Ultraviolet light induces increased T cell activation in lupus-prone mice via type I IFN-dependent inhibition of T regulatory cells

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ABSTRACT

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. 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 100 mJ/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. 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. 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.

1. Introduction

Systemic lupus erythematosus (SLE) is an autoimmune disease in which patients experience devastating organ damage mediated by immune cells and inflammatory cytokine production [1,2]. Minimal sun exposure, especially ultraviolet (UV) B wavelengths, is a prominent factor that drives cutaneous inflammation in lupus patients [3–7]. However, the mechanisms through which SLE skin is predisposed to persistent inflammation following UVB exposure are 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 [8]. In addition, CD11b$^+$Langerin$^-$ dendritic cells promote expansion of T regulatory

(T$_{Reg}$) cells [9,10]. In healthy skin, there is also activation of other suppressive populations such as neutrophils secreting IL-10 and monocytes secreting interferon (IFN) alpha. These changes result in an overall suppressive phenotype [11,12].

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 [13–15]. Reduction of Langerhans cells in SLE skin promotes UVB-mediated inflammation via suppression of epidermal growth factor receptor-mediated signaling [16]. 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 [8,17–21]. Intriguingly, despite its immunosuppressive role in healthy skin [8,11,12], SLE patients and lupus-prone mice demonstrate a rise in type I IFN signaling following UVB exposure; thus suggesting a potential pro-inflammatory

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role for type I IFNs in lupus skin [12,21]. For example, type I IFNs demonstrate a proinflammatory role in keratinocytes and promote cell death following UVB [22].

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 [12], type I IFNs exhibit a proinflammatory role in lupus-prone mice and are required for skewed immune activation following UVB exposure.

2. Methods and materials

2.1. Mice

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 10-week old female New Zealand Mixed (NZM) 2328 lupus-prone mice and iNZM (knockout of the α chain of the type I IFN receptor) mice. Both NZM 2328 and iNZM mice were a gift from Dr. Chaim Jacob, University of Southern California [23]. All mice were housed in specific pathogen-free facilities at University of Michigan and treated in accordance to our University of Michigan IACUC-approved protocol.

2.2. UVB irradiation

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 $100 \, mJ/cm^2$ 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 310 nm of UVB radiation.

2.3. Flow cytometry

10-week old female BALB/c, NZM, and iNZM mice were treated with/without UVB for 5 consecutive days followed by harvesting of 2 inguinal draining lymph nodes (dLN) passed through a 70 μm filter to generate a single cell suspension. Cells were then incubated in flow block (1% bovine serum albumin and 1% horse serum in PBS) for 30 min, followed by staining with CD8 clone: 53–6.7 (BD Bioscience, San Jose, CA), CD3 clone: 17A2, CD4 clone: GK15, CD69 clone: H12F3, CD25 clone: 3C7, and B220 clone: RA3-6B2(Biolegend, San Diego, CA), Ig (Southern Biotech, Birmingham, AL) for 45 min. Following the extracellular staining, cells were intracellularly stained for Foxp3 clone: FJK16s (Biolegend) 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: $CD4^+$ T cells: $CD3^+$, $CD4^+$, $CD8^-$; $CD8^+$ T cells: $CD3^+$, $CD8^+$, $CD4^-$; T cell activation: $CD69^+$; B cells: B220+; T_{Reg} cells: $CD4^+$, $CD3^+$, $CD25^+$, Foxp3+; Ab secreting cells: $CD4^- CD8^- IgH + L^{hi} B220^{int-low}$.

2.4. T regulatory cell 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. $CD4^+ CD25^+$ T_{Reg} 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 (T_{Reg}: $T_{Effector}$) with/without anti-CD3/CD28 beads (ThermoFisher) for 72 h in a 96-well plate. Cells were then stained, as described in the flow cytometry section, for CD3 clone: 17A2 (BioLegend) for 45 min, followed by staining with live/dead cell dye (ThermoFisher) for 30 min. 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 T_{Regs}, followed by live cell gating, then $CD3^+$ cells and lastly proliferation dye 670 to examine percent proliferation of $CD4^+$ T cells (Supplementary figure 1). Percentage proliferation was calculated using the formula: $(100 \times 1:1,1:2,$ or $1:4,$ samples)/0:1 sample.

