The Role of Type I Interferons in TLR7-Mediated Lupus and UVB-Induced Immune Cell Responses

by

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Dedication

The wolf and fortune Family

My loving husband Quenten Fortune

All of my Friends

Abby the cat

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Abstract

Systemic Lupus Erythematosus (SLE) is an autoimmune disease that affects up to .02% of the US population and nearly 90% of patients are women. These SLE patients experience devastating organ damage mediated by autoantibody production, immune complex deposition, and elevated production of type I interferons (IFNs). SLE disease activity occurs in a series of flares that incrementally damage kidneys, skin, liver and other organs. Type I IFNs are purported to be important cytokines in the development of SLE, but their role in TLR7- mediated lupus development and UVB induced immune response has not been well understood. This thesis addresses two main questions:

1) What role do type I IFNs play in TLR7 –mediated lupus development.

To investigate the role of cutaneous TLR7 signaling and IFN production in lupus flare development, we used a lupus-prone murine model NZM 2328 (NZB/W derived congenic strain) and iNZM (knockout of the α-chain on the IFNα/β receptor). In order to induce lupus flare, mice were treated on the ear with R848 (TLR 7 agonist). Importantly, we show that both NZM and iNZM mice exhibit a decline in survival after 3 to 4 weeks with R848 but not vehicle treatment. This TLR 7 cutaneous stimulation resulted in development of splenomegaly and liver inflammation in a type I IFN-dependent manner. Interestingly, development of autoantibody production in the LN occurred in the absence of type I IFN signaling. Upregulation of IL-1β, *ccl2* and renal infiltration of dendritic cells also occurred in a type I IFN-independent manner. Though upregulation of IL-1β occurred, knockout of IL-1β in NZM mice did not show to be

protective. These data suggest induction of both type I IFN dependent and independent lupus phenotypes downstream of TLR 7 cutaneous stimulation.

2) What role do type I IFNs play in UVB-induced immune response in lupus-prone mice

In order to examine UVB responses, eight-to-ten-week-old female wild-type (BALB/c), lupus-prone (NZM2328) and iNZM mice (lack a functional type I IFN receptor on NZM2328 background) were treated on their dorsal skin with 100mJ/cm^2 of UVB for 5 consecutive days. We demonstrated elevated expression of type I IFNs in lupus vs. healthy skin following UVB exposure. Further, we show that UVB treatment led to skewed T cell activation in the dLN of lupus-prone mice through type I IFNs suppression of T regulatory cells. In addition, type I IFNs increased inflammation in lupus compared to healthy skin through regulating the recruitment of differential DC populations and inducing macrophage activation. We also identified that CD103$^+$CDllb$^-$ DCs migrate into the dLN of lupus-prone mice in a type I IFN dependent manner. These data suggest type I IFNs prime lupus skin for increased inflammatory response. Thus, we propose that type I IFNs are important for UVB-induced inflammation through regulation of the innate and adaptive immune response in lupus-prone mice and may be an effective target for prevention of UVB- induced cutaneous inflammation.

Chapter 1 Introduction

The section labeled "The impact of UVB' in SLE pathogenesis" in this chapter was published in *Frontiers in Immunology* at the time of defense[1].

1.1 Systemic Lupus Erythematosus

1.1.1 Epidemiology and clinical manifestations

Systemic Lupus Erythematosus (SLE) is a chronic autoimmune disease that is heterogeneous and multi-symptomatic, occurring in a series of flares in patients. Incidence worldwide ranges from 0.3 to 23.7 per 100,000 persons each year, and prevalence ranges from 6.5 to 178.0 per 100,000 persons[2]. Geographically, higher incidence of SLE is found in Asia .0031%, Australia .093%, and the Americas .02%[3, 4]. Complex interactions including gender and ethnic background contribute to the difference in incidence rate among SLE patients[2]. Peak incidence of SLE development is higher in women than men (in a ratio of 9:1 and can be as high as 15:1). This is in part contributed by hormone differences, as before puberty the female-to-male ratio is 3:1[5]. Ethnically, non-Caucasian groups tend to have a higher incidence than Caucasians, possibly due to differences in genetic and epigenetic factors[2].

SLE patients can present with general systemic manifestations including malaise, fever, myalgias, weight changes, and headache[6]. More specific features of patients involve arthritis, cutaneous inflammation, renal disease, central nervous system involvement, splenomegaly, liver inflammation, and increased risk for cardiovascular disease[6]. Development of musculoskeletal manifestations in SLE patients is common[7]; they can exhibit joint pain and progress to

development of arthritis, which affects between 69% to 95% of patients[8-10]. Unlike rheumatoid arthritis, SLE patients develop intermittent swelling with less damage occurring in the bone and cartilage[11].

Cutaneous inflammation, termed cutaneous lupus erythematosus (CLE), is another manifestation that affects between 70- 80% of all lupus patients and up to 25% of SLE patients will develop CLE prior to SLE onset[12]. CLE is heterogeneous and contains 3 subtypes: acute, subacute, and chronic, which is divided into 4 additional subsets [6, 12]. Acute CLE (aCLE) patients display the classic malar rash on the checks and nasal bridge; these patients can frequently progress to systemic disease development[12]. Subacute CLE (sCLE) patients develop psoriasiform lesions around the neck that display increased keratinocyte apoptosis[12]. It has been reported that up to 48.7% of these patients exhibit systemic disease[13, 14]. Discoid or Chronic CLE is one of the most common clinical manifestations of CLE. These patients have scarring lesion development on the face, the neck and scalp that exhibit lymphocyte and macrophage infiltration[12]. While it is not understood what immunological factors drive the progression of the different subtypes of disease and how CLE progresses to SLE , the majority of these patients are photosensitive; this is displayed as development of skin lesions following sun exposure[1].

Lupus nephritis (LN) is also a devastating manifestation of SLE and is associated with poor prognosis. The incidence of LN in SLE patients varies ethnically: Asian (55%), African (51%), Hispanic (43%), and Caucasian (14%). 25% of LN patients go on to develop end-stage renal disease 10 years after renal compromise[15-17]. Glomerulonephritis is the most common form of renal disease in SLE patients[18]. While several factors contribute to GN development, immune complex deposition triggers damage to the glomerulus through binding of Fc receptors and

initiating complement activation[17]. This damage results in the leakage of protein into the urine, termed proteinuria, and scarring of the kidney leading to end-stage renal disease[19].

1.1.2 Treatment

The complexity in progression and development of SLE has made it challenging to create new FDA approved therapies, though promising clinical trials are on the horizon[20, 21]. Currently, general corticosteroids and immune suppressants are being used to assist patients. Only one Biologic (belimumab) has been approved (in 2011) for treatment of active SLE patients; it works through blocking the biological activity of B-lymphocyte stimulator (BLyS) to reduce the production of autoantibodies and other B-cell related functions[22, 23]. A better understanding of the mechanism involved in the pathogenesis of lupus could lead to more specific therapies.

1.1.3 Factors contributing to Lupus pathogenesis

Genetic and epigenetic factors along with environmental exposure drive the immune system to promote lupus disease development.

Genetics

Genetics is one factor that contributes to disease susceptibility; this is demonstrated through the high incidence rate observed in identical twins (29%–57%) and in familial cases (10%–12%)[24, 25]. Previous genome-wide association studies have identified more than 60 risk loci for SLE susceptibility; these loci are associated with antigen presentation, T and B cell signaling, phagocytosis and cytokine, particularly type I interferon, signaling.

In regard to antigen presentation, major histocompatibility complex (MHC), alleles for both class I and class II are associated with increased risk for SLE. Particularly, class II alleles have been shown to be involved with lupus pathogenesis and contribute to ethnic disparities in

SLE[26]. Two alleles, HLA-DR2 and 3 positively correlate with lupus development[27, 28]. Both HLA-DR and DQ alleles contribute to pathogenesis in part through anti-Ro/SSA antibody production, which is present in 25-50% of SLE patients. On the other hand, DQA1 alleles have demonstrated association with the production of anti-dsDNA antibodies, which is another pathogenic antibody expressed in lupus[29]. Though MHC I haplotypes may be relevant to lupus susceptibility, their role is not well understood. Other deficiencies in early complement components 2, 4, and C1q are also associated with SLE susceptibility[30-33] through their effect on reduced clearance of apoptotic cells allowing for prolonged autoantigen exposure in lupus patients

Gene variants regulating immune cell activation, phagocytosis, and cytokine signaling within SLE patients have also been identified. BLK variant rs922483 and CSK variant rs34933034 mediate activation of mature B cells that may be autoreactive [34, 35]. T and B cells in lupus patients are also affected by a missense mutation of PTPN22, a tyrosine phosphatase protein that affects T and B cell signaling, resulting in increased autoantibody production and T cell activation[36-38]. Reduced phagocytosis by antigen presenting cells in lupus patients is associated with variation in ITGAM, which regulates phagocytosis of complement coated antigens[39, 40]. Gene variants associated with type I IFN signaling involving STAT4, IRF5, and IRF7 have also been implicated in increased risk[41-43]. Promotion of production of type I IFNs could possibly be due to polymorphisms in TLR 7 exhibited in lupus patients[44, 45].

Epigenetics

Differential epigenetic modifications are another factor influencing lupus development. Such modifications impact methylation of DNA and acetylation of histones, but do not alter the DNA sequence. Altered DNA methylation has been found most prominently in the T cell population of SLE patients. This is in part implicated by reduced expression of DNA methyltransferase enzymes (DNMTs), which add a methyl group to cytosine in GC dinucleotides, resulting in reduced gene expression or gene silencing[46]. T cells of SLE patients also exhibit altered transcription factor networks; in particular, increased cAMP responsive element (CREM) α promotes effector T cell generation[47-50].

Several cytokines including IL-4, IL-6, IL-10, IL-17 demonstrate reduced DNA methylation resulting in increased production of these cytokines[51-56]. Type I IFNs signaling are particularly affected in SLE patients by hypomethylation of interferon regulatory factor 7 (IRF7), a transcription regulator for type I IFN production[57]. This contributes to the increased interferon signature in SLE patients and displays effects on both innate and adaptive immune cells.

Histone modifications are another level of epigenetics implicated in SLE development. Histones are comprised of H2A, H2B, H3 and H4, which in complex with DNA constitute a nucleosome. The terminal amino acid of a histone can be acetylated, citrullinated, phosphorylated, or methylated. In SLE, histone H3K9 methylation is decreased in $CD4^+$ T cells, allowing for reduced silencing of genes affecting effector function[58]. T cells also exhibit modifications at the IL-17 gene cluster, contributing to increased expression of IL-17 by T_H17 and double negative cells[56, 59]. In lupus patients, expression of the IL-2 gene is reduced due to histone deacetylation and DNA methylation , resulting in promotion of effector T cells while possibly suppressing T regulatory (T_{reg}) cells[47, 60, 61]. This importance of IL-2 is confirmed *in vivo*

as treatment of SLE patients with low-dose IL-2 for 5 days promotes increased T_{Regs} [62]; this biology is currently being pursued as a treatment for SLE.

Cell populations

Increased presence of autoantigens in SLE patients promotes loss of tolerance and increased immune cell activation. Of the innate immune cells, neutrophils have been shown to also contribute to increased autoantigens[63, 64]. SLE patients' neutrophils include low-density granulocytes that show increased formation of neutrophil extracellular traps (NETosis), the extrusion of nuclear, cytoplasmic, and granular material[65]. NETs are not properly cleared in SLE patients due to low DNase activity as a result of anti-DNase antibody production and deficiencies in complement that lead to reduced opsonization [66-68]. Self-nuclear material from NETS can form immune complexes and self-peptide complexes that in turn can lead to stimulation of endosomal TLRs followed by secretion of type I IFNs[65, 69]. SLE neutrophils exhibit impaired phagocytosis and reduced production of reactive oxygen species (ROS), which correlates with disease activity[70-72].

The increased presence of autoantigens from apoptotic cells and NETs promotes DC activation. Particularly, plasmacytoid dendritic cells (pDCs) are shown to be activated in SLE patients through exposure to self -immune complexes and self-peptide complexes[69, 73, 74]. This stimulation is through endosomal TLRs 7 and 9, resulting in increased type I IFN production[69, 73]. Their role in pathogenesis is demonstrated through depletion of pDCs in lupus-prone mice resulting in reduced disease progression[75, 76]; though they seem to be more important for the initiation stage of SLE pathogenesis, since reconstitution of pDCs later in disease does not fully reverse the disease progression. The important role for pDCs in SLE is also supported by

observations in lupus patients treated with a monoclonal antibody targeting BDCA2, which reduces skin lesion development[77].

In SLE patients and mice, conventional and monocyte derived DCs exhibit more of an inflammatory profile, promoting autoreactivity through self-nucleic acid sensing[78, 79]. Conventional DC's (cDCs) from SLE patients and mice show increased expression of costimulatory molecules (ex. CD40), possibly allowing for increased T cell activation[80-83]. They also exhibit decreased expression of death-ligand 1 allowing for increased survival of autoreactive cells[84]. There is also an increase in CDllb$^+$ DCs with CCR7 expression infiltrating into tissues and secondary lymphoid organs and correlating with lupus disease progression[85, 86].

Monocytes and macrophages also contribute to SLE pathogenesis. Reduced expression and function of Fc gamma receptors (FcγR) on monocytes from SLE patients may contribute to the induction of inflammatory cytokines and impaired phagocytosis[87-92]. Macrophages also exhibit impaired phagocytosis, which results in accumulation of apoptotic cells near the germinal center[93, 94]. In addition, SLE patients show increased expression of costimulatory and adhesion molecules on monocytes and monocyte-derived APCs, assisting in infiltration and lymphocyte activation. Monocytes/macrophages in SLE patients exhibit increased inflammasome activity, which may contribute to SLE pathogenesis[95, 96]. These unique features could be due to cytokines in SLE patient serum, as serum from lupus patients can impair phagocytosis of apoptotic cells by macrophages obtained from healthy individuals[97]. In particular, chronic type I IFN expression exhibited in SLE patients has been shown to influence the inflammatory profile of monocytes[98].

Along with interacting with self-presenting APC, T cells also exhibit alterations in T cell signaling that contribute to a loss of tolerance. For example, the CD3 ζ chain is decreased in T cells and there is increased mTOR activity resulting in hyperactivation[99-101]. Though the T cells

are hyperactive, they have impaired IL-2 production which contributes to the reduction of T_{Reg} cells, a defect seen in both lupus mouse models and human studies[47, 102]. In addition, there is skewing of polarization toward effector T_H17 cells and double negative T cells producing IL-17, IL-1β, and IFNγ[47, 56, 61].T cells are an important checkpoint for B cell activation, and in SLE, promote autoreactive B cells; this is demonstrated through somatic hypermutations seen in the genes encoding antibodies of SLE patients. The transient interactions between T and B cells in lupus patients compared to healthy individuals are believed to contribute to this pathogenic effect[103, 104]. Specifically, T follicular helper cells are implicated in promoting autoreactive B cells, as they are expanded in lupus murine models and correlate with disease activity and severity in SLE patients[105-108].

SLE patients and lupus-prone mice exhibit increased autoreactive B cells[109, 110]. Generation of autoreactive B cells in SLE patients is due to defects in surface molecules, signaling transduction, and tolerance check points. SLE patients exhibit defects in FcγRIIIB surface expression on B cells[111]; this has been shown to contribute to glomerulonephritis and autoantibody production in mice[112, 113]. There is also altered BCR signaling such as increased PI3K and Akt activation in SLE patients, which regulates class switching and differentiation[114, 115]. In particular, there is a shift in the B cell pool leading to increased pre-naïve cells and transitional cells[116, 117]. In addition, BLyS and type I IFNs, both of which are increased in SLE, have been shown to enhance BCR signaling[118-121]. SLE patients and mice also demonstrate increased T follicular helper (TFH) cells, which may regulate B cell activation in germinal centers[105, 122]; this may also contribute to enhanced somatic hypermutation displayed in B cells from SLE patients [117, 123-125].

B cells likely contribute to SLE both through autoantibody production and through antibody-independent roles. Autoantibodies in lupus patients are targeted against nuclear material including histones, chromatin, and DNA[18]. Most studies have demonstrated a correlation of anti-double stranded DNA antibodies with disease activity[126]. They contribute to disease pathogenesis through the formation of immune complexes that can activate Fc receptors and the complement pathway in tissue[127]. Autoantibodies can also bind self-nucleic acids and be endocytosed by antigen presenting cells to stimulate endosomal TLRs, resulting in the production of type I IFNs[128]. Intriguingly, autoantibodies are not required for SLE development, as mice that are incapable of secreting antibodies still develop lupus[129]. Functions independent of antibody production that may contribute to disease include antigen presentation, co-stimulation of T cells, production of cytokines and chemokines[130-132].

1.2 Type I Interferons and SLE Pathogenesis

1.2.1 Type I IFNs

Currently, there exist three classes of interferons (IFNs): type I, type II and type III. Type I IFNs include: IFN-α, IFN-β, IFN-δ, IFN-ε, IFN-κ, IFN-τ, and IFN-ω. IFN-α has 13 different subtypes, while the others have one form[133]. The type II and III IFNs are composed of IFN γ and IFNλ (4 subtypes) respectively[134]. Each class of IFNs binds to a different receptor on the target cell to induce signaling.

Type I IFNs bind to a heterodimeric transmembrane protein receptor composed of IFNAR1 and IFNAR2, resulting in phosphorylation of two associated cytoplasmic kinases, the tyrosine kinase 2(TyK2) and Janus kinase 1(Jak1)[134, 135]. These kinases then phosphorylate STAT1 and STAT2, which translocate to the nucleus along with interferon regulatory factor

9(IRF9) to bind to IFN regulatory elements; this is followed by transcription of interferon stimulated genes (ISGs)[134]. Binding of the type I IFNAR can also trigger signaling of the MAPK and PI3/AKT pathways[134].

Type I IFN production is triggered through sensing of nuclear material derived from viruses, bacteria, or self-nucleic acids by pattern recognition receptors (PRRs). Both TLRs and the cytoplasmic nucleic acid sensors, have been shown to induce type I IFN production following stimulation. Cytosolic sensors are expressed in most cells, while TLRs are expressed most highly in immune cells[135].

1.2.2 Type I IFNs and SLE

Gene expression

The majority of SLE patients display a type I IFN-regulated gene signature[136], and this appears before pathology is evident[137]. GWAS studies have revealed several genetic loci associated with increased risk for SLE that are linked to type I IFN production. In both humans and mice, type I IFNs induce lupus-like symptoms. In patients with hepatitis C, clinical lupus can develop following type I IFN treatment[138]. This is also exhibited in mice treated with adenovirus containing IFN-α, leading to the acceleration of lupus development. Further supporting a role for type I IFNs, NZM lupus-prone mice lacking the type I IFNR demonstrate a reduction in disease pathogenesis[139-141]. A cross sectional study also shows IFNs correlate with lupus disease activity; however, in longitudinal studies expression of IFN gene signatures are stable over time[142-145], indicating a role for type I IFNs in the induction of disease but maybe not disease flares. These data have led to the dogma of type I IFNs being a driving force in lupus development.

Activation

Endocytosed ICs or antimicrobial peptides containing self-nucleic acids induce type I IFN production through TLR and cytosolic nucleic acid signaling[146, 147]. A relationship between RNA-containing IC and type I IFN signature in PBMCs from lupus patients has been demonstrated in previous work[148].

pDCs are implicated as a major source of type I IFN production; this is demonstrated through reduced IFN-α production when pDCs are depleted in lupus-prone mice and lupus patients leading to disease improvement[75, 146, 147]. The uptake of ICs and antimicrobial peptides, such as LL37, can trigger type I IFN production by pDCs through TLR7 and 9 stimulation[73, 149, 150]. Neutrophil Extracellular Traps (NETosis) can also activate pDCs to produce type I IFNs through oxidized mitochondrial DNA[74]. However, previous studies have also shown IFN-α can still be produced in the absence of pDCs in PBMCs from SLE patients[151]; indicating other cell types as an additional source for IFN-α production. Other cell populations that produce type I IFNs include neutrophils from SLE patients having increased IFN-α transcripts, Ly6C^{high} proinflammatory monocytes, and keratinocytes producing IFN-κ [152-155].

Environmental triggers that promote lupus disease development are associated with induction of type I IFN production. Ultraviolet B-light (UVB) is one major environmental trigger that induces type I IFN expression in lupus patients and mice[156, 157]. UVB treatment of keratinocytes from SLE patients leads to increased IFN-κ production that is implemented in driving proinflammatory cytokine production such as IL-6[154], but the role of this pathway in UVB induced immune cell activation is not well understood. Other environmental triggers such

as procainamide and hydralazine can also induce type I IFN through the dysregulation of DNA methylation[158].

1.2.3 Cell populations and Type I IFNs

Type I IFNs affect the inflammatory profile of several immune cell populations in SLE pathogenesis. Chronic expression leads to priming of monocytes for more activation and inflammatory response[98, 159]. Monocytes can differentiate into cDCs in the presence of SLE serum and this is dependent on type I IFNs[151]. Priming of monocyte derived DCs in the presence of apoptotic blebs leads to activation of T cells, possibly through overexpression of Ifi202 and Ifit4 leading to increased IL-12 production which promotes Th1 differentiation[160-163]. Type I IFNs can also increase T cell survival and suppress T regulatory cell function[164]; this could in turn contribute to the autoreactive T cell population being activated in SLE patients. Along with T cell help, type I IFNs have been shown to promote B cell activation and isotype class switching[165].

1.3 Dissertation rationale

My dissertation research focuses on the role of type I IFNs in triggers of SLE. There are two areas of focus: TLR7-mediated lupus development and UVB-induced immune cell response. Type I IFNs have demonstrated effects on both the innate and adaptive immune cell populations and are elevated in SLE patients. Though they may be associated with several manifestations in SLE, the mechanisms involved in contributing to TLR7 mediated lupus characteristic development or UVB-induced immune response isn’t known. This project will focus on understanding which TLR-7-related lupus characteristics are regulated by type I IFNs and if type I IFNs are pro or anti-inflammatory in a UVB- induced immune cell response in lupus-prone mice.

1.4 Toll-like receptor 7 involvement in SLE pathogenesis

1.4.1 Types of TLRs

In humans, 10 TLR family members have been identified, and in mice, 13 are present; these TLRs are localized to the plasma membrane and endosome. TLRs can sense lipopolysaccharide (LPS), flagellin, lipoproteins, single-stranded RNA, or unmethylated DNA (CpG), and following sensing of their respective ligand, signal through either MyD88 or TRIF. This eventually results in the activation and translocation of IRF7, IRF3, NFkB, or JNK into the nucleus to induce gene transcription[166]. Given the genetic risk associated with TLR7 polymorphisms in SLE and numerous murine models that demonstrate a role for TLR7 in driving lupus phenotypes[167-170], a focus on TLR7 is warranted.

1.4.2 TLR 7

TLR7 is localized to the endosome and recognizes double stranded RNA that is typically presented by viruses or self-nucleic acids in autoimmunity. Expression of TLR7 is seen in macrophages, monocytes, B cells, and dendritic cells; in particular, dendritic cell subsets that express high levels of TLR7 are pDCs and cDCs. Binding of dsRNA by TLR 7 results in signal transduction that is dependent on the adaptor protein MyD88, leading to the upregulation of NFκβ regulated cytokines and increased expression of IRF7 regulated cytokines[171].

1.4.3 TLR7 and SLE

Polymorphisms in TLR7 are linked to SLE development in humans. The most prevalent SNP is rs3853839, which was identified in Eastern Asians[167]. The copy number of the TLR 7 gene has also been shown to correlate with increased IFN-α expression[172], which suggest a role for TLR7 in type I IFN production in SLE patients.

Initial observations of TLR7's role in driving lupus development in mice were made in the BXSB mouse model. The male mice in this strain harbor a Y-linked autoimmune accelerating (yaa) locus on the Y chromosome that contains 16 genes including TLR7, and this results in the male mice developing lupus characteristics[173]. Lack of TLR7 signaling in MRL^{lpr} and NZB/W lupus-prone mouse models resulted in diminished autoantibodies and attenuation of lupus nephritis[169, 170]. In addition, epicutaneous stimulation of wild-type mice with Imiquimod (TLR7 agonist) initiated increased autoantibody production, splenomegaly, and lupus nephritis[174]. The effect of TLR 7 is also exhibited in mice transgenic for an extra copy, as these mice develop glomerular nephritis, splenomegaly, liver inflammation and antinuclear antibodies[168]. Though type I IFNs are upregulated following TLR7 stimulation[174] ,their role in TLR7 mediated lupus development is not well understood.

1.5 The impact of UVB in SLE pathogenesis

1.5.1 Human Healthy and Autoimmune Skin responses to UV

UV light falls in the spectrum between visible light and gamma irradiation. UVA, UVB, and UVC are divided based on wavelength (UVA=400-320nm; UVB=320-280nm; UVC=280-100 nm), with shorter wavelengths associated with higher energy effects. In general the longer wavelengths, such as UVA, penetrate more deeply in the skin, reaching the dermis, whereas UVB is absorbed almost entirely by the keratinocytes of the epidermis[175]. UVC rarely reaches the skin as it is primarily absorbed by atmospheric ozone. Following UV exposure, the keratinocytes act as first responders, trigging inflammatory cytokine and chemokine production (fig. 1.1). If the UV exposure is substantial enough, keratinocytes also undergo apoptosis.

1.5.2 Wild type and autoimmune murine models of UV exposure

Although lupus patients experience sensitivity to UV exposure and display both local and systemic flares, understanding the mechanism is challenging due to variability between patients[176]. Thus, murine models are ideal for understanding the mechanisms regulating both the local and systemic UV response with the caveat that no one animal model will mimic every aspect of human disease. Like in humans, UVA has shown therapeutic effects for autoimmune conditions in mice [177]. However, most studies that examine the mechanism behind UV damage utilize UVB; thus, mechanisms involved in local and systemic response following UVB treatment will be reviewed below.

1.5.3 UV-induced DNA damage and apoptosis

Mice also exhibit increased apoptosis and DNA damage in the skin after UV exposure(fig.1.1). In murine skin, keratinocytes and fibroblasts are susceptible to UVB-induced apoptosis [178-181]. Both TLR and TWEAK-Fn14 signaling pathways have been shown to regulate this process. TLR 4-MyD88 signaling pushes cells to undergo apoptotic vs. necrotic cell death pathways after UVB exposure via caspase 3 activation, as mice deficient for either TLR4 or MyD88 display increased necroptosis markers and TNFα production [182]. The TWEAK-Fn14 signaling pathway has also been investigated in mice for its role in apoptosis, since Fn14 is upregulated on keratinocytes following UVB exposure. Knockout (KO) of Fn14 led to protection from UVB induced skin inflammation[183], while the addition of TWEAK led to increased apoptosis of keratinocytes from UV treated MRL/*lpr* mice[184]. UV exposure also led to increased DNA damage/ release in both wild-type mice and lupus-prone mice, though lupus-prone MRL/*lpr* mice demonstrate increased susceptibility to UV-mediated DNA release[185]. This UV induced DNA damage may play a role in lesion development, as TREX1 KO mice, which lack cytosolic DNase,

develop lupus-like lesions [186]. Further, UV-modified DNA can induce CLE-like lesions when injected into the skin of MRL/*lpr* mice [187]. Though DNA damage and apoptosis result from UV irradiation, the differences between wild-type mice and lupus-prone mice regarding mechanisms of immune activation remains understudied.

1.5.4 UV- induced autoantigen exposure

Exposure of autoantigens at the dermoepidermal junction also occurs in lupus-prone mice following UV treatment. One study identified immune complexes and antibodies to desmoglein 3 at the dermoepidermal junction in NZB/NZW F_1 mice exposed to 500 mJ/cm^2 UVB every other day [188]. While production of anti-Ro antibodies is rare in murine lupus models, UVB induces similar externalization of the Ro autoantigen in mice. Indeed, injection of Ro+ serum from patients with subacute cutaneous lupus into Balb/c mice exposed to UVB results in deposition of anti-Ro antibodies at the dermoepidermal junction [189]. Further studies should address the role of autoantibodies in murine lupus models of UV-mediated skin inflammation.

1.5.5 UV-induced inflammation

1.5.5.1 Cytokines

Murine cytokine production after UVB is similar to that seen in human skin: TNFα, IL-6, IL-1, IL-23, and type I IFNs are all increased(fig.1.1) [179, 182, 190]. Most of the cytokine induction is fairly rapid: TNFα and IL-6 production occurs 8-24 hours after UVB exposure [191]. However, data examining their role in UV-mediated changes remain limited. In lupus-prone mice, IFN-regulated gene *Ifi202* has a pro-inflammatory effect on apoptosis following UV stimulation [192], but in wild-type mice, IFNs demonstrate a protective effect in the skin as mice lacking the type I IFN receptor have greater inflammatory responses [156]. UVB induces colony-stimulating factor-1 (CSF1) which

likely enhances macrophage recruitment to the skin [193]. Following UVB, TNFα has a pro-inflammatory effect by increasing apoptosis of keratinocytes [190, 194, 195]. Though studies on the role of IL-1 family members following UV exposure are limited, mice transgenic for IL-1α demonstrate skin inflammation [196]. IL-6 -/- mice demonstrate decreased epidermal proliferation after UVB and also decreased systemic IL-10, suggesting IL-6 may have both epidermal and immune regulatory functions [197]. IL-23 in wild-type mice has a protective effect on UVB-mediated damage by reducing DNA damage [198]; however the function of this cytokine has not been examined in lupus-prone mice following UV stimulation. Intriguingly, neutralizing antibodies to IL-23 have a protective effect in lupus-prone mice, which suggests a pro-inflammatory function for this cytokine after UVB stimulation [199]. Further exploration into the role of these cytokines following UV exposure in wild-type and lupus-prone mice may yield novel data for therapeutic development for photosensitivity.

1.5.5.2 Immune Cell Recruitment

Epidermal damage from UV exposure results in upregulation of chemokines and recruitment of neutrophils, monocytes, macrophages, dendritic cells and T cells (fig.1.1) [181, 183, 200]. The dose of exposure regulates the inflammatory response. Hairless mice exposed to low dose (20 mJ/cm^2) UVB demonstrate increased epidermal thickness but not inflammation. The same mice exposed to a single high dose (400 mJ/cm^2) demonstrate neutrophil and macrophage recruitment [201]. C57BL/6 mice exposed to two doses of 500 mJ/cm^2 of UVB also demonstrate infiltration of pDCs within 24 hours and macrophages and neutrophils after 24-78 hours [157]. In wild-type mice, CD4$^+$ T cells and CD8$^+$ T cells exhibit pro-inflammatory functions through production of IFNγ following UVB stimulation [200]; this inflammation is downregulated via induction of T regulatory cells in the skin [202]. IFNα-producing monocytes are recruited to the skin in wild-type mice

following UVB exposure, and they also exhibit a negative regulatory effect on UVB-driven inflammation via type I IFN-mediated pathways [156]. Resident Langerhans cells are essential to resolution of UVB induced skin inflammation through their phagocytosis of apoptotic keratinocytes [200]; thus, they also exhibit an anti-inflammatory role.

The effect of UVB in mice with a propensity for autoimmune conditions is less well-studied. In lupus-prone MRL/*lpr* mice, markers of neutrophil and macrophage infiltration are present after UVB, but how this compares with wild-type mice was not evaluated [183]. Other studies have compared effects in lupus-prone vs. wild type mice. Increased $CD8^+$ and $CD4^+$ cells were noted in MRL/*lpr* vs. Balb/c mice after 2 and 6 days of 500 mJ/cm^2 UVB treatment [193]. Production of chimerin and recruitment of pDCs to the skin after UVB exposure is increased in MRL/*lpr* vs. wild-type mice [157]. *Ex vivo* irradiation of lymph nodes from lupus-prone (both NZB/NZW F_1 and MRL/*lpr*) vs. wild-type mice exhibited greater upregulation of ICAM-1 and LFA-1, which promotes migration of immune cells into the tissues [179]. These studies have generated a preliminary understanding of the differential effects of UVB in lupus-prone vs. wild-type mice., but additional research is needed.

1.5.6 UV-induced systemic disease flares

Anecdotal and case report data support a link between cutaneous UVB exposure and induction of systemic disease flares in patients [203, 204]. This connection between the cutaneous and systemic immune system has not been well characterized in human or murine models (reviewed in [205]). To date, the main lupus-prone mouse model that has demonstrated systemic responses to UV is BXSB male mice, which carry an additional copy of TLR7 as part of the Yaa locus [206]. In this strain, daily exposure to 400 mJ/cm^2 full spectrum UV for one week resulted in 66% of mice succumbing to death after two weeks. This level of irradiation did not impair survival in Balb/c,

MRL/*lpr* or (NZBxNZW)F_1 mice. Chronic exposure to 120 mJ/cm^2 thrice weekly also resulted in >85% lethality after 4 weeks of treatment in male BXSB mice. Death in the male BXSB mice was accompanied by changes consistent with lupus nephritis [207]. Whether it is TLR7 driving this phenotype has not been elucidated, but stimulation of TLR7 in Balb/c mice with topical TLR7 agonist for two weeks followed by UVB resulted in rising autoantibody titers compared to UVB only-treated mice [208], and TLR7 stimulation itself can promote systemic disease flares[209]. This suggests that TLR7 signaling may have a role in UVB-mediated systemic immune activation. However, epidermal damage itself may be sufficient to drive lupus flares in lupus-prone mice [210], so the effects of UVB on systemic immune activation may be multivariate.

Sensing of UVB-modified nucleic acids may contribute to systemic flare development following UVB exposure. For example, injection of UVB-induced apoptotic DNA in wild-type and lupus-prone MRL/*lpr* mice led to development of lupus-like characteristics such as increased dsDNA antibodies and proteinuria [211, 212]. Hypomethylation of DNA seems to be important for this process [212]. It is tempting to speculate that these systemic effects may be secondary to STING activation as UVB-modified DNA is resistant to degradation by TREX1 and is able to induce IFN responses and cutaneous lupus-like lesions when injected into the ear of MRL/*lpr* mice[187]. Further exploration is needed to understand the role of UVB-mediated DNA changes in driving systemic immune responses in SLE.

UVB induced epidermal injury in healthy and lupus skin results in apoptosis of keratinocytes, the upregulation of type I IFNs, and immune cell infiltration/activation. Though type I IFNs are expressed, their role in UVB-induced immune cell activation in lupus-prone mice remains unclear.

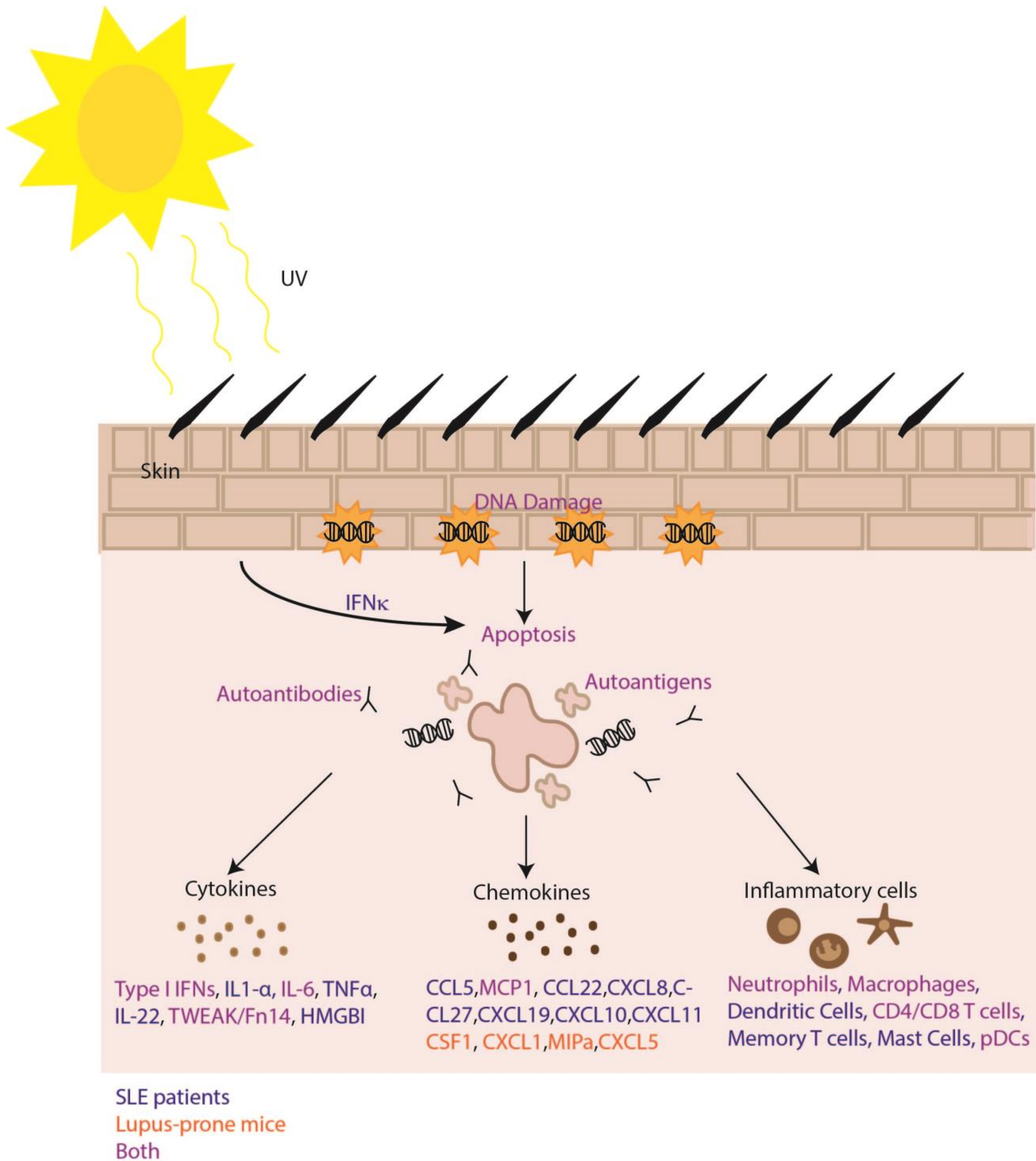


Figure 1.1 Summary of mechanisms of photosensitivity.

In lupus, increased IFN kappa promotes UV-mediated apoptosis resulting in immune complex formation, autoantigen exposure and release of numerous inflammatory cytokines and chemokines. Infiltration of inflammatory cells follows and is perpetuated by inhibition of negative regulatory mechanisms. Pathways with evidence in both human and murine systems are shown in purple. Human only pathways are shown in blue, and murine-specific pathways are shown in orange.

These chapters will examine the role of type I IFN in lupus characteristic development. Chapter 2 will describe the methods and material used. Chapter 3 will describe the role of type I IFNs in TLR7 mediated lupus. Chapter 4 will explore how type I IFNs regulate UVB-induced adaptive immune cell response in the draining lymph nodes of lupus-prone vs. wild-type mice. Chapter 5 will examine the UVB-induced immune cell response in the skin of lupus-prone vs. wild-type mice and how type I IFNs regulate this differential response. Chapter 6 will discuss results from chapter 3 to 5 and propose future directions.

Chapter 2 Methods and Materials

Portions of this chapter are published in the *Journal of Immunology*[209]

2.1 Mice

All mice were bred and housed at the University of Michigan in specific pathogen-free housing. All mice were treated according to our University of Michigan IACUC-approved protocol. 8-10-week-old female wild-type BALB/c mice obtained from Jackson Laboratory were utilized for this study. Wild-type mice were compared to NZM2328 mice and INZM (lack the α-chain of the IFNα/β receptor) mice, which were a kind gift of Dr. Chaim Jacob, University of Southern California[141]. NZM2328 IL-1β -/- mice were generated through the University of Michigan Transgenic Animal Model Core via CRISPR-Cas9 technology using IL-1β CRISPR/Cas9 KO Plasmid sc-421097 from Santa Cruz Biotechnology (Santa Cruz, CA). An 8-bp deletion resulting in a frame shift mutation was confirmed via Sanger sequencing through the University of Michigan DNA sequencing core (fig. 2.1). NZM2328 IL-1β-/- mice were backcrossed onto the NZM2328 background for three generations to eliminate off-target effects of using the CRISPR system followed by heterozygote crossing to develop mice homozygous for the IL-1β deletion. Female 10-week old mice were utilized for all experiments. The NZM mouse model has minimal autoantibody production and no nephritis at this age[141]. Males were not used as they do not achieve a lupus phenotype in this model, and lupus is predominantly a female disease[213].

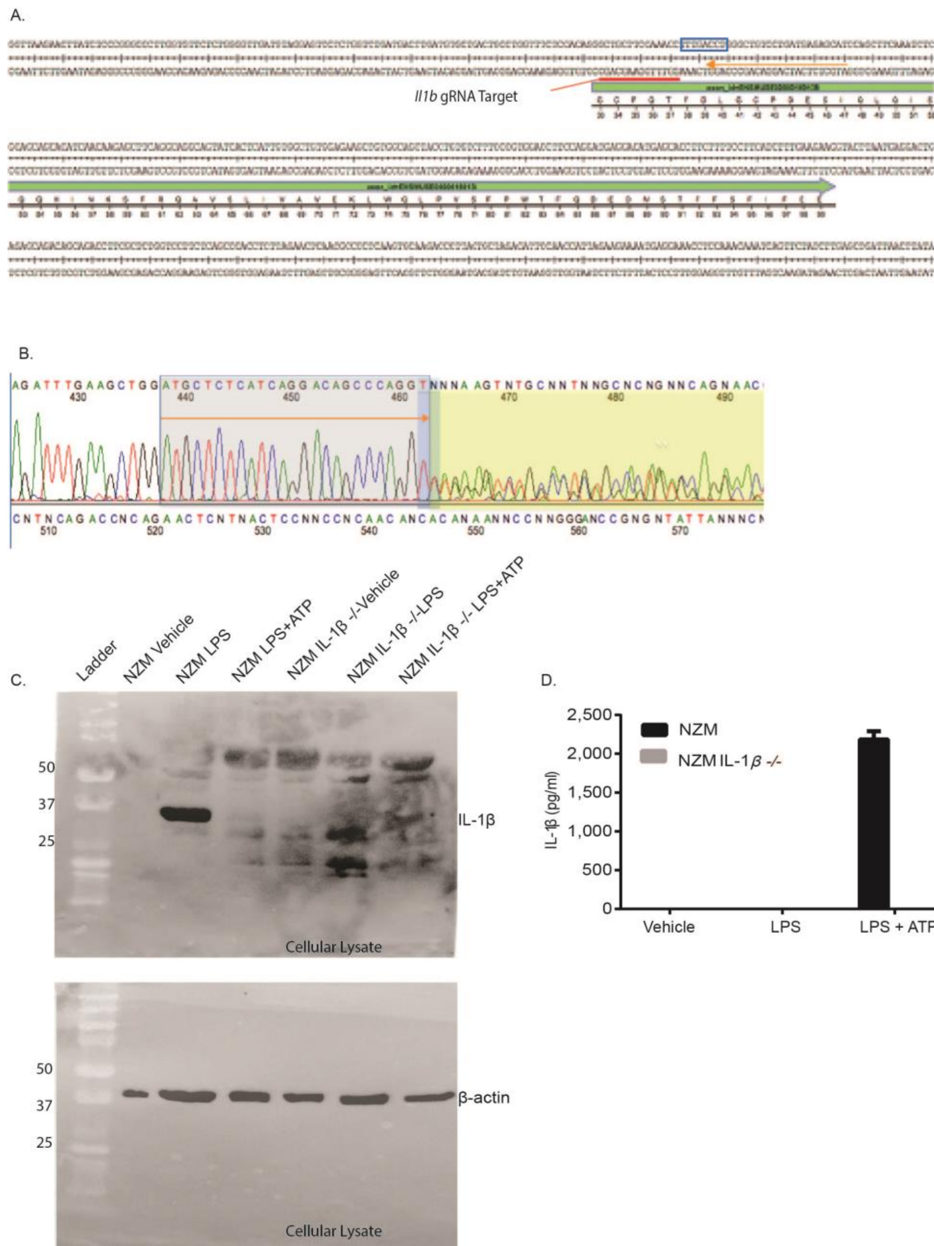


Figure 2.1 Generation of IL-1β -/- mice.

