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Developing interferon-β as a safe in vivo experimental-medicine model of human inflammation

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ABSTRACT

Background: Inflammation is increasingly implicated in a wide range of neuropsychiatric and neurodegenerative disorders from depression to dementia. Compelling evidence for an inflammatory role in these disorders includes experimental-medicine studies with IFN-α and endotoxin, alongside therapeutic benefits observed with anti-cytokine agents.

Aim: To develop and characterise a new, safe in-vivo mild inflammatory response that is titratable, elicits robust host sickness manifestations within an experimentally tractable timeframe, and has minimal cardiovascular effects, avoiding the requirement for continuous cardiac monitoring and ensuring applicability across diverse experimental contexts and participant groups, from the young to the elderly.

Methods: Using a randomized, blinded, placebo-controlled, repeated measures cross-over design, physiological, behavioural, cytokine, cellular and transcriptomic immune responses were collected from 30 healthy volunteers (15 young (18–34) and 15 older (60–75) years) on two separate occasions, once after 100 μg subcutaneous IFN-β (EXTAVIA®) and once after subcutaneous saline (placebo) injection.

Results: IFN-β increased ~15-fold at 4 h and 9-fold at 6½ hours and rapidly induced anticipated increases in negative mood, tiredness, tension and sickness symptoms and reduced vigour (all $p < 0.01$) without serious side effects. It was associated with a modest increase in temperature (mean: +1.1C) and heart rate (mean: +11 bpm) but no change in blood-pressure or cardiovascular instability. IL-6, TNF-α, neutrophil to lymphocyte ratio and monocyte count all showed significant increases (all $p < 0.05$). Transcriptomic analyses confirmed activation of classical Interferon signalling pathways as well as Toll-like Receptor (TLR), Inflammasome, Pyroptosis, MyD88 and a variety of other host response pathways that have been implicated in the pathophysiology of neuropsychiatric or neurodegenerative disorders.

Conclusions: IFN-β is a safe, robust new experimental model of mild inflammation that can be safely used to induce transient changes in systemic inflammation in healthy individuals from 18-75 years. Modulation of diverse immunological processes suggests it could be a valuable new experimental medicine tool across neuropsychiatric and neurodegenerative disorders.

1. Introduction

Converging evidence from epidemiological, experimental and pre-clinical studies has increasingly implicated systemic inflammation in a wide range of neuropsychiatric disorders, ranging from depression to age-related cognitive decline and dementia (Khandaker et al., 2021). Experience with COVID-19 has reminded us that robust host immune responses during severe systemic infections can result in sustained or

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persistent cognitive impairment even in previously healthy individuals (Wood et al., 2025). More generally, long-term cognitive deficits following critical illness and sepsis have been recognised for over a decade (Iwashyna et al., 2010).

However, of these approaches, arguably, the strongest evidence for an aetiological role for inflammation in human neuropsychiatric disorders has come from experimental medicine studies. This includes acute experimental immune-challenge models, and studies of patients receiving IFN-α injections over many months for therapeutic purposes (Capuron et al., 2007; Capuron and Miller, 2004; Harrison et al., 2014, 2015, 2016), which have predominantly focused on mechanisms of mood and motivational effects, and post-infective cohort studies after naturalistic infections, which have had a greater focus on long-term cognitive deficits (Iwashyna et al., 2010; Wood et al., 2025).

Together, these approaches have helped identify a discrete set of brain regions that are particularly sensitive to systemic inflammation, including medial temporal lobe structures such as the amygdala and hippocampus, and the hypothalamus that are integral to stress and memory circuits (Harrison et al., 2014; Wan et al., 1994; Yirmiya & Goshen, 2011); the insula, dorsal, posterior and sub-genual cingulate and *peri*-aqueductal grey (PAG) that form the interoceptive and autonomic control networks, salience and default mode networks (Harrison, Brydon, Walker, Gray, Steptoe, & Critchley, 2009; Kitzbichler et al., 2021) and dopamine-rich subcortical structures critical to motor responses, reward-related behaviours and motivational control (Brydon et al., 2008; Eisenberger et al., 2010; Harrison et al., 2015).

