly, mild lymphadenopathy, hypergammaglobulinemia, glomerulonephritis, mild vasculitis and mild leukopenia	Female: 9 months of age Male: 15 months of age
MRL/lpr	Intercross of four different strains of mice: LG/J, AKR/J, C3H/HeDi, and C57BL/6.	Anti-dsDNA , anticardiolipin, rheumatoid factors, cryoglobulin, gp70, albumin, transferrin, La, Ro, Su, ribosome P, Sm, S10 RNA polymerase I, laminin, collagen, ubiquitin, mitochondria, circulating immune complexes.	More prevalent and accelerated in females, but not as prominent as in B/W.	Splenomegaly, lymphadenopathy, arthritis, cerebritis (Cognitive dysfunction), skin rash, vasculitis and nephritis	6 months of age
BXSB	Backcross between a C57BL/6 (B6) female and a satin beige (SB/Le) male	anti-dsDNA , cryoglobulin, gp70 albumin, transferrin	Only occurs in male mice	Glomerulonephritisis	5 months of age
Induced/accelerated models of lupus					
Pristane-induced	Intraperitoneally injection of pristane into normal mouse strain like BALB/c mice	Anti-RNP, anti-DNA, Anti-Sm, anti-ribosomal P and anti-histone.	More severe in females than in males, at least in SJL strain.	Glomerulonephritis, arthritis, anemia and serositis (strain dependent).	
Graft-versus-host (GVH) disease	Injections of donor lymphocytes into a semi-allogenic recipient	AutoAb	Female	Immune complex nephritis.	

Table 1.
Main mouse models used to study lupus.

autoantibodies against autoantigens. In **Table 1**, we summarize the principal characteristics of the most extensively studied mouse strains of both spontaneous and induced murine lupus models. Additionally, there are genetically modified mouse models in which researchers inactivate, express, or overexpress a gene product or protein to recognize their single role in lupus and immunity in general such as transgenic-induced lupus and gene knockout-induced lupus [1–3]. In this chapter, we will refer in detail to the NZB/W F1 mice, which are the oldest classic spontaneous models of lupus used to study, on the one hand, the numerous susceptibility loci from which several candidate genes have emerged. Also, it has allowed to address important issues such as physiological aspects of the disease, antibody specificities, the role of antigen-presenting cells, the participation of B and T lymphocytes, and drug responses in many preclinical studies. This model was generated by the cross between the NZB and NZW strains. Both NZB and NZW display limited autoimmunity, as will be discussed here, while the NZB/W F1 hybrids develop severe lupus-like phenotypes resembling that of lupus patients. The purpose of this chapter is to summarize the contributions and significant advances in the understanding of lupus pathogenesis by the use of the NZB/W F1 murine model.

2. Histopathology characteristics of NZB/W F1 mice

In pre-autoimmune NZB/W F1 mice, *in vivo* expression of IFN-α precipitates the autoimmune process and kidney damage, leading to premature death from severe immune complex glomerulonephritis. This fact does not happen in non-autoimmune BALB/c mice. These findings support the notion that sustained IFN-α production in susceptible individuals may be sufficient to generate all the characteristics of SLE [4]. Interestingly, Liu et al. demonstrated that IFN-α accelerates murine systemic lupus erythematosus in NZB/W mice in a T cell-dependent manner [5].

The major cause of death in the NZB/W F1 female is chronic glomerulonephritis with heavy mesangial deposits before 5 months of age, tubular cast formation, proliferation of glomerular cells, prominent crescent formation, and a significant periglomerular and interstitial monocytic infiltrate. Extraglomerular renal deposits of IgG2a and C3 are present in the peritubular tissue and arterioles, and increase in frequency with age.

Diseased mice develop splenomegaly and progressive thymic cortical atrophy that begins very early in the disease and results in nearly complete loss of the thymic cortex as the disease progresses. In many mice, the loss of cortex is accompanied by medullary atrophy. Additionally, females have lymphoid hyperplasia with nodes rarely exceeding 2–3 times the average size [6].

3. Serologic characteristics of NZB/W F1 mice

Interestingly very early, it was reported that repeated administration of dsDNA or ssDNA to NZB/W F1 mice has a tolerogenic and long-lasting effect in this strain of mice that otherwise are susceptible to developing lupus [7]. Autoimmune-prone NZB mice mainly produce anti-DNA antibodies IgM and develop a mild SLE. NZB/W F1 females develop a fulminant SLE at 6–9 months associated with a decrease in IgM and an increase in anti-DNA IgG antibodies. These results helped to elucidate the role of the H-2 complex in the anti-DNA antibody production, leading to the conclusion that the production of IgG anti-DNA antibodies observed in NZB/W F1 hybrid mice is restricted to the H-2d/H-2z heterozygous mice [8].