A. Diagram showing the Il1b gene guide RNA target (red line) and resulting 8bp deletion (blue box) of Il1b. B. Sanger sequencing demonstrating loss of homozygosity in il1b exon (marked by orange arrow in A and B) in il1b-/+ progeny. Confirmation of an 8-bp deletion (AGGTCAA) was made via bi-directional sequencing of cloned amplicons. (C and D) BMDMs from 10 week-old NZM 2328 and NZM IL1β KO mice were stimulated with or without 1ug LPS for 4 hours followed by activation of the inflammasome with or without 5mM ATP for 1 hr. C. Western blot of cellular lysates showing pro-IL1β (31kDa) upregulation in NZM but not IL-1β-/- cells after LPS treatment. Active IL-1β was secreted and detected in D. β-actin (42kDa) is shown on the bottom. D. [IL-1β] in the media of BMDMs from C. was analyzed via ELISA. No secreted IL-1β was detected from NZMIL-1β-/- mice.

2.2 Mouse model justification

Lupus is a complex disease with varied manifestations that cannot be replicated by simple tissue culture or *ex vivo* experiments. Because murine models provide the ability to manipulate specific protein expression, cellular levels and environmental exposures, they provide an unparalleled system by which to study disease mechanisms. We utilized the NZM2328 mouse model as these mice lack lupus characteristics until around 35 weeks of age, which allows us to study environmental factors driving lupus development. NZM2328 mice are more associated with the C57BL/6 genetic background, but these mice have shown sensitivity to hair depletion via Veet. Our UVB studies utilize BALB/c mice as the control, because this strain does not demonstrate sensitivity to Veet similar to NZM2823 mice. In addition, following UVB exposure BALB/c mice demonstrate similar erythema development and cytokine expression compared to C57BL/6 mice[214].

2.3 TLR 7 cutaneous stimulation

Female 10-week old mice were treated via epicutaneous application of 100µg of the TLR7 agonist R848 (Enzo Life Science) dissolved in 8 µL DMSO, or DMSO alone as a control, to the right ear three times weekly until euthanasia. DMSO and R848 treated mice were housed in separate cages to avoid cross-contamination. For some studies, mice were treated for only 2 weeks before euthanasia (for evaluation pre-proteinuria); others were treated until they developed proteinuria or became moribund (for survival studies) followed by euthanasia. Control mice were harvested with their paired R848-treated littermates.

2.4 Analysis of anti-dsDNA and IgG serum levels

Serum was collected 2 weeks post UVB treatment. Anti-IgG and dsDNA IgG antibody levels were analyzed via ELISA kits (Alpha Diagnostic, San Antonio, TX, and Innovative Research, Novi, MI).

2.5 Analysis of proteinuria

Urine was collected weekly and protein was screened via dipstick followed by albumin measurements via Albuwell kits (Exocell, Philadelphia, PA) and total creatinine (Cr) via commercial kit (Bioassay Systems, Hayward, CA). Urinary protein excretion was represented by the albumin: Cr ratio.

2.6 Renal histopathology and immune complex deposition scoring

Glomerular inflammation (activity index) and scarring (chronicity index) of murine kidneys were quantified in a blinded fashion (by JBH) on perfused kidneys fixed in 10% formalin followed by 3µm sectioning and Periodic Acid Schiff (PAS) staining as previously described by us and others (19, 20). In brief, a semi-quantitative scoring system (0, no involvement; 0.5, minimal involvement of 10%; 1, mild involvement (10–30% section); 2, moderate involvement (31–60% of section); and 3, severe (60% of section)) was used to assess 13 different parameters of activity and chronicity (mesangial hypercellularity, mesangial deposits, mesangial sclerosis, endocapillary cellular infiltrate, subepithelial and subendothelial deposits, capillary thrombi, capillary sclerosis, cellular or organized crescents, synechiae, tubular atrophy, and interstitial fibrosis). The chronicity and activity index was generated by compiling the scores from groups of related parameters (for activity: mesangial hypercellularity, mesangial deposits, and endocapillary cellular infiltrate; for chronicity: interstitial fibrosis, tubular atrophy, synechiae, organized crescents, and capillary sclerosis). Glomerular immune complexes were

quantified by immunofluorescence microscopy as previously described[215]. Briefly, 6µm frozen kidney sections were stained for 1hr at 4°C with Texas-red-conjugated anti-mouse IgG (Sigma) and FITC-conjugated anti-C3 (ICL, Portland, OR) followed by Hoechst (Invitrogen, Eugene, OR) counterstain to stain DNA. Quantification of immune complex staining in 8 glomeruli per mouse was performed at the Center for Live Cell Imaging at the University of Michigan using Metamorph v7.0.6 to calculate the mean fluorescence in a defined area for each stain. Glomeruli were identified based on Dapi staining and outlined to define the area for analysis. Both FITC and Texas Red staining were calculated and shown as staining per glomerulus.

2.7 Liver scoring

Inflammation of murine livers were quantified blindly by JBH on livers fixed in 10% formalin followed by 3µm sectioning and Periodic Acid Schiff (PAS) staining as described by others[216]. In brief, a scoring system (0: <0.5 inflammatory foci/field; 1, 0: 5-1.0 foci/field; 2: 1.0-2.0 foci/field; 3: >2.0 foci/field) was used.

2.8 Microscopy

Images of H&E stained skin sections and PAS-stained kidney and liver sections were captured using an Olympus BX41 microscope with a 100x objective (total magnification=1000x). Images of kidney immune complex staining were captured at the Center for Live Cell Imaging (CLCI) at the University of Michigan Medical School using an Olympus IX70 inverted microscope (Olympus; Center Valley PA) with a 40x objective.

2.9 Flow cytometry

Following euthanasia, the lymph nodes (draining lymph node (dLN) from the cervical chain on the treated side and non-draining lymph node from the inguinal chain) and spleen were

removed, teased apart and passed through a 70μm filter to generate single cell suspensions. One of the kidneys was also removed, minced and digested as previously described[216] with 0.1 mg/mL Liberase (Roche), 200 U/mL DNAse (Roche) and 2.4 mM CaCl2 in DMEM (Invitrogen) at 37 °C in a humidified incubator for 1 h. Skin was minced with razors and transferred to RPMI10 (Gibco), which contained 1% Penicillin-Streptomycin (Gibco) and 10% fetal calf serum (Atlanta Biologicals). Each skin sample then received 2ml of skin digestion mix made of 5mg/ml Collagenase type IV (Sigma-Aldrich), 1mg/ml DNAse I (Sigma-Aldrich) and 1mg/ml Hyaluronidase type V (Sigma-Aldrich) added to Hanks' Balanced Salt Solution (Gibco). Samples were placed on a GentleMacs machine and run for 1 minute on "Spleen 1" setting before and after incubation for 2 hours at 37°C to digest the skin. The tissue was then passed through a 70 μm cell strainer (spleen, dLN and kidney) or a 40 μm cell strainer (skin) and RBCs in the spleen were lysed with multi-species RBC lysis buffer (eBioscience). Live cells were counted via trypan blue exclusion. The cells were incubated in flow block (1% horse serum and 1% bovine serum albumin in PBS) for 1hr, then stained for 1 hr on ice using the following antibodies: CD8 clone: 53-6.7 (BD Bioscience, San Jose, CA), CD3 clone: 17A2, CD4 clone: GK15, CD69 clone: H12F3, CD25 clone: 3C7, B220 clone: RA3-6B2(Biolegend, San Diego, CA), SA-Qdot 605 (Invitrogen), CDllc clone: N418, CDllb clone: M1/70, CD103 clone: 2E7, F480 clone: BM8, CD40 clone:3/23 for 45 minutes. Following the extracellular staining, cells were intracellularly stained for Foxp3 clone: FJK16s (Biolegend) and IgH+L A488 (Southern Biotech, Birmingham, AL) utilizing the Foxp3 / Transcription Factor Staining Buffer Set from eBiosciences, San Diego, California. The flow cytometry data was collected via a BD LSR II flow cytometer and analyzed using FlowJo VX.0.7 (Tree Star). For analysis, the live cells were gated for: **T cells:** $CD3^+$; **$CD4^+$ T cells:** $CD3^+CD4^+CD8^-$; **$CD8^+$ T cells:** $CD3^+CD8^+CD4^-$; **T cell activation:**

CD69^{+}; **B cells:** CD4^{-}CD8^{-}IgH^{+}L^{+}B220^{+}; **T$_{Reg}$ cells:** CD4^{+}CD3^{+}CD25^{+} Foxp3^{+}; **Ab secreting cells:** CD4^{-}CD8^{-}IgH+L^{hi}B220$^{int\text{-}low}$; **DC cells:** CDllc^{+}F480^{-}CDllb^{-}; **monocyte derived DC cells:** CDllc^{+}F480^{-}CDllb^{-}; **CD103^{+} DC cells:** CDllc^{+}F480^{-}CDllb-CD103^{+}; **Macrophages:** CDllc^{int}CDllb^{+}F480^{+}; **Macrophage activation:** CD40^{+}.

2.10 Bone marrow–derived macrophages

Bone marrow derived macrophages (BMDM) were generated as previously described[217]. Bone Marrow was flushed from the tibiae and femurs of 10 weeks old NZM2328 mice and NZM IL1β -/- mice and plated in macrophage differentiation media (59% IMDM, 10% FBS, 30% L-cell supernatant, and 1% penicillin/ streptomycin) for 7 days at 37°C in 5% CO2. BMDM were then plated 2x10^{6} cells per 6 well for detection of IL-1β activation.

2.11 IL-1β quantification

BMDM were incubated with or without 1ug LPS for 4 hours followed by 1-hour incubation with or without 5mM ATP to activate the inflammasome. Secreted IL-1β was measured via ELISA (DuoSet ELISA kit, R&D, Abingdon, UK). Cells were lysed with RIPA +PI for 10mins on ice. 10μg of each lysate was then run on a 10%SDS-polyacrylamide gel and blotted on nitrocellulose membranes (GE Healthcare). The membranes were blocked in 4% milk/TBST, followed by probing with anti-murine IL-1β Ab 1:500(Cell Signaling Technology) overnight. Then the membrane was incubated with anti-rabbit IgG-HRP 1:1,000 (Abcam). The pro-IL-1β band was detected using Western Bright Quantum (Advansta) and imaged using Omega Lum C system (Aplegen).

2.12 Real-time quantitative PCR analysis

Biopsies from the backs of mice treated with/without 100mJ/cm2 UVB were taken 3 hrs after the 5th UVB treatment. Skin biopsies were snap frozen in liquid nitrogen and stored at -80°C until further use. Skin was pulverized with the use of a mortar and pestle and placed in TRIzol (life technologies). Kidney tissue was homogenized in TriPur (Roche). RNA was isolated using the Direct-zol mini RNA prep kit (Zymo). 100ng of RNA was reverse-transcribed into cDNA, followed by quantitative real-time PCR analysis by the DNA sequencing core at University of Michigan on an ABI PRISM 7900HT (Applied Biosystems). Gene expression was calculated by fold change relative to no UV (control) group. The primers used were as follows (all listed 5′→3′): Myxovirus (influenza virus) resistance 1 (mx1) GATCCGACTTCACTTCCAGATGG (forward), CATCTCAGTGGTAGTCAACCC (reverse); β-Actin TGGAATCCTGTGGCATCCTGAAAC (forward), TAAAACGCAGCTCAGTAACAGTCCG (reverse); Interferon alpha (ifna) ATGGCTAGRCTCTGTGCTTTCCT (forward), AGGGCTCTCCAGAYTTCTGCTCTG(reverse); Interferon beta (ifnb) AGCTCCAAGAAAGGACGAACAT (forward), ATTCTTGCTTCGGCAGTTAC(reverse); Interferon gamma (ifng) AGCGGCTGACTGAACTCAGATTGTA (forward), GTCACAGTTTTCAGCTGTATAGGG (reverse) ; Interferon kappa (ifnk) ACTCCAAAGTTTTATGGCTGGT (forward), TACGATAGGAGACGGCGTTTA (reverse) ; Interferon regulatory factor (irf7) TGCTGTTTGGAGACTGGCTAT(forward), TCCAAGCTCCCGGCTAAGT(reverse); Chemokine Ligand 2 (ccl2) AGGTCCCTGTCATGCTTCTG (forward), GGATCATCTTGCTGGTGAAT (reverse); C-X-C Motif Chemokine Ligand 10(cxcl10) ATCATCCCTGCGAGCCTAT (forward),

ATTCTTGCTTCGGCAGTTAC (reverse) chemokine (C–C motif) ligand 5 (ccl5) CAATCTTGCAGTCGTGTTTG (forward), GGAGTGGGAGTAGGGGATTA (reverse); chemokine (C–C motif) ligand 4 (ccl4) AGCAACACCATGAAGCTCTG (forward), CTGTCTGCCTCTTTTGGTCA (reverse);   C-X-C motif chemokine 13 (cxcl13) AGAGGTTTGCGAGATGGACT (forward), GAGCCTGGACCTTTAAGCTG (reverse); Tumor necrosis factor alpha (tnf) CCCACTCTGACCCCTTTACT (forward), TTTGAGTCCTTGATGGTGGT (reverse); il1b CCCTGCAGCTGGAGAGTGTGGA (forward), CTGAGCGACCTGT-CTTGGCCG (reverse); il6 TAGTCCTTCCTACCCCAATTTCC (forward), TTGGTCCTTAGCCACTCCTTC (reverse). Gene expression was calculated relative to B actin (2^βactin/2^gene).

2.13 UVB irradiation and skin thickness measurement

The hair on the backs of the mice was removed via depilation with Veet and mice were placed in a restrainer with facial protection. The mice were treated with 100mJ/cm2 UVB using the UV-2 ultraviolet irradiation system (Tyler Research) for 5 consecutive days and harvested at the times indicated in the experiments. UVB light was provided by cascade-phosphor ultraviolet generators that emit 310nm of UVB radiation. Calipers (Fine Science Tools) were used to measure skin thickness.

2.14 T_{Reg} suppression assay

10-week old female BALB/c, NZM, and iNZM mice were treated with/without UVB for 5 consecutive days. The dLNs were processed into a single cell suspension, as described in flow cytometry. $CD4^{+}CD25^{+}$ TReg cells and $CD4^{+}$ T cells were isolated via $CD4^{+}$ $CD25^{+}$ Regulatory T Cell Isolation Kit and $CD4^{+}$ T Cell Isolation Kit, respectively (Miltenyi Biotec, Bergish

Gladbach, Germany). T_{Reg} cells were labeled with CFSE (ThermoFisher, Eugene, Oregon) and $CD4^+$ cells were labeled with cell proliferation dye 670 (ThermoFisher). Following labeling, the cells were co-incubated at a ratio of 0:1,1:1,1:2,1:4 (TReg: TEffector) with/without anti-CD3/CD28 beads (ThermoFisher) for 72 hrs in a 96-well plate. Cells were then stained, as described in the flow cytometry section, for CD3 clone: 17A2 (BioLegend) for 45 minutes, followed by staining with live/dead cell dye (ThermoFisher) for 30 minutes. After staining, cells were resuspended in PBS and data collected on a BD LSR II flow cytometer and analyzed using FlowJo. For analysis, samples were gated on CFSE negative cells to exclude TRegs, followed by live cell gating, then $CD3^+$ cells and lastly proliferation dye 670 to examine percent proliferation of $CD4^+$ T cells (fig. 2.2). Percentage proliferation was calculated using the formula: (100 x 1:1,1:2, or 1:4, samples) /0:1 sample.

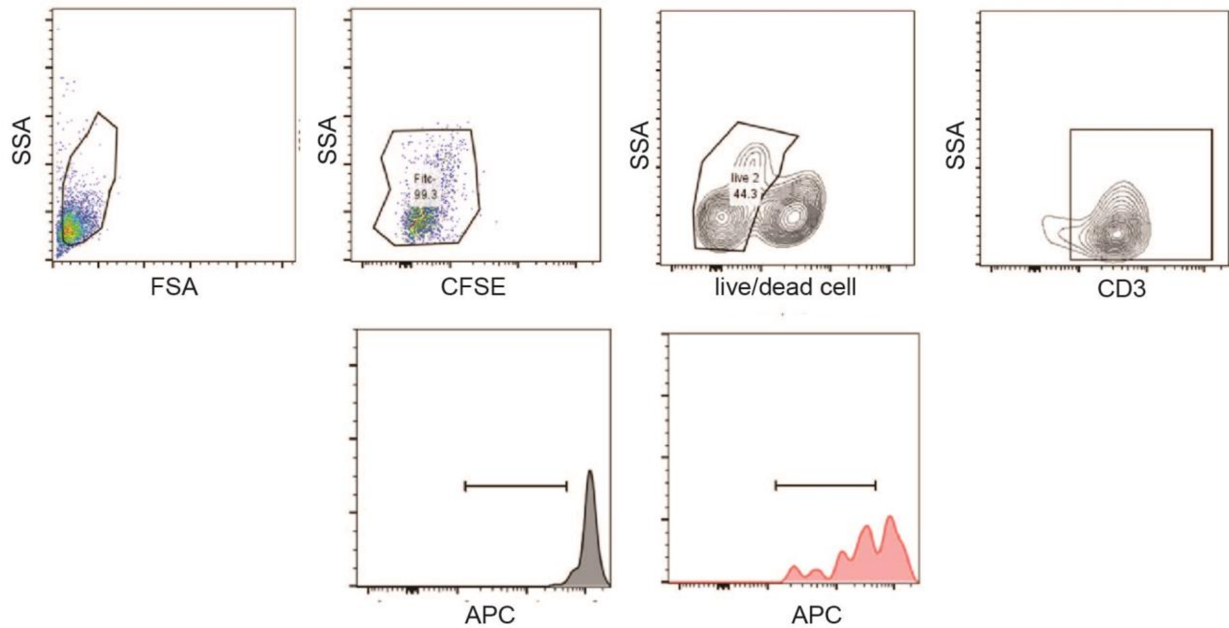


Figure 2.1 Gating strategy for CD4$^+$ T cell proliferation in T_{Reg} suppression assay.

Samples were gated on CFSE negative cells to exclude T_{Reg} cells, followed by live cell gating, then CD3$^+$ cells and lastly proliferation dye 670 to examine percent proliferation of CD4$^+$ T cells. Percent proliferation was calculated using the formula: (100 x 1:1,1:2, or 1:4, samples) /0:1 sample.

2.15 Skin histopathology

Skin inflammation was quantified (by Lorie Lowe) on skin fixed in 10% formalin followed by 3-mm sectioning and H&E staining. A semiquanitative scoring system (0-3) was used to assess the different levels of epidermal and dermal skin inflammation. 4 different parameters: hyperkeratosis, neutrophil infiltration, and hyperplasia were used to generate the epidermal inflammation sore. 3 parameters: overall inflammation, lymphocyte infiltration, and neutrophil infiltration were used to generate the dermal inflammation score.

2.16 Immunohistochemistry

To detect monocytes and macrophage, immunohistostaining was performed as follows: sections were deparaffinized, rehydrated, and heated at 100°C for 20 min in pH 6 antigen retrieval buffer. Slides were washed, treated with 3% hydrogen peroxide in phosphate-buffered saline (PBS) for 5 min, blocked and incubated with anti-Ly6c antibody (ab15627) 1:400 dilutions, anti-Ly6g antibody (ab25377) 1:100 dilutions, and Isotype controls IgG2a and IgG2bk overnight at 4°C, all antibodies were form abcam. All slides were then incubated with biotinylated secondary antibodies 1:200 dilution (Vector Laboratories, Burlingame, CA), followed by incubation with vectastain ABC reagent, and stained with peroxidase substrate, counterstained with hematoxylin, dehydrated and mounted.

2.17 Statistical analysis

All statistics were completed using GraphPad Prism 6.0. For figures where >2 comparisons were made, ANOVA testing was used. Tukey's multiple comparison test was used for normally distributed data and a Kruskal-Wallace/Dunn's multiple comparison test was used for non-parametric data. For figures where comparisons between two groups was completed, a two-tailed student's t-test for normally distributed data was used. Welch's correction was

applied when required for significant differences in variances. For non-normally distributed data, a Mann–Whitney was used. Survival analysis following R848 application was completed via Log-rank test. Pearson correlation was used for comparison of renal activity scores and albumin/Cr values.

Chapter 3 TLR7-Mediated Lupus Nephritis Is Independent of Type I IFN Signaling

This work was a collaboration with Jonathan Theros, Tammi J. Reed, Jianhua Liu, Irina L. Grigorova, Giovanny J. Martínez-Colón, Chaim O. Jacob, Jeffrey B. Hodgin, and Michelle Kahlenberg. Jonathan Theros, Tammi J. Reed, Giovanny J. Martínez-Colón and Jianhua Liu assisted me in performing experiments. Irina L. Grigorova assisted with data analysis and Chiam O. Jacob donated the NZM2328 and iNZM mice. Jeffrey B. Hodgin scored the kidney and liver histology slides. Michelle Kahlenberg helped with data analysis and all collaborates assisted with editing the manuscript. The data from this chapter are published in the Journal of Immunology[209]

3.1 Abstract

Systemic Lupus Erythematosus (SLE) is an autoimmune disease characterized by increased type I interferons (IFNs), autoantibodies, and inflammatory-mediated multi-organ damage. TLR7 activation is an important contributor to SLE pathogenesis, but the mechanisms by which type I IFNs participate in TLR7-driven pathology remain uncertain. In this study, we examined the requirement for type I IFNs in TLR-7 stimulated lupus nephritis. Lupus-prone NZM2328, INZM (which lack a functional type I IFN receptor), and NZM2328 IL-1β-/- mice were treated at 10 weeks of age on the right ear with R848 (TLR7 agonist) or control (DMSO). Autoantibody production and proteinuria were assessed throughout treatment. Multi-organ inflammation was assessed at the time of decline in health. Renal infiltrates and mRNA expression were also examined after 14 days of treatment. Both NZM2328 and INZM mice exhibited a decline in survival after 3-4 weeks of R848 but not vehicle treatment. Development

of splenomegaly and liver inflammation were dependent on type I IFN. Interestingly, autoantibody production, early renal infiltration of dendritic cells, upregulation of IL-1β, and lupus nephritis occurred independent of type I IFN signaling. Development of TLR7-driven lupus nephritis was not abolished by the deletion of IL-1β. Thus, while IFNα is sufficient to induce nephritis acceleration, our data emphasize a critical role for IFN-independent signaling in TLR7-mediated lupus nephritis. Further, despite upregulation of IL-1β after TLR7 stimulation, deletion of IL-1β is not sufficient to reduce lupus nephritis development in this model.

3.2 Introduction:

Systemic lupus erythematosus (SLE) is a devastating autoimmune disease characterized by cycles of disease flare that led to permanent organ damage[218]. Lupus nephritis is a feared complication of SLE; 25% of lupus nephritis patients go on to develop end-stage renal disease within 10 years after renal compromise[15] despite current treatment regimens[219]. Triggers that led to renal disease flares still remain unclear; thus, there is a critical need to identify factors which promote lupus nephritis in order to develop novel targets and therapies.

Polymorphisms in toll-like receptor 7 (TLR7) are linked with the development of lupus[44, 220]. Murine models of lupus have also provided genetic and experimental evidence to support a role for TLR7 activation in the pathogenesis of lupus nephritis[221, 222]. Male BXSB mice, which contain an additional copy of TLR7 on the Y chromosome, develop lupus nephritis, whereas female mice are protected[223]. Transgenic mice overexpressing TLR 7 also develop lupus [168]. Inducible murine lupus models, such as the pristane model, are dependent on TLR7 signaling for lupus pathogenesis [224]. Interestingly, even repeated TLR7 agonist epicutaneous application to wild-type mice leads to development of lupus-like characteristics including mild lupus

nephritis[174]. Despite these supportive data, the mechanisms by which TLR7 signaling leads to nephritis remain unknown.

TLR7 activation can result in both type I IFN production and NFκB activation in various cell populations such as dendritic cells, monocytes, macrophages, and B cells. Type I IFNs, including IFNα, can promote lupus development[141, 225, 226] and are sufficient to induce acceleration of nephritis in lupus prone mice[140]. In addition, various genetic and inducible models of lupus are protected by deletion of type I IFN signaling[141, 227]. Given the importance of type I IFN in lupus, we hypothesized that TLR7-induction of type I IFNs was responsible for its effects. To test this, we examined an inducible model of lupus flare via epicutaneous stimulation of TLR7 in lupus-prone mice. The NZM2328 mouse strain was selected for this study because they have spontaneous development of lupus characteristics including autoantibody production around 14 weeks of age, splenomegaly, and lupus nephritis around 35 weeks of age[228]. Surprisingly, we found that TLR7 treatment of young mice accelerated lupus nephritis development, including renal infiltration of innate immune populations, immune complex deposition, and IL-1β upregulation, independently of type I IFNs. Other manifestations, such as liver inflammation and splenomegaly required type I IFNs to occur. Further, we demonstrated that a novel IL-1β-/- mouse on the NZM2328 background was also susceptible to TLR7-accelerated nephritis. These data support IFN-independent immune activation in the presence of robust TLR7 stimulation as sufficient for acceleration of lupus nephritis.

3.3 Results

TLR7 Epicutaneous stimulation leads to an interferon-independent decline in survival.

TLR7 stimulation in the skin may serve an important role in lupus flare induction [174, 206]; thus, we first examined the effects of cutaneous TLR7 stimulation on young, pre-autoimmune

NZM2328 lupus-prone mice. These mice typically develop lupus nephritis around 35 weeks of age followed by a decline in survival [229]. Intriguingly, R848 topical treatment of 10-week old NZM2328 mice led to a rapid decline in survival when compared to DMSO-treated controls (Figure 3.1). This decline was marked by a nephrotic appearance including ascites, edema, and lethargy. Given that TLR7 stimulation is associated with increased IFN signaling, we hypothesized that this accelerated death was driven by type- I IFNs. Thus, we next examined the effects of R848 on NZM2328 mice that are deficient in the type I IFN receptor (INZM). These mice are protected from naturally-occurring development of lupus for up to 2 years of life [141]. To our surprise, treatment of these mice with R848 led to edema, ascites and a rapid decline in survival in a time frame similar to NZM2328 mice (Figure 3.1). No difference in the survival curves between NZM and INZM mice were noted via Log-rank test (p=0.723). Median survival was 30 days for both strains. These data suggest that despite absent type I IFN signaling, both INZM and NZM2328 mice exhibit a rapid decline in survival following epidermal TLR7 activation.

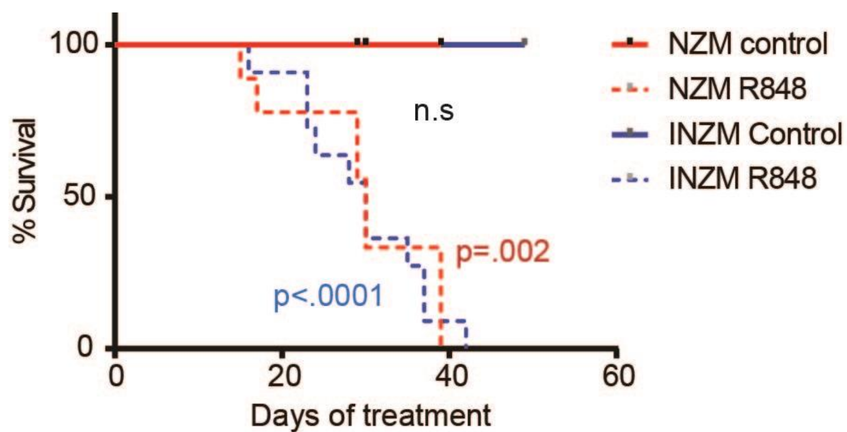
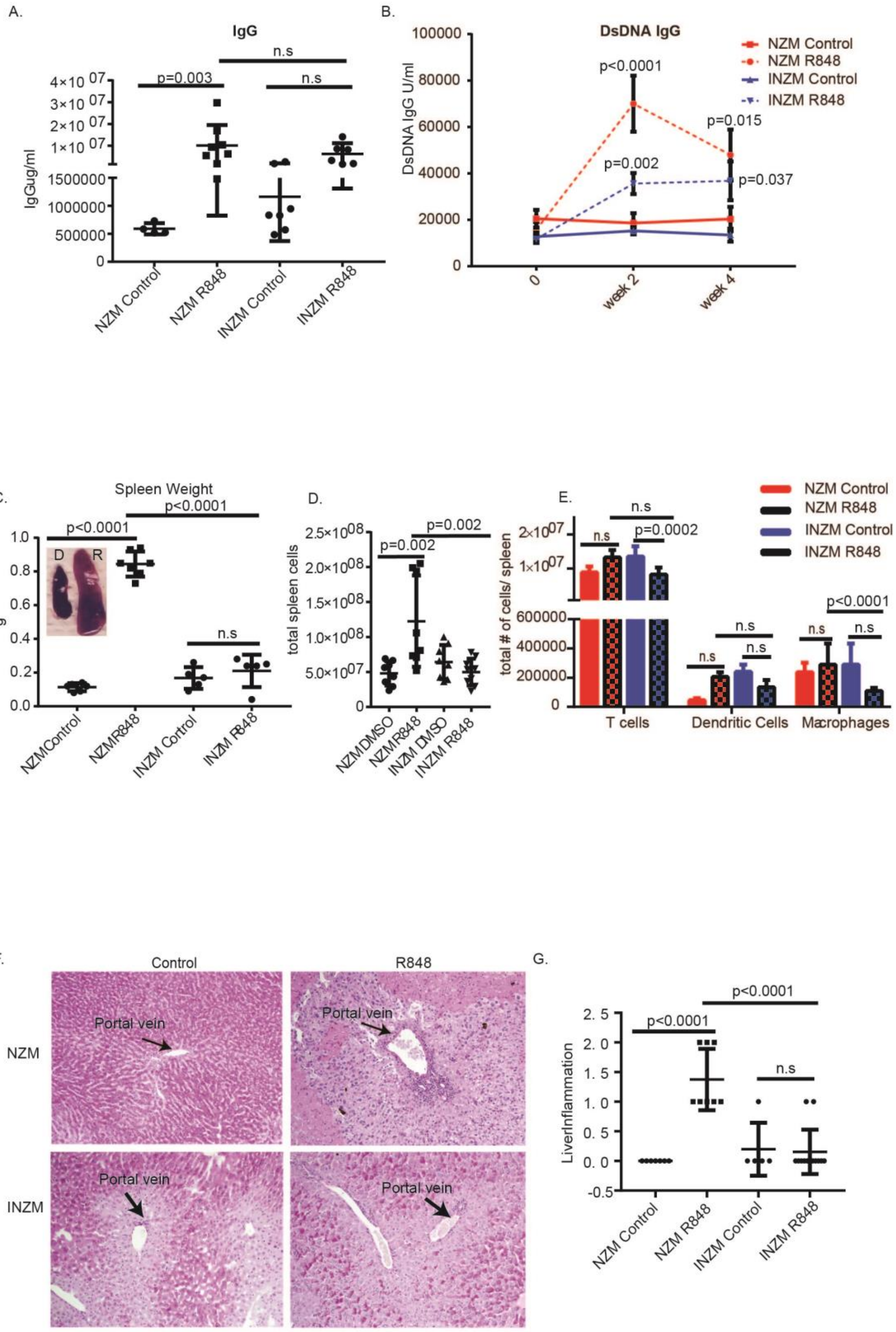


Figure 3.1 Epicutaneous TLR 7 stimulation leads to type I IFN-independent accelerated decline in survival in lupus-prone mice.

10-week-old NZM2328 and INZM mice were treated with 100µg of the TLR7 agonist R848 or control (DMSO) three times weekly. Survival curve for NZM2328 and INZM mice is shown. n=12 each for DMSO and R848 treated NZM mice. n=11 for DMSO and n=12 for R848 treated INZM mice. The p value in blue shows the difference in the survival curves between INZM DMSO vs. INZM R848 treated mice. The p value in red shows the difference in the survival curves between NZM DMSO vs. NZM R848 treated mice. The p value in black shows the difference in the survival curves between NZM R848 and INZM R848 treated mice.

TLR7 epicutaneous stimulation leads to accelerated development of murine lupus.

We next examined the development of systemic lupus characteristics following R848 stimulation. Using our assay, dsDNA antibodies typically rise to about 100,000U/ml during nephritis onset in NZM 2328 mice [215]. Importantly, while only NZM2328 treated with R848 demonstrated significant acceleration of total IgG production, both NZM2328 and INZM mice demonstrated increased dsDNA antibodies in the serum (Figure 3.2A and B); however, the rise dsDNA antibodies in the serum was greater in NZM vs. INZM mice (p=.0167 NZM vs. INZM at 2 weeks). This suggests that autoantibody production following TLR7 epicutaneous stimulation is enhanced by but not dependent on type I IFN signaling Consistent with previous literature [141, 230], splenomegaly was detected following R848 stimulation in NZM2328 but not INZM mice, supporting a role for IFNs in TLR7-driven splenomegaly (Figure 3.2C). An increase in total splenic cells following R848 stimulation was detected in only NZM2328 mice; analysis of cell subsets identified no significant changes in T cells, dendritic cells, or macrophages (Figure 3.2D and E). Further, NZM2328 mice treated with R848 demonstrated accelerated development of liver inflammation as indicated by necrotic hepatocytes and focal portal inflammation. INZM mice treated with R848 did not develop liver inflammation, indicating that type I IFNs are required for this manifestation following TLR7 activation (Figure 3.2 F and G).



A.
IgG
n.s
p=0.003
n.s
IgGug/ml
NZM Control
NZM R848
INZM Control
INZM R848
B.
DsDNA IgG
NZM Control
NZM R848
INZM Control
INZM R848
p<0.0001
p=0.015
p=0.002
p=0.037
DsDNA IgG U/ml
0
week 2
week 4
C.
Spleen Weight
p<0.0001
p<0.0001
D
R
n.s
g
NZMControl
NZMR848
INZM Control
INZM R848
D.
p=0.002
p=0.002
total spleen cells
NZMDMSO
NZMR848
INZM DMSO
INZM R848
E.
n.s
n.s
p=0.0002
total # of cells/ spleen
NZM Control
NZM R848
INZM Control
INZM R848
p<0.0001
T cells
Dendritic Cells
Macrophages
F.
Control
R848
NZM
INZM
Portal vein
G.
p<0.0001
p<0.0001
n.s
LiverInflammation
NZM Control
NZM R848
INZM Control
INZM R848

Figure 3.2 TLR 7 stimulation leads to IFN-independent elevated autoantibody production and IFN dependent splenomegaly and liver inflammation.

10 week-old NZM2328 and INZM mice treated with R848 or DMSO control were analyzed. A. Total IgG in serum of NZM2328 and INZM mice after 2 weeks of treatment. Each dot represents an individual mouse. B. dsDNA IgG in serum at 0 weeks, 2 weeks, and 4 weeks of treatment. n=13 NZM R848; n=14 NZM DMSO; n= 9 INZM DMSO; n=11 INZM R848 C. Spleen weight of NZM2328 and INZM mice from survival studies. Spleens were harvested when mice were moribund (around 20-40 days of treatment). Littermate DMSO controls were harvested at the same times as the moribund mice. Each dot represents an individual mouse. Representative photographs of DMSO and R848 NZM2328 spleens shown in inset. D. Immune cell populations in the spleen were evaluated by flow cytometry after 2 weeks of R848 or DMSO treatment. n=13 NZM R848; n=11 NZM control; n=8 INZM control; n=9 INZM R848. (E and F) 10 week- old NZM2328 and INZM mice treated with R848 or DMSO control were treated until moribund and analyzed for development of liver inflammation. E. Representative photo of the portal vein. F. Graph represents liver inflammation scoring of NZM2328 and INZM mice. Each symbol represents one mouse.

We next examined changes in B cells and antibody (Ab)-secreting cells in the spleen and lymph nodes. As shown in Figure 3, both total B cells (B220+) and Ab-secreting cells (IgH+L^{high}) increased in the spleen of NZM2328 mice but only Ab-secreting cells increased in INZM mice post R848 stimulation (Figure 3A-C), suggesting that amplification of splenic antibody production is independent of type I IFNs. We then examined the lymph node populations to determine if IFN-independent B cell activation was also occurring there. As shown in Figure 3D, stimulation with R848 resulted in increased total number of cells in the cervical (draining) lymph nodes in both NZM2328 and INZM mice. Contrarily, increased cell numbers were noted in inguinal (non-draining) lymph nodes in only NZM mice after R848 treatment. The total number of B cells ($B220^{+}$) in both NZM2328 and INZM were increased in draining but not the non-draining lymph nodes following R848 stimulation (Figure 3E). NZM2328 showed an increase in the secreting cell population in the draining and non-draining lymph node, but INZM only demonstrated an increase in the draining lymph node (Figure 3F). Of note, the overall numbers of B cells and Ab-secreting cells were significantly fewer in the INZM mice. These data indicate that TLR7 stimulation amplifies local B cell and Ab production responses in the absence of type I IFN signaling, but the responses may be diminished. However, systemic amplification of Ab-secreting cells in the non-draining lymph nodes required the presence of type I IFN signaling. Together, this suggests that the accelerated autoantibody production seen post TLR7 cutaneous stimulation is partially type I IFN-independent and that the Ab-secreting cells in the dLN and spleen may serve as a site for autoantibody production in this model.

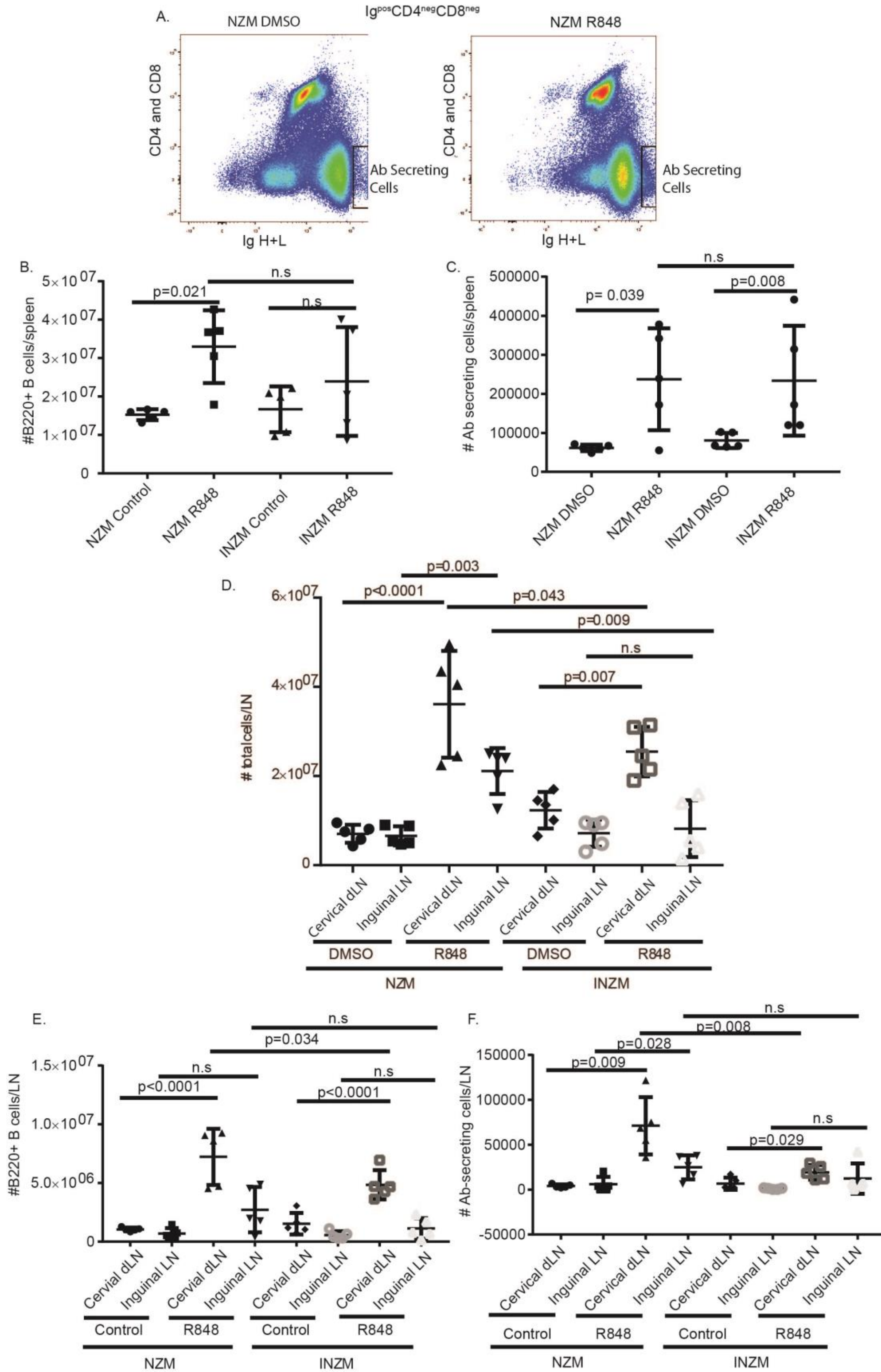
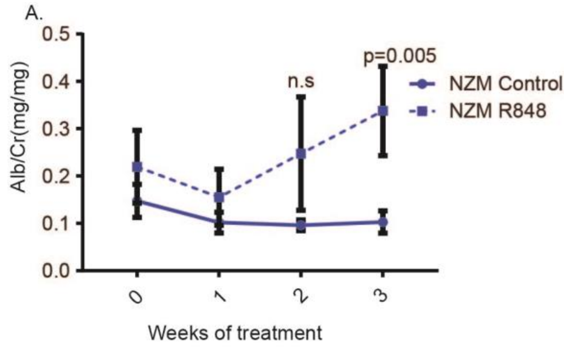

A.
NZM DMSO
Ig^pos CD4^neg CD8^neg
NZM R848
CD4 and CD8
Ab Secreting Cells
Ig H+L
B.
#B220+ B cells/spleen
p=0.021
n.s
NZM Control
NZM R848
INZM Control
INZM R848
C.
# Ab secreting cells/spleen
p= 0.039
p=0.008
NZM DMSO
NZM R848
INZM DMSO
INZM R848
D.
total cells/LN
p<0.0001
p=0.003
p=0.043
p=0.009
p=0.007
Cervical dLN
Inguinal LN
DMSO
R848
NZM
INZM
E.
#B220+ B cells/LN
p=0.034
p<0.0001
Cervial dLN
Control
F.
# Ab-secreting cells/LN
p=0.009
p=0.028
p=0.008
p=0.029

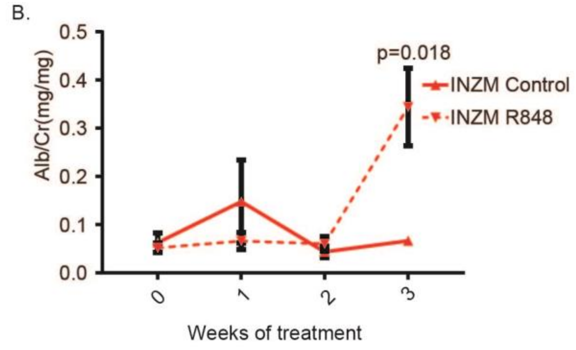
Figure 3.3 TLR 7 stimulation leads to IFN-independent increases in secreting cells in the draining lymph nodes and the spleen.