2.5. RTqPCR

Biopsies from the backs of mice treated with/without 100mJ/cm2 UVB were taken 3 h 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). RNA was isolated using the Direct-zol mini RNA prep kit (Zymo). 100 ng 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*) GATCCG ACTTCACTTCCAGATGG (forward), CATCTCAGTGGTAGTCAACCC (reverse); β-Actin TGGAATCCTGTGGCATCCTGAAAC (forward), TAA AACGCAGCTCAGTAACAGTCCG (reverse); Interferon alpha (*ifna)* ATGGCTAGRCTCTGTGCTTTCCT (forward), AGGGCTCTCCAGAYTTC- TGCTCTG(reverse); Interferon beta (*ifnb*) AGCTCCAAGAAAGGACGAA CAT (forward), ATTCTTGCTTCGGCAGTTAC(reverse); Interferon gamma (*ifng*) AGCGGCTGACTGAACTCAGATTGTA (forward), GTCAC AGTTTTCAGCTGTATAGGG (reverse); Interferon kappa (*ifnk*) ACTCC AAAGTTTTTATGGCTGGT (forward), TACGATAGGAGACGGCGTTTA (reverse); Interferon regulatory factor (*irf7*) TGCTGTTTGGAGACTGGC TAT(forward), TCCAAGCTCCCGGCTAAGT(reverse).

2.6. Analysis of anti-IgG and dsDNA IgG antibody 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).

2.7. Statistics