Building on this, studies that have simultaneously investigated acute and chronic effects of IFN-α in the same participants have shown that individuals with stress and reward systems most sensitive to acute inflammation also go on to develop the most severe depressive symptoms and motivational impairments during chronic IFN-α administration (Capuron et al., 2007; Capuron and Castanon, 2017; Davies et al., 2020; Dowell et al., 2016, 2019). In the context of fatigue-related motivational impairment, preliminary transcriptomic analyses suggest that this sensitivity may be related to early activation of m-TOR pathways (Periche-Tomas et al., 2025) which accords with the potent fatigue often associated with clinical use of mTOR-inhibitors (Peng et al., 2015).

Together, this suggests that acute and chronic immune challenges recruit and disrupt similar brain networks. Furthermore, it suggests that acute immune challenge models can serve as a reasonable proxy for predicting inter-individual differences in susceptibility to the more severe effects of intense or chronic inflammation that would be unethical to investigate experimentally outside a clinically therapeutic context. Coupled with renewed interest in developing novel immune-targeted therapies for neuropsychiatric disorders (Miller et al., 2025) and neurodegenerative disorders, this has highlighted the urgent need for a safe, reliable in-vivo experimental medicine model of inflammation that can be used across the age range. To date, this need has been partially addressed by access to short-acting forms of IFN-α or human (GMP) grade lipopolysaccharide (LPS). However, unpegylated IFN-α is no longer commercially available, and the costs and cardiovascular monitoring requirements of low-dose (i.e. 0.8–1 ng/kg) LPS restrict its use to specialised centres with substantial expertise and pose a particular challenge in older or more vulnerable populations.

Here we present a novel model of acute experimental inflammation using IFN-β, which was designed to achieve a balance between efficient sickness induction and minimal invasiveness and need for participant monitoring. Though we only report response to a single IFN-β dose here, like LPS, IFN-β is likely to offer a titratable approach to modulating immune activation. For example, when used clinically IFN-β dose is known to markedly influence cytokine and sickness symptom responses. Indeed, in MS treatment, dose-titration strategies are routinely used to minimise these responses and improve tolerability (Wroe, 2005). Similar to LPS, responses to IFN-beta also show tolerance, and in clinical practice, low-initial doses, e.g. 62 μg, are gradually titrated up to 250 μg IFN-β-1b (Prosperini et al., 2011). This capacity for stepwise dose escalation similarly offers opportunities for future studies to explore dose–response relationships in immune, neuroendocrine, cardiovascular, and psychological outcomes within experimental models.

Type-I interferons (IFNs), particularly IFNα and IFN-β, are critical antiviral cytokines that regulate inflammatory responses by activating the JAK-STAT pathway via the IFNAR1/IFNAR2 receptor, leading to the induction of interferon-stimulated genes (ISGs) and the secretion of cytokines such as IL-6, TNF-α, and IL-1ra (Bekisz et al., 2013; Kasper & Reder, 2014; Kümpfel et al., 2000). Until recently, Type-I interferons were widely used clinically, for example, IFN-α in the treatment of Hepatitis-C (IFN-α) (McHutchison et al., 1998), and IFN-β for relapse prevention in multiple sclerosis (MS) (Jacobs et al., 1981, 1982; Jacobs et al., 1996).

In this context, IFN-α impedes Hepatitis-C virus (HCV) replication by inducing an antiviral state in infected and neighbouring cells, enhancing natural killer (NK) cell activity, and disrupting HCV protein processing (Castet et al., 2002; Stegmann et al., 2010; C. Wang et al., 2003). In contrast, IFN-β is believed to reduce MS relapses through immunomodulatory effects including inhibiting T-cell activation and proliferation, suppressing pro-inflammatory cytokine production, enhancing anti-inflammatory cytokine production, reducing the expression of adhesion molecules, and promoting regulatory T-cell expansion (Cheng et al., 2015; Kieseier, 2011; Mirandola et al., 2009; Teige et al., 2006; Windhagen et al., 1995).