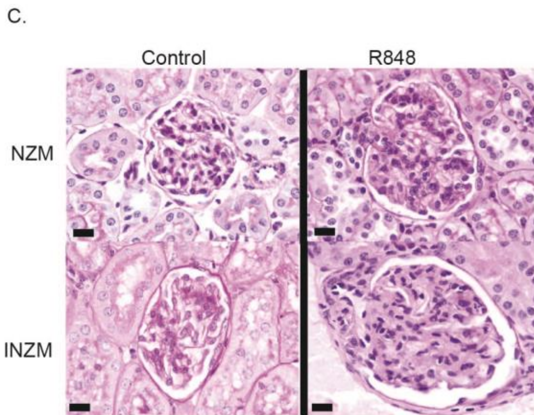
Immune cell populations in the spleen were evaluated by flow cytometry after 2 weeks of R848 or DMSO treatment. n= 5 NZM DMSO; n= 5 NZM R848; n=5 INZM DMSO; n=5 INZM R848. A. Gating strategy for Ab-secreting cells. B, C. Graphs displaying changes in B cells: $CD4^{-}CD8^{-}$ $IgH^{+}IgL^{+}B220^{+}$ (shown in B) and Ab-secreting cells: $CD4^{-}CD8^{-}$ $IgH^{+}IgL^{high}$ (shown in C). D. Total number of cells isolated from indicated lymph nodes for DMSO or R848 treated mice. E, F. Graphs displaying changes in B cells: $CD4^{-}CD8^{-}IgH^{+}IgL^{+}B220^{+}$ (shown in E) and Ab-secreting cells: $CD4^{-}CD8^{-}$ $IgH^{+}IgL^{high}$ (shown in F) for draining (cervical) and non-draining (inguinal) LN. Data is displayed as mean ± SD. Each symbol represents one mouse. LN=lymph node.

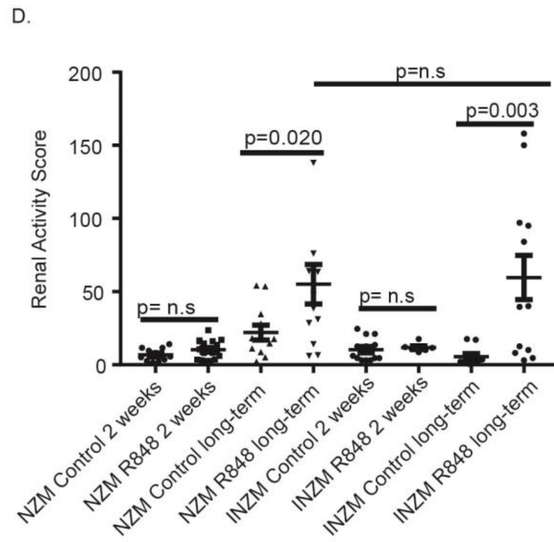
TLR7 epicutaneous stimulation leads to accelerated development of lupus nephritis in a type I IFN-independent manner

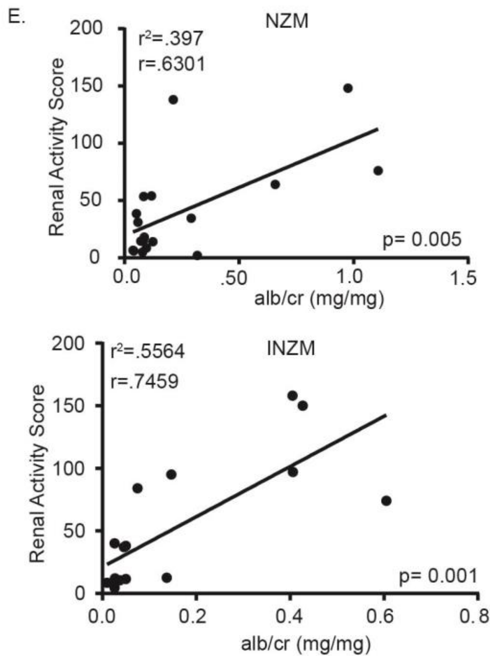
Past work has shown type I IFNs are sufficient to induce renal flares in lupus-prone mice and are required for lupus nephritis development in several murine models [140, 141, 231, 232]. We next examined whether TLR7 epicutaneous stimulation led to accelerated lupus nephritis development and whether it was dependent on type I IFNs. Surprisingly, both NZM2328 and INZM mice treated with R848 demonstrated a rise in urinary albumin:Cr ratio supportive of glomerular damage (Figure 3.4A and B). Histopathologic scoring demonstrated a significant increase in renal activity score when mice were treated with R848 until moribund (Figure 3.4C and D). A strong positive correlation was detected between renal activity score and urinary alb/cr ratio for both NZM2328 and INZM mice (Figure 3.4E), which supports renal inflammation in R848 treated mice. No significant increase in renal chronicity index score for prolonged exposure in NZM2328 and INZM mice was detected (Figure 3.4F). As immune complex deposition is a hallmark of lupus nephritis, this was also assessed. R848 treatment of the NZM2328 and INZM mice led to a significant increase in both IgG and C3 deposition within the kidney (Figure 3.5 A-C). In order to examine whether TLR7-induced lupus created similar transcriptional changes to naturally occurring lupus nephritis found in older, untreated NZM 2328 mice, we examined transcriptional signatures of the kidneys of both. As shown in Figure 3.6, identical upregulation of various inflammatory and type-I IFN associated genes were noted in both R848-induced and naturally occurring nephritis. Together, these parameters support development of accelerated lupus nephritis following cutaneous stimulation with a TLR7 agonist in a type I interferon independent manner.


A.
Alb/Cr(mg/mg)
0.5
0.4
0.3
0.2
0.1
0.0
n.s
p=0.005
NZM Control
NZM R848
0
1
2
3
Weeks of treatment


B.
Alb/Cr(mg/mg)
0.5
0.4
0.3
0.2
0.1
0.0
p=0.018
INZM Control
INZM R848
0
1
2
3
Weeks of treatment


C.
Control
R848
NZM
INZM


D.
Renal Activity Score
200
150
100
50
0
p=n.s
p=0.003
p=0.020
p= n.s
p= n.s
NZM Control 2 weeks
NZM R848 2 weeks
NZM Control long-term
NZM R848 long-term
INZM Control 2 weeks
INZM R848 2 weeks
INZM Control long-term
INZM R848 long-term


E.
NZM
r²=.397
r=.6301
Renal Activity Score
200
150
100
50
0
p= 0.005
0.0
.50
1.0
1.5
alb/cr (mg/mg)
INZM
r²=.5564
r=.7459
Renal Activity Score
200
150
100
50
0
p= 0.001
0.0
0.2
0.4
0.6
0.8
alb/cr (mg/mg)

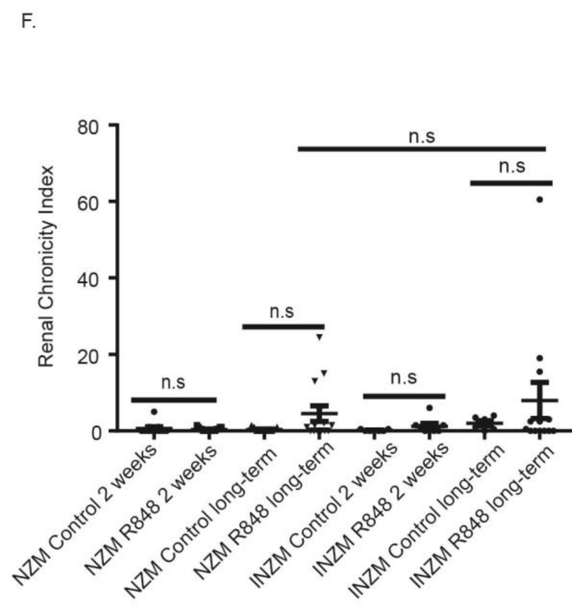

F.
Renal Chronicity Index
80
60
40
20
0
n.s
n.s
n.s
n.s
n.s
NZM Control 2 weeks
NZM R848 2 weeks
NZM Control long-term
NZM R848 long-term
INZM Control 2 weeks
INZM R848 2 weeks
INZM Control long-term
INZM R848 long-term

Figure 3.4 TLR 7- mediated lupus nephritis occurs in an interferon-independent manner.

10 week- old NZM2328 and INZM mice treated with R848 or DMSO control were analyzed for development of lupus nephritis. A. Urine alb/cr ratio was measured serially in NZM2328 treated mice n=12 NZM R848; n=15 NZM DMSO B. Urine alb/cr ratio was measured serially in INZM treated mice. n=5 INZM DMSO; n=6 INZM R848 C. Representative photo of the glomeruli in the kidney of NZM2328 and INZM mice following treatment until moribund. Littermate control mice were harvested at the time of illness in R848 treated mice. Scale bar equals 20um. D. Renal activity score for NZM2328 and INZM mice after 2 weeks of treatment or when moribund from R848 treatment (long-term treatment). E. The moribund renal activity score for NZM2328 and INZM mice treated with R848 and DMSO was plotted versus the alb/cr ratio at euthanasia and analyzed via Pearson correlation. F. Renal Chronicity index for NZM2328 and INZM mice after 2 weeks of treatment and when moribund (long-term treatment).

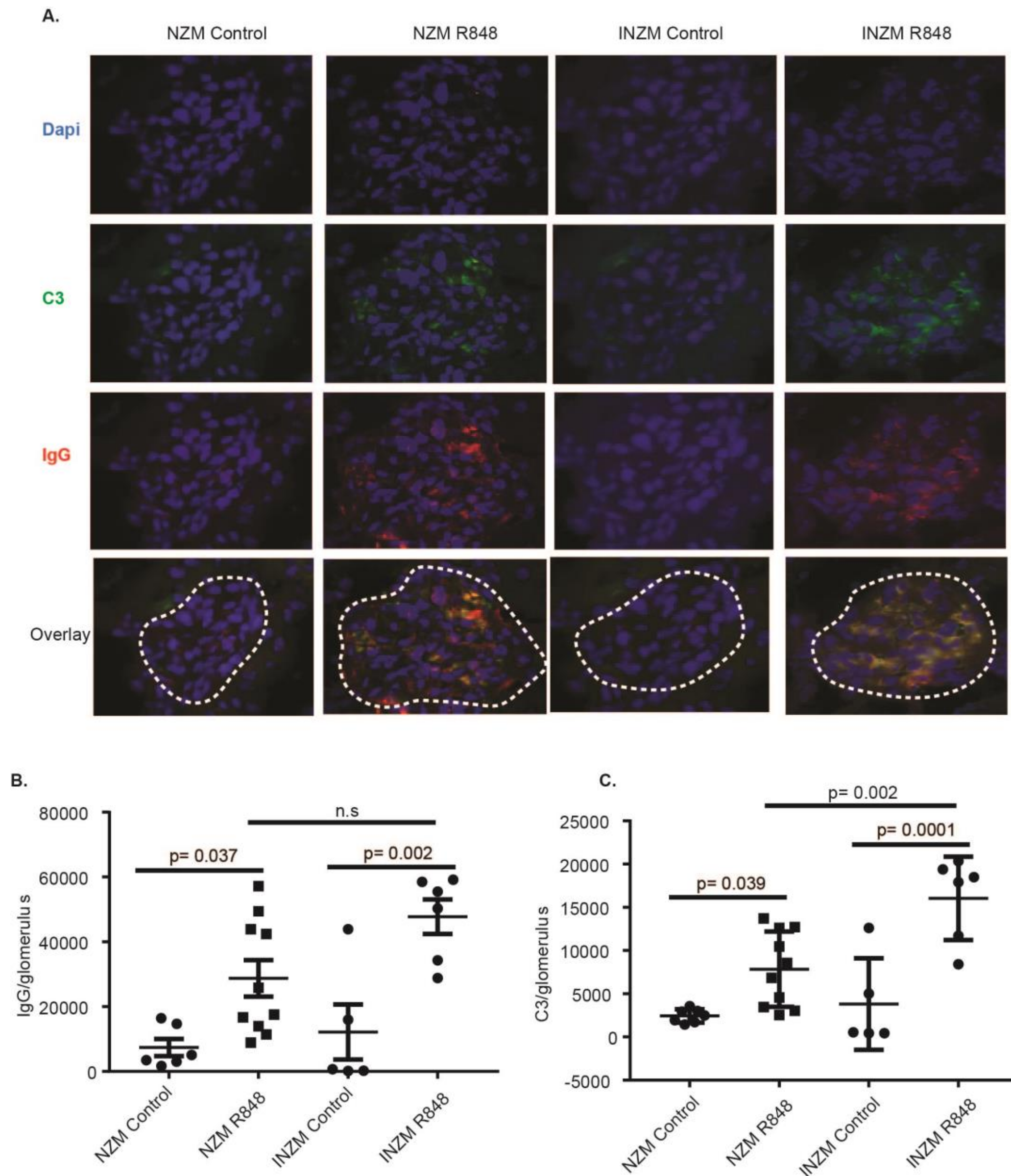


Figure 3.5 Immune complex deposition in the kidney is interferon-independent.

10 week- old NZM2328 and INZM mice treated with R848 or DMSO until moribund and were analyzed for immune complex deposition. A. Representative immunofluorescence microscopy of glomeruli (outlined by white dashed line). Texas Red- IgG, Green- C3, Blue- DAPI. (B-C). Quantification of immune complex staining/area was completed. Littermate DMSO controls were harvested when littermates were ill. B. Quantification of IgG/area. C. Quantification of C3/area. Each dot represents the average fluorescence of 8 glomeruli from a single mouse.

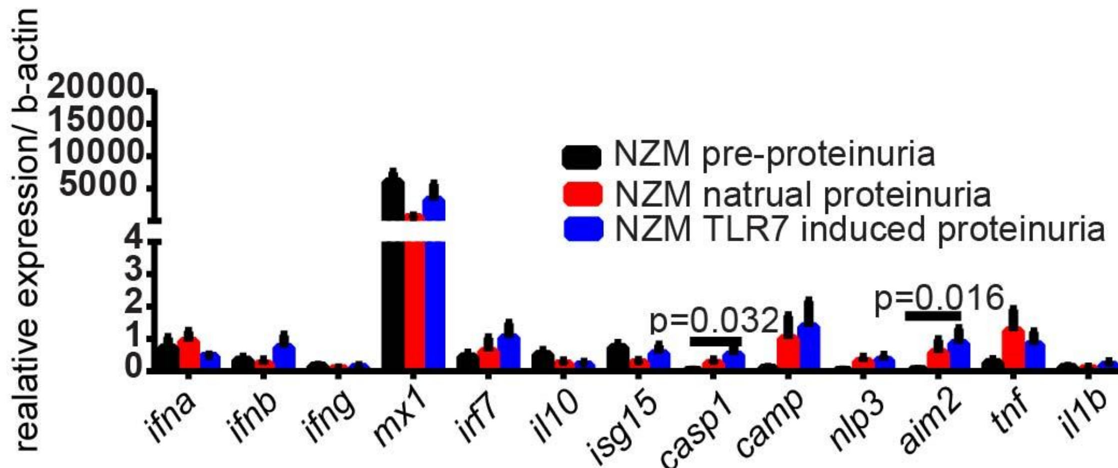


Figure 3.6 Changes in inflammatory response genes in TLR7-induced nephritis vs. natural nephritis

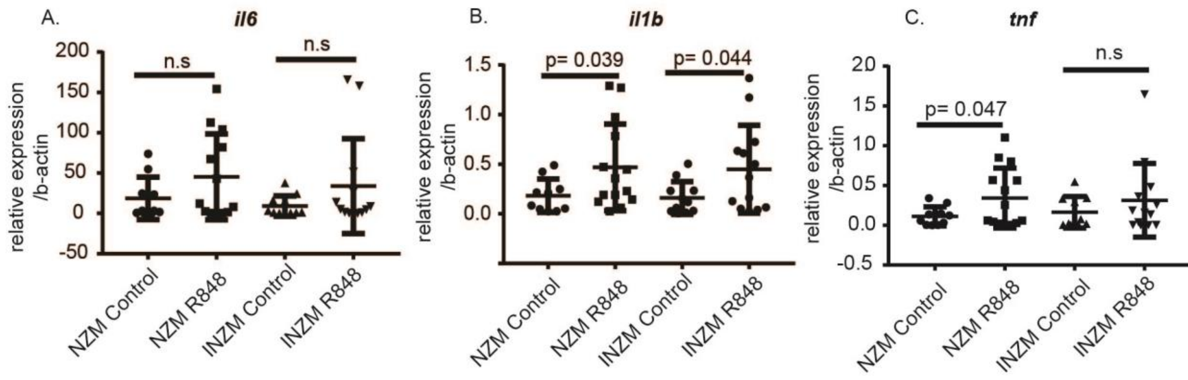
RNA was isolated from the kidney of 10 weeks-old NZM2328 mice stimulated with R848 until proteinuria(4+ by dipstick) development, from NZM2328 mice that naturally developed proteinuria (4+ by dipstick) at around 35 weeks of age, and NZM2328 mice that were pre-proteinuric (trace by dipstick). Real- time PCR was completed for analysis of the genes listed. Graphs display the mean+ SD for each gene as compared to the average of β-actin. n=4 for R848 treated, n=5 for aged NZM mice and n=5 for NZM pre-proteinurc mice. $p<0.05$ is considered significant via unpaired T test.

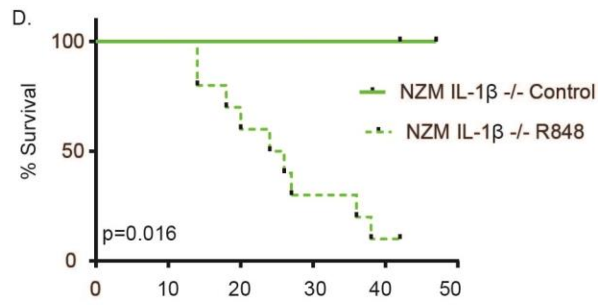
TLR7 epicutaneous stimulation leads to upregulation of IL-1β, but nephritis is not dependent on IL-1β.

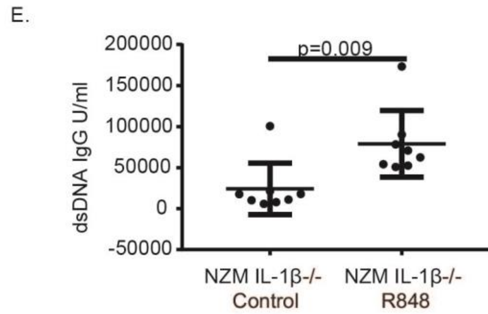
As TLR7-mediated lupus nephritis developed in the absence of type I IFN signaling, we next assessed what other pathways may play a role in TLR7-mediated lupus nephritis. We chose to examine NFκB-regulated cytokines as this transcription factor is also activated downstream of TLR7 with the hypothesis that cytokines upregulated in the kidney in both NZM and INZM mice may be important in TLR7-mediated nephritis. We examined mRNA changes in the kidney of INZM and NZM mice treated for 2 weeks with R848 or DMSO control. Following stimulation, there were significant changes in the expression of NFκB-regulated cytokines *tnf,* and *il1b*, (Fig.3.7A-C) in the NZM mice treated with R848 vs. control, but there was only a significant upregulation of *il1b* in INZM mice treated with R848 vs. DMSO (Fig.3.7B). These data support a potential common role for IL-1β in TLR7 mediated lupus nephritis flare in NZM and INZM mice.

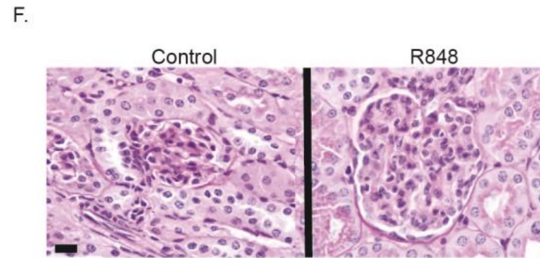
To examine the role of IL-1β in TLR7-mediated nephritis, we generated NZM2328 mice that lack IL-1β (Figure 2.1) and treated them epicutaneously with R848 or DMSO. These mice also exhibited a decline in survival (Figure 3.7D), increase in dsDNA IgG antibodies (Figure 3.7E) and development of lupus nephritis (Figure 3.7F-H). These data indicate that like type I IFNs, IL-1β is not required for TLR7-mediated nephritis.

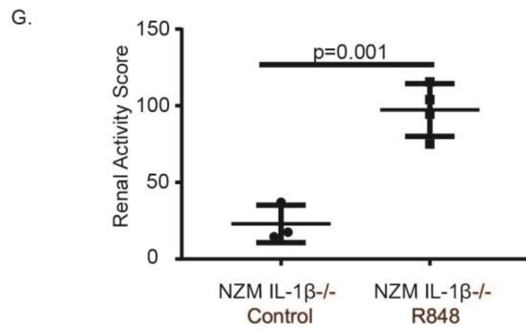
NFkB cytokines











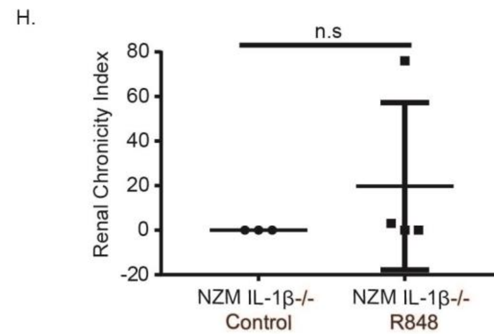


Figure 3.7 TLR 7- mediated upregulation of IL-1β is not required for lupus nephritis.

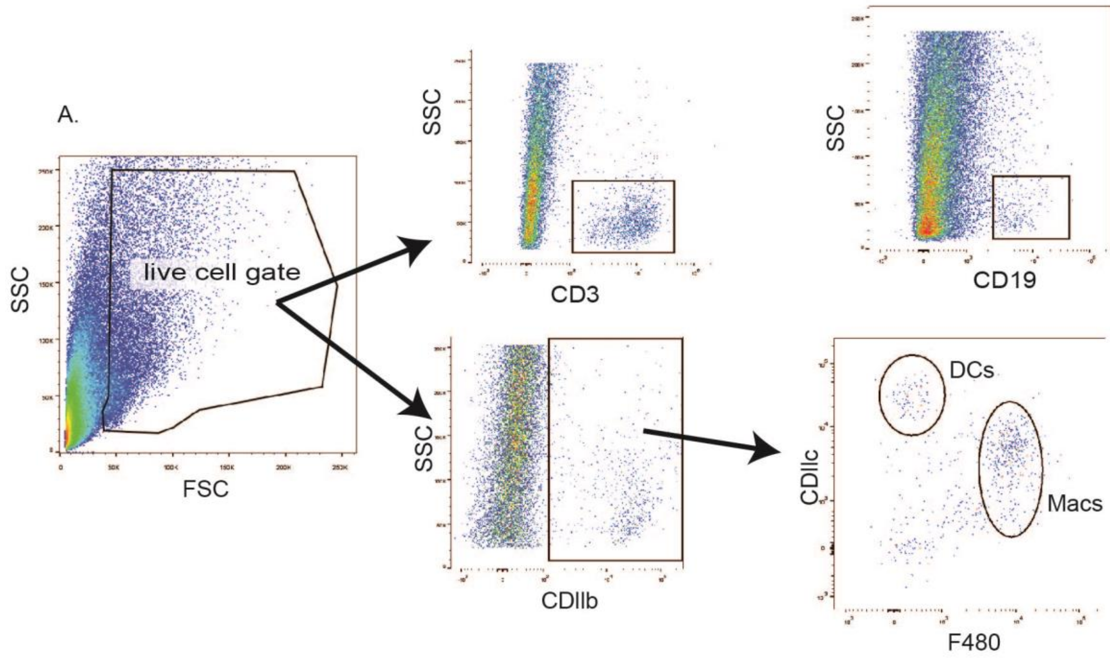
A-C. RNA was isolated from the kidney of NZM or INZM mice treated for two weeks with DMSO control or R848. Real-time PCR was completed using primers for the genes listed. Graphs display the mean± SD for each gene as compared to the average of b-actin. Each dot represents an individual mouse. D.10 week- old NZM IL-1β KO mice were treated with R848 or DMSO control and survival was plotted. n=9 NZM IL-1β -/- DMSO; n= 8 NZM IL-1β -/- R848 E. Anti-dsDNA IgG in serum at 2 weeks of treatment. n=8 NZM IL-1β -/- R848; n=8 NZM IL-1β -/- DMSO. F. Representative photomicrograph of the glomeruli in the kidney of moribund NZM IL-1β -/- mice treated with R848 or DMSO. Littermate controls were harvested when R848 treated mice were moribund. Scale bar equals 20um. G. Renal activity score for NZM IL-1β-/- mice when moribund. H. Renal chronicity index for mice in G.

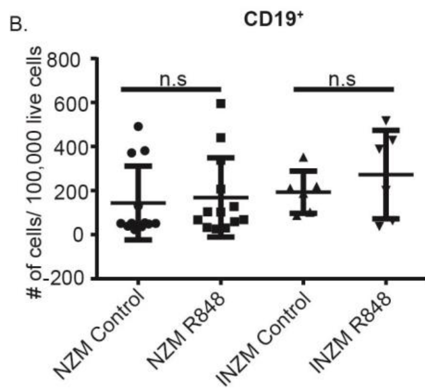
TLR7 epicutaneous stimulation leads to early infiltration of dendritic cells and upregulation of ccl2

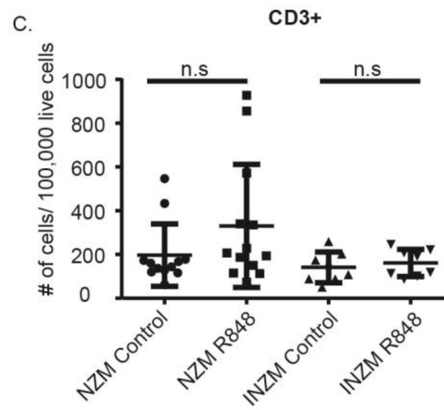
Previous work has identified important roles for macrophage and dendritic cell infiltration into the kidney prior to nephritis onset[215, 231]. We thus examined inflammatory cell recruitment into the kidney prior to lupus nephritis onset in order to determine the earliest cellular contributors to TLR7-induced nephritis. Two weeks after R848 or DMSO treatment, prior to proteinuria onset, kidneys were removed, digested and pre/early-nephritic renal immune cell populations were examined by flow cytometry. (T cell, macrophage, and dendritic cell population gating strategies are shown in Fig. 3.8A). Consistent with previous data[215], we did not see a change in the T cell ($CD3^+$) and B cell ($CD19^+$) population prior to proteinuria onset (Fig.3.8B and C). We also did not see changes in the macrophage ($CDllb^+CDllc^{int}$ $F4/80^+$) population at this time point (Fig.3.8D). However, we detected a significant infiltration of the dendritic cell population ($CDllb^+$ $CDllc^+$ $F4/80^-$) in both the NZM and INZM mice treated with R848 but not DMSO (Fig.3.8E). The number of infiltrating cells was overall fewer in INZM mice, but this was not statistically different between NZM and INZM mice ($p=0.2391$ by unpaired student's t-test). This suggests that TLR7 activation promotes early infiltration of dendritic cells into the kidney, similar to what has been observed in other genetic models of murine lupus [224], but in an interferon-independent fashion.

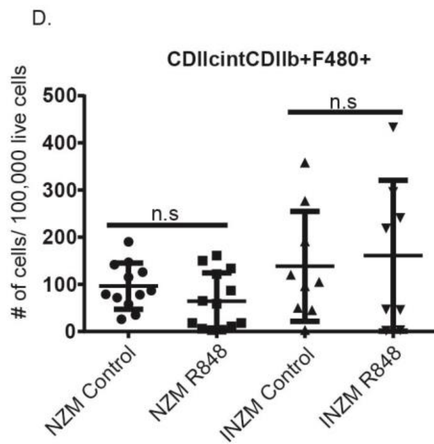
To identify potential mechanisms of chemotaxis into the kidney, we examined chemokine expression in the kidney following DMSO or R848 treatment with the hypothesis that relevant chemokines should rise in both NZM and INZM mice with R848 treatment. As shown in Figure 3.9, there were no changes in *ccl4* (MIP-2) or *cxcl13* expression in either strain following R848 treatment (Figure 3.9A, B). *ccl5* expression increased in NZM2328 but not INZM mice after

R848 exposure (Figure 3.9C). IP-10 (*cxcl10*) expression also increased only NZM2328 mice after R848 exposure (Figure 3.9D). Importantly, upregulation of *ccl2* (MCP-1), a chemokine that rises during lupus nephritis[233] and whose receptor (CCR2) is expressed on monocytes, macrophages, and dendritic cells[85, 234], was demonstrated to increase in both NZM and INZM mice after R848 treatment (Figure 3.9E). These data suggest TLR7-mediated upregulation of *ccl2* may serve as a potential trigger for induction of inflammatory cell infiltration in an interferon-dependent manner.


A.
SSC
FSC
live cell gate
SSC
CD3
SSC
CD19
SSC
CDllb
CDllc
F480
DCs
Macs


B.
CD19+
# of cells/ 100,000 live cells
n.s
n.s
NZM Control
NZM R848
INZM Control
INZM R848


C.
CD3+
# of cells/ 100,000 live cells
n.s
n.s
NZM Control
NZM R848
INZM Control
INZM R848


D.
CDllcintCDllb+F480+
# of cells/ 100,000 live cells
n.s
n.s
NZM Control
NZM R848
INZM Control
INZM R848

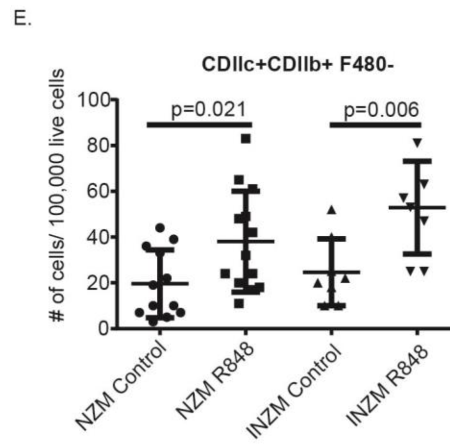

E.
CDllc+CDllb+ F480-
# of cells/ 100,000 live cells
p=0.021
p=0.006
NZM Control
NZM R848
INZM Control
INZM R848

Figure 3.8 Early dendritic cell infiltration is a common feature of TLR7 driven nephritis in NZM and INZM mice.

Immune cell population changes in the kidney of NZM and INZM mice following 2 weeks of R848 or DMSO treatment. A. Gating strategy for immune cell populations. B-E. Graphs displaying changes in T cells (B): CD3+, B cells (C): CD19+, Macrophages (D): CDllci^{nt}CDllb^{+}F480^{+}, and Dendritic Cells (E): CDllc^{+}CDllb^{+}F480^{-}. n=12 NZM DMSO; n=13 NZM R848; n=8 INZM DMSO; n=8 INZM R848. Data is displayed as mean ± SD.

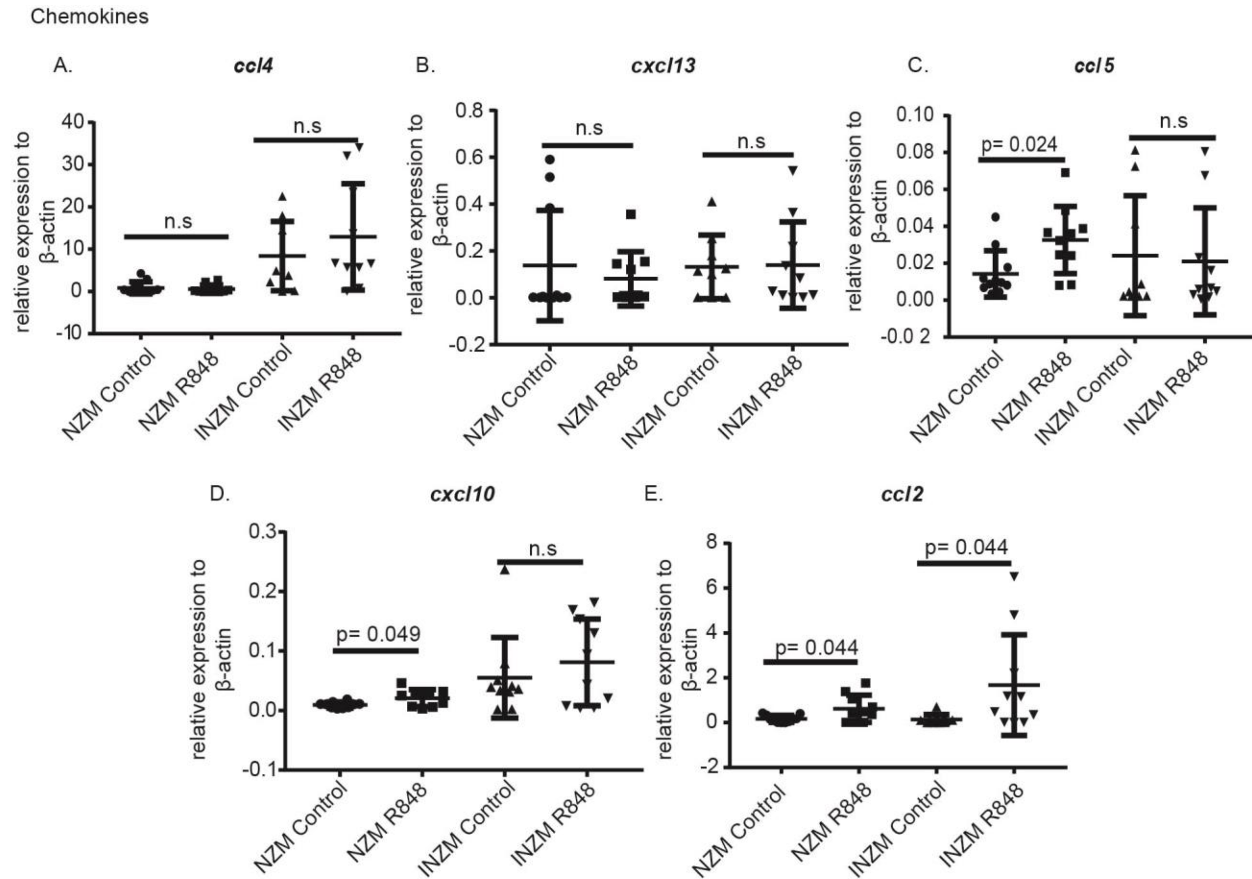


Figure 3.9 CCL2 is upregulated in NZM and INZM mice after TLR7 stimulation

NZM and INZM mice were treated with R848 or DMSO for two weeks followed by harvest of RNA from the kidney. Real-time PCR was completed for the genes listed. Graphs display the mean $\pm$ SD for each gene as compared to the average of β-actin. Each dot represents a single mouse.

3.4 Discussion

In this paper, we examine a novel model of lupus flare in which lupus nephritis is induced in genetically-prone mice after three-four weeks of epicutaneous application of a TLR7 agonist, R848. R848 treatment led to accelerated development of autoantibodies, splenomegaly, liver inflammation, and lupus nephritis. With the use of NZM mice lacking the type I IFN receptor, we demonstrated that TLR7-mediated splenomegaly and liver inflammation were dependent on type I IFN signaling. Surprisingly, however, renal injury was independent of type I IFNs. Indeed, INZM mice demonstrated proteinuria, increased renal immune complex formation, upregulation of NFκB regulated cytokines, and infiltration of $CDllb^{+}CDllc^{+}F4/80^{low}$ DCs in the kidney similar to NZM mice.

Human genetics support a role for TLR7 in lupus [44, 220], and murine data has further supported this[9, 10,174]. BXSB male mice develop lupus secondary to the Yaa locus that contains a duplication of the TLR7 gene [206]. The role of TLR 7 has also been demonstrated in transgenic mice overexpressing TLR 7[168] and in lupus inducible models such as the pristine model [224]. A recent paper also demonstrated that TLR7 epicutaneous stimulation of wild type mice led to development of mild lupus characteristics after long-term (13 to 15 weeks) treatment[174]. Our model furthers these observations and demonstrates that TLR7 stimulation rapidly (in 3-4 weeks) accelerates lupus development in young, lupus-prone mice. Further, we show that mice that are otherwise protected from lupus development in the absence of functional IFN signaling [141] are similarly susceptible to the nephritis-inducing effects of TLR7. Others have shown that overexpression of IFNα is sufficient to stimulate lupus nephritis in a similar time frame to TLR7 treatment [140]. However, in the presence of TLR7 agonist, type I IFN signaling is not required.

This observation may reflect duplicative roles of other inflammatory cytokines induced by TLR7.

Recently, the role of type I IFN has been explored in other murine models of lupus. Treatment of mice with $HgCl_2$ is able to induce autoantibody production in a lysosomal TLR-dependent but type I IFN independent fashion[235], similar to the data we see in the R848 NZM model. Development of renal disease and direct contribution of TLR7 in this xenobiotic model were not assessed[235]. In a different model, exacerbation of autoimmunity via deletion of TLR9 in the MRL/*lpr* mouse was found to be type I IFN dependent[236]. In this model, IFNs are required for development of renal disease but not anti-nucleosome antibody production. HepG-2 positive ANA antibodies and anti-RNA antibodies were dependent on type I IFN signaling[236]. Combined with our findings, these data suggest that the role for IFNs in lupus development may vary depending on the stimuli. Autoantibody production may be independent of IFNs when the trigger is strong environmental exposure (such as R848 or $HgCl_2$). Conversely, lupus that develops based on genetic factors without the need for external triggers (such as TLR9-deficient MRL/*lpr* or NZM2328 mice) may require IFN signaling for autoantibody and nephritis development.

NFκB activation occurs downstream of TLR7 stimulation and activation of this pathway is important for stimulation of lupus nephritis [237]. Thus, TLR7 activation of NFκB pathways may be a critical step in driving lupus flares. Mutations in A20, which drive NFκB activation, in WT mice led to lupus nephritis [238, 239] possibly through decreased regulation of inflammasome activity [240]. Though roles for TNF alpha and IL-6 in lupus development have been suggested [241], and IL-6 can exacerbate TLR7-driven lupus[242], we do not see these cytokines significantly upregulated in the kidney in a, interferon-independent manner in our TLR 7 stimulation model.

Intriguingly, we see an upregulation of IL-1β in the kidney prior to the onset of lupus nephritis in both NZM2328 and INZM mice. This finding coincides with data that support a potential role for IL-1β and inflammasome activation in lupus development (reviewed in [243]). However, deletion of IL-1β in NZM2328 mice did not reduce the development of lupus nephritis after TLR7 exposure. Given that TLR7 activates pleotropic pathways, generation of double or triple knockouts may be required to hinder lupus nephritis development in the presence of such a strong inflammatory activator.

Similar to others[141], we found that splenomegaly following TLR7 stimulation was type I IFN dependent. Interestingly, the massive R848-induced splenomegaly was out of proportion to the small increases in B and T cell populations in the spleen that were identified by flow. This supports a possible role for extra medullary hematopoiesis resulting in R848-mediated splenomegaly, consistent with findings by other groups [244, 245].

TLR7 epicutaneous stimulation can lead to development of autoantibody production in the absence of type I IFN signaling. We were able to see an increase in dsDNA antibodies in NZM2328 and INZM mice following R848 stimulation, although the increase in anti-dsDNA levels was less robust in INZM mice. Increases in B (B220+) cell numbers in the spleen were noted in only the NZM mice, which suggests that expansion of B cell populations in the spleen may require type I IFNs following TLR7 exposure. This would be consistent with a recently described role for splenic follicular dendritic cell production of type I IFN in promotion of autoreactive B cell populations [246, 247]. Type I IFNs also enhance the TLR7 signaling response, allowing for autoantibody production[248], and they support survival of transitional stage B cells in the spleen[249]. Thus, the more extensive systemic immune activation seen in NZM mice may be secondary to these effects of type I IFN. Overexpression of TLR7 stimulation has also been

shown to led to expansion of transitional stage B cell populations in a type I IFN independent manner[250]. Given that we see a rise in Ab-secreting cells in the spleen and dLN but not in the non-draining LN of INZM mice, this may indicate that a certain threshold of TLR7 stimulation, and possibly other cytokines, is needed to generate secreting cell expansion in a type I IFN independent manner.