All data was graphed and statistics performed using GraphPad Prism v.8.0. For data comparing multiple groups, ANOVA testing was used. Comparison between two groups was completed via a two-tailed Student's t-test for normally distributed data. When there was significant difference in variances, Welch's correction was applied. Comparisons were considered significant with a p value of < 0.05.

3. Results

3.1. 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 $100 \, mJ/cm^2$ UVB on their dorsum for 5 days, followed by harvest of the draining lymph nodes (dLN) 24 h 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. 1A–C). This response was not
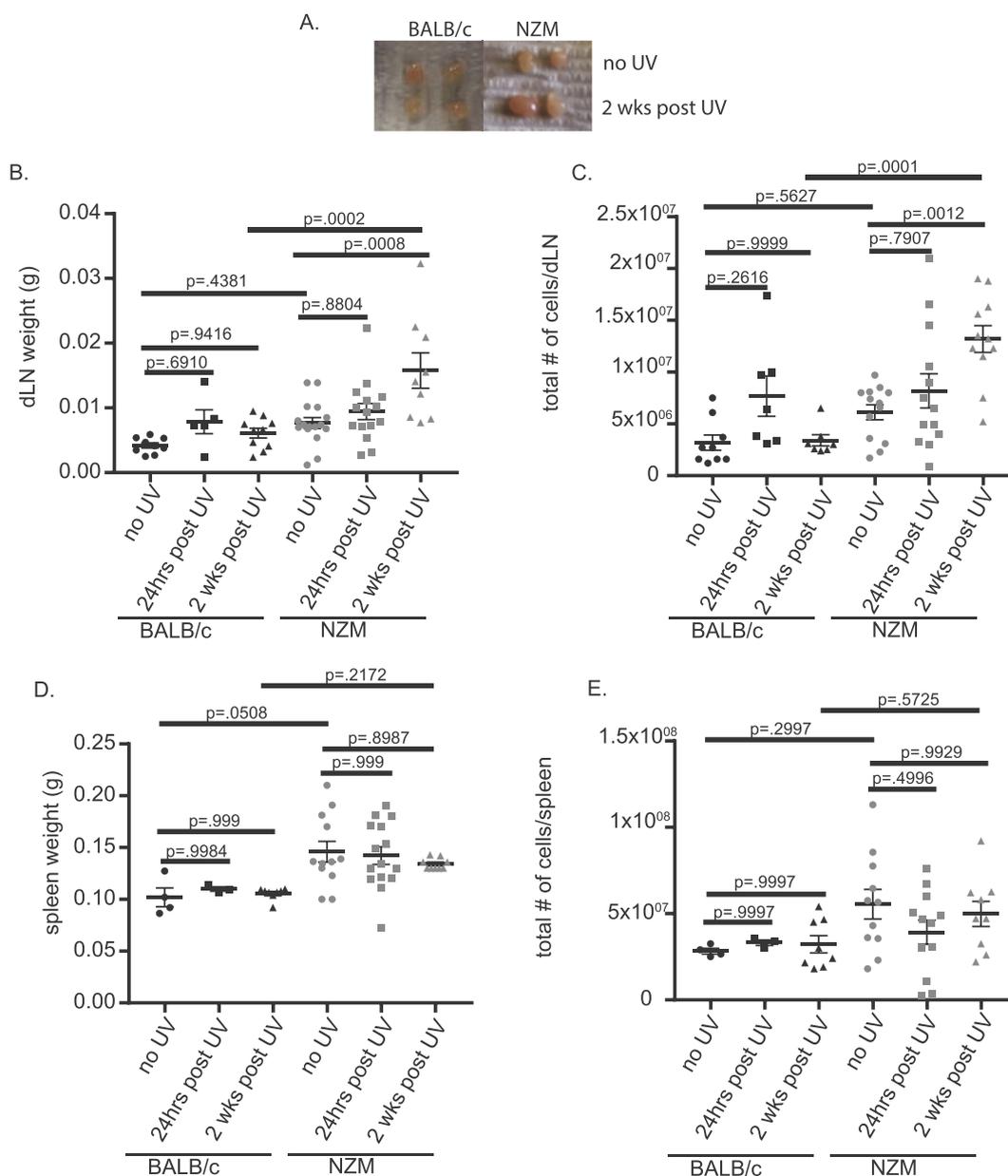
Fig. 1. UVB- induces an increase in dLN, but not splenic size, 2 weeks post treatment in lupus-prone compared to wild-type mice. Ten-week-old NZM2328 and BALB/c mice treated with 100 mJ/cm^2 on their dorsum for 5 days were analyzed 24 h s and 2 weeks post treatment. (A) Representative dLN 2 weeks post 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.