NZB/W F1 mice present high levels of circulating autoantibodies. Antibody-secreting cells (ASCs) from these mice produce antinuclear antibody (ANA) and anti-dsDNA predominantly, the majority of them being the IgG2a and IgG3 classes [3, 5, 9]. NZB/W F1 mice also produce other extractable nuclear antigens (ENA) autoantibodies such as anti-small nuclear ribonucleoprotein (snRNP) and anti-heterogeneous nuclear ribonucleoproteins (hnRNP) [10]. All these autoantibodies form immune complexes that are deposited in different organs like liver, kidney, and skin. Moreover, Brick et al. have described the presence of anti-histone antibodies in the serum of autoimmune NZB/NZW F1 mice and in MRL/lpr mice [11]. On the other hand, dietary fat affects antibody levels to lipids and cardiolipin in autoimmune-prone NZB/W F1 mice. Antibodies to cardiolipin have been reported to play an important role in thrombus formation and an increase in the rate of abortions, both in human lupus patients and in murine lupus [12].

CD5+ B-1 cells have attracted much attention, because of their involvement in both autoimmunity and B cell-type chronic lymphocytic leukemia (B-CLL). It has been demonstrated that elimination of B-1 cells prevents autoimmune symptoms in autoimmune-prone mice [13]. CD5+ B cells seem to be the precursors of CD5- anti-DNA IgG antibody-producing B cells in autoimmune-prone NZB/W F1 mice [14]. However, whether B-1 cells in the peritoneum are generally involved in the pathogenesis of the autoimmune disease remains controversial.

4. Cellular abnormalities

Systemic lupus erythematosus (SLE) produces alterations in the organism that affect cells of the innate and adaptive immune systems. In this section, we will

summarize the modifications described in diseased NZB/W F1 mice in different immune cell populations.

4.1 Dendritic cells

Dendritic cells (DCs) are the cellular sentinels of the organism, important orchestrators of immune responses, and key components in fine-tuning the balance between tolerance and immunity.

Two major subsets of DCs are described: conventional DCs (cDCs) and plasmacytoid DCs (pDCs), although other subsets of DCs have been described from DCs generated from bone marrow cultures [15]. Tissue-derived pDCs are considered to be the major IFN-α source in SLE; however, diseased NZB/W F1 mice show an increase in the frequency and absolute numbers of both cDCs and pDCs in spleen and blood compared to healthy mice. Also, compared to healthy mice, diseased mice present alterations in both types of DCs since they display an abnormal phenotype characterized by an overexpression of the co-stimulatory molecules CD80, CD86, PD-L1, and PD-L2. Homing experiments demonstrate that DCs from lupus-diseased mice migrate preferentially to the spleen compared to DCs from control mice. This preferential recruitment and retention of DCs in the spleen are related to altered expression of different chemokine and chemokine receptors on both DCs and spleen stromal cells [16]. Recently, pDCs from spleen and bone marrow have been compared in several models of lupus-prone mice without clear results concerning the role of pDC in the development of lupus [17].

In NZB/W F1 mice, the spleen is the principal organ, where nucleosome-specific T cells are stimulated. Splenic antigen-presenting cells, including macrophages, contribute significantly to the production of autoantibodies and in the development of the disease [18]. On the other hand, anti-apoptotic molecules such as Bcl-2 inhibitors selectively kill pDCs, but not cDCs, reducing IFN-α production [19].

4.2 Macrophages

Macrophages are professional antigen-presenting cells and play an essential role in the activation of the adaptative immune response. Macrophages usually eliminate circulating apoptotic bodies and pathogens. Macrophages from diseased NZB/W F1 lupus mice have reduced phagocytic capacity. The impaired ability of resident peritoneal macrophages from lupus-prone mice to engulf apoptotic cells has been demonstrated by *in vivo* and *in vitro* cell clearance assays [20, 21]. Some studies have shown defective Fc-mediated phagocytosis by peritoneal macrophages [22] making more autoantigens available that favor an autoimmune response. In this regard, it was shown that spleen F4/80^{high} macrophages could present autoantigen efficiently to T cells, thus giving help to autoantibody-producing B cells in lupus-prone mice [18].

F4/80^{high} macrophages reside in healthy kidneys. In NZB/W F1, there is an increasing number of macrophages during nephritis. However, these macrophages do not show a pro-inflammatory (M1) or anti-inflammatory (M2) phenotype upon cytokine stimulation. Instead, they acquired a mixed functional phenotype that resembles gut F4/80^{high} macrophages constitutively activated [23]. Macrophages from diseased NZB/W F1 mice differ in the expression of some inflammatory genes, chemokine receptors, and TLRs, which are consistent with their heterogeneity and variability in renal location, further supporting the idea that ineffective macrophage function may contribute to glomerulonephritis in NZB/W F1 mice.