TLR7 stimulation results in the recruitment of $CD11b^{+}CD11c^{+}F4/80^{-}$ DCs in the kidney prior to proteinuria onset without significant changes in the T cell or B cell population. Interestingly, this recruitment occurs in a type I IFN-independent manner. The DC chemoattractant MCP-1/CCL2 was increased in the kidney in both NZM and INZM mice, which supports its potential role for instigating nephritis downstream of TLR7 activation. Intriguingly, *CCL2* has been shown to rise during human lupus nephritis development[233] and is proposed as a urinary biomarker for disease[251]. Blockade of CCL2 demonstrates some efficacy in murine lupus models[252]. Macrophages, which also are recruited by CCL2, were not increased in the kidney after R848 treatment, which may reflect differential upregulation of CCL2's receptor, CCR2, on DCs and macrophages in this model. Overall, consideration of the role of CCL2 downstream of TLR7-driven lupus should be made in future studies.

In summary, we have demonstrated a novel role for TLR7 epicutaneous stimulation in mediating lupus flare in lupus-prone mice. Following stimulation, splenomegaly and liver inflammation occur in a type I IFN-dependent manner. Importantly, autoantibody production and lupus nephritis occur independent of IFN signaling. We demonstrate that IL-1β is not required for development of TLR7-activated lupus nephritis. Future studies should address the role of CCL2 in TLR7-mediated lupus nephritis. In addition, our data lend a note of caution to ongoing trials utilizing type I IFN blockade in lupus nephritis: consideration of the upstream

drivers of nephritis (which may vary in individual patients) may be important for identifying effective treatment modalities. In particular, our data would suggest that blockade of type I IFN signaling my not be effective if TLR7 is driving the phenotype

Chapter 4 UVB induces prolonged T cell activation in lupus-prone mice via type I IFN inhibition of T regulatory cells

This work was a collaboration with Shannon N. Estadt, Jonathan Theros, Tyson Moore, Jason Ellis, Jianhua Liu, Tamra J. Reed, Chaim O. Jacob, Johann E. Gudjonsson, J. Michelle Kahlenberg. Shannon N. Estadt, Jonathan Theros, Tyson Moore, Jason Ellis, Jianhua Liu, and Tamra J. Reed assisted with experiments. Chaim O. Jacob supplied the NZM2328 and iNZM mice. J. Michelle Kahlenberg assisted with data analysis and all collaborated helped with editing the manuscript. The data from this chapter was submitted to the *Journal of Autoimmunity* at the time of defense.

4.1 Abstract

Objective

Ultraviolet (UV) light is a known trigger of skin and possibly systemic inflammation in systemic lupus erythematosus (SLE) patients. Although type I interferons (IFN) are upregulated in SLE skin after UV exposure, the mechanisms to explain increased UVB-induced inflammation remain unclear. This paper compares the role of type I IFNs in regulating immune cell activation between wild-type and lupus-prone mice following UVB exposure.

Methods

10-week old female lupus-prone (NZM2328), wild-type (BALB/c) and iNZM mice (lack a functional type I IFN receptor on NZM2328 background) were treated on their dorsal skin with 100mJ/cm^2 of UVB for 5 consecutive days. Following UVB treatment, draining lymph node cell

populations were characterized via flow cytometry and suppression assays; treated skin was examined for changes in expression of type I IFN genes.

Results

Only NZM2328 mice showed an increase in T cell numbers and activation 2 weeks post UVB exposure. This was preceded by a significant increase in UVB-induced type I IFN expression in NZM2328 mice compared to BALB/c mice. Following UVB exposure, both BALB/c and iNZM mice demonstrated an increase in functional T regulatory (T_{Reg}) cells; however, this was not seen in NZM2328 mice.

Conclusions

These data suggest a skewed UVB-mediated T cell response in lupus-prone mice where activation of T cells is enhanced secondary to a type I IFN-dependent suppression of T_{Reg} cells. Thus, we propose type I IFNs are important for UVB-induced inflammation in lupus-prone mice and may be an effective target for prevention of UVB-mediated flares.

4.2 Introduction:

Systemic lupus erythematosus (SLE) is an autoimmune disease in which patients experience devastating organ damage mediated by immune cells and inflammatory cytokine production [218, 253]. Minimal sun exposure, especially ultraviolet (UV) B wavelengths, is a prominent factor that drives cutaneous inflammation in lupus patients [254-258]. However, the mechanisms through which SLE skin is predisposed to persistent inflammation following UVB exposure is unknown.

Much of our knowledge regarding the effects of UVB have been uncovered through studies of healthy skin. Classically, following UVB-mediated damage, resolution of

inflammation is promoted via several mechanisms. Langerhans cells phagocytose apoptotic cells and promote dampening of inflammatory responses[200]. In addition, $CD11b^{+}Langerin^{-}$ dendritic cells promote expansion of T regulatory (T_{Reg}) cells[202, 259]. In healthy skin, there is also activation of other suppressive populations such as: neutrophils secreting IL-10 and monocytes secreting interferon (IFN) alpha, resulting in an overall suppressive phenotype[156, 260].

UVB exposure may have differential effects in SLE patients compared to healthy controls. In SLE patients, reduced phagocytosis of apoptotic cells results in prolonged autoantigen exposure[93, 97, 261]. Reduction of Langerhans cells in SLE skin promotes UVB-mediated inflammation via suppression of epidermal growth factor receptor-mediated signaling[262]. In addition, UVB exposure in SLE patients and lupus-prone mice leads to infiltration of neutrophils, macrophages, dendritic cells, T cells, and mast cells into the skin[65, 157, 200, 263-265]. Intriguingly, despite its immunosuppressive role in healthy skin[156, 200, 260], SLE patients and lupus-prone mice demonstrate a rise in type I IFN signaling following UVB exposure; thus suggesting a potential pro-inflammatory role for type I IFNs in lupus skin[156, 157]. For example, type I IFNs demonstrate a proinflammatory role in keratinocytes and promote cell death following UVB[155].

Because of the unclear role of type I IFNs in UVB-mediated inflammation, this paper seeks to understand the differences in immune cell activation following UVB exposure of lupus-prone and wild-type mice and to elucidate the role of type I IFNs in this process. We found that lupus-prone mice demonstrate increased expansion and prolonged activation of T cells in the draining lymph nodes of UVB exposed skin that is mediated by type I IFN-dependent repression of T_{Reg} cells. Thus, in contrast to wild-type mice[156], type I IFNs exhibit a proinflammatory role and are required for skewed immune activation in lupus-prone mice following UVB exposure.

4.3 Results

UVB exposure increases the number of activated T cells in the dLN of lupus-prone mice

Ten-week-old NZM2328 (lupus-prone) and BALB/c (wild-type) mice were treated with 100mJ/cm^2 UVB on their dorsum for 5 days, followed by harvest of the draining lymph nodes (dLN) 24 hrs or 2 weeks after the last UVB treatment. Intriguingly, the size of the draining lymph nodes (dLN) was increased 2 weeks following treatment in NZM2328 compared to BALB/c mice (fig.4.1A-C). This response was not systemic, indicated by a lack of increase in spleen size following UVB exposure in either strain (fig.1D and E). In order to determine the cellular contribution to the expanded LNs, we next examined changes in adaptive immune cell populations in the dLN and observed a significant increase in T cells 2 weeks post treatment in NZM2328 mice (fig.4.2 A). This UVB dosage did not significantly increase total B cells (Fig. 4.2B) or antibody secreting cells (Figure 4.3A). In addition, no significant increase in total IgG or anti-dsDNA antibodies were detected in the serum 2 weeks after UVB exposure (Figure 4.3B,C). Further exploration of changes in the subsets of T cells revealed increases in both CD4$^+$ and CD8$^+$ T cells (fig.4.2 C and E). In addition to expansion, we also observed increased activation of both CD4$^+$ and CD8$^+$ T cell subsets in NZM, but not WT mice, as indicated by increased CD69$^+$ expression (fig.4.2D and F). These results suggest that UVB exposure induces expansion and activation of T cells in the dLN of lupus-prone but not wild-type mice.

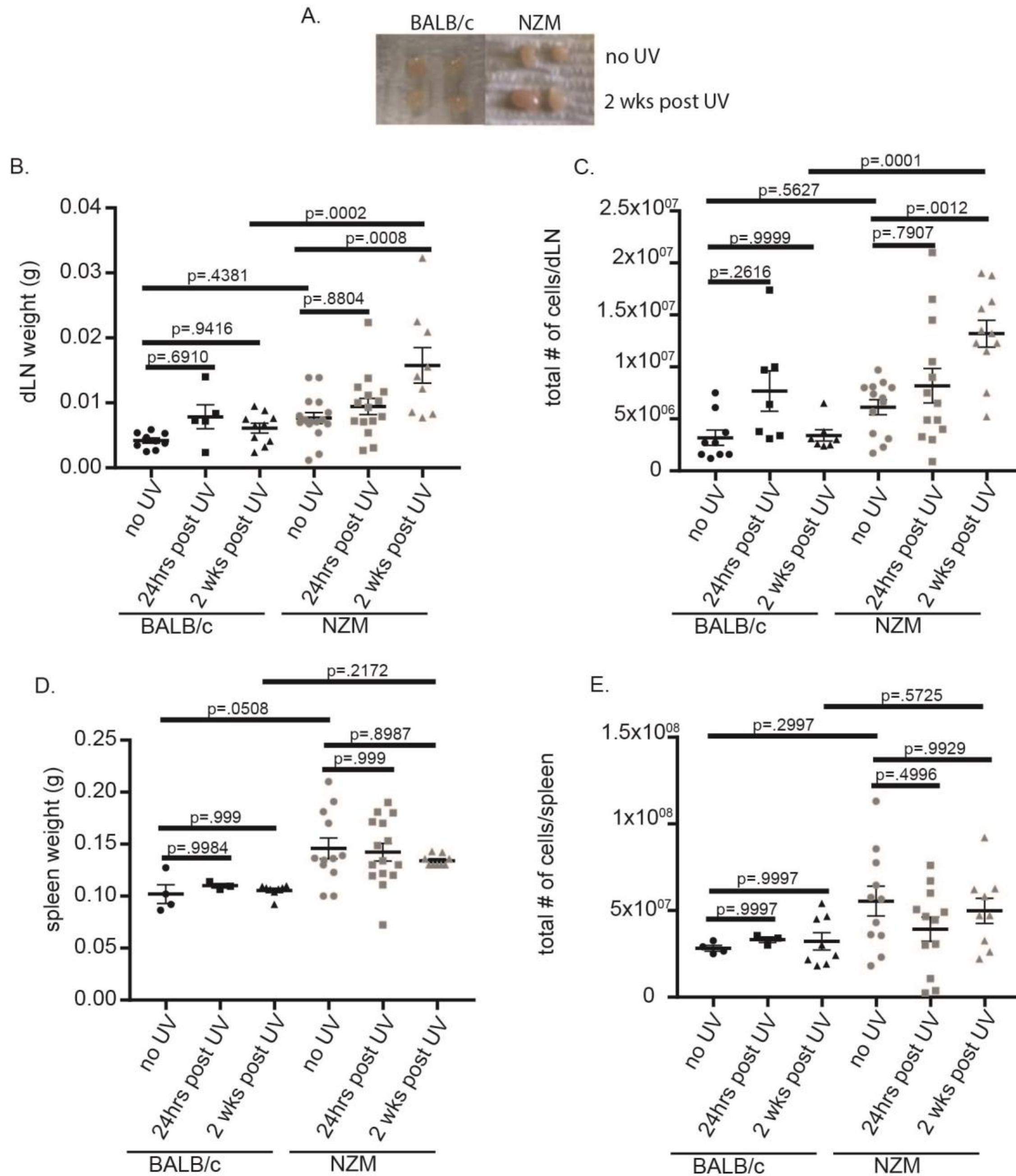


Figure 4.1 UVB- induces an increase in dLN, but not splenic size, 2 weeks post treatment in lupus-prone compared to wild-type mice.

Ten-week-old NZM2328 and BALB/c mice treated with 100mJ/cm^2 on their dorsum for 5 days were analyzed 24hrs and 2 weeks post treatment. (**A**) Representative dLN 2 weeks post treatment. (**B**) dLN weight. (**C**) Total number of dLN cells. (**D**) Spleen weight. (**E**) Total number of splenocytes. (**B-E**) Each dot represents an individual mouse. ANOVA testing was used to determine significance.

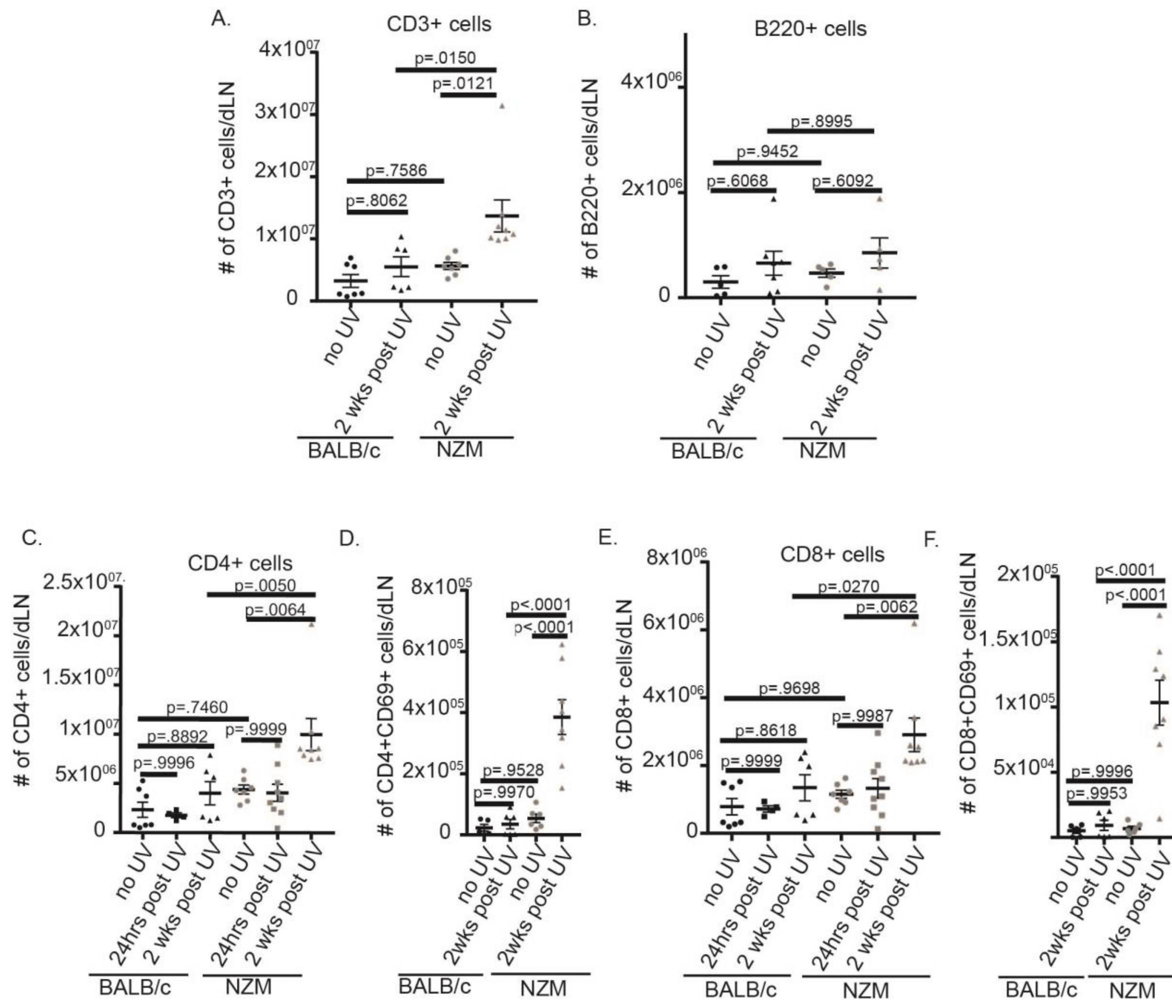


Figure 4.2 Lupus-prone mice have increased T cell activation in dLN 2 weeks post UVB treatment compared to wild-type mice.

Changes in immune cell populations in the dLN were evaluated by flow cytometry 24hrs and 2 weeks post UVB treatment. (**A**) Total T cells per dLN: $CD3^+$. (**B**) Total B cells: $B220^+$. (**C**) $CD4^+$ T cells: $CD3^+CD4^+CD8^-$. (**D**) $CD4^+$ T cell activation: $CD69^+$. (**E**) $CD8^+$ T cells: $CD3^+CD4^-CD8^+$. (**F**) $CD8^+$ T cell activation: $CD69^+$. (**A-F**) Each dot represents an individual mouse. ANOVA testing was used to determine significance.

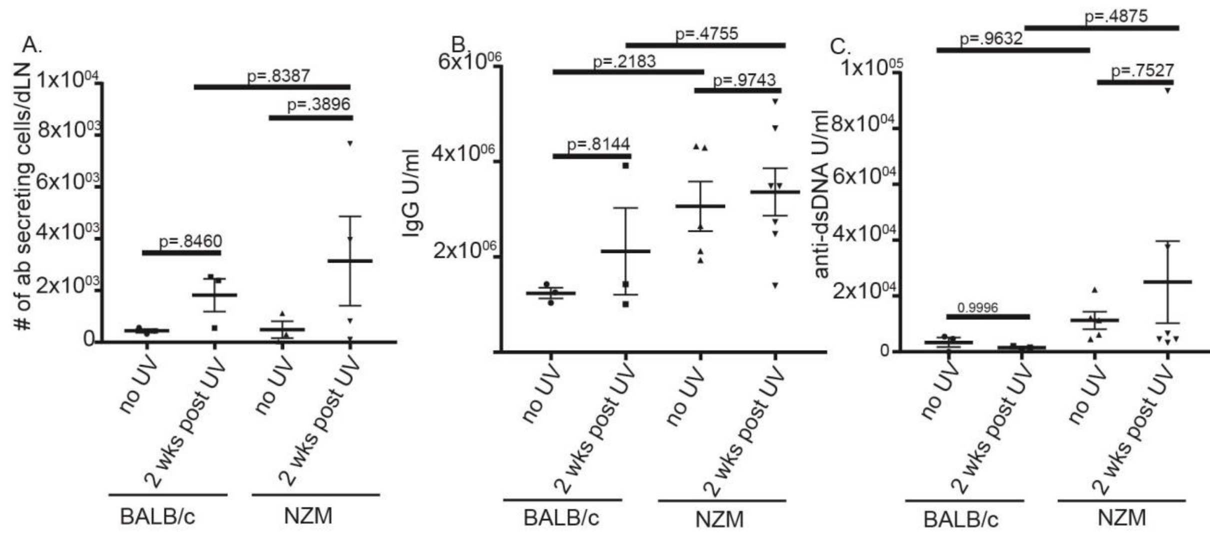
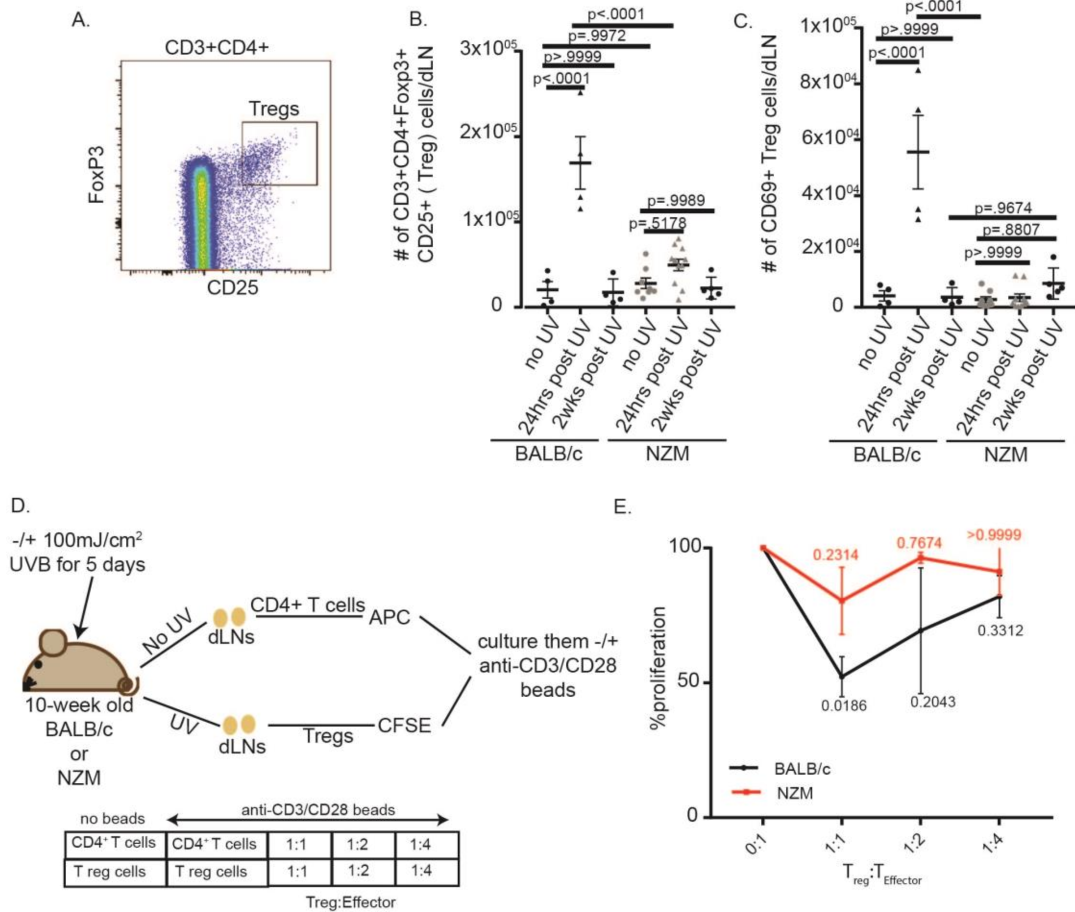


Figure 4.3 UVB fails to induce ab secreting cells and antibody production.

(**A**) Ten-week-old iNZM mice treated with 100mJ/cm2 on their dorsum for 5 days and dLNs were analyzed via flow cytometry 2 weeks post treatment. Each dot represents an individual mouse. Changes in ab secreting cells: $CD4^{-}CD8^{-}IgH+L^{hi}B220^{int\text{-}low}$ (**B**) IgG abs in the serum (C) dsDNA IgG abs in the serum. ANOVA testing was used to determine significance.

UVB exposure fails to induce functional T_{Reg} cells in the dLN of lupus-prone mice

UV exposure is known to induce T_{Reg} activation [202, 266], which is critical for preventing aberrant T cell activation, so we next examined changes in T_{Reg} populations following UVB exposure. Interestingly, BALB/c but not NZM mice demonstrated a significant increase in T_{Reg} cell numbers 24hrs post UVB treatment (fig.4.4A, B). Strikingly, a significant increase in activated, $CD69^{+}$ T_{Reg} cells was also noted in BALB/c mice following UVB exposure. In contrast, no increase in CD69 expression was noted on $Foxp3^{+}$ cells in NZM2328 mice, suggesting that T_{Reg} cells were activated only in wild-type mice after UVB (fig.4.4C). In order to confirm aberrant T_{Reg} suppressive function in NZM mice, we performed a T_{Reg} suppression assay (fig 4.4D,E) using T_{Regs} isolated from UVB-exposed mice. While T_{Regs} from BALB/c mice were able to suppress CD4+ T cell proliferation, T_{Regs} from NZM mice did not significantly inhibit proliferation at any ratio. These data indicate that T_{Reg} cells from dLN of UVB-treated lupus-prone mice have reduced functionality thus setting the stage for skewing of T cell activation following UVB exposure.



CD4⁺ T cells	CD4⁺ T cells	1:1	1:2	1:4
T reg cells	T reg cells	1:1	1:2	1:4

Figure 4.4 UVB exposure fails to induce T regulatory cell activation in lupus-prone mice.

(**A-D**) T regulatory cell changes were examined via flow cytometry in the dLN 24hrs and 2 weeks post UVB treatment. Each dot represents an individual mouse. (**A**) Changes in T regulatory cells: $CD3^{+}CD4^{+}CD25^{+}FoxP3^{+}$. (**B**) T regulatory cell activation: $CD69^{+}$. (**C**) Diagram of protocol for T regulatory suppression assay. (**D**) Percent proliferation of $CD4^{+}$ cells in T_{Reg} suppression assay. n=three independent experiments in duplicate. ANOVA testing was used to determine significance.

Type I IFN signaling is increased in NZM skin and is required for activation of T cells following UVB exposure.

Anti-inflammatory effects of type I IFNs have been described in wild-type mice post UVB exposure[156], yet SLE skin has elevated type I IFNs after UVB [156, 157]. Thus, we next characterized induction of IFNs after UVB exposure in NZM2328 vs. BALB/c mice. Examination of transcriptional changes in the skin of mice 3 hrs after UVB exposure resulted in an upregulation of type I IFNs and their downstream signaling genes in the skin of both wild-type and lupus-prone mice. Interestingly, NZM2328 mice had significantly higher expression of *ifnb* and *ifnk* as well as downstream IFN-regulated genes, indicating an elevated type I IFN response in lupus-prone mice vs. WT following UVB exposure (fig.4.5A).

In order to understand the role of type I IFNs in UVB-induced T cell activation in lupus-prone mice we studied iNZM mice, which lack a functional type I IFN receptor. Intriguingly, UVB treatment of 10-week old iNZM mice failed to induce an increase in T cell numbers 2 weeks post UVB (fig.4.5B). No difference in T cell subset numbers or activation (via $CD69^{+}$) were identified in iNZM mice (fig. 4.5C-F), similar to BALB/c mice (fig.2 C and E). These results suggest that in the absence of type I IFN signaling, activation of dLN T cells is prevented.

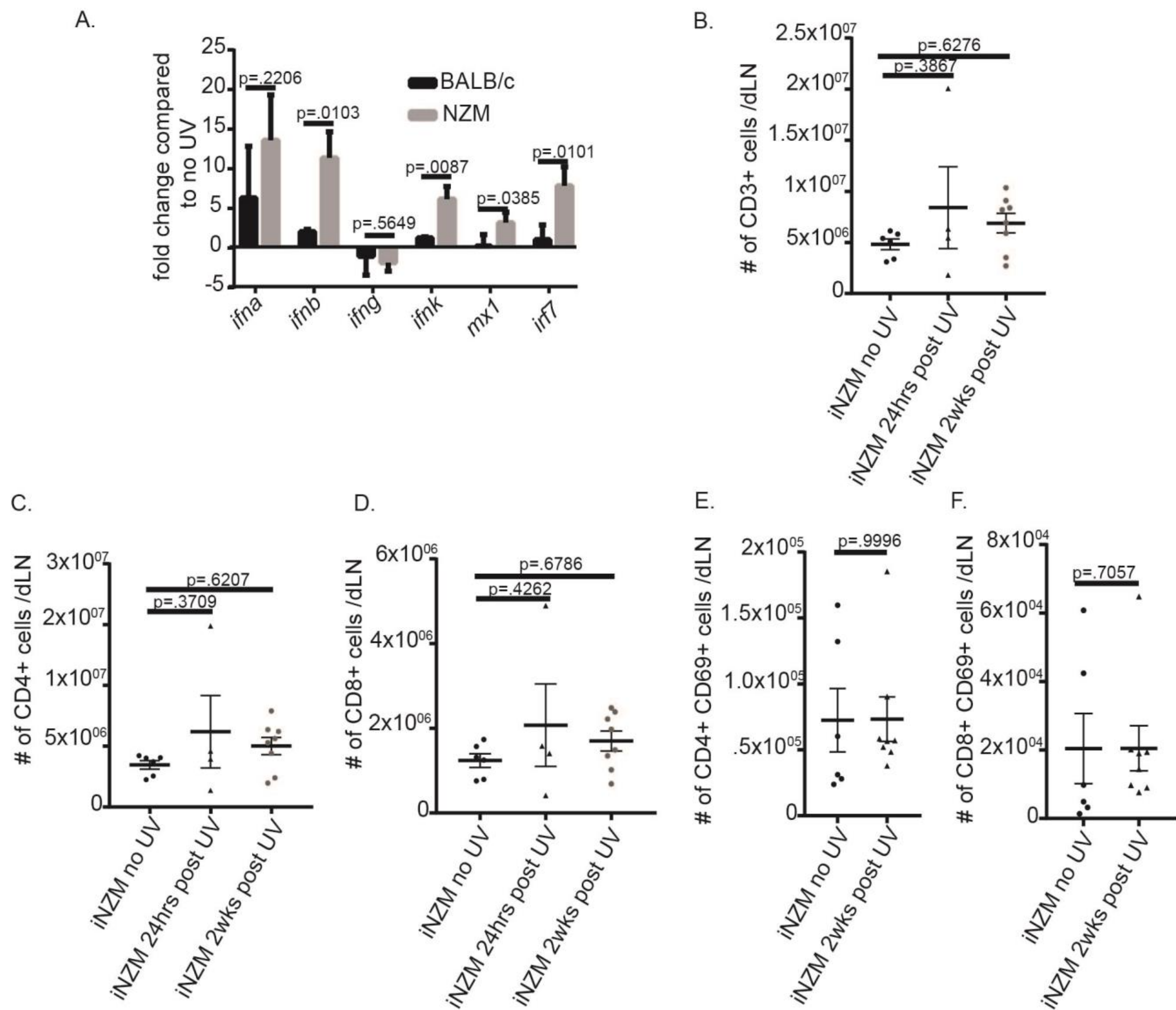


Figure 4.5 UVB induced T cell activation in lupus-prone mice is type I IFN dependent.

(**A**) RNA isolated from the skin of NZM or BALB/c mice 3hrs post UVB treatment. Real-time PCR was performed using primers of the genes listed. Graph displays the fold change for each gene compared with the respective no UV group (n = 4 NZM no UV; n = 4 NZM UV; n= 4 BALB/c no UV; n=4 BALB/c UV). A two-tailed student's t-test was used for normally distributed data and for comparisons with significant difference in variances, Welch's correction was applied. (**B-F**) Ten-week-old iNZM mice treated with 100mJ/cm^2 UVB on their dorsum for 5 days were analyzed via flow cytometry 24hrs or 2 weeks post treatment. Each dot represents an individual mouse. (**B**) Total T cells: CD3$^+$. (**C**) CD4$^+$ T cells: CD3$^+$CD4$^+$CD8$^-$. (**D**) CD8$^+$ T cells: CD3$^+$CD4$^-$CD8$^+$. (**E**) CD4$^+$ T cell activation: CD69$^+$. (**F**) CD8$^+$ T cell activation: CD69$^+$. ANOVA testing was used to determine significance.

Type I IFNs regulate T_{Reg} functionality in lupus-prone mice following UVB exposure

Past work in other disease models has shown that type I IFNs can manipulate T_{Reg} cells directly or indirectly[267, 268], so we next examined the effect of type I IFN signaling on T_{Reg} cells following UVB treatment. Consistent with a role for type I IFNs in suppression of T_{Regs}, we observed a significant increase in the number and activation of T_{Reg} cells in iNZM mice 24hrs post UVB exposure (figs.5A, B). Further characterization of T_{Reg} cells in iNZM mice showed strong suppressive function, and even suggest enhancement of suppressive function in the absence of type I IFN signaling (fig.5C). Taken together, these data indicate that lupus-prone mice display an enhanced type I IFN response to UVB that inhibits T_{Reg} function and promotes T cell activation and expansion.

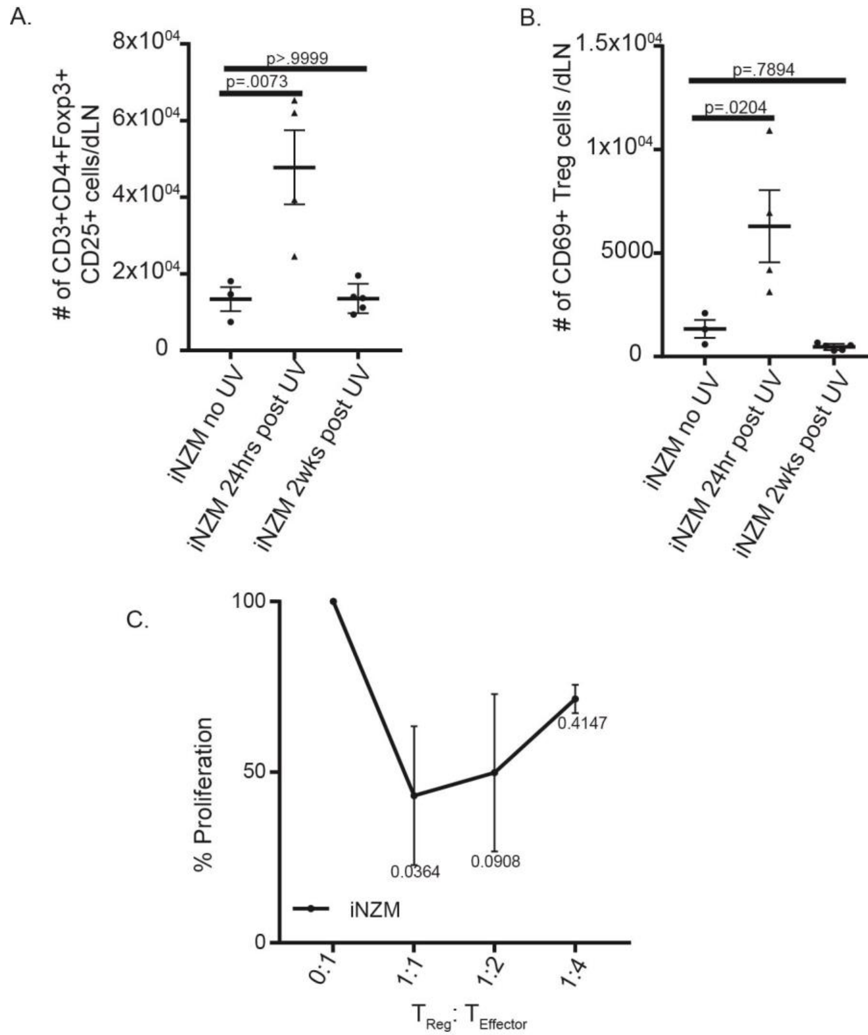


Figure 4.6 Type I IFNs suppress TReg activation and functionality following UVB exposure.

(**A and B**) Ten-week-old iNZM mice were treated with 100mJ/cm2 on their dorsum for 5 days and dLNs were analyzed via flow cytometry 24hrs or 2 weeks post treatment. Each dot represents an individual mouse. (**A**) Changes in T regulatory cells: $CD3^+CD4^+CD25^+FoxP3^+$. (**B**) T regulatory cell activation: $CD69^+$. (**C**) Percent proliferation of $CD4^+$ cells in T_{Reg} suppression assay. n=three independent experiments in duplicate. ANOVA testing was used to determine significance.

4.4 Discussion

SLE-associated skin inflammation is characterized by increased apoptosis, increased type I IFN expression, and the presence of inflammatory infiltrates, including T cells. However, the mechanisms to explain the propensity for UVB-induced inflammation remain unclear. In this paper, we examined the mechanisms involved in UVB-induced immune cell activation in wild-type vs. lupus-prone mice. UVB exposure results in increased T cell activation and decreased T_{Reg} induction in a type I IFN-dependent manner in lupus-prone vs. WT mice. Intriguingly, we also noted enhanced cutaneous type I IFN responses to UVB in lupus-prone mice, consistent with previous observations in human SLE skin[154, 269, 270].

To our knowledge, we demonstrate for the first time a differential activation of T_{regs} in wild-type vs. lupus-prone mice following UVB treatment. UVB-induced T_{regs} can migrate into healthy skin to contribute, along with resident T cells, to skewing the immune cell response towards a suppressive phenotype and possibly limit DNA damage[202, 271]. Our data also support a role for T_{reg} inhibition of T cell activation in the dLN itself. In SLE skin, activated T cells contribute to apoptosis induction through increased expression of FasL[265, 272, 273]. Whether activated Tcells in the dLN are able to migrate to the skin and contribute to inflammation following UVB exposure remains to be determined.

Some types of photosensitive cutaneous lupus lesions (especially discoid lupus) are associated with T cell infiltrates[274] and may present without significant autoantibody positivity[275]. Similarly, treatment of our lupus-prone mice with $100mJ/cm^2$ UVB was able to significantly activate T cells, but we did not identify an induction of B cell activation or antibody production. Thus, our model may reflect scenarios where T cells are the dominant contributors following UVB stimulation. Alternatively, our results may indicate a need for a higher doses of

UV to induce B cell activation, as UVB-driven autoantibody production in BXSB mice was induced at higher daily dosages of UVB (500 mJ/cm^2)[206]. Another possibility is that because we studied UVB treatment in pre-autoimmune lupus-prone mice, the autoreactive B cell populations have not yet developed sufficiently to be rapidly induced following UVB stimulation. While production of auto-antibodies can be driven via B cells in the draining lymph nodes[209], the type of stimulation (especially utilizing TLR7 activation) may be relevant as well.

Similar to others, we found an upregulation of type I IFNs in the skin of wild-type and lupus-prone mice following UVB exposure[156, 192]. The expression of type I IFNs, especially *ifnb* and *ifnk* are enhanced in lupus-prone compared to wild-type mice following UVB treatment. Sources of the IFN production may include infiltrating inflammatory monocytes[156]; however, in lupus skin, both infiltrating plasmacytoid dendritic cells and keratinocytes exhibit upregulation of type I IFNs following UVB as well[155, 263]. Keratinocyte production of IFNκ has been shown to prime lupus skin for a more inflammatory response through promotion of other proinflammatory cytokines, such as IL-6[154]. It has also been demonstrated that chronic exposure to type I IFNs results in enhanced immune cell activation, suggesting an inflammatory role for type IFNs in the skin and for priming of monocytes and dendritic cells migrating to dLNs[98, 155].

Intriguingly, we also demonstrate that type I IFNs have a proinflammatory role in T cell expansion in lupus-prone mice through the repression of T_{Reg} activation in the dLN. This is contrary to their protective role demonstrated in wild-type mice[156]. This differential effect could be due to T cells in lupus patients having decreased DNA methylation allowing for sensitization to type I IFN effects[276]. Alternatively, in lupus-prone mice or SLE patients, migratory dendritic cell populations may bring enhanced IFN production into the dLN and provide focal inhibition of T_{Regs} through type I IFNs. This is the focus of ongoing studies.

In conclusion, we demonstrate UVB-induced differential immune cell activation in lupus-prone vs. wild-type mice. Lupus-prone mice exhibit prolonged T activation following UVB exposure compared to wild-type mice. To our surprise, upregulation of Type I IFNs in lupus-prone mice drives a skewed T cell response through repression of T_{Reg} cells following UVB exposure. Future studies should address the source of type I IFNs through characterization of inflammatory profile changes in dendritic cells in the skin and dLN. Overall, our paper suggests type I IFNs may be a target to prevent UVB-induced T cell activation in the skin of SLE patients.

Chapter 5 Type I IFNs regulate the skewed innate immune response in lupus-prone mice following UVB exposure

This work was a collaboration with Shannon N. Estadt, Jonathan Theros, Tyson Moore, Lorie Lowe, Chaim O. Jacob, Johann E. Gudjonsson, J. Michelle Kahlenberg. Shannon N. Estadt, Jonathan Theros and Tyson Moore helped with experiments. Lorie Lowe scored the skin histology slides and Chaim O. Jacob donated the NZM2328 and iNZM mice. J. Michelle Kahlenberg assisted with data analysis. The data from this chapter is preliminary.

5.1 Abstract

Objective

Ultraviolet (UV) light is a known trigger of persistent cutaneous inflammation in systemic and cutaneous lupus erythematosus patients. Although UVB exposure induces elevated type I interferon (IFN) expression in lupus compared to healthy skin and skews T cell activation, how it primes the innate immune cell response remains unclear. This chapter compares the regulation of the innate immune response by type I IFNs between wild-type and lupus-prone mice following UVB exposure.

Methods

Eight to ten-week- old female wild-type (BALB/c), lupus-prone (NZM2328) and iNZM mice (lack a functional type I IFN receptor on NZM2328 background) were treated on their dorsal skin with 100mJ/cm^2 of UVB for 5 consecutive days. Skin thickness and erythema

development were tracked throughout treatment. Following UVB exposure, the skin was examined for inflammation through scoring of H&E stained slides; the skin and dLN innate immune cell population changes were characterized via flow cytometry and immunohistochemistry.

Results

NZM2328 and BALB/c mice exhibited similar skin injury follow UVB exposure. However, NZM2328 mice displayed increased skin inflammation compared to BALB/c mice that was abrogated in the absence of type I IFN signaling. UVB induced recruitment of different DC populations and triggered differential macrophage activation in the skin and dLN of NZM2328 vs. BALB/c mice. In addition, migration of $CD103^{+}CDllb^{-}$ DCs to the dLN was detected in only NZM2328 mice after UVB exposure.

Conclusions

These data suggest type I IFNs prime lupus skin for increased inflammation through regulating recruitment of DCs and macrophage activation. In addition, type I IFNs also regulate the migration of the $CD103^{+}CDllb^{-}$ DC population into the dLN. Thus, we propose type I IFNs are important for UVB-induced inflammation through regulation of the innate immune response in lupus-prone mice and may be an effective target for prevention of UVB- induced cutaneous inflammation.

5.2 Introduction

Systemic Lupus Erythematosus (SLE) is an autoimmune disease that exhibits various characteristics, including persistent skin inflammation. Up to 70% of patients can develop

Cutaneous Lupus Erythematosus (CLE), a heterogeneous manifestation displayed as rash and lesion development in the skin[6], yet the mechanism involved in the etiology of the disease is unknown.

Ultraviolet light (UV) from the sun is one major factor that leads to skin injury in healthy and lupus individuals[254-258]. Specifically, UVB exposure results in the infiltration of neutrophils, dendritic cells (DC), monocytes and macrophages. Most studies examining innate immune cell changes following UVB utilize healthy individuals and wild-type mice. In healthy skin, UVB induces recruitment of inflammatory monocytes producing IFNα, which reduce other immune cell recruitment and IL-6 production[156]. Infiltration of IL-10 producing neutrophils and migration of $CD11b^{+}langerin^{-}$ dendritic cells that promote expansion of T regulatory (T_{Reg}) cells also help to dampen inflammation[202, 259]. Overall, this results in the resolution of UVB-induced skin inflammation.

On the other hand, the recruitment and inflammatory role of innate immune cell subsets may differ in lupus patients due to effects from type I IFNs. Chronic type I IFN expression, exhibited in lupus patients, has demonstrated the ability to prime lupus monocytes, DCs and keratinocytes towards an inflammatory profile[154, 269, 270]; suggesting a role for IFNs in skewing the innate immune cell response in lupus skin following UVB exposure. We also showed in chapter 4 that UVB exposure in lupus-prone mice resulted in T cell activation in the draining lymph node (dLN) through type I IFN-mediated suppression of T regulatory cells; which DC subset is the source of type I IFNs driving this response remains to be understood.