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. 2 A). This UVB dosage did not significantly increase total B cells (Fig. 2B) or antibody secreting cells (Supplementary Figure 2A). In addition, no significant increase in total IgG or *anti*-dsDNA antibodies were detected in the serum 2 weeks after UVB exposure (Supplementary Figure 2B, C). Further exploration of changes in the subsets of T cells revealed increases in both CD4$^+$ and CD8$^+$ T cells (Fig. 2C 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. 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.

3.2. 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 [9,24], 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 UV treatment (Fig. 3A and 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. 3C). In order to confirm aberrant T_{Reg} suppressive function in NZM mice, we performed a T_{Reg} suppression assay (Fig. 3D and 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
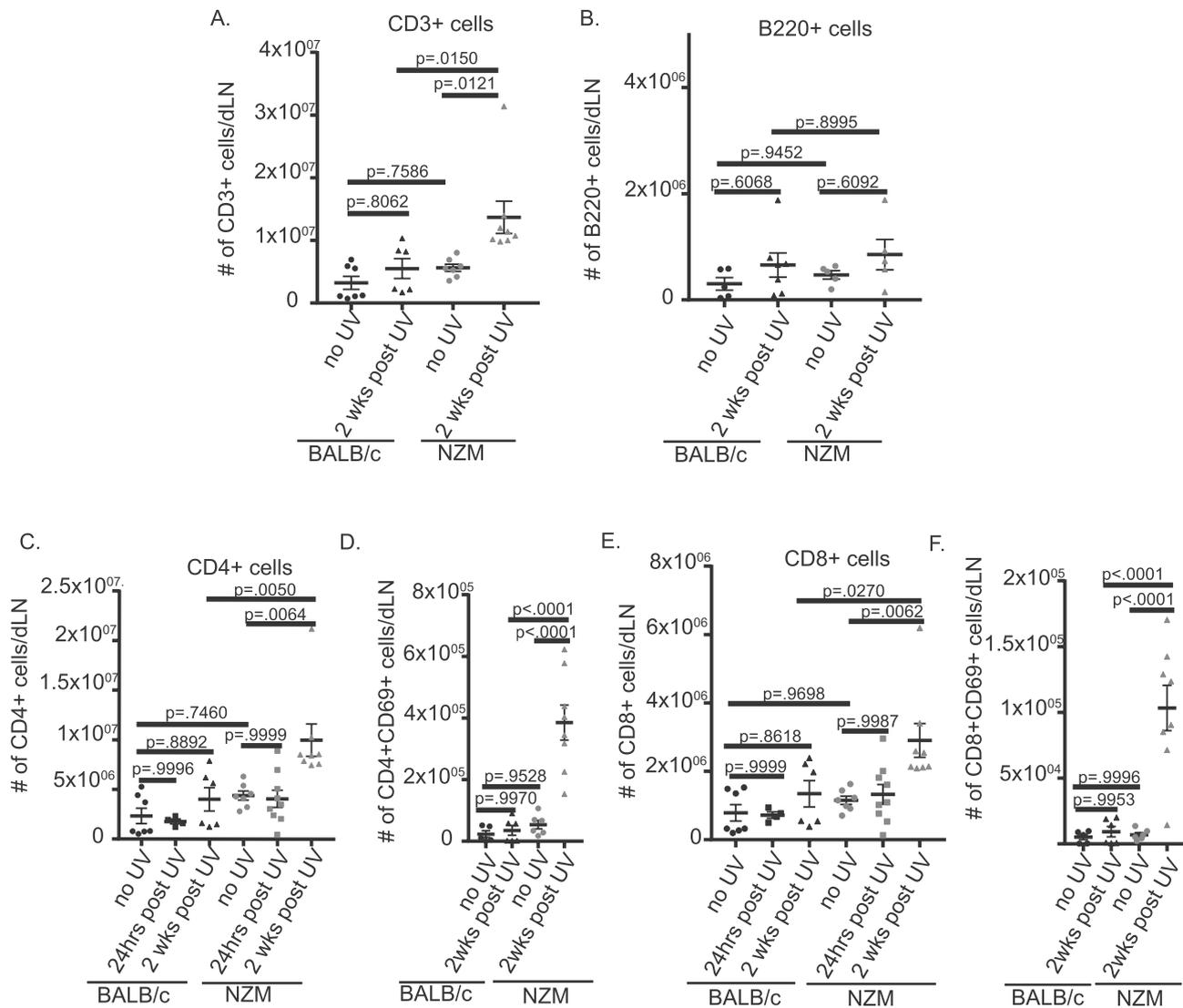
Fig. 2. Lupus-prone mice have increased T cell activation in dLN 2 weeks post UVB treatment compared to wild-type mice. Changes in immune cell populations in the dLN were evaluated by flow cytometry 24 h s and 2 weeks post UVB treatment. (A) Total T cells per dLN: CD3$^+$. (B) Total B cells: B220$^+$. (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.

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.

3.3. 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 [12], yet SLE skin has elevated type I IFNs after UVB [12,21]. 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 h after UVB exposure identified 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. 4A).

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. 4B). No difference in T cell subset numbers or activation (via CD69$^+$) were identified in iNZM mice (Fig. 4C–F), similar to BALB/c mice (Fig. 2C and E). These results suggest that in the absence of type I IFN signaling, activation of dLN T cells is prevented in a lupus-prone background.