Macrophages produce a broad array of cytokines that can affect the immune response. For example, macrophages from peritoneal cavity upon stimulation with

DNA secrete high amounts of IL-6 and TNF-α [24], two cytokines that participate in B cell proliferation and function. Very early, it was reported that IL-6 secretion by peritoneal and not by spleen macrophages have an active role in the production of anti-DNA autoantibodies in NZB/W F1 mice [25].

4.3 T cells

In the NZB/W F1 lupus mice, spleen $CD4^+$ T cells exhibit an activated phenotype characterized by high expression of PD-1, CD25, CD69 and increased secretion of IFN-γ and IL-10 [16, 26]. The primary function of T cells in lupus is to help B cells in the production of autoantibodies [27], thus, avoiding the interaction between T and B cells may decrease the signs of the disease. Treatment with an anti-CD4 monoclonal antibody dramatically reduced glomerular immunoglobulin, complemented deposition, and diminished lymphocytic infiltration and vasculitis in the kidneys [28]. CD28 blockade decreased the production of anti-ds DNA autoantibody, prevented the development of lupus nephritis, and prolonged animal survival [29].

Regulatory $CD4^+$ T cells (Tregs) are essential players in the maintenance of peripheral immune tolerance. Usually, Tregs suppress the activity of specific T helper (Th) cells, but in NZB/W F1 mice, a homeostatic state of imbalance between regulatory and effector T cells is produced due to a decrease of IL-2, an essential cytokine for the maintenance of Tregs [30]. On the other hand, the levels of the adipocytokine leptin are elevated in diseased mice and correlate with the production of autoantibodies and renal disease. Although leptin can promote effector T cell responses to self-antigens, it also inhibits Treg activity [31]. On the other hand, Likuni et al. demonstrated that Tregs could directly suppress B cells in NZB/W F1 lupus mice through cell-to-cell contact-mediated mechanisms, thus directly regulating auto-antibody-producing B cells, including those B cells that increase in number during active disease [32].

Follicular helper T cells are CD4+ T cells population that supports the activation and differentiation of previously class-switched B cells to long-lived antibody-secreting plasma cells. Recent reports show that follicular helper T cells contribute to the pathogenesis of lupus through the ICOS/ICOSL pathway in NZB/W F1 mice [33]. Also, the activation through the Ox40/Ox40L pathway increases the number of follicular helper T cells and promotes cellular and humoral autoimmune responses in NZB/W F1 mice [34]. Interestingly, Cortini et al. showed that, reciprocally, B cells support the follicular helper T cells development in NZB/W F1 mice through the OX40L expression on B cells [35].

Although $CD8^+$ T cells have not been directly implicated in SLE, sick NZB/W F1 mice show an impaired expansion of $CD8^+$ T cells, as well as the acquisition of memory, secretion of cytokine, and suppression of autoimmunity [36].

4.4 B cells

Participation of B cells in lupus implicates several of its cellular functions. Besides the secretion of autoantibody against a panoply of antigens, B cells contribute in other ways to the pathogenesis of lupus, including antigen presentation to T cells, follicular helper T cell differentiation, and cytokine secretion. Although the phenotype of resting B cells isolated from NZB/W F1, and non-autoimmune mice do not show significant differences, B cells from lupus mice are hyper-responsive to T cell-derived stimuli *in vitro.* T cell-derived cytokines and signals delivered through CD40 crosslinking induce higher levels of proliferation, IgM secretion, and enhanced expression of costimulatory molecules in NZB/W F1 B cells [37].

B cell-activating factor (BAFF) and a proliferation-inducing ligand (APRIL) play key roles in peripheral B cell survival, maturation, and differentiation. In NZB/W F1 mice, chronic activation of the immune system induced an increase in the levels of circulating BAFF and APRIL. The continuous activation of B cells and thus overexpression of BAFF and APRIL may contribute to an increase in the generation of autoreactive B cells and a thus furthering the development of autoimmune disease [38].

B cells activation by T cells leads to the differentiation of B cells into long-lived plasma cells. However, continuous activation in autoimmune NZB/W F1 mice also generates short-lived plasmablasts. The number of splenic antibody-secreting cells (ASC) increases in NZB/W F1 mice aged 1–5 months and stabilizes after this period. Less than 60% of the splenic auto-ASCs are short-lived plasmablasts, whereas 40% are non-dividing, long-lived plasma cells with a half-life of 6 months. Although anti-proliferative immunosuppressive therapy depleted short-lived plasmablasts, long-lived plasma cells survived and continued to produce autoantibodies [39]. Additionally, Cheng et al. demonstrated that autoantibodies from long-lived "memory" plasma cells of NZB/W F1 mice drive complex immune nephritis [40].

5. Genetic characteristics: susceptibility loci in NZB and NZW mice and in the NZB/W F1 hybrid

Several chromosomal regions containing genes affecting lupus susceptibility or resistance have been identified pointing that murine lupus is genetically complex and mediated by a combination of genes.