Like naturalistic viral infections, IFN injections induce a systemic inflammatory response and a broad repertoire of flu-like symptoms (Davis et al., 1989; Filipi & Jack, 2020). In healthy individuals, IFN-β exhibits a half-life of approximately 4–5 h with 30–50 % bioavailability and typically reaches peak concentrations within 1–8 h post-injection. In clinical use, repeated administration commonly produces mild flu-like side-effects such as chills, fever, fatigue, and nausea, which generally emerge around 3–4 h after dosing and are consistent across administration routes (Hu et al., 2016; Salmon et al., 1996). In healthy volunteers, IFN-β symptoms typically peak around 6–8 h post-injection (Exton et al., 2002; Salmon et al., 1996). Underpinning these effects, peripheral administration of Type-I interferons, including IFN-β, can access or modulate the brain through various mechanisms, including active transport across the blood–brain barrier (BBB), release of inflammatory mediators from the cerebral vasculature, and signalling via visceral afferent nerve fibres (Critchley & Harrison, 2013; Wang et al., 2008). In humans, in addition to increasing cytokines in the blood, IFN-α also increases cytokines such as IL-6 and TNF-α in the cerebrospinal fluid (Capuron & Miller, 2004; Raison et al., 2009; Taylor & Grossberg, 1998). Rodent studies also show rapid upregulation of interferon-stimulated genes in the brain following systemic IFN administration (Wang, 2009; Wang & Campbell, 2005).

Existing models of experimental inflammation, such as typhoid (Typhim®) vaccination or endotoxin challenges, present limitations. For example, Typhoid vaccination typically elicits a relatively modest immune response and is not titratable, while endotoxin challenges, although effective, typically require continuous cardiovascular monitoring and medical oversight. Conversely, models using influenza vaccination suffer from the fact that these vaccines change every year and vary between being trivalent or quadrivalent, making comparison between studies challenging.

Compared with lipopolysaccharide (LPS), which mimics gram-negative bacterial infection through direct activation of Toll-like receptor 4 (TLR4) on monocytes, macrophages, and dendritic cells, IFN-β more closely models a viral infection by acting downstream of TLR signalling as part of the type I interferon response (Kagan et al., 2008; Poltorak et al., 1998; Uematsu & Akira, 2007). Whereas LPS induces a broad cytokine cascade including both pro-inflammatory (e.g., TNF-α, IL-6, IL-1β) and counter-regulatory anti-inflammatory cytokines such as IL-10, IFN-β, preferentially drives expression of interferon-stimulated genes and a more restricted cytokine profile dominated by IL-6, TNF-α, and IL-1 receptor antagonist (Boxel-Dezaire et al., 2006; Dumitru