3.4. 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 [25,26], 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 24 hs post UVB exposure (Fig. 5A and B). Further characterization of T$_{Reg}$ cells in iNZM mice showed strong suppressive capacity, and even suggested 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
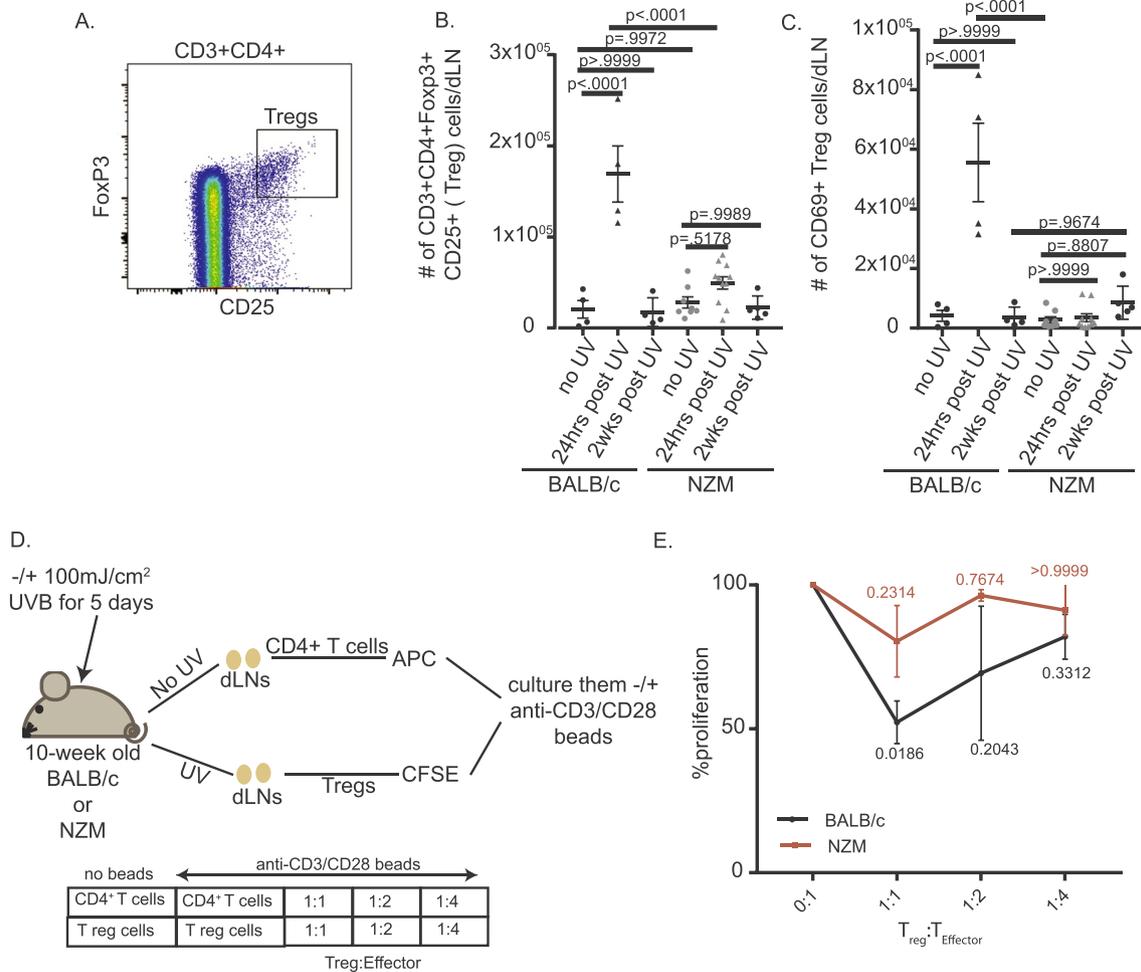
Fig. 3. UVB exposure fails to induce T regulatory cell activation in lupus-prone mice. (A–D) T regulatory cell changes were examined via flow cytometry in the dLN 24 h s and 2 weeks post UVB treatment. Each dot represents an individual mouse. (A) Changes in T regulatory cells: $CD3^+CD4^+CD25^+FoxP3^+$. (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.

to UVB that inhibits T_{Reg} function and promotes T cell activation and expansion.

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 [27–29].

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. In healthy skin, migratory UVB-induced T_{regs} contribute, along with resident T cells, to skewing of the immune cell response towards a suppressive phenotype and thus possibly limiting DNA damage [9,30]. Our data also support a role for T_{regs} in inhibition of T cell activation in the dLN following UVB exposure. Thus, in SLE, where T_{regs} are suppressed secondary to increased IFNs, activated T cells may contribute to apoptosis induction through increased expression of FasL