In NZB/W F1 hybrids, genetic interactions between alleles present in NZB and NZW are the causes of the severe systemic autoimmunity found in these mice, due to the generation of a phenotype that is absent in both parental strains.

To search for contributing loci in this model of SLE, investigators backcrossed NZB/W F1 mice to NZW, then used brother-sister mattings to generate 27 substrains, termed New Zealand mixed (NZM) mice [41]. Further analysis of these 27 substrains led to the selection of NZM2410 as a lupus model. Susceptibility to lupus in NZM2410 is predominantly due to genes localized to the telomeric region of chromosome 1 (Sle1), the middle of chromosome 4 (Sle2), and the centromeric segment of chromosome 7 (Sle3) [42]. To study the contribution of each of these loci to pathogenesis, congenic strain construction was performed by transferring each of these intervals from NZM2410 onto the B6 background. Phenotypic analysis of congenic mice revealed that each locus contributes a unique component phenotype to the disease [43]. Although the B6.Sle congenic strains express phenotypes relevant to autoimmunity, none develop severe pathology, indicating that individual genes are not sufficient to cause lupus. The co-expression of these three major loci is necessary and sufficient for the development of a fully penetrant disease. These studies demonstrated that susceptibility to lupus involves both genetic interactions and additive effects of individual genes.

Additionally, to the Sle susceptibility loci, other loci present on chromosomes 1, 4, 7, and 17 have been associated with susceptibility in multiple lupus-prone strains including the NZB/W F1 model, an indication that genes in these regions may be necessary for immune regulation and function.

5.1 Susceptibility loci for systemic lupus on chromosome 1: Sle1, Nba2, Lbw7, Sbw1, and Cgnz1

The congenic strain, B6.Sle1, develops autoantibodies against nuclear autoantigens and displays spontaneous T cell activation without developing

glomerulonephritis [44]. Fine mapping of the Sle1 locus determined that four loci within this congenic interval, termed Sle1a, Sle1b, Sle1c, and Sle1d, are implicated in the loss of tolerance to chromatin [45, 46].

Analyses of NZB congenic mice, (NZB X SM/J)F1 X NZB, revealed that the Nba2 lupus susceptibility locus is associated with hypergammaglobulinemia and the development of various autoantibodies, including anti-DNA, anti-chromatin, and anti-gp70 [47]. In these studies, mice congenic for the Nba2 locus did not develop significant renal disease on a B6 background but developed severe lupus nephritis when crossed with NZW mice [48], consistent with the need of multiple susceptibility genes for full expression of lupus.

The susceptibility loci, Sle1 and Nba2, overlap in the same region of chromosome 1, suggesting that some susceptibility genes may be shared among lupus-prone strains. Within The Nba2 and Sle1 genetic segment there are genes encoding for the inhibitor type IIFcγR (FcγR IIB) [49], members of the SLAM/CD2 family of immunomodulatory receptors (Cd244, Cd229, Cs1, Cd48, Cd150, Ly108, and Cd84) [45] and members of the IFN-inducible (Ifi) family [48] all of which can regulate cell proliferation and survival. Analysis of congenic strains demonstrated that the presence of nuclear antigens and the severity of renal disease are linked with the FcγR and SLAM gene clusters with little involvement from the Ifi interval [50].

The inhibitory receptor for IgG, FcγRIIB, appears to be a fundamental regulator of B cell as well as myeloid cell activation [51]. Deficiencies in these routes result in heightened humoral and inflammatory responses, further contributing to lupus pathology [52].

The complement receptor 2 (CR2) gene, which encodes the complement receptor type 2 that acts as a B cell co-receptor is also in the Sle1c interval [53].

Theofilopoulos and colleagues identified Sbw1 and Lbw7 in chromosome 1 during their original linkage analysis of (NZB X NZW) F2 progeny [54]. Sbw1 defines a locus associated with splenomegaly, while Lbw7 defines a locus associated with anti-chromatin autoantibodies. Lbw7 of NZW origin is likely to be identical to Nba2 from NZB [54]. Additionally, Cgnz1 was detected in lupus-prone NZM2338 mice and significantly linked to chronic glomerulonephritis, severe proteinuria, and early mortality in female mice [55].

5.2 Susceptibility loci for systemic lupus on chromosome 4: Sle2, Nba1, Sgp4, Lbw2, Sbw2, and Adnz1

The congenic strain, B6.Sle2, displays lowered B cell activation thresholds coincident with the appearance of polyclonal IgM in the sera and expansion of the B1a cell compartment, in the absence of glomerulonephritis [43]. Interestingly, combining this locus with Sle1, resulted in glomerulonephritis and enhanced mortality compared with the single congenic strains alone [56].