In this chapter we examine how type I IFNs regulate UVB-induced innate immune cell responses in healthy and lupus-prone mice. In order to examine this, we treated BALB/c (wild-type), New Zealand Mixed 2328 (lupus-prone) mice, and iNZM mice (lack a functional type I

IFN receptor on NZM2328 background) on dorsal skin with 100mJ/cm^2 UVB for 5 consecutive days. Interestingly, we identified differential DC infiltration and macrophage activation in lupus-prone compared to healthy skin that is regulated by type I IFNs following UVB exposure. Lupus-prone mice also exhibit type I IFN dependent CD103$^+$CDllb$^-$ DC migration into the dLN, suggesting these cells participate in skewing UVB- induced T cell activation.

5.3 Results

UVB induces increased skin inflammation in lupus-prone mice that is type I IFN dependent

Eight to ten-week-old BALB/c (wild-type) and NZM2328 (lupus-prone) mice, were treated on dorsal skin with 100mJ/cm^2 UVB for 5 consecutive days. Both strains of mice developed erythema (redness in skin) and increased skin thickness by the 5th day of treatment (Fig. 5.1). Interestingly, when inflammatory infiltrates were quantified by a dermatopathologist, NZM2328 mice exhibited increased epidermal and dermal inflammation compared to BALB/c mice; this indicates UVB exposure results in increased inflammation in lupus-prone mice (Fig. 5.2A and B). Since type I IFNs are elevated following UVB exposure in lupus compared to healthy skin, we next examined if they were playing a role in skewing the inflammatory response by comparing UVB responses with those in iNZM mice (lack a functional type I IFNR). UVB treatment of iNZM mice resulted in reduced skin inflammation compared to NZM2328 mice (Fig. 5.2A and B), showing that type I IFNs drive UVB induced lupus cutaneous inflammation. In addition, NZM2328 mice treated with UVB exhibited increased infiltration of neutrophils in the skin compared to BALB/c mice and iNZM mice (Fig. 5.2C), demonstrating the enhanced infiltration of neutrophils in lupus skin is type I IFN dependent. These data indicate UVB

exposure leads to similar injury in lupus and healthy skin, but lupus- prone mice display enhanced skin inflammation that is type I IFN-dependent.

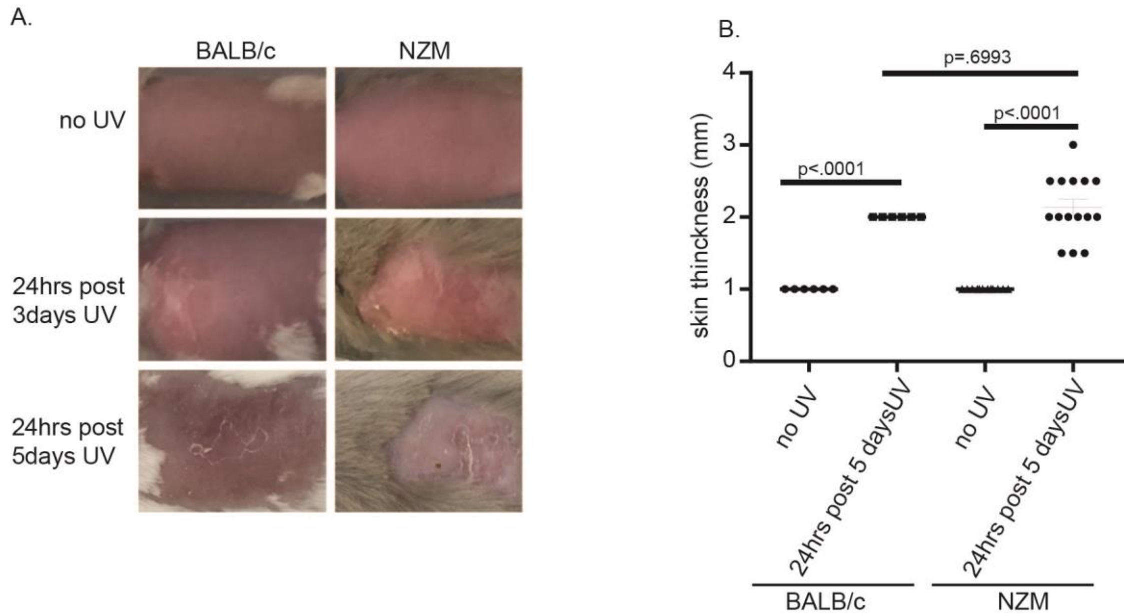
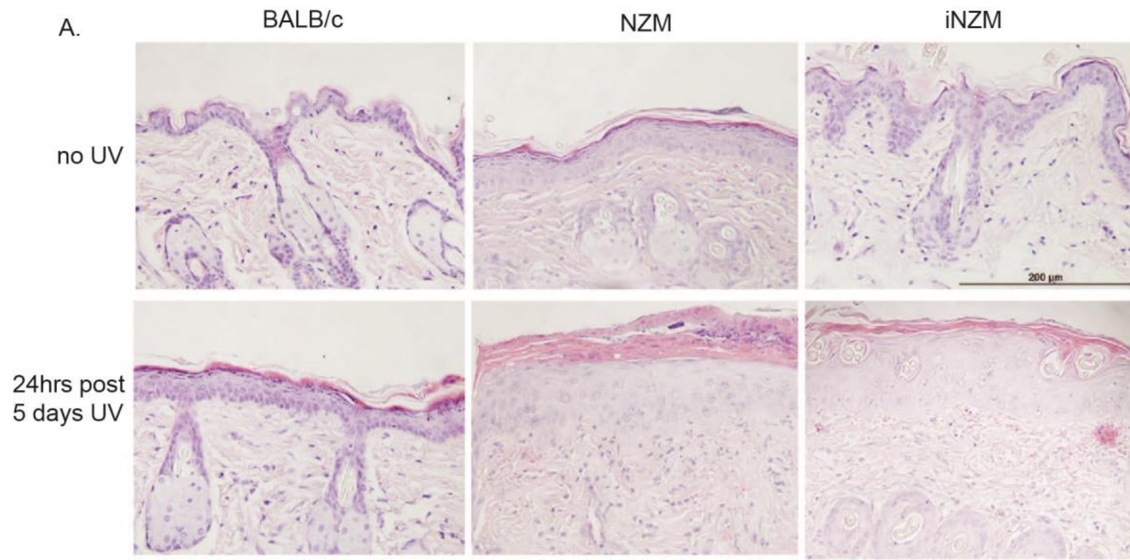
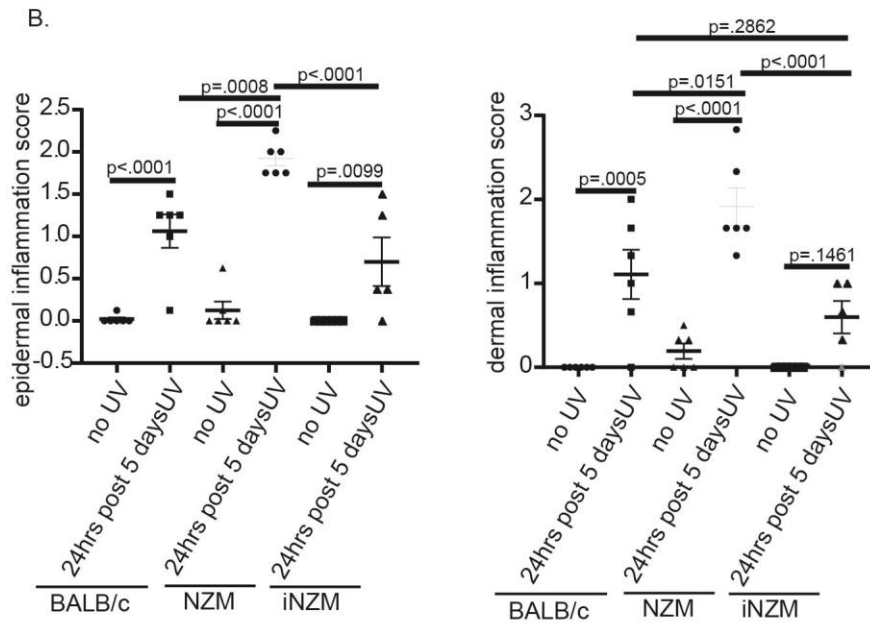


Figure 5.1 UVB exposure leads to skin injury in lupus-prone and wild-type mice.

Eight- to- ten-week-old BALB/c and NZM2328 mice were treated with 100mJ/cm^2 on their dorsum for 5 days. (A) Representative picture of erythema development 24hrs after 3days and 5days UVB. BALB/c n=5 and NZM2328 n= 6 (B) skin thickness 24hrs post 5 days UVB. Each dot represents one mouse and ANOVA testing was used to determine significance. BALB/c no UV n= 6 , BALB/c UV n=6, NZM no UV n=12, NZM UV n= 15.


A.
BALB/c
NZM
iNZM
no UV
24hrs post
5 days UV
200 µm


B.
epidermal inflammation score
p<.0001
p=.0008
p<.0001
p<.0001
p=.0099
no UV
24hrs post 5 daysUV
BALB/c
NZM
iNZM
dermal inflammation score
p=.2862
p<.0001
p=.0151
p<.0001
p=.0005
p=.1461

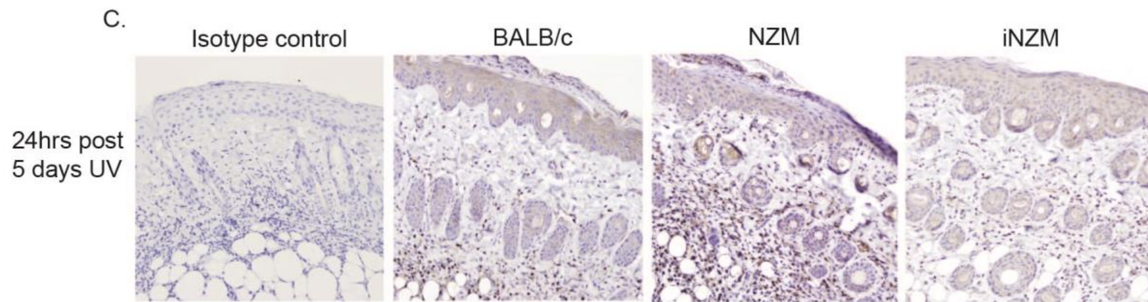

C.
Isotype control
BALB/c
NZM
iNZM
24hrs post
5 days UV

Figure 5.2 Increased skin inflammation in lupus-prone vs. wild-type mice is type I IFN dependent.

NZM 2328, BALB/c and iNZM mice were harvested 24hrs post 5 days UVB treatment. (A) representative H&E stained slides of skin sections n=3 in each group (B) epidermal and dermal inflammation score. Each dot represents one mouse and ANOVA testing was used to determine significance. BALB/c no UV n= 6 BALB/c UV n=6; NZM no UV n=6; NZM UV n=6; iNZM no UV n=6; iNZM UV n=5. (C) Representative of immunohistochemistry stained slides for Ly6G (neutrophils). BALB/c UV n=2; NZM UV n=2; iNZM UV n=2.

Type I IFN signaling partially suppresses macrophage activation in lupus skin following UVB exposure

Previous literature demonstrates type I IFNs can influence the activity of the monocyte and macrophage populations[98, 156, 277], so we examined their differential changes following UVB exposure. BALB/c mice displayed an enhanced monocyte (Ly6C+) infiltration into the skin compared to NZM2328 and iNZM mice (Fig. 5.3A), indicating this population is preferentially enhanced in healthy skin following UVB treatment. On the other hand, NZM2328 mice demonstrate a significant increase in macrophages in the skin (Fig 5.3B). However, our preliminary data shows macrophages in the BALB/c mice display increased CD40 expression (Fig 5.3C); which suggest impaired macrophage activation in lupus skin. Further, lack of the type I IFN signaling in lupus-prone mice partially restored CD40 expression, suggesting type I IFNs can suppress macrophage activation in lupus-prone mice following UVB exposure (Fig.5.3C). All of these data suggest that type I IFNs regulate the balance of macrophage infiltration and activation while not directly impacting monocyte infiltration.

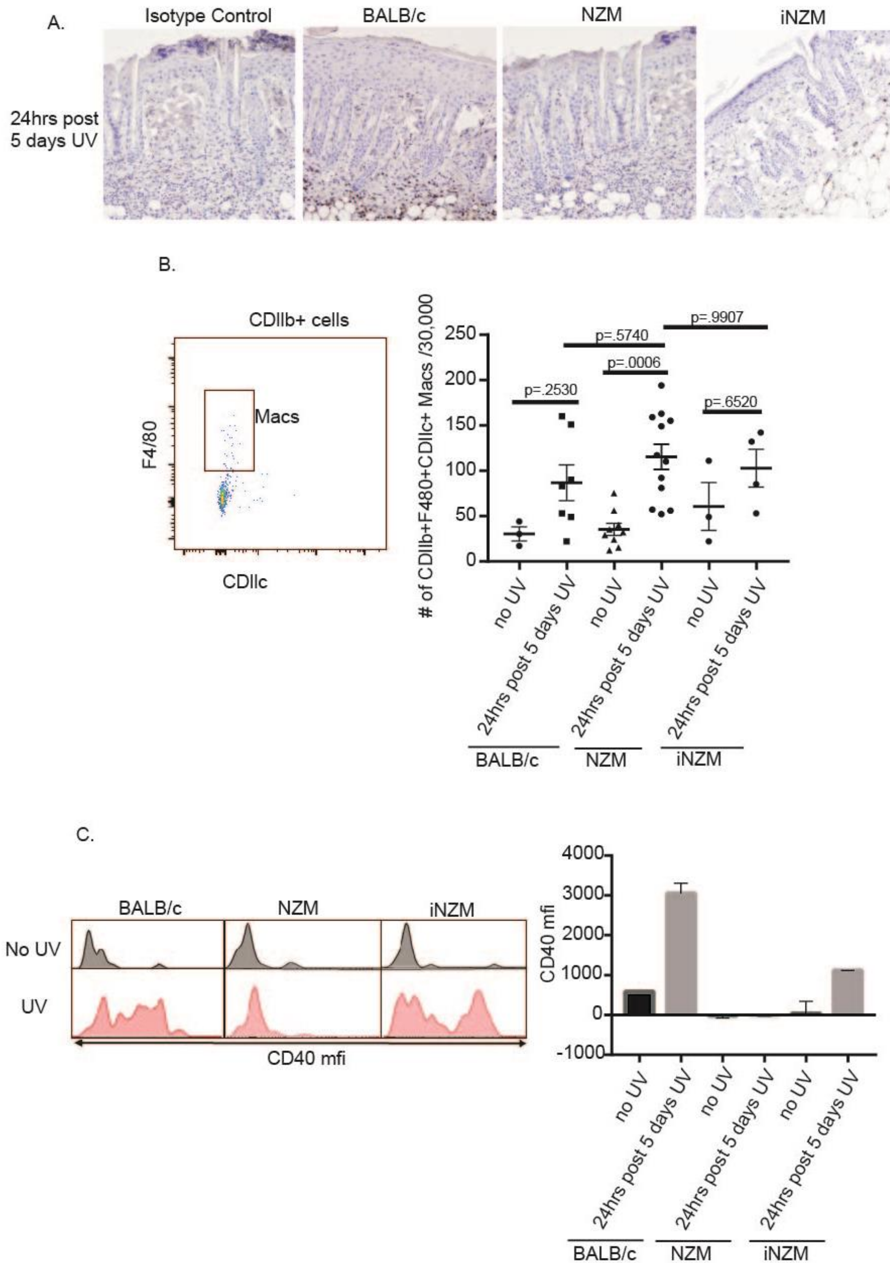


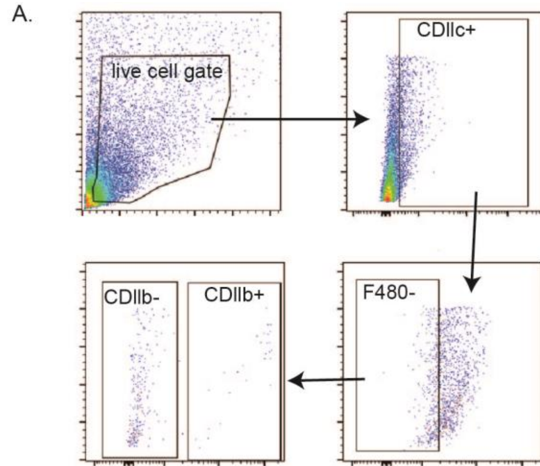
Figure 5.3 UVB induces differential macrophage activation and monocyte infiltration into the skin of lupus vs. healthy skin.

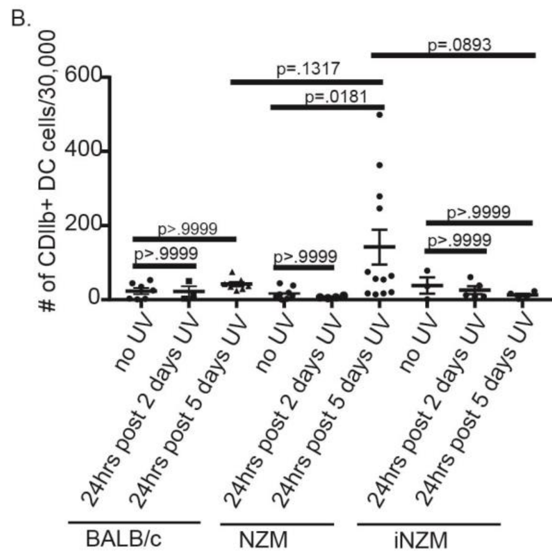
(A) Representative immunohistochemistry of Ly6C (for monocytes) stained skin slides. BALB/c UV n=3; NZM UV n=3; iNZM UV n=2. (B) Macrophages: CDllb^{+}F480^{+}CDllc^{+} in the skin. Each dot represents one mouse and ANOVA testing was used to determine significance. BALB/c no UV n= 3 BALB/c UV n=7; NZM no UV n=9; NZM UV n=13; iNZM no UV n=3; iNZM UV n=4. (C) CD40 mfi expression on macrophages BALB/c no UV n=2; BALB/c UV n= 2; NZM2328 no UV n=2; NZM UV n= 2; iNZM no UV n=2; iNZM UV n=2.

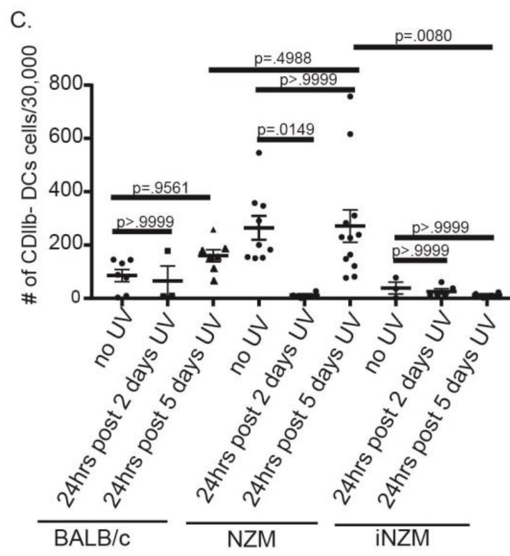
UVB induces a differential DC response in the skin and dLN of lupus-prone mice that is type I IFN dependent.

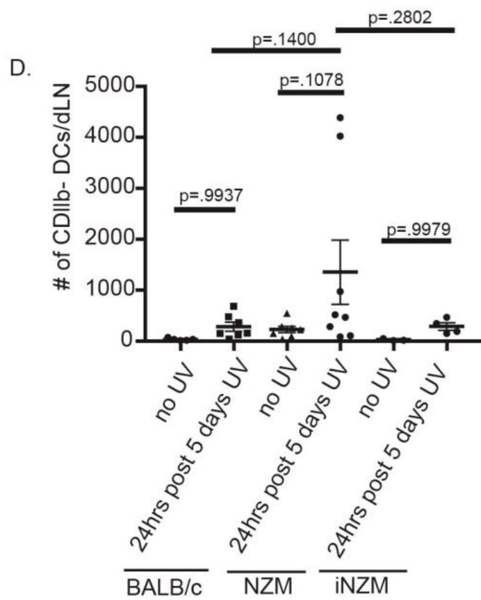
Past literature has demonstrated UVB exposure results in increased infiltration of dendritic cell populations[156, 259], so we next examined which DC subsets were being recruited utilizing the gating strategy in figure 5.4A. NZM2328 mice displayed a significant increase in monocyte derived DCs (CDllb^{+}) 24hrs after the 5th UVB treatment that was not exhibited in BALB/c or iNZM mice (Fig. 5.4B), indicating that UVB induces increased infiltration of monocyte derived DCs in lupus-prone mice that is type I IFN dependent. In addition, following the 2nd UVB treatment, NZM2328 mice also displayed a significant decrease in conventional DCs (CDllb^{-}) compared to BALB/c mice (Fig. 5.4C), suggesting that conventional DCs leave lupus skin following UVB exposure. This decrease is type I IFN dependent, as this is not displayed in iNZM mice (Fig 5.4C).

Since lupus-prone mice display a decrease in the CDllb^{-} DC population following UVB exposure, we further explored if this dendritic cell population was infiltrating into the dLN. Intriguingly, we show a trending increase of CDllb^{-} DCs into the dLN of NZM2328 mice compared to BALB/c and iNZM mice (Fig 5.4D). In particular, the CD103^{+}CDllb^{-} DC subset infiltrates into the dLN of NZM2328 mice and this infiltration is regulated by type I IFN signaling, as this is not demonstrated in iNZM mice (Fig 5.4E). These data indicate that UVB exposure in lupus-prone mice leads to increased infiltration of monocyte derived DCs and migration of CD103^{+}CDllb^{-} DCs into the dLN in a type I IFN dependent manner, contributing to skin inflammation and possibly skewing T cell activation in the dLN.


A.
live cell gate
CDllc+
F480-
CDllb-
CDllb+


B.
# of CDllb+ DC cells/30,000
p=.1317
p=.0181
p=.0893
p>.9999
p>.9999
p>.9999
p>.9999
p>.9999
no UV
24hrs post 2 days UV
24hrs post 5 days UV
BALB/c
NZM
iNZM


C.
# of CDllb- DCs cells/30,000
p=.4988
p>.9999
p=.0149
p=.0080
p=.9561
p>.9999
p>.9999
p>.9999
no UV
24hrs post 2 days UV
24hrs post 5 days UV
BALB/c
NZM
iNZM


D.
# of CDllb- DCs/dLN
p=.1400
p=.1078
p=.2802
p=.9937
p=.9979
no UV
24hrs post 5 days UV
BALB/c
NZM
iNZM

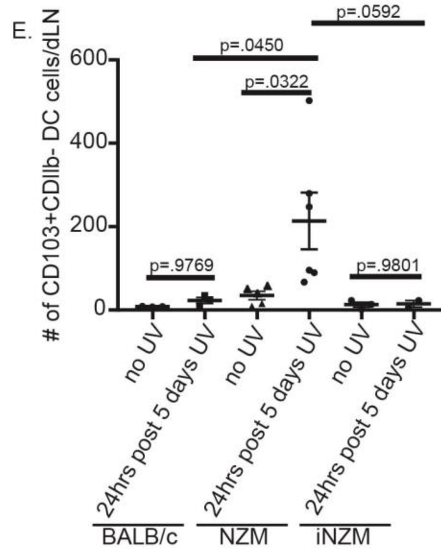

E.
# of CD103+CDllb- DC cells/dLN
p=.0450
p=.0322
p=.0592
p=.9769
p=.9801
no UV
24hrs post 5 days UV
BALB/c
NZM
iNZM

Figure 5.4 Differential DC response in the skin and dLN of lupus-prone mice that is dependent on type I IFN signaling following UVB exposure.

(A- E) Mice were harvest 24hrs after 2 days or 5 days of UVB treatment. (A) Gating strategy for CDllb$^-$ DCs and CDllb$^+$ DCs in the skin. (B) CDllb$^+$ DCs in the skin. (C) CDllb$^-$ DCs in the skin. (D) CDllb$^-$ DCs in the dLN. (E) CD103$^+$CDllb$^-$ DCs in the dLN. Each dot represents one mouse and ANOVA testing was used to determine significance.

5.4 Discussion

UVB exposure leads to persistent skin inflammation in lupus patients that manifest as lesion development in the skin. While we show in chapter 4 that type I IFN expression is elevated in lupus skin and regulates T cell activation in the dLN, how it primes the innate immune response following UVB exposure is not understood. In this chapter we examine UVB-induced innate immune cell response in lupus compared to wild-type mice, and how type I IFNs contribute to this response. For the first time, we show that UVB exposure induces increased inflammation in lupus compared to healthy skin that results from recruitment of differential DC populations and macrophage activation. We also demonstrate that $CD103^{+}CDllb^{-}$ DCs migrate into the dLN of lupus-prone mice. Intriguingly, this differential DC and macrophage response is regulated by type I IFNs, as NZM2328 mice lacking type I IFN signaling exhibit similar changes as wild-type mice.

In this chapter we demonstrate UVB exposure leads to similar injury in lupus and healthy skin; however, lupus-prone mice exhibit enhanced skin inflammation. Multiple factors are suggested to contribute to persistent lupus skin inflammation including apoptosis, upregulation of proinflammatory cytokines, and infiltration of immune cells. In particular, we show for the first time type I IFNs play a proinflammatory role in UVB-induced skin inflammation; this could be through type I IFNs ability to prime both innate and adaptive immune cell responses[164, 165122, 123].

Interestingly, we demonstrate neutrophils are increased in lupus compared to healthy skin following UVB exposure. These neutrophils have been shown to release neutrophil extraceullular traps (NETs) in CLE lesions [65]; this can contribute to an increase in autoantigen presence and proinflammatory cytokine production[74]. Further we demonstrate that the

recruitment of neutrophils following UVB exposure is dependent on type I IFN signaling, possibly perpetuating a positive feedforward loop as NETs induce type I IFN production[278]. Neutrophils in lupus patients are also shown to correlates with disease activity[71]; thus continued exploration into the inflammatory profile of these cells following UVB exposure is warranted.

Similar to others, we show that monocytes are recruited into healthy skin following UVB exposure[156]; these monocytes in healthy mice have been shown to produce IFN-α that can reduce other immune cell infiltration and IL-6 production[156], contributing to resolution of UVB-induced skin inflammation.

While we demonstrate lupus-prone mice display a significant increase in macrophages infiltrating into lupus skin following UVB exposure, they may exhibit impaired activation compared to wild-type mice. This altered macrophage activation is demonstrated in inactive and active SLE patients directing them toward a M1 inflammatory phenotype[279]. In addition, we show type I IFN signaling partially suppresses this activation and past studies have shown type I IFNs skew macrophages to a M1 inflammatory phenotype[280], so examination of whether these macrophages are M1 or M2 should be further evaluated.

We also show infiltration of monocyte derived DCs (CDllb^{+}) into lupus compared to healthy skin following UVB exposure; this could be due to increased expression of CCR7 allowing for increased infiltration into tissues and secondary lymphoid organs correlating with disease activity [85, 86]. In addition, we see decreased conventional DCs (CDllb^{-}) in the skin following UVB exposure, possibly migrating to the dLN contributing to T cell activation through increased expression of costimulatory molecules[80-83]. Further, we show changes in these DC subsets is dependent on type I IFN signaling; this could occur through type I IFN-mediated

priming of the skin for increased chemokine production to recruit DCs[281], though further examination into this mechanism should take place.

To our knowledge we show for the first time that there is migration of CD103$^+$CDllb$^-$ DCs into the dLN of lupus-prone vs wild-type mice following UVB exposure; these cells can display increased phagocytosis and presentation of apoptotic cells possibly driving a loss in tolerance[282]. We also see this infiltration is type I IFN dependent, which has been demonstrated in tumor models. These cells could be contributing to the skewed T cell activation in the dLN 2 weeks post treatment, thus further exploration into the inflammatory profile of this cells are warranted.

In conclusion, we demonstrate UVB-induces differential skin inflammation in lupus-prone vs. wild-type mice. Lupus-prone mice exhibit differential dendritic cell and macrophage recruitment into the skin compared to wild-type mice. DC recruitment to the skin and dLN along with macrophage activation is regulated by type I IFN signaling. In particular, lupus-prone mice also show migration of CD103$^+$CDllb$^-$ DCs into the dLN following UVB exposure, possibly providing the source of type I IFNs skewing T cell activation. Future studies should characterize the inflammatory profile of macrophages and dendritic cells in the skin and dLN to understand how they are contributing to differential skin inflammation. Overall, our paper suggests type I IFNs may be a target to prevent UVB-induced skin inflammation in SLE patients.

Chapter 6 Discussion and Future Directions

These chapters have explored the role of type I IFNs in TLR7 mediated lupus and UVB induced immune response. We showed that TLR7 cutaneous stimulation led to accelerated development of autoantibodies, splenomegaly, liver inflammation and lupus nephritis (fig 6.1). While splenomegaly and liver inflammation development are type I IFN dependent, lupus nephritis and autoantibody production could occur in the absence of type I IFNs. Further, we demonstrated elevated expression of type I IFNs in lupus vs. healthy skin following UVB exposure and that they exhibit a proinflammatory role. We further showed that UVB treatment led to skewed T cell activation in the dLN of lupus-prone mice through type I IFNs suppression of T regulatory cells (fig 6.2). In addition, type I IFNs increased inflammation in lupus compared to healthy skin through regulating the recruitment of neutrophils, differential DC populations and macrophage activation. We also identified that $CD103^{+}CDllb^{-}$ DCs migrate into the dLN of lupus-prone mice in a type I IFN dependent manner. Although we have uncovered a lot about how type I IFNs regulate TLR7 mediated lupus and UVB induced immune response there are still unanswered questions left to explore including further clarification of the role of $CD103^{+}$ DCs in UVB-induced immune cell response, how UVB-induced type I IFNs manipulate polarization of T cells, and the role of TLR7 in UVB-induced immune responses.

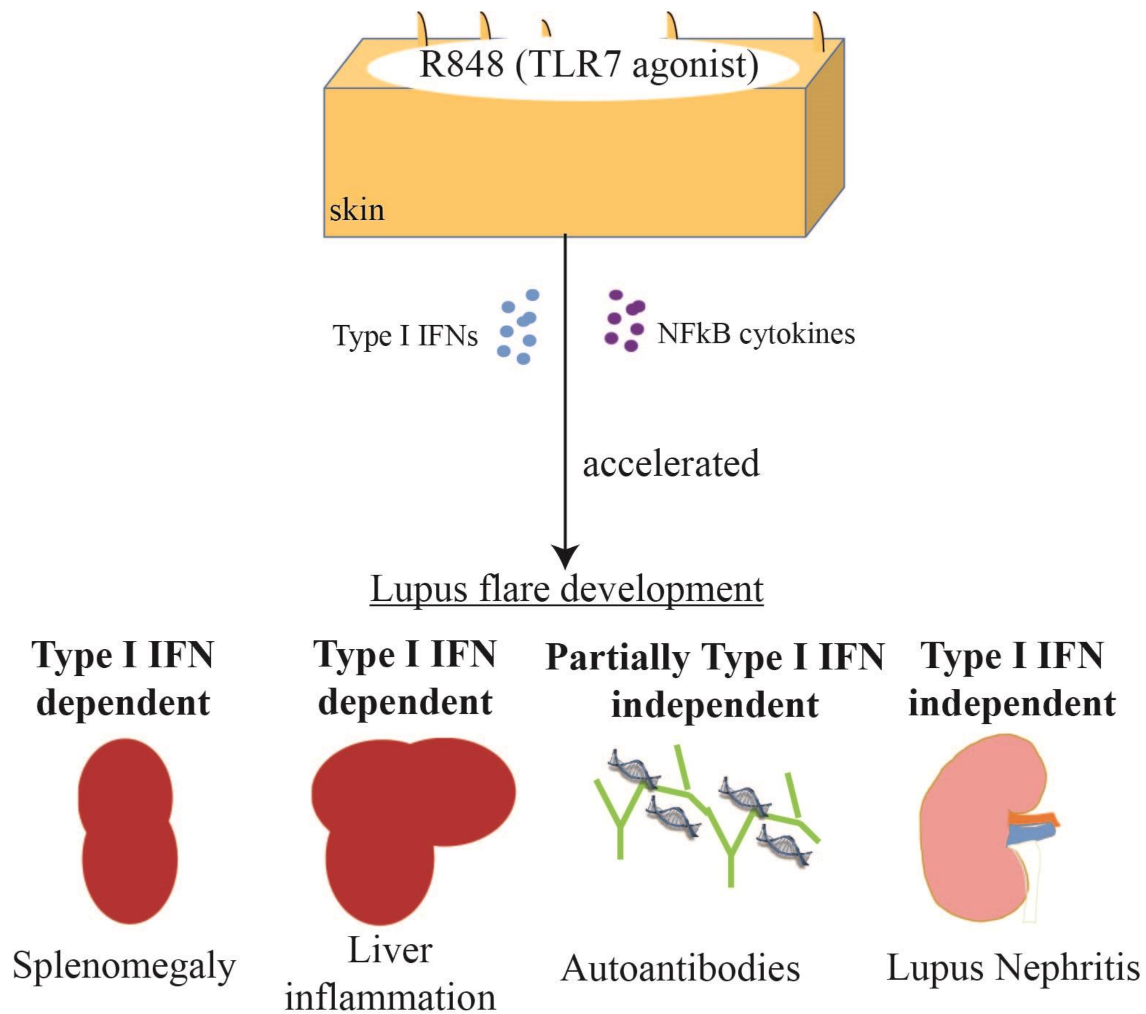
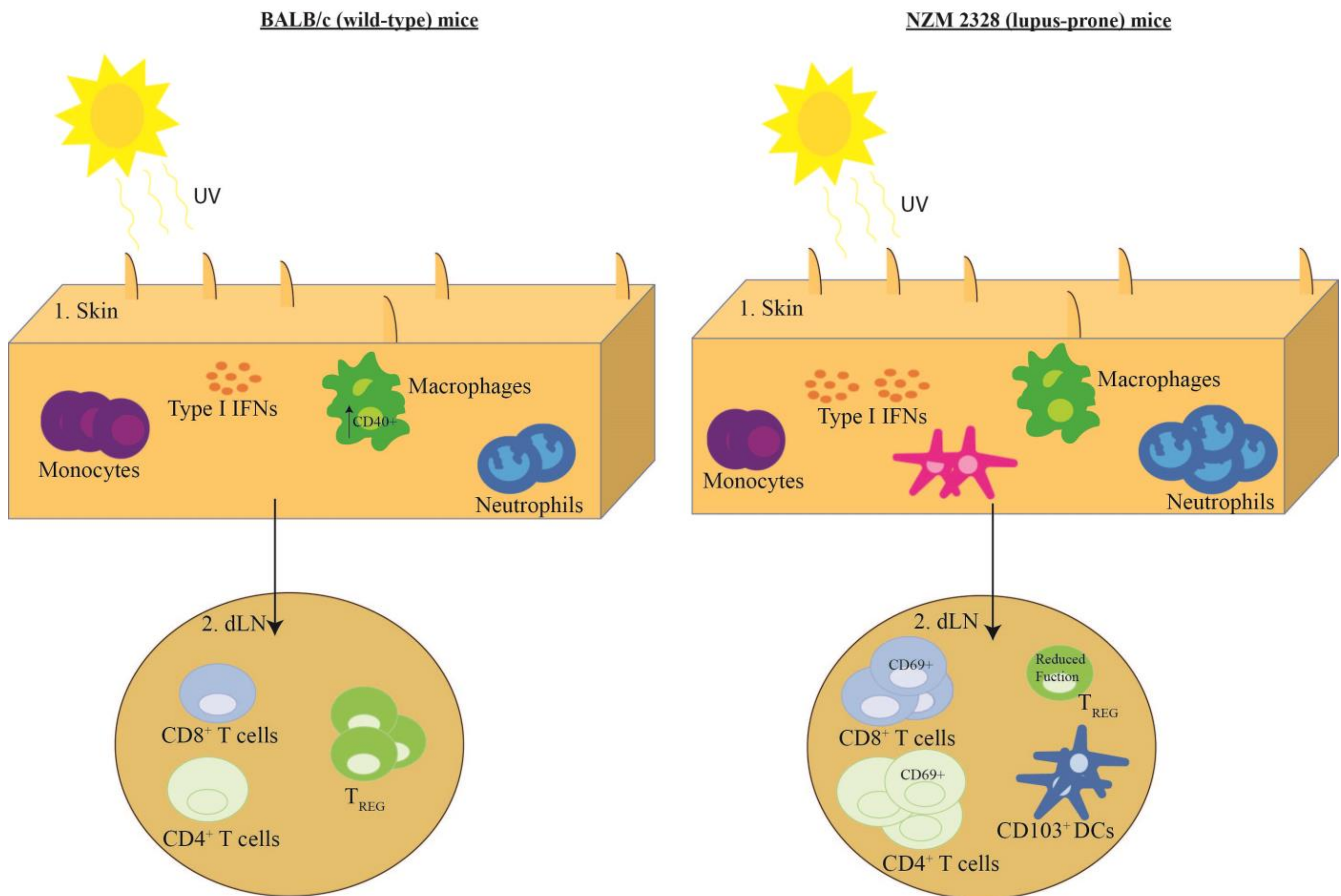


Figure 6.1 TLR7 cutaneous stimulation leads to accelerated lupus characteristic development that is type I IFN dependent and independent.

TLR7 cutaneous stimulation in NZM2328 lupus-prone mice accelerated lupus flare development. Type I IFNs regulated the development of splenomegaly and liver inflammation, but autoantibody production and lupus nephritis were able occur in its absence.



1. Skin
- Elevated expression of type I IFNs in lupus vs. healthy skin

- Type I IFNs regulate the recruitment of neutrophils, differential DC populations and macrophage activation in lupus skin

2. dLN
- Skewed T cell activation in the dLN of lupus-prone mice through type I IFNs suppression of T regulatory cells

- CD103+CDllb- DCs migrate into the dLN of lupus-prone mice in a type I IFN dependent manner

Figure 6.2 UVB induces a differential immune response that is regulated by type I IFNs.

UVB exposure drives increased skin inflammation in lupus-prone mice compared wild-type mice through recruitment of neutrophils, differential DC populations, and macrophage activation that is regulated type I IFN signaling. In addition, $CD103^{+}$ DCs infiltrate into the dLN of lupus-prone mice in a type I IFN dependent manner. Lupus-prone mice vs. wild-type mice also exhibit skewed T cell activation in the dLN driven by type I IFNs suppression of T regulatory cells.

6.1 Are CD103^{+} DCs the source of type I IFNs needed for UVB- induced T cell activation?

We demonstrated that CD103^{+} DCs infiltrate into the dLN following UVB exposure in lupus-prone mice prior to induction of a skewed to T cell response in the dLN. Interestingly, we identified that the skewed T cell activation is type I IFN-dependent, but whether CD103^{+} DCs are the source of type I IFNs that drive skewing of T cell activation should be studied.

Currently the number of DC subsets is ever growing and now requires the use of several phenotypic and morphological markers to separate out the various types. More recently, the integrin E_{α} (CD103) marker has been used to subset a class of DCs that are located in lymphoid and non-lymphoid compartments such as skin, spleen, dLN, and lung among other organs[283-287]. CD103^{+} DCs have demonstrated importance in regulating mucosa, lung and skin immunity[288, 289]. They are known for their ability to cross-present foreign and self-antigens[287, 290-294]. Specifically, CD103^{+} DCs are involved in the uptake of apoptotic bodies and promotion of tolerance [295-297]; however, in autoimmune disorders this subset of DCs may promote loss of tolerance. This is demonstrated by a murine asthma model in which depletion of CD103^{+} DCs led to a reduced inflammatory response[298] . Thus, depleting this cell population in lupus-prone mice and examining changes in T cell activation following UVB exposure could reveal if this population is playing a role in skewing T cell activation. This could also be examined through the transfer of CD103^{+} DCs from UVB exposed lupus skin into non-UVB treated lupus-prone mice and examining changes in T cell activation. We could also consider isolating out CD103^{+} DCs from the DL of UVB treated lupus prone mice and co-culturing them with T cells to measure T cell activation and IFN production.

CD103$^+$ DCs can migrate into the dLN, induce effector T cells and regulate the induction of T_{Reg} cells[287, 299, 300]. This DC subset has been shown to present viral antigens to naïve CD4$^+$ or CD8$^+$ T cells and they can cross present antigen to CD8$^+$ T cells [301, 302]. Further, they demonstrate the ability to induce differentiation of CD4$^+$ T cells in other disease models. Following MOG subcutaneous immunization, CD103$^+$ DCs accumulate in the skin and induce effector T cell differentiation into Th1/Th17 cells[303]. In an allergic asthma model, CD103$^+$DC in females exhibit greater antigen uptake and when cultured with CD4$^+$ T cells induce Th2 cytokines[304]. Mouse lung CD103$^+$ DCs have been shown to prime T cells toward a Th1 and Th17 response[305]. CD103$^+$ DCs in a herpes simplex virus skin infection model induce polarization toward a Th17 phenotype[306]. Since we see skewed T cell activation in lupus-prone mice following UVB exposure, examination of whether CD103$^+$ DCs are polarizing T cells toward a Th1 or Th7 phenotype should be pursued. This can be examined through understanding the cytokine profile of T cells in the dLN of lupus-prone and CD103$^+$ DC depleted mice following UVB exposure.

While we demonstrated that the skewing of T cell activation in the dLN of lupus-prone mice following UVB exposure is dependent on type I IFN-mediated suppression of T_{REGs}, which cytokines are produced by CD103$^+$ DC and how they might play a role in skewing T cell activation still has yet to be understood. CD103$^+$ DCs exhibit production of several cytokines in other disease models. In a mouse model of invasive pulmonary aspergillosis, IL-2 producing CD103$^+$ cells drive a protective response through T_{Reg} induction; however, defects in IL-2 production promote IL-23 production by CD103$^+$ DCs thus polarizing T cells toward a Th17 phenotype creating a hyper-inflammatory response[307]. In lupus patients, IL-2 expression is reduced, suggesting T cells are skewed toward a Th17 phenotype. Immune complex stimulation

through Fc receptor (FcR) can convert $CD103^+$ DCs from a tolerogenic response to an inflammatory response[48, 61, 62]. These DCs, upon FCR stimulation, secrete TNFα, IL-1β, and IL-23 and promote Th17 polarization[308]. Interestingly, $CD103^+$ DCs also demonstrate the ability to produce type I IFNs; this has been exhibited in Histoplasma lung infections where $CD103^+$ DCs are the main type I IFN producing cells to induce T cell activation[309]. Since type I IFNs are elevated in lupus-prone mice, exploration into whether $CD103^+$ DCs are a source of type I IFNs and how this influences T cell activation should be characterized. This can be tested by first examining mRNA expression in flow sorted $CD103^+$ DCs through qPCR or single cell sequencing, then co-culturing $CD103^+$ DCs from UVB treated lupus-prone mice and $CD4^+$ T cells with/without a functional type I IFN receptor and examining T cell activation.