[20,31,32]. Whether activated T cells 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 [33] and may present without significant autoantibody positivity [34]. Similarly, treatment of our lupus-prone mice with $100 \, \text{mJ/cm}^2$ 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 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 ($500 \, \text{mJ/cm}^2$) [35]. In addition, because we studied UVB treatment in pre-autoimmune lupus-prone mice, the autoreactive B cell populations may not have 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 [36], 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 [12,37]. 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 [12]; however, in lupus skin, both infiltrating plasmacytoid
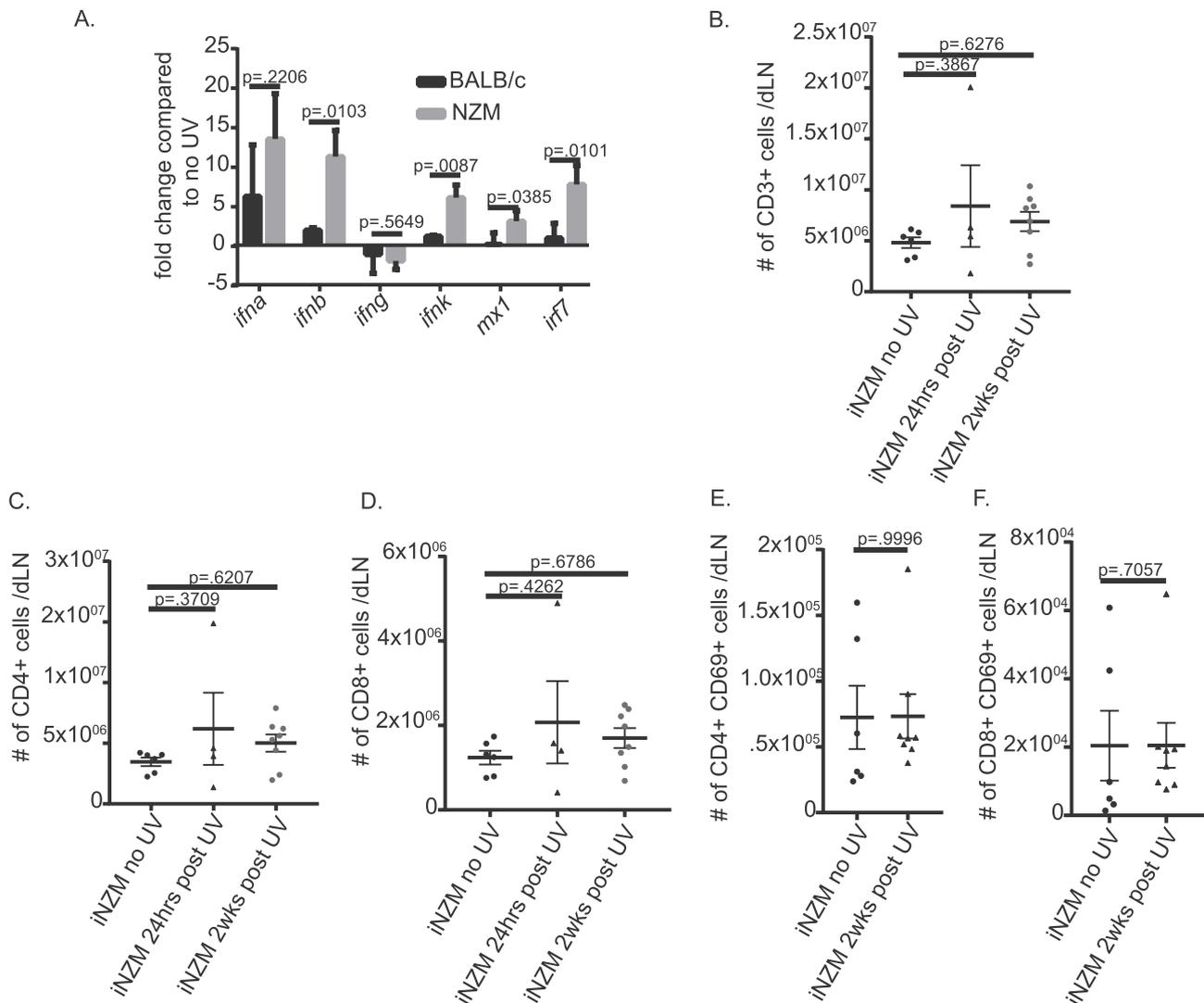
Fig. 4. UVB induced T cell activation in lupus-prone mice is type I IFN dependent. (A) RNA isolated from the skin of NZM or BALB/c mice 3 h s post UVB treatment. Real-time PCR was performed using primers of the genes listed. Graph displays the fold change for each gene compared with the respective no 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 $100 \, \text{mJ/cm}^2$ UVB on their dorsum for 5 days were analyzed via flow cytometry 24 h s 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.

dendritic cells and keratinocytes exhibit upregulation of type I IFNs following UVB as well [18,22], possibly secondary to UVB effects on immunostimulatory nucleic acids [38,39]. 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 [28]. 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 [22,40]. 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 [12]. This differential effect could be due to T cells in lupus patients having decreased DNA methylation allowing for sensitization to type I IFN effects [41]. 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.