Another susceptibility locus present on chromosome 4 is the Nba1 locus from NZB and the Lbw2 susceptibility locus from NZB/W F1. Both are associated with kidney disease, while another locus, sbw2, is associated with splenomegaly. The Sbw2 locus mapped to the same region as Lbw2, suggesting a single locus with pleiotropic effects [54]. The Nba1/Lbw2 interval contains the C1qa gene encoding the first component of complement C1q. It has been shown that an insertion polymorphism in the NZB sequence upstream of C1q gene may be related to a limited degree of C1q production, which may confer a risk for lupus nephritis by reducing IC clearance and promoting IC deposition in the glomeruli [57].

Overlapping with the Nba1 locus, there is a locus designated Sgp4, which was linked to the production of nephritogenic gp70 antigens. Production of autoantibodies to the retroviral envelope glycoprotein gp70, and the generation of

gp70-anti-gp70 immune complexes (gp70 IC) have been implicated in the development of nephritis in these lupus models [58, 59].

An additional study using NZM2328 mice found that the NZB-derived locus Adnz1 also contributed to the production of anti-DNA autoantibodies but not to lupus nephritis [55].

5.3 Susceptibility loci for systemic lupus on chromosome 7: Sle3, Lbw5, Nba5, and Aia3

Chromosome 7 contains several susceptibility genes regulating nephritis and autoantibodies. Among them are the Sle3 and Lbw5 loci, both derived from the NZW strain and the Nba5 locus from the NBW strain. A candidate gene present in this region is Cd22, which functions as a negative regulator of BCR signaling transduction.

Sle3 appears to be responsible for the hyperactive and pro-inflammatory antigen-presenting capacity of dendritic cells and macrophages [60].

The Nba5 susceptibility locus was associated with higher titers of anti-gp70 autoantibodies [61], while Aia3 with autoimmune hemolytic autoimmunity in a linkage analysis of NZB [62].

5.4 Susceptibility loci for systemic lupus on chromosome 17: Lbw1 (MHC)

The contribution of MHC haplotype to disease was first reported in the NZB/NZW F1 model [63]. These genes are located in chromosome 17. Several studies demonstrated a strong association of H2d/z heterozygosity with the development of SLE, indicating a co-dominant contribution from each strain, H2d from NZB and H2z from NZW [64].

6. Influence of sex

Differences between female and male responses to foreign and self-antigens have been well-documented. It was suggested that genes and hormones are involved in the differences found in their innate and adaptive immune responses. Generally, females mount higher immune responses than males, which can contribute to the increased susceptibility to autoimmune diseases in females [65].

Similar to humans, within the NZB/W F1 mouse model lupus develops primarily in females with a lesser percentage and severity in male mice. In female mice, lupus signs appear after 6 months of age, with 50% mortality at 8.5 months and 90% mortality at 12.8 months. Male mice develop the disease after a year of age with 50% mortality at about 15 months of age [66]. Accordingly, early studies performed in NZB/W F1 mice showed that estrogen supplementation is associated with a worsening disease and shorter lifespan than untreated littermate. In contrast, supplementation of a female with the male sex hormone 5α-dihydrotestosterone reduce immune complex deposition and prolong survival despite the presence of high levels of IgG antibodies to DNA. Additionally, castrated or 17β-estradiol-treated NZB/W F1 male mice have an earlier onset of lupus and accelerated mortality, suggesting a suppressive effect of androgen [67, 68]. Data accumulated during the past few years provide evidence that female hormones, particularly estrogens, promote lupus pathogenesis. However, some opposite results are suggesting that sexual dichotomy is due to protective effects of androgens. The mortality induced by estrogens may be due to toxic effects rather than accelerated autoimmunity [69].

Cells of the immune system, including B cells, express the cellular receptors for estrogens, estrogen receptor-α (ERα), and estrogen receptor-β [70]. Global disruption of the ERα gene in NZB/W F1 causes a significant reduction in the concentration of anti-histone/DNA and anti-double-stranded DNA IgG antibodies, which are associated with glomerulonephritis. This loss of tolerance was observed in female mice whereas, more modest effects are seen in males [71] suggesting that the ability of ERα signaling to enhance autoantibody production and lupus pathogenesis is more pronounced in females than in males. Additionally, specific deletion of ERα in B cells retards the production of autoantibodies and the development of nephritis in NZB/W F1 mice, demonstrating that ERα acts in a B cell-intrinsic manner to control B cell activation, autoantibody production, and lupus nephritis [72].

B cells with the CD5 marker, which spontaneously produce IgM, are found in higher numbers in NZB mice and have been implicated in lupus [73]. Treatment of lupus-prone female NZB/W F1 mice with tamoxifen (TAM), a synthetic antiestrogen with high affinity for the estrogen receptor, decreases the percentage of B cells and CD5+ B cells in the spleen. Also, TAM-treated mice had less severe proteinuria and increased survival rate compared to controls [74].

