SCIENTIFIC REPORT

Glucocorticoids (GC) have been the mainstay treatment of SLE for over 60 years. This is despite their devasting side effects including organ damage, heart disease, osteoporosis and the worsening of lupus itself. Type I IFNs play a large role in SLE pathogenesis and critically are resistant to GC. Thus, there is a lack of an effective treatment for SLE, particularly one which can target the underlying source of the disease.

In this study we identified a novel regulator of type I IFN, a glucocorticoid-induced leucine zipper called GILZ. We demonstrated that GILZ has the ability to regulate proinflammatory cytokines associated with SLE pathogenesis and importantly type I IFNs. Additionally, our research has shown that GILZ does not appear to recapitulate the adverse metabolic side effects of GC but does display multiple beneficial effects similar to GC (reviewed in Flynn et al 2019).

In SLE patients we demonstrated that GILZ was able to regulate IFN, through its negative correlation with IFN Score and through correlations with key components of the IFN pathway. GILZ was also found to correlate with SLE disease severity. Using our GILZ KO mouse model of autoimmunity we demonstrated that GILZ regulated IFN production in response to TLR stimulation and showed increased ISG expression in GILZ KO mice. Taken together, these results highlight the ability of GILZ to regulate IFN in SLE and shed new light on the potential of a GILZ based therapy for SLE.

Published Papers

- **Flynn JK.,** Dankers W and Morand EF. **Could GILZ Be the Answer to Glucocorticoid Toxicity in Lupus?** Frontiers in Immunology 2019; 10, 1684 https://www.frontiersin.org/articles/10.3389/fimmu.2019.01684/full

Conference Presentations

- **Glucocorticoid-Induced Leucine Zipper (GILZ) is a novel regulator of type I Interferon**
  Flynn JK et al. 2019 14th World Congress on Inflammation
  Abstract number 140
  https://wci2019.org/program-overview.php

- **The Glucocorticoid-Induced Protein GILZ Represent a Checkpoint in the IFN Program in SLE**
  Eric Morand et al. 2019 ACR/ARP Annual Meeting
  Abstract number 1787
  https://acrabstracts.org/abstract/the-glucocorticoid-induced-protein-gilz-represent-a-checkpoint-in-the-ifn-program-in-sle/
  https://onlinelibrary.wiley.com/toc/23265205/2019/71/S10

- **Lyn-Deficient Murine Lupus Is Exacerbated by Glucocorticoid-Induced Leucine Zipper (GILZ) Deficiency**
  Nataraja C et al. 2019 ACR/ARP Annual Meeting

Abstract number 61
https://acrabstracts.org/abstract/lyn-deficient-murine-lupus-is-exacerbated-by-glucocorticoid-induced-leucine-zipper-gilz-deficiency/
https://onlinelibrary.wiley.com/toc/23265205/2019/71/S10

Papers to be submitted 2020

- **Flynn JK et al. GILZ is a novel regulator of pro-inflammatory cytokines and type I IFN.** Frontiers immunology or Arthritis Research.