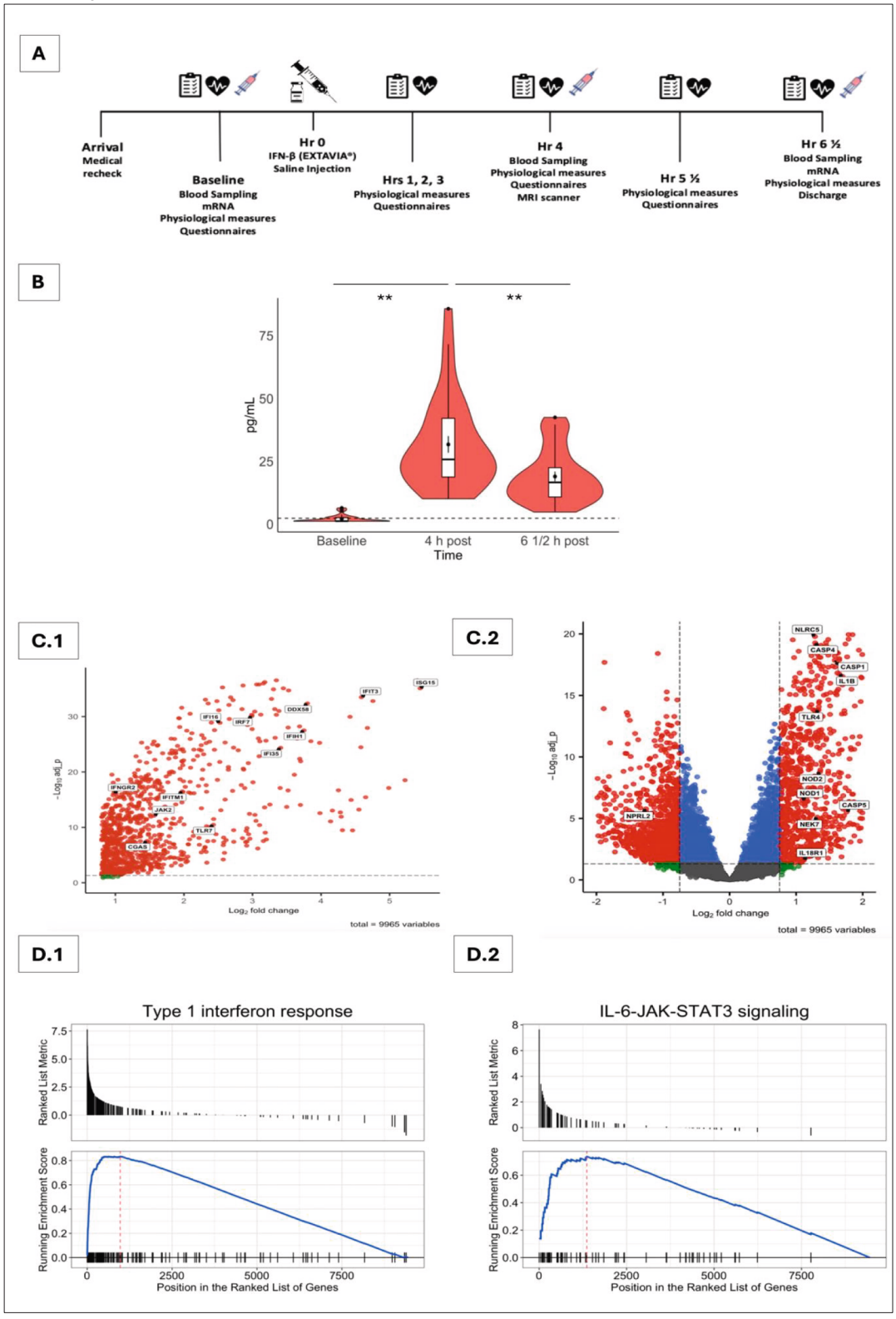

Fig. 1. Subcutaneous administration of IFN-β promotes resolving hallmarks of anti-viral immunity in humans. (A) Experimental session flowchart. (B) IFN-β plasma concentrations. Significant values show paired sample *t*-test results (**$p < 0.001$). Dashed line shows lower limit of detection (2.3 pg/mL). (C) Volcano plots of RNA-seq data showing differential gene expression following IFN-β intervention. Highlighted are the genes involved in the interferon (C.1) and inflammasome pathways (C.2). (D) Gen Set Enrichment Analysis (GSEA) for (D.1) Type-I Interferon-Responsive genes and (D.2) JAK-STAT signalling. The vertical black lines indicate the positions of individual genes from the gene set within the ranked list, while the blue curve represents the enrichment score (ES), calculated as a running sum that reflects the concentration of gene set members at the top or bottom of the list.