On the other hand, it has been described that NZB/W F1 males have higher levels of a population of $Gr1^{high}$Ly-6G + CD11b + myeloid cells that protect them against lupus development [75]. This population is testosterone-regulated and suppresses autoantibody production *in vivo*. Additionally, Gr1+ cells from NZB/W F1 males suppress the differentiation and effector function of CXCR5+ PD-1+ T follicular helper cells, germinal center formation, and plasma cell differentiation [76].

Since sex hormones can bind transcription factors, they might affect autoimmunity via their effects on gene transcription. Accordingly, it has been demonstrated that estrogen upregulates the expression of IFN-γ through the ERα [71].

Additionally, the expression of interferon regulatory factor 5 (IRF5), a lupus susceptibility factor that controls the expression of type I IFNs, is higher in NZB/W F1 females than in males. IRF5 expression also depends on ERα expression, because of splenic cells from ERα knockout female express lower levels of IRF5 [77]. This suggests a (positive) feedback loop between the IFNs and estrogens since activation of type I IFNs or IFN-γ signaling upregulates the expression of ERα [78].

Other studies have provided evidence that lupus-associated miRNAs are differentially expressed in splenocytes of NZB/W F1 male and female mice. Additionally, these miRNAs were upregulated by estrogen treatment [79]. miRNAs regulate the expression, mainly at the post-transcriptional level, of some genes that are important in the development of the innate and adaptive immune system and the maintenance of immune homeostasis. Dysregulation of miRNAs impacts the function of different types of immune cells causing a breakdown of immune tolerance and ultimately the development of autoimmune-related disorders such as SLE [80].

7. Treatment of murine SLE

Different treatments to improve lupus have been evaluated in the NZB/W F1 murine model. In this section, we will review some well-documented procedures.

Interleukin-6 (IL-6) is a multifunctional cytokine synthetized by macrophages, monocytes, and B and T cells. IL-6 is critical for B cell differentiation and maturation, immunoglobulin secretion, cytotoxic T cell differentiation, acute-phase protein production, bone marrow progenitor stimulation, renal mesangial cell

proliferation, and macrophage/monocyte functions. Lupus mice treated with anti-IL-6 mAb reduce B cell proliferation, the ds-DNA antibodies production, and kidney damage [81]. Additionally, treatment with antibodies against the IL-6 receptor (IL6R-mAb) inhibits the production of anti-DNA and anti-TNP IgGs antibodies, and consequently, this treatment increases the survival of the mice [82]. Tocilizumab, an anti-IL6R-mAb commercialized mainly for the treatment of rheumatoid arthritis [83], has been evaluated in SLE patients. This procedure decreases anti-dsDNA antibody levels and circulating plasma cells and improves arthritis and medical scores [84].

Interleukin-10 (IL-10) is a cytokine produced by subsets of activated T cells and macrophages. It mediates a variety of both immunostimulatory and immunosuppressive properties. IL-10 neutralization with anti-IL-10 delays the onset of the disease, increasing survival from 10 to 80% in mice at 9 months. Autoimmunity protection by IL-10 antagonism appeared to be due to an upregulation of endogenous tumor necrosis factor alpha (TNF-α) [85].

TNF-α is a pleiotropic cytokine with immunostimulatory and proinflammatory activities. TNF-α stimulates T and B cell proliferation, immunoglobulin synthesis, enhances natural killer (NK) cell activity, and boosts neutrophil activation. The NZB/W F1 mice have reduced levels of TNF-α, and their treatment with recombinant TNF-α increased their survival [86]. Infliximab, a TNF-α blocking antibody, was evaluated in short- and long-term therapy in SLE patients showing several adverse effects in long-term therapy [87]. Infliximab and Etanercept are another TNF-α blockers commercialized mainly to treat rheumatoid arthritis [88, 89].

Type I interferons (IFN) are primarily regarded as inhibitors of viral replication. However, type I IFN, mainly IFN-α, plays a major role in activation of both the innate and adaptive immune system [90]. IFN-α signature precedes the onset of lupus in NZB/W F1 mice and in humans. Treatment with a vaccine that induces the secretion of anti-IFN-α neutralizing antibodies causes a delay in proteinuria development, low deposits of immune complexes, and increases survival [91]. Two antibodies against IFN-α, Sifalimumab and Rontalizumab, evaluated in SLE patients correlate with improvements in disease activity [92, 93].