Since we see that the migration of $CD103^+$ DCs into the dLN is type I IFN dependent, an alternative thought is that $CD103^+$ DCs are not producing type I IFNs, but are being primed by type I IFNs towards an inflammatory phenotype to induce T cell activation. Given the increase in type I IFNs we identified in the skin of NZM2328 mice shortly after UVB exposure, this is a feasible scenario. Previous studies have shown type I IFNs produced by DCs are important for intrinsic signaling affecting DC activation, migration, and localization with T cells[310-318]. In addition, IFNs have been shown to affect DC cross presentation of viral and tumor antigen[314, 319, 320]; this IFN effect on DC inflammatory phenotypes could be context dependent. Previous literature has shown that timing of IFNβ production can affect DC activity. For example, if IFNβ exposure takes place during DC maturation it primes the DC to promote a Th1 response, but if it takes place during DC stimulation of naïve T cells it primes the DC to inhibit the Th1 response; this response is through regulation of IL-12 family cytokines in DCs (IL-12, IL-23, IL-27 and IL-18) by IFNβ[321]. Further, type I IFNs have demonstrated an ability to regulate CCR7

expression on DCs[311]. Thus, examination into how type I IFNs might be affecting $CD103^+$ DC inflammatory profile should also be considered.

6.2 Are UVB-induced type I IFNs skewing T cells to a Th1 or Th17 phenotype through manipulation of transcription factors in lupus- prone mice?

Our data demonstrate a skewing of T cell activation away from the induction of T_{Reg} cells in a type I IFN dependent manner, but which inflammatory T cell subsets are being induced and how type I IFNs are regulating their development should be further examined.

Type I IFNs have been shown to directly affect T_{Reg} function and survival. Type I IFNs demonstrate an anti-proliferative effect on T cells in vitro[322, 323]. In particular, IFN alpha treatment in chronic hepatitis C patients leads to anti-proliferative and pro-apoptotic effects on T_{Regs}[324]. Further, in autoimmune diseases and cancers, type I IFNs have been shown to inhibit T_{Reg} function directly and indirectly[325]; this results in the inhibition of T_{Reg} suppression of antigen specific $CD4^+$ T cells[326, 327].

While type I IFNs seem to play a role in Treg suppression, they also demonstrate intrinsic signaling to promote T cell survival and differentiation; this effect is exhibited in other disease models. In LCMV infections, intrinsic signaling by type I IFNs has been shown to be important for $CD4^+$ T cell survival[328, 329]. Type I IFNs are also important for differentiation of $CD4^+$ T cells into T follicular helper (TFh) cells in vivo following immunization[330-332]. In an EAE mouse model, Type I and II IFN signaling play a role in modulating Th17 responses[333]. In humans, Type I IFNs polarized $CD4^+$ T cells to a Th1 phenotype[334]. The role of type I IFNs in T cell polarization could be through regulation of master transcription factors. Type I IFNs have been shown to be needed for initial T cell differentiation through manipulation of Bcl6 expression in TFh cells and T-bet in Th1 cells[335]. Alternatively, IFNs have also been shown to

drive Th1 polarization through the manipulation of the β2-chain of the IL-12 receptor[336-338]. For example, in Listeria monocytogenes infections type I IFNs have been shown to drive Th1 differentiation through synergistic effects with IL-12[339]. Examining how type I IFNs are regulating T cell polarization following UVB exposure in lupus-prone mice can be characterized through examining changes in T-bet, Bl6, RORγt and the IL-12 receptor in T cells with/without functional type I IFN receptor following UVB exposure.

6.3 Is TLR 7 important for CDllb^{-} DC migration in lupus-prone mice?

Our preliminary data from chapter 5 demonstrates UVB exposure in lupus-prone mice leads to CDllb^{-} DC migration from the skin to the dLN. This migration may be increased with epicutatious application of 100μg R848, a TLR7 agonist prior to UVB treatment (fig. 6.1).

TLR7 has demonstrated a role in driving lupus phenotypes in patients and lupus-prone mice[167-170, 173, 174]. It has also been suggested to contribute to the UVB-induced systemic response in lupus. This is seen in BXSB male mice, which carry an additional copy of TLR7 on the Yaa locus, are treated with 400mJ/cm^2 UV leading to accelerated lupus characteristic development and death [207]. In addition, wild-type mice treated epicutaneously with TLR7 for 2 weeks prior to UVB treatment developed increased skin inflammation [208]. Yet, the mechanism behind how TLR7 may contribute to the UVB response in lupus isn't clear.

Expression of TLR7 is seen in macrophages, monocytes, B cells, and dendritic cells; in particular, dendritic cell subsets that express high levels of TLR7 are pDCs and cDCs. Previous literature in other disease models show TLR7 stimulation is important for DC migration and increased function. *In vivo*, DC stimulation through TLR7 leads to migration of DCs to the T cell

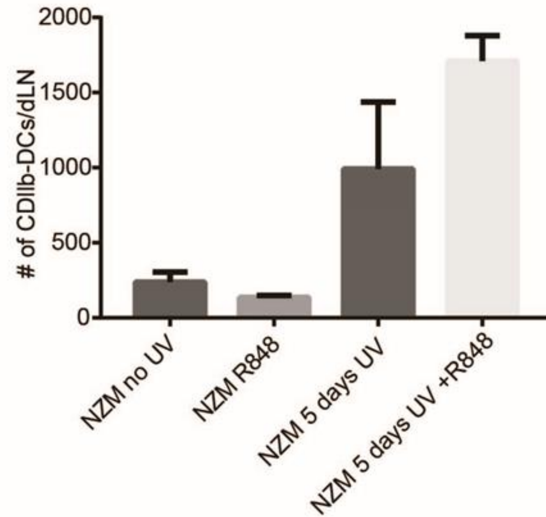


Figure 6.3 Enhanced migration of CDllb^{-} DCs into dLN with R848 stimulation prior to UVB treatment.

Eight-week-old NZM 2328 mice were treated for 2weeks with 100µg R848 followed by 5 days 100mJ/cm^{2} UVB exposure. CDllc^{+}CDllb^{-} DC population evaluated by flow cytometry 24hrs after the 5th UVB treatment. NZM no UV n=9; NZM days UV n=12; NZM 2 days UV + DMSO n=2; NZM 2 days UV + R848 n=2. (B)dLN 24hrs following 5 days UVB treatment. NZM no UV n=9; NZM R848 n=2; NZM 5 days UV n=12; NZM 5 days UV + R848 n=2.

zone in the lymph node[340]. Further, different DC subsets have been shown to migrate following TLR7 stimulation. Oral administration of 10µg R848 led to increased migration of conventional DC subsets such as $CD8^+$ DCs in wild-type mice[341]. Monocyte derived DCs migrated out of endocervical tissue blocks exposed to R848 for 24hrs[342]. It has also been shown that following topical R848 stimulation Langerhans cells migrate out of the skin[343]. Interestingly, R848 stimulation has also been shown to result in increased $CD103^+$ DCs migration in Peyer's patches[342]. Since we see identified enhanced $CDllb^-$ DC migration into the dLN with R848 and UVB treatment, examining which $CDllb^-$ DC subsets migrate in a TLR7 dependent manner in lupus-prone mice should be examined with the use of flow cytometry or CyTOF.

In addition to affecting DC migration, TLR7 stimulation also increases DC function. Oral administration of R848 led to increased DC activation that was dependent on type I IFNs[342]; this was also demonstrated in murine splenic DCs, which produced increased cytokines following R848 treatment[340, 344]. TLR7 stimulation also resulted in increased conventional DCs and pDC activation that was type I IFN-dependent in wild-type mice[345]. In addition, R848 stimulation in vitro leads to increased IL-12p70 production by DCs, which is involved in polarizing T cells to a Th1 response[346]. Further, DC TLR7 stimulation can lead to increased cross-presentation[347]. Following UVB exposure, examining if DCs have increased inflammatory cytokine production and antigen presentation in a TLR7 dependent manner in lupus-prone mice should be explored. This can be tested through examining IL-23, IL-12p70, type I IFNs production in DCs following UVB treatment and through the use of co-culture experiments with T cells to measure T cell activation and polarization.

6.4 Is Keratinocyte IFNκ production regulating CDllb$^-$ DC migration in lupus-prone mice in a TLR7 dependent manner?

We demonstrate in chapter 5 that UVB induces type I IFN expression in the skin and we show that R848 cutaneous stimulation also induces type I IFN expression in the skin, particularly IFN κ (fig. 2). While CDllb$^-$ DC migration into the dLN is type I IFN-dependent and can be enhanced with TLR7 stimulation, whether keratinocytes are the source of type I IFNs driving this migration should be further characterized.

Keratinocytes are self-regenerating cells that create the epidermis through a coordinated program of duplication and differentiation, they also demonstrate an immune regulatory role. Keratinocytes produce antimicrobial peptides such as LL37, which has demonstrated a pathogenic role in lupus through the induction of pDC activation[348]. They also have been shown to express some surface and endosomal TLRs. The expression of TLR7 in keratinocytes is controversial; however, a paper has demonstrated its expression and activation[349]. Upon exposure to environment triggers, keratinocytes are capable of producing type I IFNs, IL-1β, IL-18, and TNF alpha[350]. Interestingly, in SLE patients' keratinocytes display increased IFNκ expression at baseline that is further increased following UVB treatment[155]. Since treatment of lupus-prone mice with R848 also leads to increased IFNκ expression in the skin, examining if TLR7 plays a role in keratinocyte production of IFNκ following UVB treatment should be explored. This can be examined through UVB treating keratinocytes isolated from lupus-prone mice with/ without R848 and characterizing the mRNA changes through RNA sequencing.

UVB-induced IFN production by keratinocytes has also been shown to stimulate DC activation [155]. Further, type I IFNs have demonstrated an ability to increase TLR7-mediated IFN α production by pDC and regulate CCR7 expression on monocyte derived DCs[311, 351], but

whether UVB induced keratinocyte production of IFNκ is regulating CDllb$^-$DCs activation and migration needs to be further studied. One option would be to use lupus-prone mice with an epidermal-specific knockout of IFNκ. Following UVB exposure, changes in CDllb$^-$ DCs in the dLN can be examined with the use of flow cytometry.

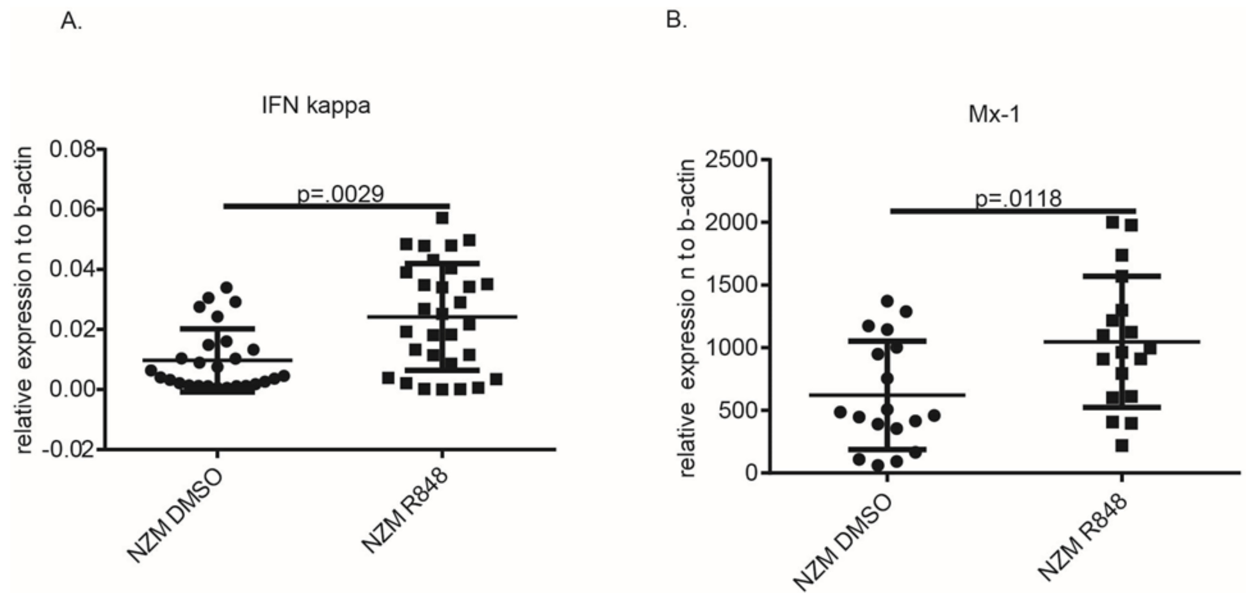


Figure 6.4 R848 treatment leads to increased IFN kappa and Mx-1 in the skin of lupus-prone mice 2 weeks post treatment.

Eight-week-old NZM 2328 mice were treated for 2weeks with 100µg R848 followed by harvest of skin for RNA. Real-time PCR was completed for *ifnk* and mx1. A. *ifnk*. B. *mx1*. Each dot represents one mouse and ANOVA testing was used to determine significance.

6.5 Conclusion

This thesis examines the role of type I IFNs in TLR7 mediated lupus characteristic development and UVB induced immune response. We showed type I IFNs regulate TLR7 mediated splenomegaly development and liver inflammation. Further, we demonstrated type I IFNs exhibit a proinflammatory role in UVB induced immune response. The additional ideas proposed in this chapter will allow for novel understanding of the role of type I IFNs in lupus characteristic development in order to develop novel treatment for SLE patients. Given that type I IFN inhibition drugs are available (baricitinib) or in development (anifrolumab) this work would also inform us on how we can use these medications smartly and in a more efficacious manner.

Bibliography

1. Wolf SJ, Estadt SN, Gudjonsson JE, Kahlenberg JM. Human and Murine Evidence for Mechanisms Driving Autoimmune Photosensitivity. Frontiers in immunology. 2018;9:2430. PMCID: PMC6205973.
2. Pons-Estel GJ, Ugarte-Gil MF, Alarcon GS. Epidemiology of systemic lupus erythematosus. Expert review of clinical immunology. 2017 Aug;13(8):799-814. PMCID: PMC.
3. Rees F, Doherty M, Grainge MJ, Lanyon P, Zhang W. The worldwide incidence and prevalence of systemic lupus erythematosus: a systematic review of epidemiological studies. Rheumatology (Oxford, England). 2017 Nov 1;56(11):1945-61. PMCID: PMC.
4. Stojan G, Petri M. Epidemiology of systemic lupus erythematosus: an update. Current opinion in rheumatology. 2018 Mar;30(2):144-50. PMCID: PMC6026543.
5. Lahita RG. The role of sex hormones in systemic lupus erythematosus. Current opinion in rheumatology. 1999 Sep;11(5):352-6. PMCID: PMC.
6. Cojocaru M, Cojocaru IM, Silosi I, Vrabie CD. Manifestations of systemic lupus erythematosus. Maedica. 2011 Oct;6(4):330-6. PMCID: PMC3391953.
7. Zoma A. Musculoskeletal involvement in systemic lupus erythematosus. Lupus. 2004;13(11):851-3. PMCID: PMC.
8. Grossman JM. Lupus arthritis. Best practice & research Clinical rheumatology. 2009 Aug;23(4):495-506. PMCID: PMC.
9. Nzeusseu Toukap A, Galant C, Theate I, Maudoux AL, Lories RJ, Houssiau FA, Lauwerys BR. Identification of distinct gene expression profiles in the synovium of patients with systemic lupus erythematosus. Arthritis and rheumatism. 2007 May;56(5):1579-88. PMCID: PMC.
10. Ceccarelli F, Perricone C, Cipriano E, Massaro L, Natalucci F, Capalbo G, Leccese I, Bogdanos D, Spinelli FR, Alessandri C, Valesini G, Conti F. Joint involvement in systemic lupus erythematosus: From pathogenesis to clinical assessment. Seminars in arthritis and rheumatism. 2017 Aug;47(1):53-64. PMCID: PMC.
11. Abu-Shakra M, Buskila D, Shoenfeld Y. Osteonecrosis in patients with SLE. Clinical reviews in allergy & immunology. 2003 Aug;25(1):13-24. PMCID: PMC.
12. Stannard JN, Kahlenberg JM. Cutaneous lupus erythematosus: updates on pathogenesis and associations with systemic lupus. Current opinion in rheumatology. 2016 Sep;28(5):453-9. PMCID: PMC4965280.
13. Wieczorek IT, Propert KJ, Okawa J, Werth VP. Systemic symptoms in the progression of cutaneous to systemic lupus erythematosus. JAMA dermatology. 2014 Mar;150(3):291-6. PMCID: PMC.
14. Gronhagen CM, Fored CM, Granath F, Nyberg F. Cutaneous lupus erythematosus and the association with systemic lupus erythematosus: a population-based cohort of 1088 patients in Sweden. The British journal of dermatology. 2011 Jun;164(6):1335-41. PMCID: PMC.

15. de Zubiria Salgado A, Herrera-Diaz C. Lupus nephritis: an overview of recent findings. Autoimmune diseases. 2012;2012:849684. PMCID: PMC3318208.
16. Ben-Menachem E. Review article: systemic lupus erythematosus: a review for anesthesiologists. Anesthesia and analgesia. 2010 Sep;111(3):665-76. PMCID: PMC.
17. Mohan C, Putterman C. Genetics and pathogenesis of systemic lupus erythematosus and lupus nephritis. Nature reviews Nephrology. 2015 Jun;11(6):329-41. PMCID: PMC.
18. Borchers AT, Leibushor N, Naguwa SM, Cheema GS, Shoenfeld Y, Gershwin ME. Lupus nephritis: a critical review. Autoimmunity reviews. 2012 Dec;12(2):174-94. PMCID: PMC.
19. Lech M, Anders HJ. The pathogenesis of lupus nephritis. Journal of the American Society of Nephrology : JASN. 2013 Sep;24(9):1357-66. PMCID: PMC3752952.
20. Furie R, Khamashta M, Merrill JT, Werth VP, Kalunian K, Brohawn P, Illei GG, Drappa J, Wang L, Yoo S, Investigators CDS. Anifrolumab, an Anti-Interferon-alpha Receptor Monoclonal Antibody, in Moderate-to-Severe Systemic Lupus Erythematosus. Arthritis & rheumatology. 2017 Feb;69(2):376-86. PMCID: PMC5299497.
21. van Vollenhoven RF, Hahn BH, Tsokos GC, Wagner CL, Lipsky P, Touma Z, Werth VP, Gordon RM, Zhou B, Hsu B, Chevrier M, Triebel M, Jordan JL, Rose S. Efficacy and safety of ustekinumab, an IL-12 and IL-23 inhibitor, in patients with active systemic lupus erythematosus: results of a multicentre, double-blind, phase 2, randomised, controlled study. Lancet. 2018 Oct 13;392(10155):1330-9. PMCID: PMC.
22. Furie R, Petri M, Zamani O, Cervera R, Wallace DJ, Tegzova D, Sanchez-Guerrero J, Schwarting A, Merrill JT, Chatham WW, Stohl W, Ginzler EM, Hough DR, Zhong ZJ, Freimuth W, van Vollenhoven RF, Group B-S. A phase III, randomized, placebo-controlled study of belimumab, a monoclonal antibody that inhibits B lymphocyte stimulator, in patients with systemic lupus erythematosus. Arthritis and rheumatism. 2011 Dec;63(12):3918-30. PMCID: PMC5007058.
23. Navarra SV, Guzman RM, Gallacher AE, Hall S, Levy RA, Jimenez RE, Li EK, Thomas M, Kim HY, Leon MG, Tanasescu C, Nasonov E, Lan JL, Pineda L, Zhong ZJ, Freimuth W, Petri MA, Group B-S. Efficacy and safety of belimumab in patients with active systemic lupus erythematosus: a randomised, placebo-controlled, phase 3 trial. Lancet. 2011 Feb 26;377(9767):721-31. PMCID: PMC.
24. Deapen D, Escalante A, Weinrib L, Horwitz D, Bachman B, Roy-Burman P, Walker A, Mack TM. A revised estimate of twin concordance in systemic lupus erythematosus. Arthritis and rheumatism. 1992 Mar;35(3):311-8. PMCID: PMC.
25. Alarcon-Segovia D, Alarcon-Riquelme ME, Cardiel MH, Caeiro F, Massardo L, Villa AR, Pons-Estel BA, Grupo Latinoamericano de Estudio del Lupus E. Familial aggregation of systemic lupus erythematosus, rheumatoid arthritis, and other autoimmune diseases in 1,177 lupus patients from the GLADEL cohort. Arthritis and rheumatism. 2005 Apr;52(4):1138-47. PMCID: PMC.
26. Ruiz-Narvaez EA, Fraser PA, Palmer JR, Cupples LA, Reich D, Wang YA, Rioux JD, Rosenberg L. MHC region and risk of systemic lupus erythematosus in African American women. Human genetics. 2011 Dec;130(6):807-15. PMCID: PMC3215804.
27. Smolen JS, Klippel JH, Penner E, Reichlin M, Steinberg AD, Chused TM, Scherak O, Graninger W, Hartter E, Zielinski CC, et al. HLA-DR antigens in systemic lupus erythematosus: association with specificity of autoantibody responses to nuclear antigens. Annals of the rheumatic diseases. 1987 Jun;46(6):457-62. PMCID: PMC1002164.

28. Eroglu GE, Kohler PF. Familial systemic lupus erythematosus: the role of genetic and environmental factors. Annals of the rheumatic diseases. 2002 Jan;61(1):29-31. PMCID: PMC1753892.
29. Azizah MR, Ainoi SS, Kuak SH, Kong NC, Normaznah Y, Rahim MN. The association of the HLA class II antigens with clinical and autoantibody expression in Malaysian Chinese patients with systemic lupus erythematosus. Asian Pacific journal of allergy and immunology. 2001 Jun;19(2):93-100. PMCID: PMC.
30. Sarma JV, Ward PA. The complement system. Cell and tissue research. 2011 Jan;343(1):227-35. PMCID: PMC3097465.
31. Pickering MC, Botto M, Taylor PR, Lachmann PJ, Walport MJ. Systemic lupus erythematosus, complement deficiency, and apoptosis. Advances in immunology. 2000;76:227-324. PMCID: PMC.
32. Unsworth DJ. Complement deficiency and disease. Journal of clinical pathology. 2008 Sep;61(9):1013-7. PMCID: PMC.
33. Boteva L, Morris DL, Cortes-Hernandez J, Martin J, Vyse TJ, Fernando MM. Genetically determined partial complement C4 deficiency states are not independent risk factors for SLE in UK and Spanish populations. American journal of human genetics. 2012 Mar 9;90(3):445-56. PMCID: PMC3309188.
34. Manjarrez-Orduno N, Marasco E, Chung SA, Katz MS, Kiridly JF, Simpfendorfer KR, Freudenberg J, Ballard DH, Nashi E, Hopkins TJ, Cunninghame Graham DS, Lee AT, Coenen MJ, Franke B, Swinkels DW, Graham RR, Kimberly RP, Gaffney PM, Vyse TJ, Behrens TW, Criswell LA, Diamond B, Gregersen PK. CSK regulatory polymorphism is associated with systemic lupus erythematosus and influences B-cell signaling and activation. Nature genetics. 2012 Nov;44(11):1227-30. PMCID: PMC3715052.
35. Hom G, Graham RR, Modrek B, Taylor KE, Ortmann W, Garnier S, Lee AT, Chung SA, Ferreira RC, Pant PV, Ballinger DG, Kosoy R, Demirci FY, Kamboh MI, Kao AH, Tian C, Gunnarsson I, Bengtsson AA, Rantapaa-Dahlqvist S, Petri M, Manzi S, Seldin MF, Ronnblom L, Syvanen AC, Criswell LA, Gregersen PK, Behrens TW. Association of systemic lupus erythematosus with C8orf13-BLK and ITGAM-ITGAX. The New England journal of medicine. 2008 Feb 28;358(9):900-9. PMCID: PMC.
36. Arechiga AF, Habib T, He Y, Zhang X, Zhang ZY, Funk A, Buckner JH. Cutting edge: the PTPN22 allelic variant associated with autoimmunity impairs B cell signaling. Journal of immunology. 2009 Mar 15;182(6):3343-7. PMCID: PMC2797545.
37. Dai X, James RG, Habib T, Singh S, Jackson S, Khim S, Moon RT, Liggitt D, Wolf-Yadlin A, Buckner JH, Rawlings DJ. A disease-associated PTPN22 variant promotes systemic autoimmunity in murine models. The Journal of clinical investigation. 2013 May;123(5):2024-36. PMCID: PMC3638909.
38. Menard L, Saadoun D, Isnardi I, Ng YS, Meyers G, Massad C, Price C, Abraham C, Motaghedi R, Buckner JH, Gregersen PK, Meffre E. The PTPN22 allele encoding an R620W variant interferes with the removal of developing autoreactive B cells in humans. The Journal of clinical investigation. 2011 Sep;121(9):3635-44. PMCID: PMC3163953.
39. Rhodes B, Furnrohr BG, Roberts AL, Tzircotis G, Schett G, Spector TD, Vyse TJ. The rs1143679 (R77H) lupus associated variant of ITGAM (CD11b) impairs complement receptor 3 mediated functions in human monocytes. Annals of the rheumatic diseases. 2012 Dec;71(12):2028-34. PMCID: PMC3488763.

40. Nath SK, Han S, Kim-Howard X, Kelly JA, Viswanathan P, Gilkeson GS, Chen W, Zhu C, McEver RP, Kimberly RP, Alarcon-Riquelme ME, Vyse TJ, Li QZ, Wakeland EK, Merrill JT, James JA, Kaufman KM, Guthridge JM, Harley JB. A nonsynonymous functional variant in integrin-alpha(M) (encoded by ITGAM) is associated with systemic lupus erythematosus. Nature genetics. 2008 Feb;40(2):152-4. PMCID: PMC.
41. Fu Q, Zhao J, Qian X, Wong JL, Kaufman KM, Yu CY, Hwee Siew H, Tan Tock Seng Hospital Lupus Study G, Mok MY, Harley JB, Guthridge JM, Song YW, Cho SK, Bae SC, Grossman JM, Hahn BH, Arnett FC, Shen N, Tsao BP. Association of a functional IRF7 variant with systemic lupus erythematosus. Arthritis and rheumatism. 2011 Mar;63(3):749-54. PMCID: PMC3063317.
42. Graham RR, Kozyrev SV, Baechler EC, Reddy MV, Plenge RM, Bauer JW, Ortmann WA, Koeuth T, Gonzalez Escribano MF, Argentine, Spanish Collaborative G, Pons-Estel B, Petri M, Daly M, Gregersen PK, Martin J, Altshuler D, Behrens TW, Alarcon-Riquelme ME. A common haplotype of interferon regulatory factor 5 (IRF5) regulates splicing and expression and is associated with increased risk of systemic lupus erythematosus. Nature genetics. 2006 May;38(5):550-5. PMCID: PMC.
43. Raj P, Rai E, Song R, Khan S, Wakeland BE, Viswanathan K, Arana C, Liang C, Zhang B, Dozmorov I, Carr-Johnson F, Mitrovic M, Wiley GB, Kelly JA, Lauwerys BR, Olsen NJ, Cotsapas C, Garcia CK, Wise CA, Harley JB, Nath SK, James JA, Jacob CO, Tsao BP, Pasare C, Karp DR, Li QZ, Gaffney PM, Wakeland EK. Regulatory polymorphisms modulate the expression of HLA class II molecules and promote autoimmunity. eLife. 2016 Feb 15;5. PMCID: PMC4811771.
44. Shen N, Fu Q, Deng Y, Qian X, Zhao J, Kaufman KM, Wu YL, Yu CY, Tang Y, Chen JY, Yang W, Wong M, Kawasaki A, Tsuchiya N, Sumida T, Kawaguchi Y, Howe HS, Mok MY, Bang SY, Liu FL, Chang DM, Takasaki Y, Hashimoto H, Harley JB, Guthridge JM, Grossman JM, Cantor RM, Song YW, Bae SC, Chen S, Hahn BH, Lau YL, Tsao BP. Sex-specific association of X-linked Toll-like receptor 7 (TLR7) with male systemic lupus erythematosus. Proceedings of the National Academy of Sciences of the United States of America. 2010 Sep 07;107(36):15838-43. PMCID: PMC2936646.
45. Deng Y, Zhao J, Sakurai D, Kaufman KM, Edberg JC, Kimberly RP, Kamen DL, Gilkeson GS, Jacob CO, Scofield RH, Langefeld CD, Kelly JA, Ramsey-Goldman R, Petri MA, Reveille JD, Vila LM, Alarcon GS, Vyse TJ, Pons-Estel BA, Argentine Collaborative G, Freedman BI, Gaffney PM, Sivils KM, James JA, Gregersen PK, Anaya JM, Niewold TB, Merrill JT, Criswell LA, Stevens AM, Boackle SA, Cantor RM, Chen W, Grossman JM, Hahn BH, Harley JB, Alarcomicronn-Riquelme ME, Biolupus, networks G, Brown EE, Tsao BP. MicroRNA-3148 modulates allelic expression of toll-like receptor 7 variant associated with systemic lupus erythematosus. PLoS genetics. 2013;9(2):e1003336. PMCID: PMC3585142.
46. Lei W, Luo Y, Lei W, Luo Y, Yan K, Zhao S, Li Y, Qiu X, Zhou Y, Long H, Zhao M, Liang Y, Su Y, Lu Q. Abnormal DNA methylation in CD4+ T cells from patients with systemic lupus erythematosus, systemic sclerosis, and dermatomyositis. Scand J Rheumatol. 2009;38(5):369-74. PMCID: PMC.
47. Hedrich CM, Crispin JC, Rauen T, Ioannidis C, Apostolidis SA, Lo MS, Kyttaris VC, Tsokos GC. cAMP response element modulator alpha controls IL2 and IL17A expression during CD4 lineage commitment and subset distribution in lupus. Proceedings of the National Academy of Sciences of the United States of America. 2012 Oct 9;109(41):16606-11. PMCID: PMC3478624.

48. Hedrich CM, Crispin JC, Rauen T, Ioannidis C, Koga T, Rodriguez Rodriguez N, Apostolidis SA, Kyttaris VC, Tsokos GC. cAMP responsive element modulator (CREM) alpha mediates chromatin remodeling of CD8 during the generation of CD3+ CD4- CD8- T cells. The Journal of biological chemistry. 2014 Jan 24;289(4):2361-70. PMCID: PMC3900979.
49. Hedrich CM, Rauen T, Crispin JC, Koga T, Ioannidis C, Zajdel M, Kyttaris VC, Tsokos GC. cAMP-responsive element modulator alpha (CREMalpha) trans-represses the transmembrane glycoprotein CD8 and contributes to the generation of CD3+CD4-CD8- T cells in health and disease. The Journal of biological chemistry. 2013 Nov 1;288(44):31880-7. PMCID: PMC3814780.
50. Hedrich CM, Rauen T, Kis-Toth K, Kyttaris VC, Tsokos GC. cAMP-responsive element modulator alpha (CREMalpha) suppresses IL-17F protein expression in T lymphocytes from patients with systemic lupus erythematosus (SLE). The Journal of biological chemistry. 2012 Feb 10;287(7):4715-25. PMCID: PMC3281648.
51. Liu Y, Chen Y, Richardson B. Decreased DNA methyltransferase levels contribute to abnormal gene expression in "senescent" CD4(+)CD28(-) T cells. Clinical immunology. 2009 Aug;132(2):257-65. PMCID: PMC2710420.
52. Nile CJ, Read RC, Akil M, Duff GW, Wilson AG. Methylation status of a single CpG site in the IL6 promoter is related to IL6 messenger RNA levels and rheumatoid arthritis. Arthritis and rheumatism. 2008 Sep;58(9):2686-93. PMCID: PMC.
53. Hedrich CM, Ramakrishnan A, Dabitao D, Wang F, Ranatunga D, Bream JH. Dynamic DNA methylation patterns across the mouse and human IL10 genes during CD4+ T cell activation; influence of IL-27. Molecular immunology. 2010 Nov-Dec;48(1-3):73-81. PMCID: PMC2993837.
54. Hofmann SR, Moller J, Rauen T, Paul D, Gahr M, Rosen-Wolff Z, Brenner S, Hedrich CM. Dynamic CpG-DNA methylation of Il10 and Il19 in CD4+ T lymphocytes and macrophages: effects on tissue-specific gene expression. Klinische Padiatrie. 2012 Mar;224(2):53-60. PMCID: PMC.
55. Zhao M, Tang J, Gao F, Wu X, Liang Y, Yin H, Lu Q. Hypomethylation of IL10 and IL13 promoters in CD4+ T cells of patients with systemic lupus erythematosus. Journal of biomedicine & biotechnology. 2010;2010:931018. PMCID: PMC2879555.
56. Rauen T, Hedrich CM, Juang YT, Tenbrock K, Tsokos GC. cAMP-responsive element modulator (CREM)alpha protein induces interleukin 17A expression and mediates epigenetic alterations at the interleukin-17A gene locus in patients with systemic lupus erythematosus. The Journal of biological chemistry. 2011 Dec 16;286(50):43437-46. PMCID: PMC3234851.
57. Absher DM, Li X, Waite LL, Gibson A, Roberts K, Edberg J, Chatham WW, Kimberly RP. Genome-wide DNA methylation analysis of systemic lupus erythematosus reveals persistent hypomethylation of interferon genes and compositional changes to CD4+ T-cell populations. PLoS genetics. 2013;9(8):e1003678. PMCID: PMC3738443.
58. Hu N, Qiu X, Luo Y, Yuan J, Li Y, Lei W, Zhang G, Zhou Y, Su Y, Lu Q. Abnormal histone modification patterns in lupus CD4+ T cells. The Journal of rheumatology. 2008 May;35(5):804-10. PMCID: PMC.
59. Apostolidis SA, Crispin JC, Tsokos GC. IL-17-producing T cells in lupus nephritis. Lupus. 2011 Feb;20(2):120-4. PMCID: PMC.
60. Hedrich CM, Rauen T, Tsokos GC. cAMP-responsive element modulator (CREM)alpha protein signaling mediates epigenetic remodeling of the human interleukin-2 gene: implications

in systemic lupus erythematosus. The Journal of biological chemistry. 2011 Dec 16;286(50):43429-36. PMCID: PMC3234875.
61. Crispin JC, Oukka M, Bayliss G, Cohen RA, Van Beek CA, Stillman IE, Kyttaris VC, Juang YT, Tsokos GC. Expanded double negative T cells in patients with systemic lupus erythematosus produce IL-17 and infiltrate the kidneys. Journal of immunology. 2008 Dec 15;181(12):8761-6. PMCID: PMC2596652.
62. von Spee-Mayer C, Siegert E, Abdirama D, Rose A, Klaus A, Alexander T, Enghard P, Sawitzki B, Hiepe F, Radbruch A, Burmester GR, Riemekasten G, Humrich JY. Low-dose interleukin-2 selectively corrects regulatory T cell defects in patients with systemic lupus erythematosus. Annals of the rheumatic diseases. 2016 Jul;75(7):1407-15. PMCID: PMC.
63. Courtney PA, Crockard AD, Williamson K, Irvine AE, Kennedy RJ, Bell AL. Increased apoptotic peripheral blood neutrophils in systemic lupus erythematosus: relations with disease activity, antibodies to double stranded DNA, and neutropenia. Annals of the rheumatic diseases. 1999 May;58(5):309-14. PMCID: PMC1752888.
64. McConnell JR, Crockard AD, Cairns AP, Bell AL. Neutrophils from systemic lupus erythematosus patients demonstrate increased nuclear DNA damage. Clinical and experimental rheumatology. 2002 Sep-Oct;20(5):653-60. PMCID: PMC.
65. Villanueva E, Yalavarthi S, Berthier CC, Hodgin JB, Khandpur R, Lin AM, Rubin CJ, Zhao W, Olsen SH, Klinker M, Shealy D, Denny MF, Plumas J, Chaperot L, Kretzler M, Bruce AT, Kaplan MJ. Netting neutrophils induce endothelial damage, infiltrate tissues, and expose immunostimulatory molecules in systemic lupus erythematosus. Journal of immunology. 2011 Jul 1;187(1):538-52. PMCID: PMC3119769.
66. Manderson AP, Botto M, Walport MJ. The role of complement in the development of systemic lupus erythematosus. Annual review of immunology. 2004;22:431-56. PMCID: PMC.
67. Yeh TM, Chang HC, Liang CC, Wu JJ, Liu MF. Deoxyribonuclease-inhibitory antibodies in systemic lupus erythematosus. Journal of biomedical science. 2003 Sep-Oct;10(5):544-51. PMCID: PMC.
68. Gaipl US, Beyer TD, Heyder P, Kuenkele S, Bottcher A, Voll RE, Kalden JR, Herrmann M. Cooperation between C1q and DNase I in the clearance of necrotic cell-derived chromatin. Arthritis and rheumatism. 2004 Feb;50(2):640-9. PMCID: PMC.
69. Lande R, Ganguly D, Facchinetti V, Frasca L, Conrad C, Gregorio J, Meller S, Chamilos G, Sebasigari R, Riccieri V, Bassett R, Amuro H, Fukuhara S, Ito T, Liu YJ, Gilliet M. Neutrophils activate plasmacytoid dendritic cells by releasing self-DNA-peptide complexes in systemic lupus erythematosus. Science translational medicine. 2011 Mar 9;3(73):73ra19. PMCID: PMC3399524.
70. Hashimoto Y, Ziff M, Hurd ER. Increased endothelial cell adherence, aggregation, and superoxide generation by neutrophils incubated in systemic lupus erythematosus and Felty's syndrome sera. Arthritis and rheumatism. 1982 Dec;25(12):1409-18. PMCID: PMC.
71. Bengtsson AA, Pettersson A, Wichert S, Gullstrand B, Hansson M, Hellmark T, Johansson AC. Low production of reactive oxygen species in granulocytes is associated with organ damage in systemic lupus erythematosus. Arthritis research & therapy. 2014 Jun 5;16(3):R120. PMCID: PMC4075132.
72. Wu SA, Yeh KW, Lee WI, Yao TC, Kuo ML, Huang B, Huang JL. Impaired phagocytosis and susceptibility to infection in pediatric-onset systemic lupus erythematosus. Lupus. 2013 Mar;22(3):279-88. PMCID: PMC.