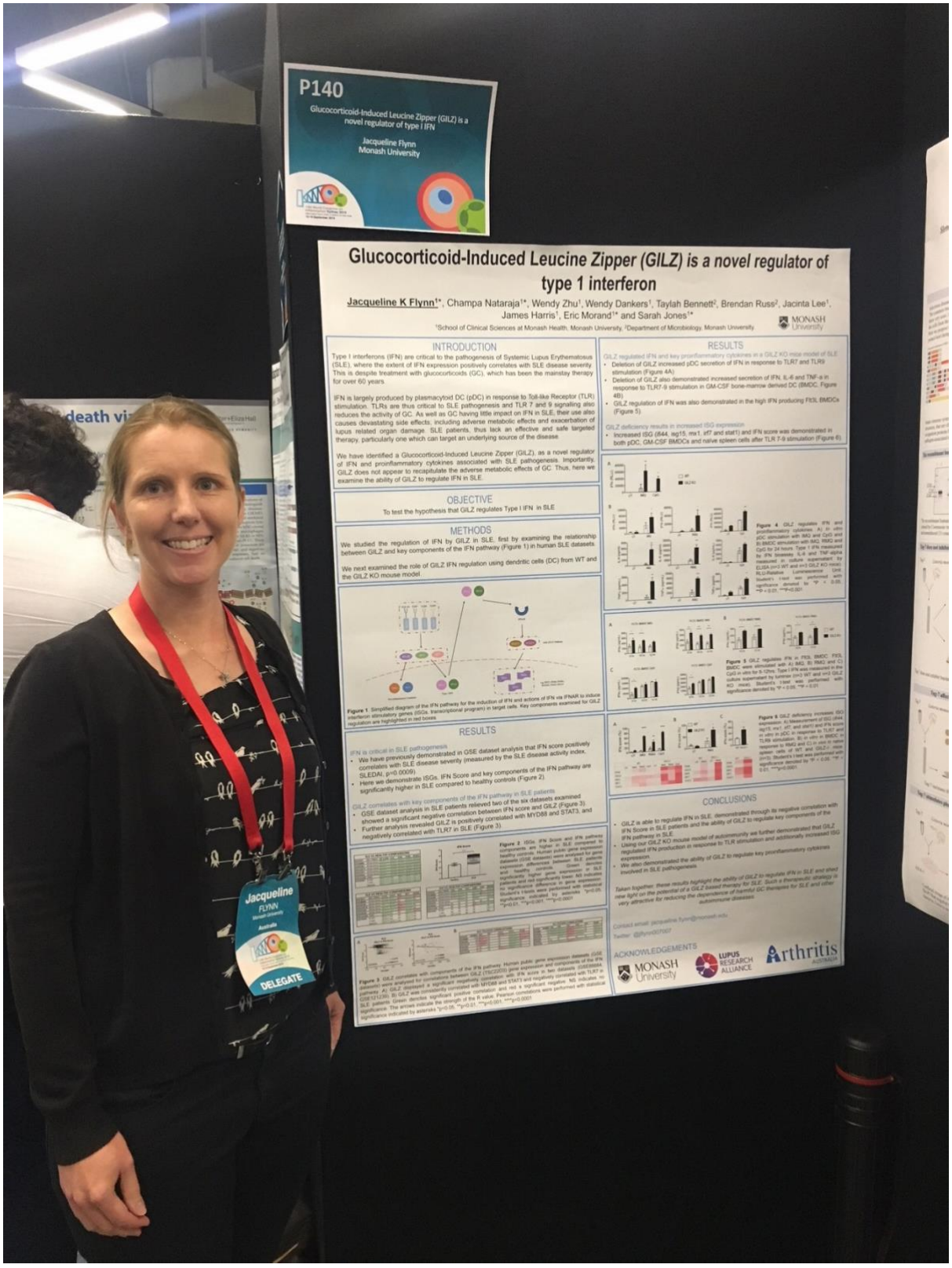
Could GILZ Be the Answer to Glucocorticoid Toxicity in Lupus?

Jacqueline K. Flynn, Wendy Dankers and Eric F. Morand**

School of Clinical Sciences at Monash Health, Monash University, Melbourne, VIC, Australia

Glucocorticoids (GC) are used globally to treat autoimmune and inflammatory disorders. Their anti-inflammatory actions are mainly mediated via binding to the glucocorticoid receptor (GR), creating a GC/GR complex, which acts in both the cytoplasm and nucleus to regulate the transcription of a host of target genes. As a result, signaling pathways such as NF-κB and AP-1 are inhibited, and cell activation, differentiation and survival and cytokine and chemokine production are suppressed. However, the gene regulation by GC can also cause severe side effects in patients. Systemic lupus erythematosus (SLE or lupus) is a multisystem autoimmune disease, characterized by a poorly regulated immune response leading to chronic inflammation and dysfunction of multiple organs, for which GC is the major current therapy. Long-term GC use, however, can cause debilitating adverse consequences for patients including diabetes, cardiovascular disease and osteoporosis and contributes to irreversible organ damage. To date, there is no alternative treatment which can replicate the rapid effects of GC across multiple immune cell functions, effecting disease control during disease flares. Research efforts have focused on finding alternatives to GC, which display similar immunoregulatory actions, without the devastating adverse metabolic effects. One potential candidate is the glucocorticoid-induced leucine zipper (GILZ). GILZ is induced by low concentrations of GC and is shown to mimic the action of GC in several inflammatory processes, reducing immunity and inflammation in *in vitro* and *in vivo* studies. Additionally, GILZ has, similar to the GC-GR complex, the ability to bind to both NF-κB and AP-1 as well as DNA directly, to regulate immune cell function, while potentially lacking the GC-related side effects. Importantly, in SLE patients GILZ is under-expressed and correlates negatively with disease activity, suggesting an important regulatory role of GILZ in SLE. Here we provide an overview of the actions and use of GC in lupus, and discuss whether the regulatory mechanisms of GILZ could lead to the development of a novel therapeutic for lupus. Increased understanding of the mechanisms of action of GILZ, and its ability to regulate immune events leading to lupus disease activity has important clinical implications for the development of safer anti-inflammatory therapies.