BAFF is a B cell-activating factor essential for the survival of B cells. BAFF is produced predominantly by myeloid cells and binds to three distinct receptors on the B cell surface; the transmembrane activator and calcium modulator ligand interactor (TACI), the B cell maturation antigen (BCMA), and the BAFF receptor. Treatment with soluble TACI-Ig fusion protein inhibits the development of proteinuria and prolongs animal survival [94]. Besides, a short course of TACI-Ig and CTLA4-Ig induces a profound depletion of splenic B cells, prolong life, and even reverse proteinuria in aged NZB/W F1 mice [95]. Atacicept is a recombinant fusion protein that blocks activation of B cells by binding to TACI ligands. In SLE patients, the Atacicept treatment favors the reductions in disease activity and severe flares [96].

CD20 is a transmembrane phosphoprotein specifically expressed on B cells. Depletion of B cells with a monoclonal antibody against CD20 favors the survival of aged NZB/W F1 mice [97]. Rituximab, an anti-CD20 monoclonal antibody frequently used in SLE patients improves lupus nephritis, arthritis, serositis, cutaneous vasculitis, mucositis, rashes, fatigue, and neurologic symptoms [98]. Although rituximab's mechanisms of action are not known, its effects are likely mediated by antibody-dependent cell-mediated cytotoxicity and the induction of apoptosis on B cells [99].

Mammalian target of rapamycin (mTOR) is a protein kinase that regulates different cellular processes such as cell proliferation, growth, motility, cell survival,

protein synthesis, and transcription. NZB/W F1 mice treated with rapamycin (a drug used in rejection prophylaxis in solid organ transplantation) from 12 to 37 weeks of age inhibit the production of autoantibodies, development of proteinuria, and prolong mouse survival [100]. Moreover, in mice with established nephritis, rapamycin suppressed the interstitial infiltration of T cells, B cells, and macrophages [101].

Antigen presentation process involves costimulatory molecules CD28, and CTLA4 expressed on T cells, representing activation or inhibitory signals to T cells. CD28 and CTLA4 bind with medium or high affinity, respectively to B7, i.e., expressed on antigen-presenting cells (APCs) [102]. Abatacept is a fusion CTLA4-Ig protein that interrupts the interaction of B7 with CD28. NZB/W F1 mice that express murine CTLA4-Ig exhibit an improvement in all of lupus symptoms increasing survival [103]. In humans, Abatacept is mainly used in rheumatoid arthritis [104], although there are some SLE studies, one of them showing improvement in skin lesions in SLE patient [105].

Based on studies done in mouse models, most clinical trials have focused on agents that control B and T lymphocytes activations and functions. **Figure 1** shows some therapeutic targets investigated in mouse models of SLE (as described in [82, 85, 91, 95, 97, 103, 106–110]), many of which where then follow up in clinical trials [88, 89, 92, 98, 104, 111–118].

