

## Interferon- $\alpha$ enhances the IL-12-induced STAT4 activation selectively in carriers of the *STAT4* SLE risk allele rs7574865[T]

Intronic single nucleotide polymorphisms (SNP) in *STAT4*, tagged by rs7574865[T], are associated with increased susceptibility to develop systemic lupus erythematosus (SLE) and a more severe disease phenotype with nephritis and stroke.<sup>1,2</sup> Recently, we demonstrated that T cells from patients with SLE carrying the *STAT4* risk allele rs7574865[T] have an enhanced induction of STAT4 protein following PHA/interleukin (IL)-2 activation, which results in increased IL-12-induced phosphorylation of STAT4 (pSTAT4) and interferon (IFN)- $\gamma$  production.<sup>3</sup> As the majority of *STAT4* risk allele carriers do not develop disease, we asked whether the *STAT4* risk allele exerts the same effect in healthy individuals. In studies of IL-12-stimulated cells from 72 healthy women (online supplementary materials and methods) we unexpectedly found that *STAT4* risk allele carriership was associated with a decreased pSTAT4 in CD8<sup>+</sup> and CD4<sup>+</sup> T cells from healthy individuals (figure 1A). Notably, in the absence of *STAT4* risk alleles healthy individuals and patients with SLE phosphorylated STAT4 to the same extent (figure 1B). However, with increasing numbers of *STAT4* risk alleles, patients with SLE responded stronger to IL-12 stimulation. The decreased IL-12 response in healthy *STAT4* risk allele carriers resulted in a decreased production of IFN- $\gamma$  in CD8<sup>+</sup> T cells and a slight, but non-significant, decrease in CD4<sup>+</sup> T cells (figure 1C). Demonstrating the specificity for IL-12, phorbol 12-myristate 13-acetate-induced IFN- $\gamma$  production was not affected (online supplementary figure 1). Thus, the *STAT4* risk allele rs7574865[T] associates with opposite directional effects in cells from healthy donors compared with patients with SLE.

Opposing directional effects have been described for SNPs in terms of neurotransmitter receptor binding in patients with psychiatric diseases and healthy individuals,<sup>4,5</sup> but to the best of our knowledge this is the first time such an effect is described in patients with SLE. Another example of this phenomenon is the differential regulation of mRNA expression in different cell types, and in *in vitro* stimulated cells compared with unstimulated cells.<sup>6</sup> The underlying mechanisms for these observations are not completely known, but may include cell-type-specific expression or context-dependent induction of transcription factors.

When exploring these possibilities, we observed that basal mRNA levels of *STAT4alpha*, *STAT4beta* and *STAT1alpha* did not correlate with *STAT4* genotype in healthy donor CD8<sup>+</sup> memory T cells. However, following PHA/IL-2 stimulation, homozygous risk individuals displayed a defective induction of *STAT4alpha* mRNA expression, which is essential for IL-12-induced IFN- $\gamma$  production,<sup>7</sup> whereas *STAT4beta* and *STAT1alpha* mRNA were normally induced (figure 1D).

Given the important role of type I IFN in SLE, we hypothesised that IFN- $\alpha$  may be an environmental modulator of the *STAT4* risk allele. Indeed, preactivation of healthy donor cells with IFN- $\alpha$  for 24 hours before PHA/IL-2 stimulation augmented the IL-12-induced pSTAT4 in CD8<sup>+</sup> and CD4<sup>+</sup> T cells from risk allele carriers, but not in non-risk allele carriers (figure 1E). In contrast, preactivation of cells with tumour necrosis factor- $\alpha$  did not affect IL-12-induced pSTAT4 (figure 1F). In support of an interaction with IFN- $\alpha$  and the

*STAT4* risk allele in patients with SLE *in vivo*, stratification of the patients from our previous study<sup>3</sup> into those with plasma levels of IFN- $\alpha$  above or below the detection limit 0.5 U/mL revealed that the increased IL-12-induced pSTAT4 in CD8<sup>+</sup> T cells from risk allele carriers was specific to the IFN- $\alpha$ <sup>+</sup> patients (figure 1G).

In conclusion, the *STAT4* risk allele rs7574865[T] has opposite effects in healthy individuals compared with patients with SLE and we identify IFN- $\alpha$  as an environmental modifier of the *STAT4* risk allele. This finding may have implications for why the majority of risk allele carriers do not develop disease, and may also suggest a mechanism where *STAT4* risk allele carriers are at risk to develop SLE when IFN is produced, for instance, during a viral infection.

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**Contributors** NH performed the experiments and analysed the data. NH and LR designed the study, interpreted the data and wrote the manuscript.