Keywords: GILZ, glucorticoids, lupus (SLE), transcription factor, treatment, regulation


P140
Glucocorticoid-Induced Leucine Zipper (GILZ) is a novel regulator of type I IFN
Jacqueline Flynn
Monash University
Glucocorticoid-Induced Leucine Zipper (GILZ) is a novel regulator of type 1 interferon
Jacqueline K Flynn[1*], Champa Nataraja[1*], Wendy Zhu[1], Wendy Dankers[1], Taylah Bennett[2], Brendan Russ[2], Jacinta Lee[1], James Harris[1], Eric Morand[1*] and Sarah Jones[1*]
[1]School of Clinical Sciences at Monash Health, Monash University, [2]Department of Microbiology, Monash University
MONASH University
INTRODUCTION
Type I interferons (IFN) are critical to the pathogenesis of Systemic Lupus Erythematosus (SLE), where the extent of IFN expression positively correlates with SLE disease severity. This is despite treatment with glucocorticoids (GC), which has been the mainstay therapy for over 60 years.
IFN is largely produced by plasmacytoid DC (pDC) in response to Toll-like Receptor (TLR) stimulation. TLRs are thus critical to SLE pathogenesis and TLR 7 and 9 signalling also reduces the activity of GC. As well as GC having little impact on IFN in SLE, their use also causes devastating side effects, including adverse metabolic effects and exacerbation of lupus related organ damage. SLE patients, thus lack an effective and safe targeted therapy, particularly one which can target an underlying source of the disease.
We have identified a Glucocorticoid-Induced Leucine Zipper (GILZ), as a novel regulator of IFN and proinflammatory cytokines associated with SLE pathogenesis. Importantly, GILZ does not appear to recapitulate the adverse metabolic effects of GC. Thus, here we examine the ability of GILZ to regulate IFN in SLE.
OBJECTIVE
To test the hypothesis that GILZ regulates Type I IFN in SLE
METHODS
We studied the regulation of IFN by GILZ in SLE, first by examining the relationship between GILZ and key components of the IFN pathway (Figure 1) in human SLE datasets.
We next examined the role of GILZ IFN regulation using dendritic cells (DC) from WT and the GILZ KO mouse model.
Figure 1 Simplified diagram of the IFN pathway for the induction of IFN and actions of IFN via IFNAR to induce interferon stimulatory genes (ISGs, transcriptional program) in target cells. Key components examined for GILZ regulation are highlighted in red boxes.
RESULTS
IFN is critical in SLE pathogenesis
We have previously demonstrated in GSE dataset analysis that IFN score positively correlates with SLE disease severity (measured by the SLE disease activity index, SLEDAI, p=0.0009)
Here we demonstrate ISGs, IFN Score and key components of the IFN pathway are significantly higher in SLE compared to healthy controls (Figure 2).
GILZ correlates with key components of the IFN pathway in SLE patients
GSE dataset analysis in SLE patients relieved two of the six datasets examined showed a significant negative correlation between IFN score and GILZ (Figure 3).
Further analysis revealed GILZ is positively correlated with MYD88 and STAT3, and negatively correlated with TLR7 in SLE (Figure 3).
Figure 2 ISGs, IFN Score and IFN pathway components are higher in SLE compared to healthy controls. Human public gene expression datasets (GSE datasets) were analysed for gene expression differences between SLE patients and healthy controls. Green denotes significantly higher gene expression in SLE patients and red significantly lower. NS indicates no significance difference in gene expression. Student's t-tests were performed with statistical significance indicated by asterisks *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001