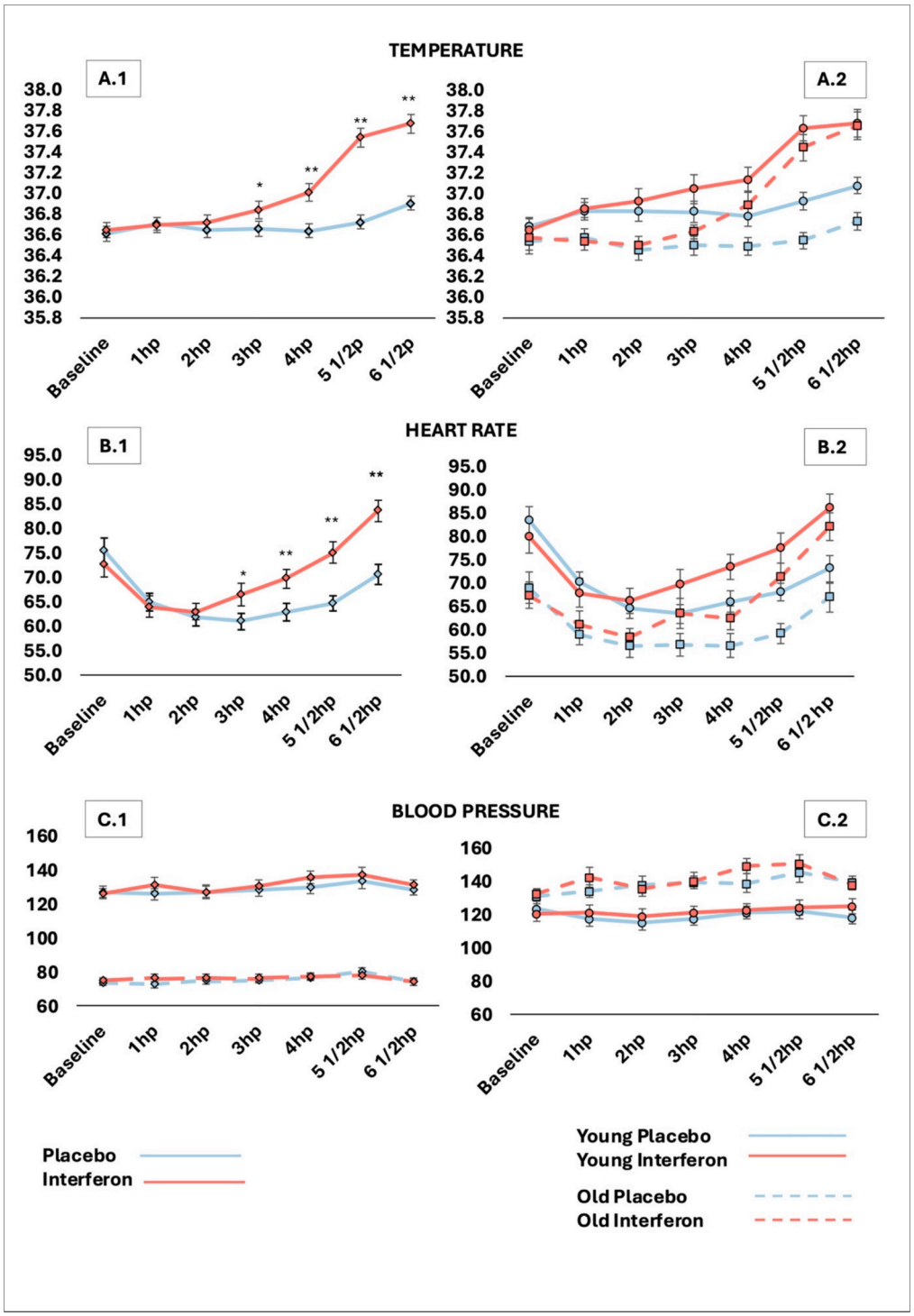

Fig. 2. Effects of IFN-β on physiological measures. Panels A1–C1 show the effects of IFN-β (red lines) versus placebo (blue lines) on (A) temperature (°C), (B) heart rate (bpm), and (C) blood pressure (mmHg) over time (left panels). Dotted lines in C1 represent diastolic blood pressure. Panels A2 and B2 display age-associated effects on temperature and heart rate, respectively (right panels), while C2 shows a significant age-related effect on systolic blood pressure; dotted lines indicate older individuals. Error bars represent SEM. Significant values show the effect of IFN-β compared to placebo (*$p < 0.01$, **$p < 0.001$).

et al., 2000; Sheikh et al., 2014). These differences are relevant to experimental medicine models, since converging evidence suggests that the brain can distinguish pathogen-associated immune signals and mount pathogen-specific behavioural and neural responses (Dantzer, 2009; Jin et al., 2024; Salvador et al., 2021). Accordingly, using IFN-β provides an opportunity to probe neuroimmune mechanisms triggered by viral-like inflammation, complementing LPS which models responses to gram-negative bacterial infections. Importantly, this favourable safety and tolerability profile makes IFN-β particularly well suited for studies spanning younger and older adults, where alternative models such as LPS may pose greater risks or monitoring burdens.

Here, we characterise the cellular, immune, cytokine, transcriptomic, behavioural and wider physiological response to peripheral IFN-β administration in healthy young (18–34) and older (60–75) adults. In a randomised, placebo-controlled, repeated measures cross-over design, thirty participants were each tested on two separate