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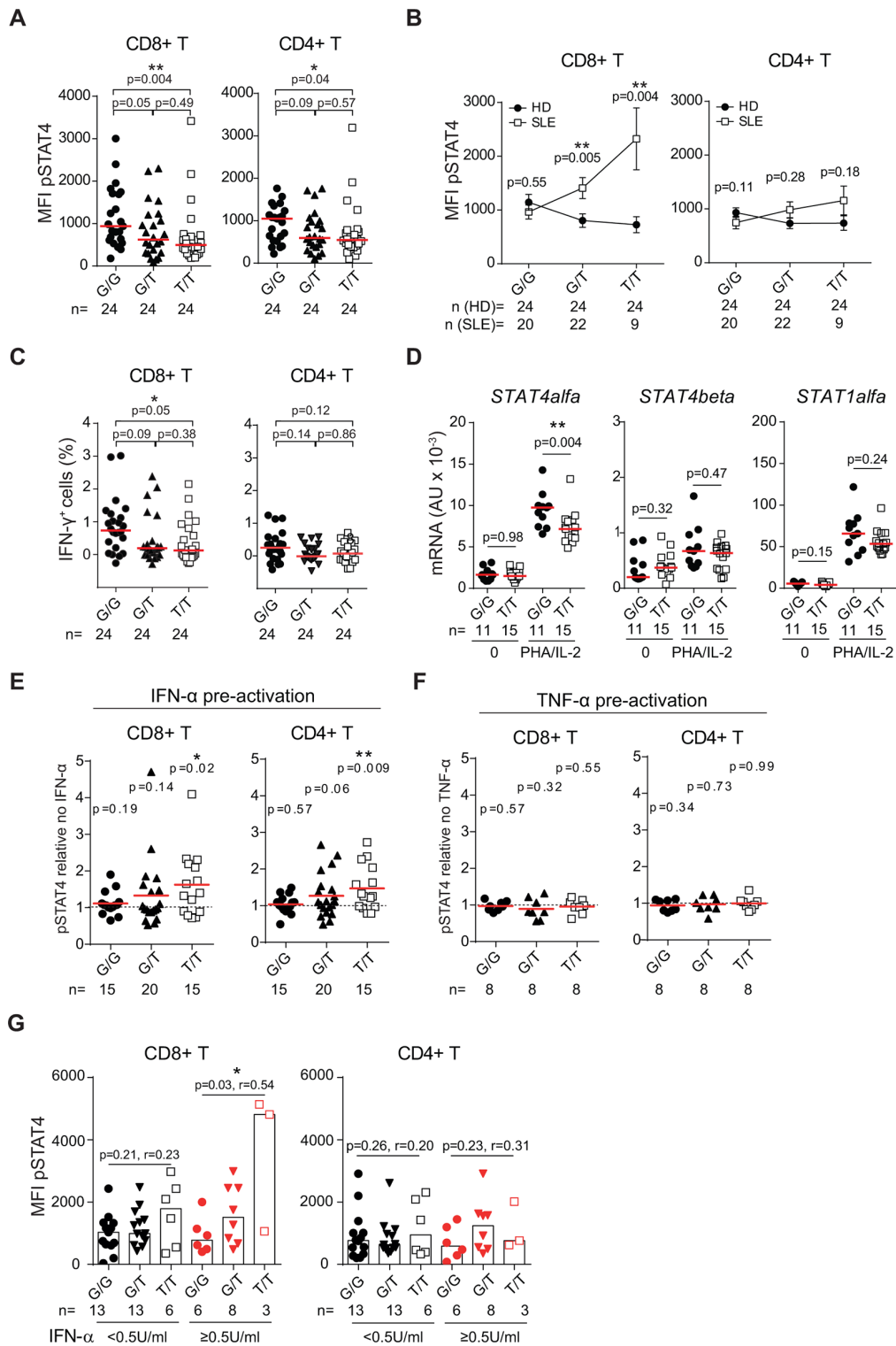
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**Figure 1** IFN- $\alpha$  enhances the IL-12 response in T cells from *STAT4* risk allele carriers. (A–C) PHA/IL-2 preactivated peripheral blood mononuclear cells (PBMC) from healthy donors (HD) were restimulated with IL-12. (A, B) Phosphorylation of *STAT4* (pSTAT4) (50 ng/mL IL-12, 20 min) and (C) frequency of IFN- $\gamma^+$  cells (5 ng/mL IL-12, 15 hours) were determined using flow cytometry. (B) Mean with error bars depicting the SEM. Data from patients with SLE are previously published.<sup>3</sup> (D) mRNA expression levels of *STAT4alpha*, *STAT4beta* and *STAT1alpha* in CD8<sup>+</sup> memory T cells from healthy individuals before and after 6 hours of stimulation with PHA/IL-2 were determined with qRT-PCR. (A–D) Statistical comparisons were made using two-tailed Mann-Whitney U tests. (E, F) PBMCs from healthy individuals were preactivated with (E) 100 U/mL IFN $\alpha$ 2, (F) 1 ng/mL TNF- $\alpha$ , or mock treated for 24 hours before PHA/IL-2 stimulation. IL-12-induced pSTAT4 (100 ng/mL, 20 min) in cytokine pretreated cells was divided by IL-12-induced pSTAT4 in mock-treated cells and two-tailed one-sample t-tests were used to assess whether the means differ from 1. (G) IL-12-induced pSTAT4 in patients with SLE stratified for plasma levels of IFN- $\alpha$  below (black) or above (red) the detection limit of 0.5 U/mL. P values for two-tailed Spearman’s correlation tests and the Spearman’s correlation coefficient (r) are denoted. (A–G) G/G, homozygous protective; G/T, heterozygous; T/T, homozygous risk for rs7574865. Red horizontal lines depict the (A–D) median or (E, F) mean values. \*P<0.05; \*\* P<0.01. IFN, interferon; IL, interleukin; n, number of individuals in each group; SLE, systemic lupus erythematosus; TNF, tumour necrosis factor.

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