5. Conclusions

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. This skewing is driven by upregulation of Type I IFNs in lupus-prone mice which are required for repression of T_{Reg} cells following UVB exposure. Future studies should address the source of type I IFNs following UVB in the skin and dLN. Overall, our work suggests that targeting of type I IFNs may be an important strategy to prevent skin inflammation and systemic immune activation following UV exposure in SLE patients.

Conflicts of interest

J.E.G. serves as an Advisory Board member for Novartis and MiRagen, and has received research support from AbbVie, SunPharma, and Genentech. J.M.K serves on advisory boards for AstraZeneca and Eli Lilly.
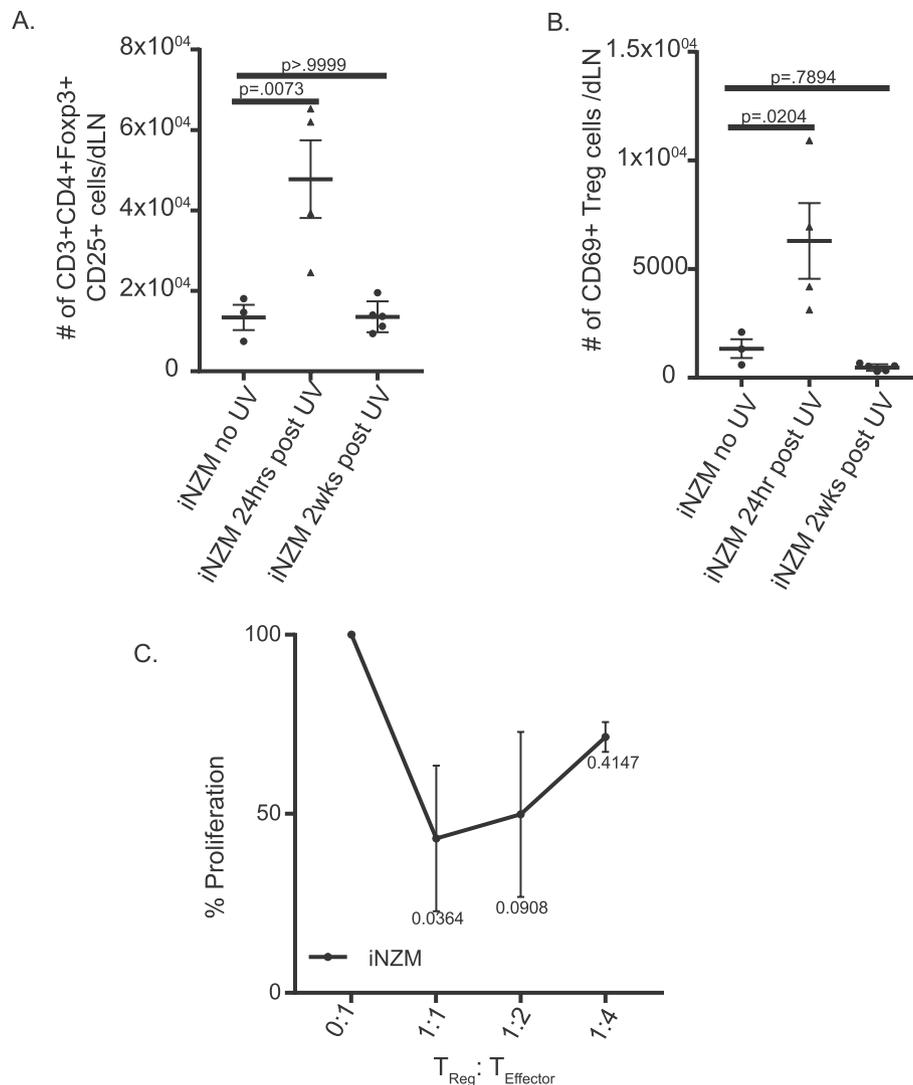
Fig. 5. Type I IFNs suppress T_{Reg} activation and functionality following UVB exposure. (A and B) Ten-week-old iNZM mice were treated with 100mJ/cm2 on their dorsum for 5 days and dLNs were analyzed via flow cytometry 24 h s or 2 weeks post treatment. Each dot represents an individual mouse. (A) Changes 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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jaut.2019.06.002

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