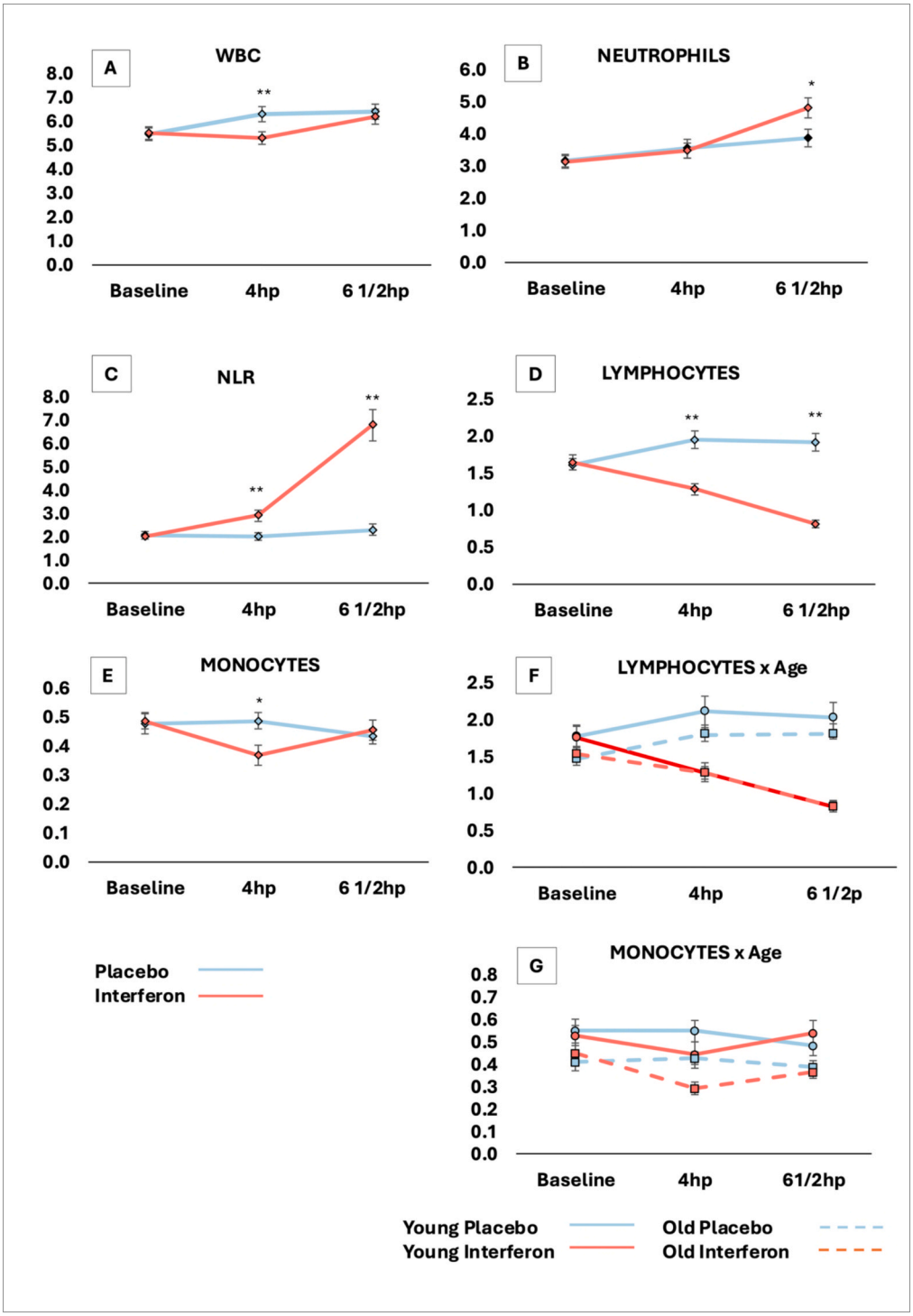

Fig. 3. Effects of IFN-β on cellular immune response. Panels A–E show the effects of IFN-β (red lines) versus placebo (blue lines) on (A) total white blood cell count (WBC), (B) neutrophils, (C) neutrophil-to-lymphocyte ratio (NLR), (D) lymphocytes, and (E) monocytes (all in 10^9/L). Panels F and G display age-associated effects on Monocytes and condition x age interaction on lymphocyte responses, respectively; dotted lines indicate older participants. Error bars represent SEM. Significant values show the effect of IFN-β relative to placebo (*$p < 0.01$, **$p < 0.001$).

occasions, once after IFN-β EXTAVIA® and once after saline (placebo). By presenting this new model, we aim to provide a method for studying host antiviral responses and their downstream immune, physiological, and psychological effects, thereby aiding mechanistic understanding and informing treatment of neuroinflammatory and neuropsychiatric conditions.