73. Savarese E, Chae OW, Trowitzsch S, Weber G, Kastner B, Akira S, Wagner H, Schmid RM, Bauer S, Krug A. U1 small nuclear ribonucleoprotein immune complexes induce type I interferon in plasmacytoid dendritic cells through TLR7. Blood. 2006 Apr 15;107(8):3229-34. PMCID: PMC.
74. Wang H, Li T, Chen S, Gu Y, Ye S. Neutrophil Extracellular Trap Mitochondrial DNA and Its Autoantibody in Systemic Lupus Erythematosus and a Proof-of-Concept Trial of Metformin. Arthritis & rheumatology. 2015 Dec;67(12):3190-200. PMCID: PMC.
75. Rowland SL, Riggs JM, Gilfillan S, Bugatti M, Vermi W, Kolbeck R, Unanue ER, Sanjuan MA, Colonna M. Early, transient depletion of plasmacytoid dendritic cells ameliorates autoimmunity in a lupus model. The Journal of experimental medicine. 2014 Sep 22;211(10):1977-91. PMCID: PMC4172228.
76. Davison LM, Jorgensen TN. Sialic acid-binding immunoglobulin-type lectin H-positive plasmacytoid dendritic cells drive spontaneous lupus-like disease development in B6.Nba2 mice. Arthritis & rheumatology. 2015 Apr;67(4):1012-22. PMCID: PMC.
77. Furie R, Werth VP, Merola JF, Stevenson L, Reynolds TL, Naik H, Wang W, Christmann R, Gardet A, Pellerin A, Hamann S, Auluck P, Barbey C, Gulati P, Rabah D, Franchimont N. Monoclonal antibody targeting BDCA2 ameliorates skin lesions in systemic lupus erythematosus. The Journal of clinical investigation. 2019 Mar 1;129(3):1359-71. PMCID: PMC.
78. Fransen JH, Hilbrands LB, Ruben J, Stoffels M, Adema GJ, van der Vlag J, Berden JH. Mouse dendritic cells matured by ingestion of apoptotic blebs induce T cells to produce interleukin-17. Arthritis and rheumatism. 2009 Aug;60(8):2304-13. PMCID: PMC.
79. Fransen JH, Hilbrands LB, Jacobs CW, Adema GJ, Berden JH, Van der Vlag J. Both early and late apoptotic blebs are taken up by DC and induce IL-6 production. Autoimmunity. 2009 May;42(4):325-7. PMCID: PMC.
80. Crispin JC, Vargas-Rojas MI, Monsivais-Urenda A, Alcocer-Varela J. Phenotype and function of dendritic cells of patients with systemic lupus erythematosus. Clinical immunology. 2012 Apr;143(1):45-50. PMCID: PMC.
81. Ding D, Mehta H, McCune WJ, Kaplan MJ. Aberrant phenotype and function of myeloid dendritic cells in systemic lupus erythematosus. Journal of immunology. 2006 Nov 1;177(9):5878-89. PMCID: PMC.
82. Colonna L, Dinnall JA, Shivers DK, Frisoni L, Caricchio R, Gallucci S. Abnormal costimulatory phenotype and function of dendritic cells before and after the onset of severe murine lupus. Arthritis research & therapy. 2006;8(2):R49. PMCID: PMC1526610.
83. Gleisner MA, Reyes P, Alfaro J, Solanes P, Simon V, Crisostomo N, Sauma D, Rosemblatt M, Bono MR. Dendritic and stromal cells from the spleen of lupic mice present phenotypic and functional abnormalities. Molecular immunology. 2013 Jul;54(3-4):423-34. PMCID: PMC.
84. Mozaffarian N, Wiedeman AE, Stevens AM. Active systemic lupus erythematosus is associated with failure of antigen-presenting cells to express programmed death ligand-1. Rheumatology (Oxford, England). 2008 Sep;47(9):1335-41. PMCID: PMC2722808.
85. Sahu R, Bethunaickan R, Singh S, Davidson A. Structure and function of renal macrophages and dendritic cells from lupus-prone mice. Arthritis & rheumatology. 2014 Jun;66(6):1596-607. PMCID: PMC4547797.
86. Schiffer L, Bethunaickan R, Ramanujam M, Huang W, Schiffer M, Tao H, Madaio MP, Bottinger EP, Davidson A. Activated renal macrophages are markers of disease onset and

disease remission in lupus nephritis. Journal of immunology. 2008 Feb 1;180(3):1938-47. PMCID: PMC2587994.
87. Nimmerjahn F, Ravetch JV. Fcgamma receptors as regulators of immune responses. Nature reviews Immunology. 2008 Jan;8(1):34-47. PMCID: PMC.
88. Blank MC, Stefanescu RN, Masuda E, Marti F, King PD, Redecha PB, Wurzburger RJ, Peterson MG, Tanaka S, Pricop L. Decreased transcription of the human FCGR2B gene mediated by the -343 G/C promoter polymorphism and association with systemic lupus erythematosus. Human genetics. 2005 Jul;117(2-3):220-7. PMCID: PMC.
89. Floto RA, Clatworthy MR, Heilbronn KR, Rosner DR, MacAry PA, Rankin A, Lehner PJ, Ouwehand WH, Allen JM, Watkins NA, Smith KG. Loss of function of a lupus-associated FcgammaRIIb polymorphism through exclusion from lipid rafts. Nature medicine. 2005 Oct;11(10):1056-8. PMCID: PMC.
90. Su K, Yang H, Li X, Li X, Gibson AW, Cafardi JM, Zhou T, Edberg JC, Kimberly RP. Expression profile of FcgammaRIIb on leukocytes and its dysregulation in systemic lupus erythematosus. Journal of immunology. 2007 Mar 1;178(5):3272-80. PMCID: PMC2824439.
91. Li X, Ptacek TS, Brown EE, Edberg JC. Fcgamma receptors: structure, function and role as genetic risk factors in SLE. Genes and immunity. 2009 Jul;10(5):380-9. PMCID: PMC2830794.
92. Hepburn AL, Mason JC, Davies KA. Expression of Fcgamma and complement receptors on peripheral blood monocytes in systemic lupus erythematosus and rheumatoid arthritis. Rheumatology (Oxford, England). 2004 May;43(5):547-54. PMCID: PMC.
93. Herrmann M, Voll RE, Zoller OM, Hagenhofer M, Ponner BB, Kalden JR. Impaired phagocytosis of apoptotic cell material by monocyte-derived macrophages from patients with systemic lupus erythematosus. Arthritis and rheumatism. 1998 Jul;41(7):1241-50. PMCID: PMC.
94. Baumann I, Kolowos W, Voll RE, Manger B, Gaipl U, Neuhuber WL, Kirchner T, Kalden JR, Herrmann M. Impaired uptake of apoptotic cells into tingible body macrophages in germinal centers of patients with systemic lupus erythematosus. Arthritis and rheumatism. 2002 Jan;46(1):191-201. PMCID: PMC.
95. Zhang H, Fu R, Guo C, Huang Y, Wang H, Wang S, Zhao J, Yang N. Anti-dsDNA antibodies bind to TLR4 and activate NLRP3 inflammasome in lupus monocytes/macrophages. Journal of translational medicine. 2016 Jun 1;14(1):156. PMCID: PMC4888250.
96. Kahlenberg JM, Carmona-Rivera C, Smith CK, Kaplan MJ. Neutrophil extracellular trap-associated protein activation of the NLRP3 inflammasome is enhanced in lupus macrophages. Journal of immunology. 2013 Feb 1;190(3):1217-26. PMCID: PMC3552129.
97. Ren Y, Tang J, Mok MY, Chan AW, Wu A, Lau CS. Increased apoptotic neutrophils and macrophages and impaired macrophage phagocytic clearance of apoptotic neutrophils in systemic lupus erythematosus. Arthritis and rheumatism. 2003 Oct;48(10):2888-97. PMCID: PMC.
98. Liu J, Berthier CC, Kahlenberg JM. Enhanced Inflammasome Activity in Systemic Lupus Erythematosus Is Mediated via Type I Interferon-Induced Up-Regulation of Interferon Regulatory Factor 1. Arthritis & rheumatology. 2017 Sep;69(9):1840-9. PMCID: PMC5575977.
99. Perl A. Activation of mTOR (mechanistic target of rapamycin) in rheumatic diseases. Nature reviews Rheumatology. 2016 Mar;12(3):169-82. PMCID: PMC5314913.
100. Gorman CL, Russell AI, Zhang Z, Cunninghame Graham D, Cope AP, Vyse TJ. Polymorphisms in the CD3Z gene influence TCRzeta expression in systemic lupus

erythematosus patients and healthy controls. Journal of immunology. 2008 Jan 15;180(2):1060-70. PMCID: PMC.
101. Liossis SN, Ding XZ, Dennis GJ, Tsokos GC. Altered pattern of TCR/CD3-mediated protein-tyrosyl phosphorylation in T cells from patients with systemic lupus erythematosus. Deficient expression of the T cell receptor zeta chain. The Journal of clinical investigation. 1998 Apr 1;101(7):1448-57. PMCID: PMC508723.
102. Katsiari CG, Kyttaris VC, Juang YT, Tsokos GC. Protein phosphatase 2A is a negative regulator of IL-2 production in patients with systemic lupus erythematosus. The Journal of clinical investigation. 2005 Nov;115(11):3193-204. PMCID: PMC1253625.
103. Sinai P, Dozmorov IM, Song R, Schwartzberg PL, Wakeland EK, Wulfing C. T/B-cell interactions are more transient in response to weak stimuli in SLE-prone mice. European journal of immunology. 2014 Dec;44(12):3522-31. PMCID: PMC4261040.
104. Mietzner B, Tsuiji M, Scheid J, Velinzon K, Tiller T, Abraham K, Gonzalez JB, Pascual V, Stichweh D, Wardemann H, Nussenzweig MC. Autoreactive IgG memory antibodies in patients with systemic lupus erythematosus arise from nonreactive and polyreactive precursors. Proceedings of the National Academy of Sciences of the United States of America. 2008 Jul 15;105(28):9727-32. PMCID: PMC2474524.
105. Choi JY, Ho JH, Pasoto SG, Bunin V, Kim ST, Carrasco S, Borba EF, Goncalves CR, Costa PR, Kallas EG, Bonfa E, Craft J. Circulating follicular helper-like T cells in systemic lupus erythematosus: association with disease activity. Arthritis & rheumatology. 2015 Apr;67(4):988-99. PMCID: PMC4450082.
106. Szabo K, Papp G, Szanto A, Tarr T, Zeher M. A comprehensive investigation on the distribution of circulating follicular T helper cells and B cell subsets in primary Sjogren's syndrome and systemic lupus erythematosus. Clin Exp Immunol. 2016 Jan;183(1):76-89. PMCID: PMC4687513.
107. Keszei M, Detre C, Castro W, Magelky E, O'Keeffe M, Kis-Toth K, Tsokos GC, Wang N, Terhorst C. Expansion of an osteopontin-expressing T follicular helper cell subset correlates with autoimmunity in B6.Sle1b mice and is suppressed by the H1-isoform of the Slamf6 receptor. FASEB journal : official publication of the Federation of American Societies for Experimental Biology. 2013 Aug;27(8):3123-31. PMCID: PMC3714581.
108. Yang X, Yang J, Chu Y, Wang J, Guan M, Zhu X, Xue Y, Zou H. T follicular helper cells mediate expansion of regulatory B cells via IL-21 in Lupus-prone MRL/lpr mice. PloS one. 2013;8(4):e62855. PMCID: PMC3634758.
109. Quach TD, Manjarrez-Orduno N, Adlowitz DG, Silver L, Yang H, Wei C, Milner EC, Sanz I. Anergic responses characterize a large fraction of human autoreactive naive B cells expressing low levels of surface IgM. Journal of immunology. 2011 Apr 15;186(8):4640-8. PMCID: PMC3095097.
110. Rodriguez-Bayona B, Ramos-Amaya A, Perez-Venegas JJ, Rodriguez C, Brieva JA. Decreased frequency and activated phenotype of blood CD27 IgD IgM B lymphocytes is a permanent abnormality in systemic lupus erythematosus patients. Arthritis research & therapy. 2010;12(3):R108. PMCID: PMC2911899.
111. Mackay M, Stanevsky A, Wang T, Aranow C, Li M, Koenig S, Ravetch JV, Diamond B. Selective dysregulation of the FcgammaIIB receptor on memory B cells in SLE. The Journal of experimental medicine. 2006 Sep 4;203(9):2157-64. PMCID: PMC2118390.
112. Tiller T, Kofer J, Kreschel C, Busse CE, Riebel S, Wickert S, Oden F, Mertes MM, Ehlers M, Wardemann H. Development of self-reactive germinal center B cells and plasma cells

in autoimmune Fc gammaRIIB-deficient mice. The Journal of experimental medicine. 2010 Nov 22;207(12):2767-78. PMCID: PMC2989760.
113. Fukuyama H, Nimmerjahn F, Ravetch JV. The inhibitory Fcgamma receptor modulates autoimmunity by limiting the accumulation of immunoglobulin G+ anti-DNA plasma cells. Nature immunology. 2005 Jan;6(1):99-106. PMCID: PMC.
114. Suarez-Fueyo A, Barber DF, Martinez-Ara J, Zea-Mendoza AC, Carrera AC. Enhanced phosphoinositide 3-kinase delta activity is a frequent event in systemic lupus erythematosus that confers resistance to activation-induced T cell death. Journal of immunology. 2011 Sep 1;187(5):2376-85. PMCID: PMC.
115. Wu XN, Ye YX, Niu JW, Li Y, Li X, You X, Chen H, Zhao LD, Zeng XF, Zhang FC, Tang FL, He W, Cao XT, Zhang X, Lipsky PE. Defective PTEN regulation contributes to B cell hyperresponsiveness in systemic lupus erythematosus. Science translational medicine. 2014 Jul 23;6(246):246ra99. PMCID: PMC.
116. Arce E, Jackson DG, Gill MA, Bennett LB, Banchereau J, Pascual V. Increased frequency of pre-germinal center B cells and plasma cell precursors in the blood of children with systemic lupus erythematosus. Journal of immunology. 2001 Aug 15;167(4):2361-9. PMCID: PMC.
117. Lee J, Kuchen S, Fischer R, Chang S, Lipsky PE. Identification and characterization of a human CD5+ pre-naive B cell population. Journal of immunology. 2009 Apr 1;182(7):4116-26. PMCID: PMC.
118. Yurasov S, Tiller T, Tsuiji M, Velinzon K, Pascual V, Wardemann H, Nussenzweig MC. Persistent expression of autoantibodies in SLE patients in remission. The Journal of experimental medicine. 2006 Oct 2;203(10):2255-61. PMCID: PMC2118096.
119. Vazquez MI, Catalan-Dibene J, Zlotnik A. B cells responses and cytokine production are regulated by their immune microenvironment. Cytokine. 2015 Aug;74(2):318-26. PMCID: PMC4475485.
120. Stohl W, Hilbert DM. The discovery and development of belimumab: the anti-BLyS-lupus connection. Nature biotechnology. 2012 Jan 9;30(1):69-77. PMCID: PMC3264947.
121. Jacob N, Guo S, Mathian A, Koss MN, Gindea S, Putterman C, Jacob CO, Stohl W. B Cell and BAFF dependence of IFN-alpha-exaggerated disease in systemic lupus erythematosus-prone NZM 2328 mice. Journal of immunology. 2011 Apr 15;186(8):4984-93. PMCID: PMC3074466.
122. Xu H, Liu J, Cui X, Zuo Y, Zhang Z, Li Y, Tao R, Li Y, Pang J. Increased frequency of circulating follicular helper T cells in lupus patients is associated with autoantibody production in a CD40L-dependent manner. Cellular immunology. 2015 May;295(1):46-51. PMCID: PMC.
123. Fleischer SJ, Giesecke C, Mei HE, Lipsky PE, Daridon C, Dorner T. Increased frequency of a unique spleen tyrosine kinase bright memory B cell population in systemic lupus erythematosus. Arthritis & rheumatology. 2014 Dec;66(12):3424-35. PMCID: PMC.
124. Meffre E, Wardemann H. B-cell tolerance checkpoints in health and autoimmunity. Current opinion in immunology. 2008 Dec;20(6):632-8. PMCID: PMC.
125. Odendahl M, Jacobi A, Hansen A, Feist E, Hiepe F, Burmester GR, Lipsky PE, Radbruch A, Dorner T. Disturbed peripheral B lymphocyte homeostasis in systemic lupus erythematosus. Journal of immunology. 2000 Nov 15;165(10):5970-9. PMCID: PMC.
126. Gutierrez-Adrianzen OA, Koutouzov S, Mota RM, das Chagas Medeiros MM, Bach JF, de Holanda Campos H. Diagnostic value of anti-nucleosome antibodies in the assessment of disease activity of systemic lupus erythematosus: a prospective study comparing anti-

nucleosome with anti-dsDNA antibodies. The Journal of rheumatology. 2006 Aug;33(8):1538-44. PMCID: PMC.
127. Rekvig OP, Putterman C, Casu C, Gao HX, Ghirardello A, Mortensen ES, Tincani A, Doria A. Autoantibodies in lupus: culprits or passive bystanders? Autoimmunity reviews. 2012 Jun;11(8):596-603. PMCID: PMC.
128. Batteux F, Palmer P, Daeron M, Weill B, Lebon P. FCgammaRII (CD32)-dependent induction of interferon-alpha by serum from patients with lupus erythematosus. European cytokine network. 1999 Dec;10(4):509-14. PMCID: PMC.
129. Chan OT, Hannum LG, Haberman AM, Madaio MP, Shlomchik MJ. A novel mouse with B cells but lacking serum antibody reveals an antibody-independent role for B cells in murine lupus. The Journal of experimental medicine. 1999 May 17;189(10):1639-48. PMCID: PMC2193634.
130. Jacob N, Stohl W. Autoantibody-dependent and autoantibody-independent roles for B cells in systemic lupus erythematosus: past, present, and future. Autoimmunity. 2010 Feb;43(1):84-97. PMCID: PMC2809122.
131. Crawford A, Macleod M, Schumacher T, Corlett L, Gray D. Primary T cell expansion and differentiation in vivo requires antigen presentation by B cells. Journal of immunology. 2006 Mar 15;176(6):3498-506. PMCID: PMC.
132. Chan O, Shlomchik MJ. A new role for B cells in systemic autoimmunity: B cells promote spontaneous T cell activation in MRL-lpr/lpr mice. Journal of immunology. 1998 Jan 1;160(1):51-9. PMCID: PMC.
133. Pestka S, Krause CD, Walter MR. Interferons, interferon-like cytokines, and their receptors. Immunol Rev. 2004 Dec;202:8-32. PMCID: PMC.
134. Bengtsson AA, Ronnblom L. Role of interferons in SLE. Best practice & research Clinical rheumatology. 2017 Jun;31(3):415-28. PMCID: PMC.
135. Elkon KB, Stone VV. Type I interferon and systemic lupus erythematosus. Journal of interferon & cytokine research : the official journal of the International Society for Interferon and Cytokine Research. 2011 Nov;31(11):803-12. PMCID: PMC3216059.
136. Yao Y, Higgs BW, Morehouse C, de Los Reyes M, Trigona W, Brohawn P, White W, Zhang J, White B, Coyle AJ, Kiener PA, Jallal B. Development of Potential Pharmacodynamic and Diagnostic Markers for Anti-IFN-alpha Monoclonal Antibody Trials in Systemic Lupus Erythematosus. Human genomics and proteomics : HGP. 2009 Nov 17;2009. PMCID: PMC2950308.
137. Steinberg AD, Baron S, Talal N. The pathogenesis of autoimmunity in New Zealand mice, I. Induction of antinucleic acid antibodies by polyinosinic-polycytidylic acid. Proceedings of the National Academy of Sciences of the United States of America. 1969 Aug;63(4):1102-7. PMCID: PMC223434.
138. Gota C, Calabrese L. Induction of clinical autoimmune disease by therapeutic interferon-alpha. Autoimmunity. 2003 Dec;36(8):511-8. PMCID: PMC.
139. Mathian A, Weinberg A, Gallegos M, Banchereau J, Koutouzov S. IFN-alpha induces early lethal lupus in preautoimmune (New Zealand Black x New Zealand White) F1 but not in BALB/c mice. Journal of immunology. 2005 Mar 1;174(5):2499-506. PMCID: PMC.
140. Liu Z, Bethunaickan R, Huang W, Lodhi U, Solano I, Madaio MP, Davidson A. Interferon-alpha accelerates murine systemic lupus erythematosus in a T cell-dependent manner. Arthritis and rheumatism. 2011 Jan;63(1):219-29. PMCID: PMC3014995.

141. Agrawal H, Jacob N, Carreras E, Bajana S, Putterman C, Turner S, Neas B, Mathian A, Koss MN, Stohl W, Kovats S, Jacob CO. Deficiency of type I IFN receptor in lupus-prone New Zealand mixed 2328 mice decreases dendritic cell numbers and activation and protects from disease. Journal of immunology. 2009 Nov 1;183(9):6021-9. PMCID: PMC2766036.
142. Bauer JW, Petri M, Batliwalla FM, Koeuth T, Wilson J, Slattery C, Panoskaltsis-Mortari A, Gregersen PK, Behrens TW, Baechler EC. Interferon-regulated chemokines as biomarkers of systemic lupus erythematosus disease activity: a validation study. Arthritis and rheumatism. 2009 Oct;60(10):3098-107. PMCID: PMC2842939.
143. Feng X, Wu H, Grossman JM, Hanvivadhanakul P, FitzGerald JD, Park GS, Dong X, Chen W, Kim MH, Weng HH, Furst DE, Gorn A, McMahon M, Taylor M, Brahn E, Hahn BH, Tsao BP. Association of increased interferon-inducible gene expression with disease activity and lupus nephritis in patients with systemic lupus erythematosus. Arthritis and rheumatism. 2006 Sep;54(9):2951-62. PMCID: PMC.
144. Chaussabel D, Quinn C, Shen J, Patel P, Glaser C, Baldwin N, Stichweh D, Blankenship D, Li L, Munagala I, Bennett L, Allantaz F, Mejias A, Ardura M, Kaizer E, Monnet L, Allman W, Randall H, Johnson D, Lanier A, Punaro M, Wittkowski KM, White P, Fay J, Klintmalm G, Ramilo O, Palucka AK, Banchereau J, Pascual V. A modular analysis framework for blood genomics studies: application to systemic lupus erythematosus. Immunity. 2008 Jul 18;29(1):150-64. PMCID: PMC2727981.
145. Petri M, Singh S, Tesfasyone H, Dedrick R, Fry K, Lal P, Williams G, Bauer J, Gregersen P, Behrens T, Baechler E. Longitudinal expression of type I interferon responsive genes in systemic lupus erythematosus. Lupus. 2009 Oct;18(11):980-9. PMCID: PMC4752166.
146. Bennett L, Palucka AK, Arce E, Cantrell V, Borvak J, Banchereau J, Pascual V. Interferon and granulopoiesis signatures in systemic lupus erythematosus blood. The Journal of experimental medicine. 2003 Mar 17;197(6):711-23. PMCID: PMC2193846.
147. Hirai M, Kadowaki N, Kitawaki T, Fujita H, Takaori-Kondo A, Fukui R, Miyake K, Maeda T, Kamihira S, Miyachi Y, Uchiyama T. Bortezomib suppresses function and survival of plasmacytoid dendritic cells by targeting intracellular trafficking of Toll-like receptors and endoplasmic reticulum homeostasis. Blood. 2011 Jan 13;117(2):500-9. PMCID: PMC.
148. Kirou KA, Lee C, George S, Louca K, Peterson MG, Crow MK. Activation of the interferon-alpha pathway identifies a subgroup of systemic lupus erythematosus patients with distinct serologic features and active disease. Arthritis and rheumatism. 2005 May;52(5):1491-503. PMCID: PMC.
149. Ganguly D, Chamilos G, Lande R, Gregorio J, Meller S, Facchinetti V, Homey B, Barrat FJ, Zal T, Gilliet M. Self-RNA-antimicrobial peptide complexes activate human dendritic cells through TLR7 and TLR8. The Journal of experimental medicine. 2009 Aug 31;206(9):1983-94. PMCID: PMC2737167.
150. Lande R, Gregorio J, Facchinetti V, Chatterjee B, Wang YH, Homey B, Cao W, Wang YH, Su B, Nestle FO, Zal T, Mellman I, Schroder JM, Liu YJ, Gilliet M. Plasmacytoid dendritic cells sense self-DNA coupled with antimicrobial peptide. Nature. 2007 Oct 4;449(7162):564-9. PMCID: PMC.
151. Blanco P, Palucka AK, Gill M, Pascual V, Banchereau J. Induction of dendritic cell differentiation by IFN-alpha in systemic lupus erythematosus. Science. 2001 Nov 16;294(5546):1540-3. PMCID: PMC.
152. Lindau D, Mussard J, Rabsteyn A, Ribon M, Kotter I, Igney A, Adema GJ, Boissier MC, Rammensee HG, Decker P. TLR9 independent interferon alpha production by neutrophils on

NETosis in response to circulating chromatin, a key lupus autoantigen. Annals of the rheumatic diseases. 2014 Dec;73(12):2199-207. PMCID: PMC.
153. Lee PY, Weinstein JS, Nacionales DC, Scumpia PO, Li Y, Butfiloski E, van Rooijen N, Moldawer L, Satoh M, Reeves WH. A novel type I IFN-producing cell subset in murine lupus. Journal of immunology. 2008 Apr 1;180(7):5101-8. PMCID: PMC2909121.
154. Stannard JN, Reed TJ, Myers E, Lowe L, Sarkar MK, Xing X, Gudjonsson JE, Kahlenberg JM. Lupus Skin Is Primed for IL-6 Inflammatory Responses through a Keratinocyte-Mediated Autocrine Type I Interferon Loop. The Journal of investigative dermatology. 2017 Jan;137(1):115-22. PMCID: PMC5183476.
155. Sarkar MK, Hile GA, Tsoi LC, Xing X, Liu J, Liang Y, Berthier CC, Swindell WR, Patrick MT, Shao S, Tsou PS, Uppala R, Beamer MA, Srivastava A, Bielas SL, Harms PW, Getsios S, Elder JT, Voorhees JJ, Gudjonsson JE, Kahlenberg JM. Photosensitivity and type I IFN responses in cutaneous lupus are driven by epidermal-derived interferon kappa. Annals of the rheumatic diseases. 2018 Nov;77(11):1653-64. PMCID: PMC6185784.
156. Sontheimer C, Liggitt D, Elkon KB. Ultraviolet B Irradiation Causes Stimulator of Interferon Genes-Dependent Production of Protective Type I Interferon in Mouse Skin by Recruited Inflammatory Monocytes. Arthritis Rheumatol. 2017 Apr;69(4):826-36. PMCID: PMCPMC5659322.
157. Yin Q, Xu X, Lin Y, Lv J, Zhao L, He R. Ultraviolet B irradiation induces skin accumulation of plasmacytoid dendritic cells: A possible role for chemerin. Autoimmunity. 2014;47(3):185-92. PMCID: PMC.
158. Richardson B, Scheinbart L, Strahler J, Gross L, Hanash S, Johnson M. Evidence for impaired T cell DNA methylation in systemic lupus erythematosus and rheumatoid arthritis. Arthritis and rheumatism. 1990 Nov;33(11):1665-73. PMCID: PMC.
159. Santer DM, Wiedeman AE, Teal TH, Ghosh P, Elkon KB. Plasmacytoid dendritic cells and C1q differentially regulate inflammatory gene induction by lupus immune complexes. Journal of immunology. 2012 Jan 15;188(2):902-15. PMCID: PMC3238790.
160. Fehr EM, Spoerl S, Heyder P, Herrmann M, Bekeredjian-Ding I, Blank N, Lorenz HM, Schiller M. Apoptotic-cell-derived membrane vesicles induce an alternative maturation of human dendritic cells which is disturbed in SLE. Journal of autoimmunity. 2013 Feb;40:86-95. PMCID: PMC.
161. Abeler-Dorner L, Rieger CC, Berger B, Weyd H, Graf D, Pfrang S, Tarner IH, Schwarting A, Lorenz HM, Muller-Ladner U, Krammer PH, Kuhn A. Interferon-alpha abrogates the suppressive effect of apoptotic cells on dendritic cells in an in vitro model of systemic lupus erythematosus pathogenesis. The Journal of rheumatology. 2013 Oct;40(10):1683-96. PMCID: PMC.
162. Yamauchi M, Hashimoto M, Ichiyama K, Yoshida R, Hanada T, Muta T, Komune S, Kobayashi T, Yoshimura A. Ifi202, an IFN-inducible candidate gene for lupus susceptibility in NZB/W F1 mice, is a positive regulator for NF-kappaB activation in dendritic cells. International immunology. 2007 Aug;19(8):935-42. PMCID: PMC.
163. Huang X, Shen N, Bao C, Gu Y, Wu L, Chen S. Interferon-induced protein IFIT4 is associated with systemic lupus erythematosus and promotes differentiation of monocytes into dendritic cell-like cells. Arthritis research & therapy. 2008;10(4):R91. PMCID: PMC2575605.
164. Alunno A, Bartoloni E, Bistoni O, Nocentini G, Ronchetti S, Caterbi S, Valentini V, Riccardi C, Gerli R. Balance between regulatory T and Th17 cells in systemic lupus

erythematosus: the old and the new. Clinical & developmental immunology. 2012;2012:823085. PMCID: PMC3386568.
165. Lopez P, Rodriguez-Carrio J, Caminal-Montero L, Mozo L, Suarez A. A pathogenic IFNalpha, BLyS and IL-17 axis in Systemic Lupus Erythematosus patients. Scientific reports. 2016 Feb 5;6:20651. PMCID: PMC4742957.
166. Lim KH, Staudt LM. Toll-like receptor signaling. Cold Spring Harbor perspectives in biology. 2013 Jan 1;5(1):a011247. PMCID: PMC3579400.
167. Kawasaki A, Furukawa H, Kondo Y, Ito S, Hayashi T, Kusaoi M, Matsumoto I, Tohma S, Takasaki Y, Hashimoto H, Sumida T, Tsuchiya N. TLR7 single-nucleotide polymorphisms in the 3' untranslated region and intron 2 independently contribute to systemic lupus erythematosus in Japanese women: a case-control association study. Arthritis research & therapy. 2011 Mar 11;13(2):R41. PMCID: PMC3132023.
168. Deane JA, Pisitkun P, Barrett RS, Feigenbaum L, Town T, Ward JM, Flavell RA, Bolland S. Control of toll-like receptor 7 expression is essential to restrict autoimmunity and dendritic cell proliferation. Immunity. 2007 Nov;27(5):801-10. PMCID: PMC2706502.
169. Christensen SR, Shupe J, Nickerson K, Kashgarian M, Flavell RA, Shlomchik MJ. Toll-like receptor 7 and TLR9 dictate autoantibody specificity and have opposing inflammatory and regulatory roles in a murine model of lupus. Immunity. 2006 Sep;25(3):417-28. PMCID: PMC.
170. Nickerson KM, Christensen SR, Shupe J, Kashgarian M, Kim D, Elkon K, Shlomchik MJ. TLR9 regulates TLR7- and MyD88-dependent autoantibody production and disease in a murine model of lupus. Journal of immunology. 2010 Feb 15;184(4):1840-8. PMCID: PMC4098568.
171. Celhar T, Magalhaes R, Fairhurst AM. TLR7 and TLR9 in SLE: when sensing self goes wrong. Immunologic research. 2012 Sep;53(1-3):58-77. PMCID: PMC.
172. Garcia-Ortiz H, Velazquez-Cruz R, Espinosa-Rosales F, Jimenez-Morales S, Baca V, Orozco L. Association of TLR7 copy number variation with susceptibility to childhood-onset systemic lupus erythematosus in Mexican population. Annals of the rheumatic diseases. 2010 Oct;69(10):1861-5. PMCID: PMC.
173. Andrews BS, Eisenberg RA, Theofilopoulos AN, Izui S, Wilson CB, McConahey PJ, Murphy ED, Roths JB, Dixon FJ. Spontaneous murine lupus-like syndromes. Clinical and immunopathological manifestations in several strains. The Journal of experimental medicine. 1978 Nov 1;148(5):1198-215. PMCID: PMC2185049.
174. Yokogawa M, Takaishi M, Nakajima K, Kamijima R, Fujimoto C, Kataoka S, Terada Y, Sano S. Epicutaneous application of toll-like receptor 7 agonists leads to systemic autoimmunity in wild-type mice: a new model of systemic Lupus erythematosus. Arthritis & rheumatology. 2014 Mar;66(3):694-706. PMCID: PMC.
175. D'Orazio J, Jarrett S, Amaro-Ortiz A, Scott T. UV Radiation and the Skin. International journal of molecular sciences. 2013 06/07
04/25/received
05/18/revised
05/24/accepted;14(6):12222-48. PMCID: PMC.
176. Leone J, Pennaforte JL, Delhinger V, Detour J, Lefondre K, Eschard JP, Etienne JC. [Influence of seasons on risk of flare-up of systemic lupus: retrospective study of 66 patients]. La Revue de medecine interne. 1997;18(4):286-91. PMCID: PMC.

177. McGrath H, Jr., Bak E, Michalski JP. Ultraviolet-A light prolongs survival and improves immune function in (New Zealand black x New Zealand white)F1 hybrid mice. Arthritis and rheumatism. 1987 May;30(5):557-61. PMCID: PMC.
178. Okamoto H, Mizuno K, Itoh T, Tanaka K, Horio T. Evaluation of apoptotic cells induced by ultraviolet light B radiation in epidermal sheets stained by the TUNEL technique. The Journal of investigative dermatology. 1999 Nov;113(5):802-7. PMCID: PMC.
179. Foltyn VN, Golan TD. In vitro ultraviolet irradiation induces pro-inflammatory responses in cells from premorbid SLE mice. Lupus. 2001;10(4):272-83. PMCID: PMC.
180. Theofilopoulos AN, Baccala R, Beutler B, Kono DH. Type I interferons (alpha/beta) in immunity and autoimmunity. Annual review of immunology. 2005;23:307-36. PMCID: PMC.
181. Choubey D, Kotzin BL. Interferon-inducible p202 in the susceptibility to systemic lupus. Frontiers in bioscience : a journal and virtual library. 2002 May 1;7:e252-62. PMCID: PMC.
182. Harberts E, Fishelevich R, Liu J, Atamas SP, Gaspari AA. MyD88 mediates the decision to die by apoptosis or necroptosis after UV irradiation. Innate immunity. 2014 Jul;20(5):529-39. PMCID: PMC4041851.
183. Doerner J, Chalmers SA, Friedman A, Putterman C. Fn14 deficiency protects lupus-prone mice from histological lupus erythematosus-like skin inflammation induced by ultraviolet light. Experimental dermatology. 2016 Dec;25(12):969-76. PMCID: PMC5127760.
184. Doerner JL, Wen J, Xia Y, Paz KB, Schairer D, Wu L, Chalmers SA, Izmirly P, Michaelson JS, Burkly LC, Friedman AJ, Putterman C. TWEAK/Fn14 Signaling Involvement in the Pathogenesis of Cutaneous Disease in the MRL/lpr Model of Spontaneous Lupus. The Journal of investigative dermatology. 2015 Aug;135(8):1986-95. PMCID: PMC4504782.
185. Golan DT, Borel Y. Increased photosensitivity to near-ultraviolet light in murine SLE. Journal of immunology. 1984 Feb;132(2):705-10. PMCID: PMC.
186. Scholtissek B, Zahn S, Maier J, Klaeschen S, Braegelmann C, Hoelzel M, Bieber T, Barchet W, Wenzel J. Immunostimulatory Endogenous Nucleic Acids Drive the Lesional Inflammation in Cutaneous Lupus Erythematosus. The Journal of investigative dermatology. 2017 Jul;137(7):1484-92. PMCID: PMC.
187. Gehrke N, Mertens C, Zillinger T, Wenzel J, Bald T, Zahn S, Tuting T, Hartmann G, Barchet W. Oxidative damage of DNA confers resistance to cytosolic nuclease TREX1 degradation and potentiates STING-dependent immune sensing. Immunity. 2013 Sep 19;39(3):482-95. PMCID: PMC.
188. Lee YF, Cheng CC, Lan JL, Hsieh TY, Lin NN, Lin HY, Chiu YT. Effects of mycophenolate mofetil on cutaneous lupus erythematosus in (NZB x NZW) F1 mice. Journal of the Chinese Medical Association : JCMA. 2013 Nov;76(11):615-23. PMCID: PMC.
189. Herrera-Esparza R, Villalobos R, Bollain YGJJ, Ramirez-Sandoval R, Sanchez-Rodriguez SH, Pacheco-Tovar G, Avalos-Diaz E. Apoptosis and redistribution of the Ro autoantigen in Balb/c mouse like in subacute cutaneous lupus erythematosus. Clinical & developmental immunology. 2006 Jun-Dec;13(2-4):163-6. PMCID: PMC2270769.
190. Lu KQ, Brenneman S, Burns R, Jr., Vink A, Gaines E, Haake A, Gaspari A. Thalidomide inhibits UVB-induced mouse keratinocyte apoptosis by both TNF-alpha-dependent and TNF-alpha-independent pathways. Photodermatology, photoimmunology & photomedicine. 2003 Dec;19(6):272-80. PMCID: PMC.
191. Paz ML, Ferrari A, Weill FS, Leoni J, Maglio DH. Time-course evaluation and treatment of skin inflammatory immune response after ultraviolet B irradiation. Cytokine. 2008 Oct;44(1):70-7. PMCID: PMC.

192. Xin H, D'Souza S, Jorgensen TN, Vaughan AT, Lengyel P, Kotzin BL, Choubey D. Increased expression of Ifi202, an IFN-activatable gene, in B6.Nba2 lupus susceptible mice inhibits p53-mediated apoptosis. Journal of immunology. 2006 May 15;176(10):5863-70. PMCID: PMC.
193. Menke J, Hsu MY, Byrne KT, Lucas JA, Rabacal WA, Croker BP, Zong XH, Stanley ER, Kelley VR. Sunlight triggers cutaneous lupus through a CSF-1-dependent mechanism in MRL-Fas(lpr) mice. Journal of immunology. 2008 Nov 15;181(10):7367-79. PMCID: PMCPMC2607048.
194. Schwarz A, Bhardwaj R, Aragane Y, Mahnke K, Riemann H, Metze D, Luger TA, Schwarz T. Ultraviolet-B-induced apoptosis of keratinocytes: evidence for partial involvement of tumor necrosis factor-alpha in the formation of sunburn cells. The Journal of investigative dermatology. 1995 Jun;104(6):922-7. PMCID: PMC.
195. Zhuang L, Wang B, Shinder GA, Shivji GM, Mak TW, Sauder DN. TNF receptor p55 plays a pivotal role in murine keratinocyte apoptosis induced by ultraviolet B irradiation. Journal of immunology. 1999 Feb 01;162(3):1440-7. PMCID: PMC.
196. Groves RW, Mizutani H, Kieffer JD, Kupper TS. Inflammatory skin disease in transgenic mice that express high levels of interleukin 1 alpha in basal epidermis. Proceedings of the National Academy of Sciences of the United States of America. 1995 Dec 5;92(25):11874-8. PMCID: PMC40505.
197. Nishimura N, Tohyama C, Satoh M, Nishimura H, Reeve VE. Defective immune response and severe skin damage following UVB irradiation in interleukin-6-deficient mice. Immunology. 1999 09/29/received
11/30/revised
11/30/accepted;97(1):77-83. PMCID: PMC.
198. Majewski S, Jantschitsch C, Maeda A, Schwarz T, Schwarz A. IL-23 antagonizes UVR-induced immunosuppression through two mechanisms: reduction of UVR-induced DNA damage and inhibition of UVR-induced regulatory T cells. The Journal of investigative dermatology. 2010 Feb;130(2):554-62. PMCID: PMC.
199. Kyttaris VC, Kampagianni O, Tsokos GC. Treatment with anti-interleukin 23 antibody ameliorates disease in lupus-prone mice. BioMed research international. 2013;2013:861028. PMCID: PMC3690216.
200. Hatakeyama M, Fukunaga A, Washio K, Taguchi K, Oda Y, Ogura K, Nishigori C. Anti-Inflammatory Role of Langerhans Cells and Apoptotic Keratinocytes in Ultraviolet-B-Induced Cutaneous Inflammation. Journal of immunology. 2017 Oct 15;199(8):2937-47. PMCID: PMC.
201. Cela EM, Friedrich A, Paz ML, Vanzulli SI, Leoni J, Gonzalez Maglio DH. Time-course study of different innate immune mediators produced by UV-irradiated skin: comparative effects of short and daily versus a single harmful UV exposure. Immunology. 2015 May;145(1):82-93. PMCID: PMCPMC4405326.
202. Schwarz A, Maeda A, Wild MK, Kernebeck K, Gross N, Aragane Y, Beissert S, Vestweber D, Schwarz T. Ultraviolet radiation-induced regulatory T cells not only inhibit the induction but can suppress the effector phase of contact hypersensitivity. Journal of immunology. 2004 Jan 15;172(2):1036-43. PMCID: PMC.
203. Fruchter O, Edoute Y. First presentation of systemic lupus erythematosus following ultraviolet radiation exposure in an artificial tanning device. Rheumatology. 2005;44(4):558-9. PMCID: PMC.

204. Pirner K, Rubbert A, Salinger R, Kalden JR, Manger B. [Significance of ultraviolet light in the pathogenesis of systemic lupus erythematosus: case report and discussion of the literature]. Zeitschrift fur Rheumatologie. 1992 Jan-Feb;51(1):20-4. PMCID: PMC.
205. Ghoreishi M, Dutz JP. Murine models of cutaneous involvement in lupus erythematosus. Autoimmunity reviews. 2009 May;8(6):484-7. PMCID: PMC.
206. Ansel JC, Mountz J, Steinberg AD, DeFabo E, Green I. Effects of UV radiation on autoimmune strains of mice: increased mortality and accelerated autoimmunity in BXSB male mice. The Journal of investigative dermatology. 1985 Sep;85(3):181-6. PMCID: PMC.
207. Ansel JC, Mountz J, Steinberg AD, DeFabo E, Green I. Effects of UV Radiation on Autoimmune Strains of Mice: Increased Mortality and Accelerated Autoimmunity in BXSB Male Mice. J Investig Dermatol. 1985;85(3):181-6. PMCID: PMC.
208. Sano S, Kataoka S, Kamijima R, Fujimoto C, Ohko K, Takaishi M, Nakajima K. Ultraviolet B irradiation accelerates the development of lupus-like autoimmunity in wild-type mice induced by topical treatment with TLR7 agonist (BA4P.212). The Journal of Immunology. 2014;192(1 Supplement):46.3-.3. PMCID: PMC.
209. Wolf SJ, Theros J, Reed TJ, Liu J, Grigorova IL, Martinez-Colon G, Jacob CO, Hodgin JB, Kahlenberg JM. TLR7-Mediated Lupus Nephritis Is Independent of Type I IFN Signaling. Journal of immunology. 2018 Jul 15;201(2):393-405. PMCID: PMCPMC6039244.
210. Clark KL, Reed TJ, Wolf SJ, Lowe L, Hodgin JB, Kahlenberg JM. Epidermal injury promotes nephritis flare in lupus-prone mice. J Autoimmun. 2015 Aug 21;65(December 2015):38-48. PMCID: PMC.
211. Mevorach D, Zhou JL, Song X, Elkon KB. Systemic exposure to irradiated apoptotic cells induces autoantibody production. The Journal of experimental medicine. 1998 Jul 20;188(2):387-92. PMCID: PMC2212450.
212. Wen ZK, Xu W, Xu L, Cao QH, Wang Y, Chu YW, Xiong SD. DNA hypomethylation is crucial for apoptotic DNA to induce systemic lupus erythematosus-like autoimmune disease in SLE-non-susceptible mice. Rheumatology (Oxford, England). 2007 Dec;46(12):1796-803. PMCID: PMC.
213. Petri M. Epidemiology of systemic lupus erythematosus. Best practice & research Clinical rheumatology. 2002 Dec;16(5):847-58. PMCID: PMC.
214. Gyongyosi N, Lorincz K, Keszeg A, Haluszka D, Banvolgyi A, Tatrai E, Karpati S, Wikonkal NM. Photosensitivity of murine skin greatly depends on the genetic background: clinically relevant dose as a new measure to replace minimal erythema dose in mouse studies. Experimental dermatology. 2016 Jul;25(7):519-25. PMCID: PMC.
215. Clark KL, Reed TJ, Wolf SJ, Lowe L, Hodgin JB, Kahlenberg JM. Epidermal injury promotes nephritis flare in lupus-prone mice. Journal of autoimmunity. 2015 Dec;65:38-48. PMCID: PMC4679658.
216. Liang W, Menke AL, Driessen A, Koek GH, Lindeman JH, Stoop R, Havekes LM, Kleemann R, van den Hoek AM. Establishment of a general NAFLD scoring system for rodent models and comparison to human liver pathology. PloS one. 2014;9(12):e115922. PMCID: PMC4275274.
217. Trouplin V, Boucherit N, Gorvel L, Conti F, Mottola G, Ghigo E. Bone marrow-derived macrophage production. Journal of visualized experiments : JoVE. 2013 Nov 22(81):e50966. PMCID: PMC3991821.
218. Bagavant H, Fu SM. Pathogenesis of kidney disease in systemic lupus erythematosus. Current opinion in rheumatology. 2009 Sep;21(5):489-94. PMCID: PMC2841319.