Figure 3 GILZ correlates with components of the IFN pathway. Human public gene expression datasets (GSE datasets) were analysed for correlations between GILZ (TSC22D3) gene expression and components of the IFN pathway. A) GILZ displayed a significant negative correlation with IFN score in two datasets (GSE39088 & GSE121239). B) GILZ was consistently correlated with MYD88 and STAT3 and negatively correlated with TLR7 in SLE patients. Green denotes significant positive correlation and red a significant negative. NS indicates no significance. The arrows indicate the strength of the R value. Pearson correlations were performed with statistical significance indicated by asterisks *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001
RESULTS
GILZ regulated IFN and key proinflammatory cytokines in a GILZ KO mice model of SLE
Deletion of GILZ increased pDC secretion of IFN in response to TLR7 and TLR9 stimulation (Figure 4A)
Deletion of GILZ also demonstrated increased secretion of IFN, IL-6 and TNF-α in response to TLR7-9 stimulation in GM-CSF bone-marrow derived DC (BMDC, Figure 4B)
GILZ regulation of IFN was also demonstrated in the high IFN producing Flt3L BMDCs (Figure 5)
GILZ deficiency results in increased ISG expression
Increased ISG (ifi44, isg15, mx1, irf7 and stat1) and IFN score was demonstrated in both pDC, GM-CSF BMDCs and naive spleen cells after TLR 7-9 stimulation (Figure 6).
Figure 4 GILZ regulates IFN and proinflammatory cytokines. A) In vitro pDC stimulation with IMQ and CpG and B) BMDC stimulation with IMQ, R848 and CpG for 24 hours. Type I IFN measured by IFN bioassay. IL-6 and TNF-alpha measured in culture supernatant by ELISA (n=3 WT and n=3 GILZ KO mice). RLU-Relative Luminescence Unit. Student's t-test was performed with significance denoted by *P < 0.05, **P < 0.01, ***P<0.001
Figure 5 GILZ regulates IFN in Flt3L BMDC. Flt3L BMDC were stimulated with A) IMQ, B) R848 and C) CpG in vitro for 6-12hrs. Type I IFN was measured in the culture supernatant by luminex (n=3 WT and n=3 GILZ KO mice). Student's t-test was performed with significance denoted by *P < 0.05, **P < 0.01
Figure 6 GILZ deficiency increases ISG expression. A) Measurement of ISG (ifi44, isg15, mx1, irf7, and stat1) and IFN scores in vitro in pDC in response to TLR7 and TLR9 stimulation. B) in vitro in BMDC in response to R842 and C) in vivo in naive spleen cells of WT and GILZ-/- mice (n=3). Student's t-test was performed with significance denoted by *P < 0.05, **P < 0.01, ***p<0.0001
CONCLUSIONS
GILZ is able to regulate IFN in SLE, demonstrated through its negative correlation with IFN Score in SLE patients and the ability of GILZ to regulate key components of the IFN pathway in SLE.
Using our GILZ KO mouse model of autoimmunity we further demonstrated that GILZ regulated IFN production in response to TLR stimulation and additionally increased ISG expression.
We also demonstrated the ability of GILZ to regulate key proinflammatory cytokines involved in SLE pathogenesis.
Taken together, these results highlight the ability of GILZ to regulate IFN in SLE and shed new light on the potential of a GILZ based therapy for SLE. Such a therapeutic strategy is very attractive for reducing the dependence of harmful GC therapies for SLE and other autoimmune diseases.
Contact email: jacqueline.flynn@monash.edu
Twitter: @flynn007007
ACKNOWLEDGEMENTS
MONASH University
LUPUS RESEARCH ALLIANCE
Arthritis AUSTRALIA
Jacqueline
FLYNN
Monash University
Australia
DELEGATE