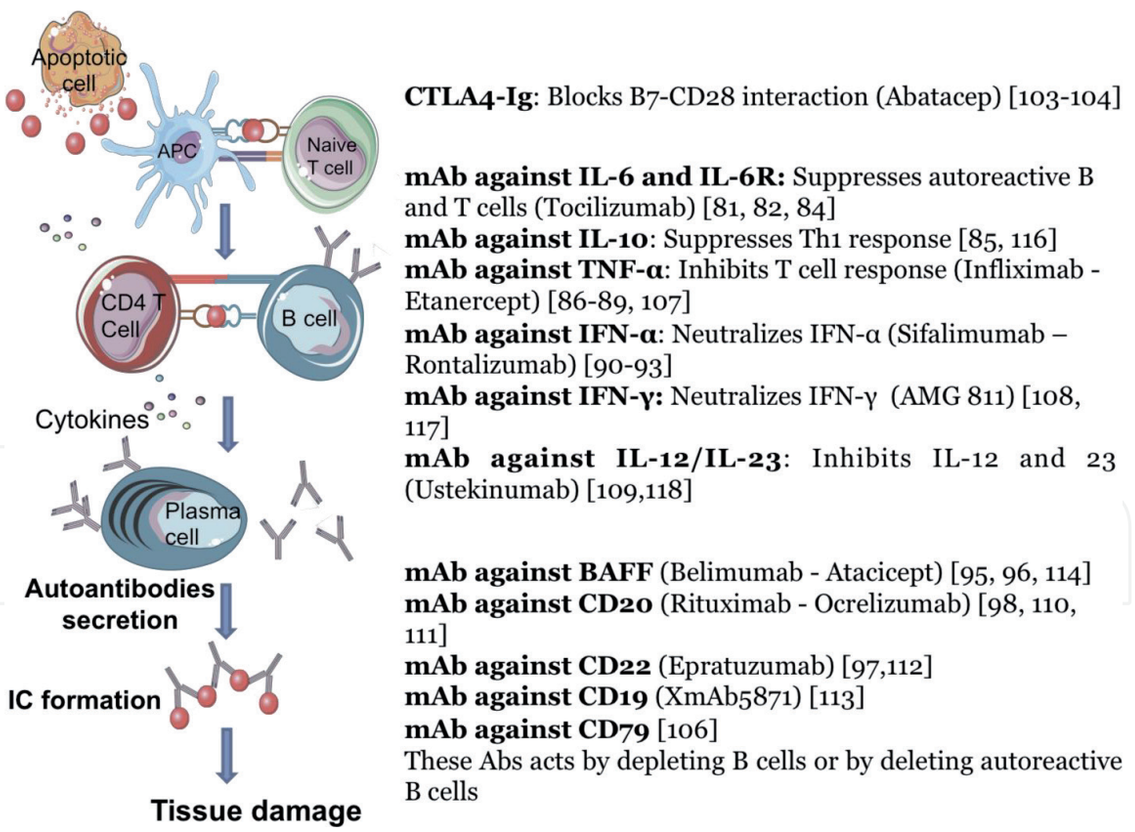

Figure 1.
Immune cells contribution to SLE and potential targets for lupus therapies, as tested in mouse models: Defects in phagocytosis of apoptotic cells leads to the presentation of autoantigens by APC to naive CD4 T cells. Activated T cells help the differentiation of B cell into plasma cells that secrete high levels of autoantibodies. These autoantibodies form immune complexes by binding to autoantigens, and engaging Fcγ receptors on different cell types. This supports inflammation and tissue destruction through the recruitment of inflammatory cells to tissues. APC: Antigen-presenting cell, IC: Immune complexes, mAb: monoclonal antibody. Texts on the right side of the figure show the different targets tested for lupus therapy. Drug names are shown in brackets

8. Conclusions

The spontaneous mouse model of lupus NZB/W F1 has been important to elucidate the pathogenesis of SLE. In this model, the lupus-like phenotypes include lymphadenopathy, splenomegaly, elevated serum antinuclear autoantibodies including anti-dsDNA IgG, and immune complex-mediated glomerulonephritis that are remarkably similar to the pathology described in human lupus. Consequently, it has provided a powerful tool to our knowledge on human lupus disease and the development of novel therapies. Additionally, similar to humans, lupus develops primarily in female NZB/W F1 mice with lesser percentage and severity in male. The female predominance of the disease remains poorly understood; however, hormonal contributions to immune system activation and X chromosome gene-dose effect have been proposed to be the important contributor to sex bias [66]. On the other hand, unlike SLE patients, NZB/W F1 mice do not manifest skin disease or arthritis [3].

Furthermore, human and murine lupus is characterized by a deregulation in autoreactive T helper cells, B and DC cells activation, and cytokine production. Defective function of regulatory T cells, inefficient clearance of immune complex and biological waste, nucleic acid sensing and IFN production pathways are also involved in the loss of tolerance and tissue damage associated to lupus [119]. The use of mouse models has allowed the study of the mechanisms involved in the cellular immune abnormalities, providing a powerful tool to identify novel pathways and targets for disease therapies. Several components of the immune system, such as cytokines, B cells, T cells, and hormones have been identified as potential targets for novel drugs. The side effects, dosage regimens, and response to treatment are first tested on murine models of lupus prior they go to clinical trials. Murine models of disease represent genetically homogeneous populations and in contrast to humans that take chronic doses of immunosuppressant, they allow for examination in the absence of any therap. Despite favorable results in mouse studies, many therapies have failed to meet clinical end points. This is probably because of the complexity of the disease, which involves the contribution of environmental and genetic susceptibility factors [119]. However, some of the therapeutic approaches have been successful recommended for SLE treatment, like Belimumab, a humanized monoclonal antibody directed against B cell activating factor. Additionally, other available agents such as rituximab, tacrolimus, azathioprine, methotrexate, cyclophosphamide, and mycophenolate mofetil are widely used off-label in SLE [9, 120].

The use of murine models has identified several novel candidate genes, and some of them have been associated to SLE in humans. An important contribution of the genetic studies in NZB/W F1 was the identification, in chromosome 1, of Sle1 and Nba2 loci, which are responsible for the production of autoantibodies. Sle1 and Nba2 encode members of the FcγR, SLAM, and IFN-inducible receptor families.

As sustained above, all the mouse models, and specifically the NZB/W F1, have the benefit of having a shorter evolution of the disease, allowing to investigate the full progression of the disorder and its pathophysiology and to test for possible therapies in a much shorter time period. In spite of their limitations and the fact that one cannot readily extrapolate to the human disease, mouse models of lupus have significantly helped researchers to advance our knowledge on this syndrome, adding relevant data on the pathogenesis of lupus and providing investigators with a valuable preclinical model for the design of future therapies. In spite of the various differences found between the human and mouse immune systems, there are sufficient similarities in the manifestation of the disease to be optimistic regarding

the use of this mouse model to further advance in our understanding of the physiology of the human disease and the formulation of creative new therapies.

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Conflict of interest

The authors declare no competing or financial interests.

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