219. Tektonidou MG, Dasgupta A, Ward MM. Risk of End-Stage Renal Disease in Patients With Lupus Nephritis, 1971-2015: A Systematic Review and Bayesian Meta-Analysis. Arthritis & rheumatology. 2016 Jun;68(6):1432-41. PMCID: PMC.
220. Tian J, Ma Y, Li J, Cen H, Wang DG, Feng CC, Li RJ, Leng RX, Pan HF, Ye DQ. The TLR7 7926A>G polymorphism is associated with susceptibility to systemic lupus erythematosus. Molecular medicine reports. 2012 Jul;6(1):105-10. PMCID: PMC.
221. Horton CG, Farris AD. Toll-like receptors in systemic lupus erythematosus: potential targets for therapeutic intervention. Current allergy and asthma reports. 2012 Feb;12(1):1-7. PMCID: PMC3307336.
222. Horton CG, Pan ZJ, Farris AD. Targeting Toll-like receptors for treatment of SLE. Mediators of inflammation. 2010;2010. PMCID: PMC2945668.
223. Murphy ED, Roths JB. A Y chromosome associated factor in strain BXSB producing accelerated autoimmunity and lymphoproliferation. Arthritis and rheumatism. 1979 Nov;22(11):1188-94. PMCID: PMC.
224. Lee PY, Kumagai Y, Li Y, Takeuchi O, Yoshida H, Weinstein J, Kellner ES, Nacionales D, Barker T, Kelly-Scumpia K, van Rooijen N, Kumar H, Kawai T, Satoh M, Akira S, Reeves WH. TLR7-dependent and FcgammaR-independent production of type I interferon in experimental mouse lupus. The Journal of experimental medicine. 2008 Dec 22;205(13):2995-3006. PMCID: PMC2605237.
225. Crow MK. Type I interferon in the pathogenesis of lupus. Journal of immunology. 2014 Jun 15;192(12):5459-68. PMCID: PMC4083591.
226. Fairhurst AM, Xie C, Fu Y, Wang A, Boudreaux C, Zhou XJ, Cibotti R, Coyle A, Connolly JE, Wakeland EK, Mohan C. Type I interferons produced by resident renal cells may promote end-organ disease in autoantibody-mediated glomerulonephritis. Journal of immunology. 2009 Nov 15;183(10):6831-8. PMCID: PMC2876821.
227. Nacionales D, Kelly-Scumpia K, Lee P, Weinstein J, Lyons R, Sobel E, Satoh M, Reeves W. Deficiency of the type I interferon receptor protects mice from experimental lupus. Arthritis and rheumatism. 2007;56(11):3770-83. PMCID: PMC.
228. Rudofsky UH, Lawrence DA. New Zealand mixed mice: a genetic systemic lupus erythematosus model for assessing environmental effects. Environmental health perspectives. 1999 Oct;107 Suppl 5:713-21. PMCID: PMC1566260.
229. Jacob CO, Zang S, Li L, Ciobanu V, Quismorio F, Mizutani A, Satoh M, Koss M. Pivotal role of Stat4 and Stat6 in the pathogenesis of the lupus-like disease in the New Zealand mixed 2328 mice. Journal of immunology. 2003 Aug 01;171(3):1564-71. PMCID: PMC.
230. Sun X, Wiedeman A, Agrawal N, Teal TH, Tanaka L, Hudkins KL, Alpers CE, Bolland S, Buechler MB, Hamerman JA, Ledbetter JA, Liggitt D, Elkon KB. Increased ribonuclease expression reduces inflammation and prolongs survival in TLR7 transgenic mice. Journal of immunology. 2013 Mar 15;190(6):2536-43. PMCID: PMC3594466.
231. Dai C, Wang H, Sung SS, Sharma R, Kannapell C, Han W, Wang Q, Davidson A, Gaskin F, Fu SM. Interferon alpha on NZM2328.Lc1R27: enhancing autoimmunity and immune complex-mediated glomerulonephritis without end stage renal failure. Clinical immunology. 2014 Sep;154(1):66-71. PMCID: PMC4167638.
232. Nacionales DC, Kelly-Scumpia KM, Lee PY, Weinstein JS, Lyons R, Sobel E, Satoh M, Reeves WH. Deficiency of the type I interferon receptor protects mice from experimental lupus. Arthritis and rheumatism. 2007 Nov;56(11):3770-83. PMCID: PMC2909118.

233. Lee YH, Song GG. Urinary MCP-1 as a biomarker for lupus nephritis: a meta-analysis. Zeitschrift fur Rheumatologie. 2017 May;76(4):357-63. PMCID: PMC.
234. Liao X, Pirapakaran T, Luo XM. Chemokines and Chemokine Receptors in the Development of Lupus Nephritis. Mediators of inflammation. 2016;2016:6012715. PMCID: PMC4923605.
235. Pollard KM, Escalante GM, Huang H, Haraldsson KM, Hultman P, Christy JM, Pawar RD, Mayeux JM, Gonzalez-Quintial R, Baccala R, Beutler B, Theofilopoulos AN, Kono DH. Induction of Systemic Autoimmunity by a Xenobiotic Requires Endosomal TLR Trafficking and Signaling from the Late Endosome and Endolysosome but Not Type I IFN. Journal of immunology. 2017 Dec 1;199(11):3739-47. PMCID: PMCPMC5698107.
236. Nickerson KM, Cullen JL, Kashgarian M, Shlomchik MJ. Exacerbated Autoimmunity in the Absence of TLR9 in MRL.Faslpr Mice Depends on Ifnar1. The Journal of Immunology. 2013 April 15, 2013;190(8):3889-94. PMCID: PMC.
237. Nanda SK, Lopez-Pelaez M, Arthur JS, Marchesi F, Cohen P. Suppression of IRAK1 or IRAK4 Catalytic Activity, but Not Type 1 IFN Signaling, Prevents Lupus Nephritis in Mice Expressing a Ubiquitin Binding-Defective Mutant of ABIN1. Journal of immunology. 2016 Dec 01;197(11):4266-73. PMCID: PMC5114882.
238. Nanda SK, Venigalla RK, Ordureau A, Patterson-Kane JC, Powell DW, Toth R, Arthur JS, Cohen P. Polyubiquitin binding to ABIN1 is required to prevent autoimmunity. The Journal of experimental medicine. 2011 Jun 06;208(6):1215-28. PMCID: PMC3173241.
239. Caster DJ, Korte EA, Nanda SK, McLeish KR, Oliver RK, G'Sell R T, Sheehan RM, Freeman DW, Coventry SC, Kelly JA, Guthridge JM, James JA, Sivils KL, Alarcon-Riquelme ME, Scofield RH, Adrianto I, Gaffney PM, Stevens AM, Freedman BI, Langefeld CD, Tsao BP, Pons-Estel BA, Jacob CO, Kamen DL, Gilkeson GS, Brown EE, Alarcon GS, Edberg JC, Kimberly RP, Martin J, Merrill JT, Harley JB, Kaufman KM, Reveille JD, Anaya JM, Criswell LA, Vila LM, Petri M, Ramsey-Goldman R, Bae SC, Boackle SA, Vyse TJ, Niewold TB, Cohen P, Powell DW. ABIN1 dysfunction as a genetic basis for lupus nephritis. Journal of the American Society of Nephrology : JASN. 2013 Nov;24(11):1743-54. PMCID: PMC3810087.
240. Li M, Shi X, Qian T, Li J, Tian Z, Ni B, Hao F. A20 overexpression alleviates pristine-induced lupus nephritis by inhibiting the NF-kappaB and NLRP3 inflammasome activation in macrophages of mice. International journal of clinical and experimental medicine. 2015;8(10):17430-40. PMCID: PMCPMC4694233.
241. Jacob N, Yang H, Pricop L, Liu Y, Gao X, Zheng SG, Wang J, Gao HX, Putterman C, Koss MN, Stohl W, Jacob CO. Accelerated pathological and clinical nephritis in systemic lupus erythematosus-prone New Zealand Mixed 2328 mice doubly deficient in TNF receptor 1 and TNF receptor 2 via a Th17-associated pathway. Journal of immunology. 2009 Feb 15;182(4):2532-41. PMCID: PMC2790862.
242. Jain S, Park G, Sproule TJ, Christianson GJ, Leeth CM, Wang H, Roopenian DC, Morse HC, 3rd. Interleukin 6 Accelerates Mortality by Promoting the Progression of the Systemic Lupus Erythematosus-Like Disease of BXSB.Yaa Mice. PloS one. 2016;11(4):e0153059. PMCID: PMC4822786.
243. Kahlenberg JM, Kaplan MJ. The inflammasome and lupus: another innate immune mechanism contributing to disease pathogenesis? Current opinion in rheumatology. 2014 Sep;26(5):475-81. PMCID: PMC4153426.

244. Weindel CG, Richey LJ, Bolland S, Mehta AJ, Kearney JF, Huber BT. B cell autophagy mediates TLR7-dependent autoimmunity and inflammation. Autophagy. 2015;11(7):1010-24. PMCID: PMC4590645.
245. Zhuang H, Han S, Xu Y, Li Y, Wang H, Yang LJ, Reeves WH. Toll-like receptor 7-stimulated tumor necrosis factor alpha causes bone marrow damage in systemic lupus erythematosus. Arthritis & rheumatology. 2014 Jan;66(1):140-51. PMCID: PMC3990233.
246. Das A, Heesters BA, Bialas A, O'Flynn J, Rifkin IR, Ochando J, Mittereder N, Carlesso G, Herbst R, Carroll MC. Follicular Dendritic Cell Activation by TLR Ligands Promotes Autoreactive B Cell Responses. Immunity. 2017 Jan 17;46(1):106-19. PMCID: PMC.
247. Chang NH, Li TT, Kim JJ, Landolt-Marticorena C, Fortin PR, Gladman DD, Urowitz MB, Wither JE. Interferon-alpha induces altered transitional B cell signaling and function in Systemic Lupus Erythematosus. Journal of autoimmunity. 2015 Apr;58:100-10. PMCID: PMC.
248. Green NM, Moody KS, Debatis M, Marshak-Rothstein A. Activation of autoreactive B cells by endogenous TLR7 and TLR3 RNA ligands. The Journal of biological chemistry. 2012 Nov 16;287(47):39789-99. PMCID: PMC3501070.
249. Hamilton JA, Wu Q, Yang P, Luo B, Liu S, Hong H, Li J, Walter MR, Fish EN, Hsu HC, Mountz JD. Cutting Edge: Endogenous IFN-beta Regulates Survival and Development of Transitional B Cells. Journal of immunology. 2017 Oct 15;199(8):2618-23. PMCID: PMCPMC5636672.
250. Giltiay NV, Chappell CP, Sun X, Kolhatkar N, Teal TH, Wiedeman AE, Kim J, Tanaka L, Buechler MB, Hamerman JA, Imanishi-Kari T, Clark EA, Elkon KB. Overexpression of TLR7 promotes cell-intrinsic expansion and autoantibody production by transitional T1 B cells. The Journal of experimental medicine. 2013 Nov 18;210(12):2773-89. PMCID: PMC3832927.
251. Gupta R, Yadav A, Aggarwal A. Longitudinal assessment of monocyte chemoattractant protein-1 in lupus nephritis as a biomarker of disease activity. Clinical rheumatology. 2016 Nov;35(11):2707-14. PMCID: PMC.
252. Devarapu SK, Kumar Vr S, Rupanagudi KV, Kulkarni OP, Eulberg D, Klussmann S, Anders HJ. Reprint of "Dual blockade of the pro-inflammatory chemokine CCL2 and the homeostatic chemokine CXCL12 is as effective as high dose cyclophosphamide in murine proliferative lupus nephritis". Clinical immunology. 2017 Oct 27. PMCID: PMC.
253. Kaul A, Gordon C, Crow MK, Touma Z, Urowitz MB, van Vollenhoven R, Ruiz-Irastorza G, Hughes G. Systemic lupus erythematosus. Nature reviews Disease primers. 2016 Jun 16;2:16039. PMCID: PMC.
254. Sanders CJ, Van Weelden H, Kazzaz GA, Sigurdsson V, Toonstra J, Bruijnzeel-Koomen CA. Photosensitivity in patients with lupus erythematosus: a clinical and photobiological study of 100 patients using a prolonged phototest protocol. The British journal of dermatology. 2003 Jul;149(1):131-7. PMCID: PMC.
255. Furukawa F. Photosensitivity in cutaneous lupus erythematosus: lessons from mice and men. Journal of dermatological science. 2003 Nov;33(2):81-9. PMCID: PMC.
256. Foering K, Goreshi R, Klein R, Okawa J, Rose M, Cucchiara A, Werth VP. Prevalence of self-report photosensitivity in cutaneous lupus erythematosus. Journal of the American Academy of Dermatology. 2012 Feb;66(2):220-8. PMCID: PMC3193852.
257. Wysenbeek AJ, Block DA, Fries JF. Prevalence and expression of photosensitivity in systemic lupus erythematosus. Annals of the rheumatic diseases. 1989 Jun;48(6):461-3. PMCID: PMC1003788.

258. Kuhn A, Sonntag M, Richter-Hintz D, Oslislo C, Megahed M, Ruzicka T, Lehmann P. Phototesting in lupus erythematosus tumidus--review of 60 patients. Photochemistry and photobiology. 2001 May;73(5):532-6. PMCID: PMC.
259. Yamazaki S, Odanaka M, Nishioka A, Kasuya S, Shime H, Hemmi H, Imai M, Riethmacher D, Kaisho T, Ohkura N, Sakaguchi S, Morita A. Ultraviolet B-Induced Maturation of CD11b-Type Langerin(-) Dendritic Cells Controls the Expansion of Foxp3(+) Regulatory T Cells in the Skin. Journal of immunology. 2018 Jan 1;200(1):119-29. PMCID: PMC.
260. Aubin F. Mechanisms involved in ultraviolet light-induced immunosuppression. European journal of dermatology : EJD. 2003 Nov-Dec;13(6):515-23. PMCID: PMC.
261. Schaper F, de Leeuw K, Horst G, Bootsma H, Limburg PC, Heeringa P, Bijl M, Westra J. High mobility group box 1 skews macrophage polarization and negatively influences phagocytosis of apoptotic cells. Rheumatology (Oxford, England). 2016 Dec;55(12):2260-70. PMCID: PMC.
262. Shipman WD, Chyou S, Ramanathan A, Izmirly PM, Sharma S, Pannellini T, Dasoveanu DC, Qing X, Magro CM, Granstein RD, Lowes MA, Pamer EG, Kaplan DH, Salmon JE, Mehrara BJ, Young JW, Clancy RM, Blobel CP, Lu TT. A protective Langerhans cell-keratinocyte axis that is dysfunctional in photosensitivity. Science translational medicine. 2018 Aug 15;10(454). PMCID: PMC6365282.
263. Farkas L, Beiske K, Lund-Johansen F, Brandtzaeg P, Jahnsen FL. Plasmacytoid dendritic cells (natural interferon- alpha/beta-producing cells) accumulate in cutaneous lupus erythematosus lesions. The American journal of pathology. 2001 Jul;159(1):237-43. PMCID: PMC1850412.
264. Mawrin C, Brunn A, Rocken C, Schroder JM. Peripheral neuropathy in systemic lupus erythematosus: pathomorphological features and distribution pattern of matrix metalloproteinases. Acta neuropathologica. 2003 Apr;105(4):365-72. PMCID: PMC.
265. Kind P, Lehmann P, Plewig G. Phototesting in lupus erythematosus. The Journal of investigative dermatology. 1993 Jan;100(1):53S-7S. PMCID: PMC.
266. Bruhs A, Schwarz T. Ultraviolet Radiation-Induced Immunosuppression: Induction of Regulatory T Cells. Methods in molecular biology. 2017;1559:63-73. PMCID: PMC.
267. Glick AB, Wodzinski A, Fu P, Levine AD, Wald DN. Impairment of regulatory T-cell function in autoimmune thyroid disease. Thyroid : official journal of the American Thyroid Association. 2013 Jul;23(7):871-8. PMCID: PMC3704106.
268. Mao C, Wang S, Xiao Y, Xu J, Jiang Q, Jin M, Jiang X, Guo H, Ning G, Zhang Y. Impairment of regulatory capacity of CD4+CD25+ regulatory T cells mediated by dendritic cell polarization and hyperthyroidism in Graves' disease. Journal of immunology. 2011 Apr 15;186(8):4734-43. PMCID: PMC.
269. Sarkar M, Hile G, Tsoi L, Xing X, Liu J, Liang Y, Berthier C, Swindell W, Patrick M, Shuai S, Tsou P, Uppala R, Beamer M, Srivastava A, Bielas S, Harms P, Getsios S, Elder J, Voorhees J, Gudjonsson J, Kahlenberg J. Photosensitivity and type I IFN responses in cutaneous lupus are driven by epidermal derived interferon kappa. Annals of Rheumatic Diseases. 2018;epub ahead of print. PMCID: PMC.
270. Zahn S, Graef M, Patsinakidis N, Landmann A, Surber C, Wenzel J, Kuhn A. Ultraviolet light protection by a sunscreen prevents interferon-driven skin inflammation in cutaneous lupus erythematosus. Experimental Dermatology. 2014;23(7):516-8. PMCID: PMC.

271. MacLeod AS, Rudolph R, Corriden R, Ye I, Garijo O, Havran WL. Skin-resident T cells sense ultraviolet radiation-induced injury and contribute to DNA repair. Journal of immunology. 2014 Jun 15;192(12):5695-702. PMCID: PMC4048764.
272. Sole C, Gimenez-Barcons M, Ferrer B, Ordi-Ros J, Cortes-Hernandez J. Microarray study reveals a transforming growth factor-beta-dependent mechanism of fibrosis in discoid lupus erythematosus. The British journal of dermatology. 2016 Aug;175(2):302-13. PMCID: PMC.
273. Mande P, Zirak B, Ko WC, Taravati K, Bride KL, Brodeur TY, Deng A, Dresser K, Jiang Z, Ettinger R, Fitzgerald KA, Rosenblum MD, Harris JE, Marshak-Rothstein A. Fas ligand promotes an inducible TLR-dependent model of cutaneous lupus-like inflammation. The Journal of clinical investigation. 2018 Jul 2;128(7):2966-78. PMCID: PMC6025993.
274. Sole C, Gimenez-Barcons M, Ferrer B, Ordi-Ros J, Cortes-Hernandez J. Microarray study reveals a TGFbeta-Dependent mechanism of fibrosis in discoid lupus erythematosus. The British journal of dermatology. 2016 Mar 12. PMCID: PMC.
275. Callen JP, Fowler JF, Kulick KB. Serologic and clinical features of patients with discoid lupus erythematosus: relationship of antibodies to single-stranded deoxyribonucleic acid and of other antinuclear antibody subsets to clinical manifestations. Journal of the American Academy of Dermatology. 1985 Nov;13(5 Pt 1):748-55. PMCID: PMC.
276. Jeffries MA, Dozmorov M, Tang Y, Merrill JT, Wren JD, Sawalha AH. Genome-wide DNA methylation patterns in CD4+ T cells from patients with systemic lupus erythematosus. Epigenetics. 2011 May;6(5):593-601. PMCID: PMC3121972.
277. Mauri C, Menon M. The many faces of type I interferon in systemic lupus erythematosus. The Journal of clinical investigation. 2015 Jul 1;125(7):2562-4. PMCID: PMC4563695.
278. Lood C, Blanco LP, Purmalek MM, Carmona-Rivera C, De Ravin SS, Smith CK, Malech HL, Ledbetter JA, Elkon KB, Kaplan MJ. Neutrophil extracellular traps enriched in oxidized mitochondrial DNA are interferogenic and contribute to lupus-like disease. Nature medicine. 2016 Feb;22(2):146-53. PMCID: PMC4742415.
279. Labonte AC, Kegerreis B, Geraci NS, Bachali P, Madamanchi S, Robl R, Catalina MD, Lipsky PE, Grammer AC. Identification of alterations in macrophage activation associated with disease activity in systemic lupus erythematosus. PloS one. 2018;13(12):e0208132. PMCID: PMC6298676 Institute. This does not alter our adherence to PLoS One policies on data sharing and materials. The work presented in this study is not part of any patents or marketed products.
280. Wang N, Liang H, Zen K. Molecular mechanisms that influence the macrophage m1-m2 polarization balance. Frontiers in immunology. 2014;5:614. PMCID: PMC4246889.
281. Meller S, Winterberg F, Gilliet M, Muller A, Lauceviciute I, Rieker J, Neumann NJ, Kubitza R, Gombert M, Bunemann E, Wiesner U, Franken-Kunkel P, Kanzler H, Dieu-Nosjean MC, Amara A, Ruzicka T, Lehmann P, Zlotnik A, Homey B. Ultraviolet radiation-induced injury, chemokines, and leukocyte recruitment: An amplification cycle triggering cutaneous lupus erythematosus. Arthritis and rheumatism. 2005 May;52(5):1504-16. PMCID: PMC.
282. Desch AN, Randolph GJ, Murphy K, Gautier EL, Kedl RM, Lahoud MH, Caminschi I, Shortman K, Henson PM, Jakubzick CV. CD103+ pulmonary dendritic cells preferentially acquire and present apoptotic cell-associated antigen. The Journal of experimental medicine. 2011 Aug 29;208(9):1789-97. PMCID: PMC3171085.
283. Ritter U, Wiede F, Mielenz D, Kiafard Z, Zwirner J, Korner H. Analysis of the CCR7 expression on murine bone marrow-derived and spleen dendritic cells. Journal of leukocyte biology. 2004 Aug;76(2):472-6. PMCID: PMC.

284. Vremec D, Zorbas M, Scollay R, Saunders DJ, Ardavin CF, Wu L, Shortman K. The surface phenotype of dendritic cells purified from mouse thymus and spleen: investigation of the CD8 expression by a subpopulation of dendritic cells. The Journal of experimental medicine. 1992 Jul 1;176(1):47-58. PMCID: PMC2119290.
285. Merad M, Ginhoux F, Collin M. Origin, homeostasis and function of Langerhans cells and other langerin-expressing dendritic cells. Nature reviews Immunology. 2008 Dec;8(12):935-47. PMCID: PMC.
286. Sung SS, Fu SM, Rose CE, Jr., Gaskin F, Ju ST, Beaty SR. A major lung CD103 (alphaE)-beta7 integrin-positive epithelial dendritic cell population expressing Langerin and tight junction proteins. Journal of immunology. 2006 Feb 15;176(4):2161-72. PMCID: PMC.
287. Jaensson E, Uronen-Hansson H, Pabst O, Eksteen B, Tian J, Coombes JL, Berg PL, Davidsson T, Powrie F, Johansson-Lindbom B, Agace WW. Small intestinal CD103+ dendritic cells display unique functional properties that are conserved between mice and humans. The Journal of experimental medicine. 2008 Sep 1;205(9):2139-49. PMCID: PMC2526207.
288. Scott CL, Aumeunier AM, Mowat AM. Intestinal CD103+ dendritic cells: master regulators of tolerance? Trends in immunology. 2011 Sep;32(9):412-9. PMCID: PMC.
289. del Rio ML, Bernhardt G, Rodriguez-Barbosa JI, Forster R. Development and functional specialization of CD103+ dendritic cells. Immunol Rev. 2010 Mar;234(1):268-81. PMCID: PMC.
290. Qiu CH, Miyake Y, Kaise H, Kitamura H, Ohara O, Tanaka M. Novel subset of CD8{alpha}+ dendritic cells localized in the marginal zone is responsible for tolerance to cell-associated antigens. Journal of immunology. 2009 Apr 1;182(7):4127-36. PMCID: PMC.
291. Bedoui S, Whitney PG, Waithman J, Eidsmo L, Wakim L, Caminschi I, Allan RS, Wojtasiak M, Shortman K, Carbone FR, Brooks AG, Heath WR. Cross-presentation of viral and self antigens by skin-derived CD103+ dendritic cells. Nature immunology. 2009 May;10(5):488-95. PMCID: PMC.
292. Jakubzick C, Tacke F, Ginhoux F, Wagers AJ, van Rooijen N, Mack M, Merad M, Randolph GJ. Blood monocyte subsets differentially give rise to CD103+ and CD103- pulmonary dendritic cell populations. Journal of immunology. 2008 Mar 1;180(5):3019-27. PMCID: PMC.
293. del Rio ML, Rodriguez-Barbosa JI, Bolter J, Ballmaier M, Dittrich-Breiholz O, Kracht M, Jung S, Forster R. CX3CR1+ c-kit+ bone marrow cells give rise to CD103+ and CD103- dendritic cells with distinct functional properties. Journal of immunology. 2008 Nov 1;181(9):6178-88. PMCID: PMC.
294. del Rio ML, Rodriguez-Barbosa JI, Kremmer E, Forster R. CD103- and CD103+ bronchial lymph node dendritic cells are specialized in presenting and cross-presenting innocuous antigen to CD4+ and CD8+ T cells. Journal of immunology. 2007 Jun 1;178(11):6861-6. PMCID: PMC.
295. Iyoda T, Shimoyama S, Liu K, Omatsu Y, Akiyama Y, Maeda Y, Takahara K, Steinman RM, Inaba K. The CD8+ dendritic cell subset selectively endocytoses dying cells in culture and in vivo. The Journal of experimental medicine. 2002 May 20;195(10):1289-302. PMCID: PMC2193756.
296. Miyake Y, Asano K, Kaise H, Uemura M, Nakayama M, Tanaka M. Critical role of macrophages in the marginal zone in the suppression of immune responses to apoptotic cell-associated antigens. The Journal of clinical investigation. 2007 Aug;117(8):2268-78. PMCID: PMC1924497.

297. Hanayama R, Tanaka M, Miyasaka K, Aozasa K, Koike M, Uchiyama Y, Nagata S. Autoimmune disease and impaired uptake of apoptotic cells in MFG-E8-deficient mice. Science. 2004 May 21;304(5674):1147-50. PMCID: PMC.
298. Nakano H, Free ME, Whitehead GS, Maruoka S, Wilson RH, Nakano K, Cook DN. Pulmonary CD103(+) dendritic cells prime Th2 responses to inhaled allergens. Mucosal immunology. 2012 Jan;5(1):53-65. PMCID: PMC3697034.
299. Coombes JL, Siddiqui KR, Arancibia-Carcamo CV, Hall J, Sun CM, Belkaid Y, Powrie F. A functionally specialized population of mucosal CD103+ DCs induces Foxp3+ regulatory T cells via a TGF-beta and retinoic acid-dependent mechanism. The Journal of experimental medicine. 2007 Aug 6;204(8):1757-64. PMCID: PMC2118683.
300. Johansson-Lindbom B, Svensson M, Pabst O, Palmqvist C, Marquez G, Forster R, Agace WW. Functional specialization of gut CD103+ dendritic cells in the regulation of tissue-selective T cell homing. The Journal of experimental medicine. 2005 Oct 17;202(8):1063-73. PMCID: PMC2213212.
301. Kim TS, Braciale TJ. Respiratory dendritic cell subsets differ in their capacity to support the induction of virus-specific cytotoxic CD8+ T cell responses. PloS one. 2009;4(1):e4204. PMCID: PMC2615220.
302. Ho AW, Prabhu N, Betts RJ, Ge MQ, Dai X, Hutchinson PE, Lew FC, Wong KL, Hanson BJ, Macary PA, Kemeny DM. Lung CD103+ dendritic cells efficiently transport influenza virus to the lymph node and load viral antigen onto MHC class I for presentation to CD8 T cells. Journal of immunology. 2011 Dec 1;187(11):6011-21. PMCID: PMC.
303. King IL, Kroenke MA, Segal BM. GM-CSF-dependent, CD103+ dermal dendritic cells play a critical role in Th effector cell differentiation after subcutaneous immunization. The Journal of experimental medicine. 2010 May 10;207(5):953-61. PMCID: PMC2867280.
304. Masuda C, Miyasaka T, Kawakami K, Inokuchi J, Kawano T, Dobashi-Okuyama K, Takahashi T, Takayanagi M, Ohno I. Sex-based differences in CD103(+) dendritic cells promote female-predominant Th2 cytokine production during allergic asthma. Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology. 2018 Apr;48(4):379-93. PMCID: PMC.
305. Furuhashi K, Suda T, Hasegawa H, Suzuki Y, Hashimoto D, Enomoto N, Fujisawa T, Nakamura Y, Inui N, Shibata K, Nakamura H, Chida K. Mouse lung CD103+ and CD11bhigh dendritic cells preferentially induce distinct CD4+ T-cell responses. American journal of respiratory cell and molecular biology. 2012 Feb;46(2):165-72. PMCID: PMC.
306. Jiao Z, Bedoui S, Brady JL, Walter A, Chopin M, Carrington EM, Sutherland RM, Nutt SL, Zhang Y, Ko HJ, Wu L, Lew AM, Zhan Y. The closely related CD103+ dendritic cells (DCs) and lymphoid-resident CD8+ DCs differ in their inflammatory functions. PloS one. 2014;9(3):e91126. PMCID: PMC3956455.
307. Zelante T, Wong AY, Ping TJ, Chen J, Sumatoh HR, Vigano E, Hong Bing Y, Lee B, Zolezzi F, Fric J, Newell EW, Mortellaro A, Poidinger M, Puccetti P, Ricciardi-Castagnoli P. CD103(+) Dendritic Cells Control Th17 Cell Function in the Lung. Cell reports. 2015 Sep 22;12(11):1789-801. PMCID: PMC.
308. Hansen IS, Krabbendam L, Bernink JH, Loayza-Puch F, Hoepel W, van Burgsteden JA, Kuijper EC, Buskens CJ, Bemelman WA, Zaat SAJ, Agami R, Vidarsson G, van den Brink GR, de Jong EC, Wildenberg ME, Baeten DLP, Everts B, den Dunnen J. FcalphaRI co-stimulation converts human intestinal CD103(+) dendritic cells into pro-inflammatory cells through

glycolytic reprogramming. Nature communications. 2018 Feb 28;9(1):863. PMCID: PMC5830413.
309. Van Prooyen N, Henderson CA, Hocking Murray D, Sil A. CD103+ Conventional Dendritic Cells Are Critical for TLR7/9-Dependent Host Defense against Histoplasma capsulatum, an Endemic Fungal Pathogen of Humans. PLoS pathogens. 2016 Jul;12(7):e1005749. PMCID: PMC4961300.
310. Webb LM, Lundie RJ, Borger JG, Brown SL, Connor LM, Cartwright AN, Dougall AM, Wilbers RH, Cook PC, Jackson-Jones LH, Phythian-Adams AT, Johansson C, Davis DM, Dewals BG, Ronchese F, MacDonald AS. Type I interferon is required for T helper (Th) 2 induction by dendritic cells. The EMBO journal. 2017 Aug 15;36(16):2404-18. PMCID: PMC5556270.
311. Parlato S, Santini SM, Lapenta C, Di Pucchio T, Logozzi M, Spada M, Giammarioli AM, Malorni W, Fais S, Belardelli F. Expression of CCR-7, MIP-3beta, and Th-1 chemokines in type I IFN-induced monocyte-derived dendritic cells: importance for the rapid acquisition of potent migratory and functional activities. Blood. 2001 Nov 15;98(10):3022-9. PMCID: PMC.
312. Montoya M, Schiavoni G, Mattei F, Gresser I, Belardelli F, Borrow P, Tough DF. Type I interferons produced by dendritic cells promote their phenotypic and functional activation. Blood. 2002 May 1;99(9):3263-71. PMCID: PMC.
313. Braunstein I, Klein R, Okawa J, Werth VP. The interferon-regulated gene signature is elevated in subacute cutaneous lupus erythematosus and discoid lupus erythematosus and correlates with the cutaneous lupus area and severity index score. The British journal of dermatology. 2012 May;166(5):971-5. PMCID: PMC3336025.
314. Diamond MS, Kinder M, Matsushita H, Mashayekhi M, Dunn GP, Archambault JM, Lee H, Arthur CD, White JM, Kalinke U, Murphy KM, Schreiber RD. Type I interferon is selectively required by dendritic cells for immune rejection of tumors. The Journal of experimental medicine. 2011 Sep 26;208(10):1989-2003. PMCID: PMC3182061.
315. Luft T, Pang KC, Thomas E, Hertzog P, Hart DN, Trapani J, Cebon J. Type I IFNs enhance the terminal differentiation of dendritic cells. Journal of immunology. 1998 Aug 15;161(4):1947-53. PMCID: PMC.
316. Buelens C, Bartholome EJ, Amraoui Z, Boutriaux M, Salmon I, Thielemans K, Willems F, Goldman M. Interleukin-3 and interferon beta cooperate to induce differentiation of monocytes into dendritic cells with potent helper T-cell stimulatory properties. Blood. 2002 Feb 1;99(3):993-8. PMCID: PMC.
317. Santini SM, Lapenta C, Logozzi M, Parlato S, Spada M, Di Pucchio T, Belardelli F. Type I interferon as a powerful adjuvant for monocyte-derived dendritic cell development and activity in vitro and in Hu-PBL-SCID mice. The Journal of experimental medicine. 2000 May 15;191(10):1777-88. PMCID: PMC2193160.
318. Ito T, Amakawa R, Inaba M, Ikehara S, Inaba K, Fukuhara S. Differential regulation of human blood dendritic cell subsets by IFNs. Journal of immunology. 2001 Mar 1;166(5):2961-9. PMCID: PMC.
319. Ivashkiv LB, Donlin LT. Regulation of type I interferon responses. Nature reviews Immunology. 2014 Jan;14(1):36-49. PMCID: PMC4084561.
320. Pinto AK, Daffis S, Brien JD, Gainey MD, Yokoyama WM, Sheehan KC, Murphy KM, Schreiber RD, Diamond MS. A temporal role of type I interferon signaling in CD8+ T cell maturation during acute West Nile virus infection. PLoS pathogens. 2011 Dec;7(12):e1002407. PMCID: PMC3228803.

321. Nagai T, Devergne O, Mueller TF, Perkins DL, van Seventer JM, van Seventer GA. Timing of IFN-beta exposure during human dendritic cell maturation and naive Th cell stimulation has contrasting effects on Th1 subset generation: a role for IFN-beta-mediated regulation of IL-12 family cytokines and IL-18 in naive Th cell differentiation. Journal of immunology. 2003 Nov 15;171(10):5233-43. PMCID: PMC.
322. Dondi E, Rogge L, Lutfalla G, Uze G, Pellegrini S. Down-modulation of responses to type I IFN upon T cell activation. Journal of immunology. 2003 Jan 15;170(2):749-56. PMCID: PMC.
323. Havenar-Daughton C, Kolumam GA, Murali-Krishna K. Cutting Edge: The direct action of type I IFN on CD4 T cells is critical for sustaining clonal expansion in response to a viral but not a bacterial infection. Journal of immunology. 2006 Mar 15;176(6):3315-9. PMCID: PMC.
324. Pacella I, Timperi E, Accapezzato D, Martire C, Labbadia G, Cavallari EN, D'Ettorre G, Calvo L, Rizzo F, Severa M, Coccia EM, Vullo V, Barnaba V, Piconese S. IFN-alpha promotes rapid human Treg contraction and late Th1-like Treg decrease. Journal of leukocyte biology. 2016 Sep;100(3):613-23. PMCID: PMC.
325. Piconese S, Pacella I, Timperi E, Barnaba V. Divergent effects of type-I interferons on regulatory T cells. Cytokine & growth factor reviews. 2015 Apr;26(2):133-41. PMCID: PMC.
326. Gangaplara A, Martens C, Dahlstrom E, Metidji A, Gokhale AS, Glass DD, Lopez-Ocasio M, Baur R, Kanakabandi K, Porcella SF, Shevach EM. Type I interferon signaling attenuates regulatory T cell function in viral infection and in the tumor microenvironment. PLoS pathogens. 2018 Apr;14(4):e1006985. PMCID: PMC5929570.
327. Srivastava S, Koch MA, Pepper M, Campbell DJ. Type I interferons directly inhibit regulatory T cells to allow optimal antiviral T cell responses during acute LCMV infection. The Journal of experimental medicine. 2014 May 5;211(5):961-74. PMCID: PMC4010906.
328. Xu HC, Grusdat M, Pandyra AA, Polz R, Huang J, Sharma P, Deenen R, Kohrer K, Rahbar R, Diefenbach A, Gibbert K, Lohning M, Hocker L, Waibler Z, Haussinger D, Mak TW, Ohashi PS, Lang KS, Lang PA. Type I interferon protects antiviral CD8+ T cells from NK cell cytotoxicity. Immunity. 2014 Jun 19;40(6):949-60. PMCID: PMC.
329. Crouse J, Bedenikovic G, Wiesel M, Ibberson M, Xenarios I, Von Laer D, Kalinke U, Vivier E, Jonjic S, Oxenius A. Type I interferons protect T cells against NK cell attack mediated by the activating receptor NCR1. Immunity. 2014 Jun 19;40(6):961-73. PMCID: PMC.
330. Barbet G, Sander LE, Geswell M, Leonardi I, Cerutti A, Iliev I, Blander JM. Sensing Microbial Viability through Bacterial RNA Augments T Follicular Helper Cell and Antibody Responses. Immunity. 2018 Mar 20;48(3):584-98 e5. PMCID: PMC5924674.
331. Cucak H, Yrlid U, Reizis B, Kalinke U, Johansson-Lindbom B. Type I interferon signaling in dendritic cells stimulates the development of lymph-node-resident T follicular helper cells. Immunity. 2009 Sep 18;31(3):491-501. PMCID: PMC.
332. Riteau N, Radtke AJ, Shenderov K, Mittereder L, Oland SD, Hieny S, Jankovic D, Sher A. Water-in-Oil-Only Adjuvants Selectively Promote T Follicular Helper Cell Polarization through a Type I IFN and IL-6-Dependent Pathway. Journal of immunology. 2016 Nov 15;197(10):3884-93. PMCID: PMC5104203.
333. Naves R, Singh SP, Cashman KS, Rowse AL, Axtell RC, Steinman L, Mountz JD, Steele C, De Sarno P, Raman C. The interdependent, overlapping, and differential roles of type I and II IFNs in the pathogenesis of experimental autoimmune encephalomyelitis. Journal of immunology. 2013 Sep 15;191(6):2967-77. PMCID: PMC3779698.

334. Brinkmann V, Geiger T, Alkan S, Heusser CH. Interferon alpha increases the frequency of interferon gamma-producing human CD4+ T cells. The Journal of experimental medicine. 1993 Nov 1;178(5):1655-63. PMCID: PMC2191249.
335. Nakayamada S, Poholek AC, Lu KT, Takahashi H, Kato M, Iwata S, Hirahara K, Cannons JL, Schwartzberg PL, Vahedi G, Sun HW, Kanno Y, O'Shea JJ. Type I IFN induces binding of STAT1 to Bcl6: divergent roles of STAT family transcription factors in the T follicular helper cell genetic program. Journal of immunology. 2014 Mar 1;192(5):2156-66. PMCID: PMC3967131.
336. Rogge L, Barberis-Maino L, Biffi M, Passini N, Presky DH, Gubler U, Sinigaglia F. Selective expression of an interleukin-12 receptor component by human T helper 1 cells. The Journal of experimental medicine. 1997 Mar 3;185(5):825-31. PMCID: PMC2196163.
337. Kadowaki N, Liu YJ. Natural type I interferon-producing cells as a link between innate and adaptive immunity. Human immunology. 2002 Dec;63(12):1126-32. PMCID: PMC.
338. Cella M, Facchetti F, Lanzavecchia A, Colonna M. Plasmacytoid dendritic cells activated by influenza virus and CD40L drive a potent TH1 polarization. Nature immunology. 2000 Oct;1(4):305-10. PMCID: PMC.
339. Way SS, Havenar-Daughton C, Kolumam GA, Orgun NN, Murali-Krishna K. IL-12 and type-I IFN synergize for IFN-gamma production by CD4 T cells, whereas neither are required for IFN-gamma production by CD8 T cells after Listeria monocytogenes infection. Journal of immunology. 2007 Apr 1;178(7):4498-505. PMCID: PMC2626161.
340. Doxsee CL, Riter TR, Reiter MJ, Gibson SJ, Vasilakos JP, Kedl RM. The immune response modifier and Toll-like receptor 7 agonist S-27609 selectively induces IL-12 and TNF-alpha production in CD11c+CD11b+CD8- dendritic cells. Journal of immunology. 2003 Aug 1;171(3):1156-63. PMCID: PMC.
341. Bonnardel J, Da Silva C, Wagner C, Bonifay R, Chasson L, Masse M, Pollet E, Dalod M, Gorvel JP, Lelouard H. Distribution, location, and transcriptional profile of Peyer's patch conventional DC subsets at steady state and under TLR7 ligand stimulation. Mucosal immunology. 2017 Nov;10(6):1412-30. PMCID: PMC.
342. Yrlid U, Milling SW, Miller JL, Cartland S, Jenkins CD, MacPherson GG. Regulation of intestinal dendritic cell migration and activation by plasmacytoid dendritic cells, TNF-alpha and type 1 IFNs after feeding a TLR7/8 ligand. Journal of immunology. 2006 May 1;176(9):5205-12. PMCID: PMC.
343. Suzuki H, Wang B, Shivji GM, Toto P, Amerio P, Tomai MA, Miller RL, Sauder DN. Imiquimod, a topical immune response modifier, induces migration of Langerhans cells. The Journal of investigative dermatology. 2000 Jan;114(1):135-41. PMCID: PMC.
344. Edwards AD, Diebold SS, Slack EM, Tomizawa H, Hemmi H, Kaisho T, Akira S, Reis e Sousa C. Toll-like receptor expression in murine DC subsets: lack of TLR7 expression by CD8 alpha+ DC correlates with unresponsiveness to imidazoquinolines. European journal of immunology. 2003 Apr;33(4):827-33. PMCID: PMC.
345. Asselin-Paturel C, Brizard G, Chemin K, Boonstra A, O'Garra A, Vicari A, Trinchieri G. Type I interferon dependence of plasmacytoid dendritic cell activation and migration. The Journal of experimental medicine. 2005 Apr 4;201(7):1157-67. PMCID: PMC2213121.
346. Boullart AC, Aarntzen EH, Verdijk P, Jacobs JF, Schuurhuis DH, Benitez-Ribas D, Schreibelt G, van de Rakt MW, Scharenborg NM, de Boer A, Kramer M, Figdor CG, Punt CJ, Adema GJ, de Vries IJ. Maturation of monocyte-derived dendritic cells with Toll-like receptor 3 and 7/8 ligands combined with prostaglandin E2 results in high interleukin-12 production and

cell migration. Cancer immunology, immunotherapy : CII. 2008 Nov;57(11):1589-97. PMCID: PMC2522299.
347. Crespo MI, Zacca ER, Nunez NG, Ranocchia RP, Maccioni M, Maletto BA, Pistoresi-Palencia MC, Moron G. TLR7 triggering with polyuridylic acid promotes cross-presentation in CD8alpha+ conventional dendritic cells by enhancing antigen preservation and MHC class I antigen permanence on the dendritic cell surface. Journal of immunology. 2013 Feb 1;190(3):948-60. PMCID: PMC.
348. Miller LS. Toll-like receptors in skin. Advances in dermatology. 2008;24:71-87. PMCID: PMC2633625.
349. Li ZJ, Sohn KC, Choi DK, Shi G, Hong D, Lee HE, Whang KU, Lee YH, Im M, Lee Y, Seo YJ, Kim CD, Lee JH. Roles of TLR7 in activation of NF-kappaB signaling of keratinocytes by imiquimod. PloS one. 2013;8(10):e77159. PMCID: PMC3795621.
350. Black AP, Ardern-Jones MR, Kasprowicz V, Bowness P, Jones L, Bailey AS, Ogg GS. Human keratinocyte induction of rapid effector function in antigen-specific memory CD4+ and CD8+ T cells. European journal of immunology. 2007 Jun;37(6):1485-93. PMCID: PMC.
351. Sakata K, Nakayamada S, Miyazaki Y, Kubo S, Ishii A, Nakano K, Tanaka Y. Up-Regulation of TLR7-Mediated IFN-alpha Production by Plasmacytoid Dendritic Cells in Patients With Systemic Lupus Erythematosus. Frontiers in immunology. 2018;9:1957. PMCID: PMC6121190.