2. Methods

2.1. Participants

Thirty healthy participants (15 young [6 male, mean age 25.2 ± 5.1 years], 15 old [6 male, mean age 65.6 ± 4.5 years]) were recruited from around the Cardiff area. Volunteers had to be non-smokers and in good health, as determined by their medical history, physical and psychiatric

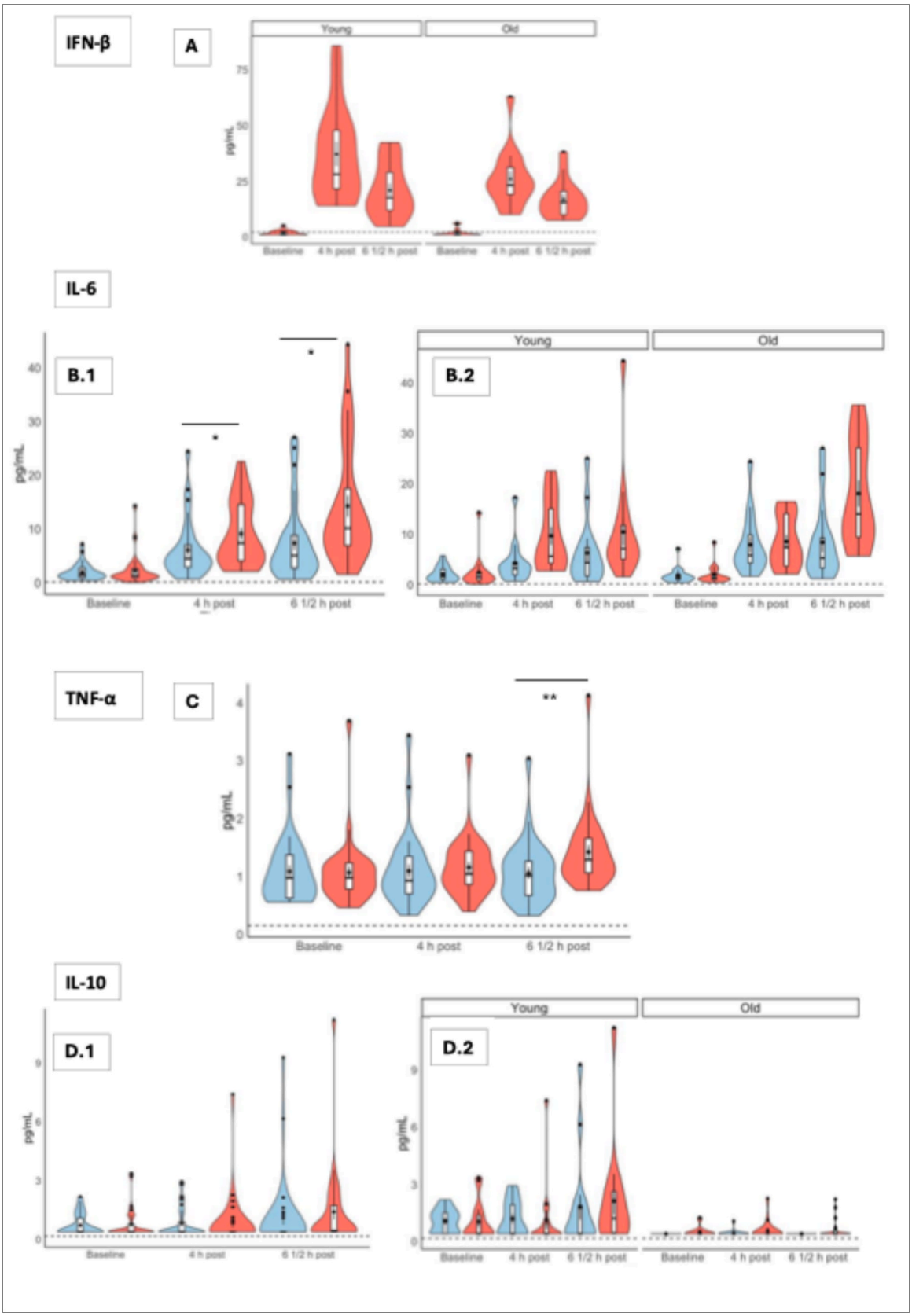

Fig. 4. Distribution of cytokine plasma concentrations. Panel A shows IFN-β concentrations split by age group. Panels B1 and B2 show IL-6 concentrations overall and by age, respectively. Panel C shows TNF-α concentrations, and panels D1 and D2 depict IL-10 concentrations overall and by age. In all panels, red denotes IFN-β and blue denotes placebo, and dashed lines indicate the lower limit of detection (IFN-β: 2.3 pg/mL; IL-6 and TNF-α: 0.156 pg/mL; IL-10: 0.78 pg/mL). Significant differences between conditions were assessed using paired-sample t-tests (*$p < 0.05$; **$p < 0.001$).

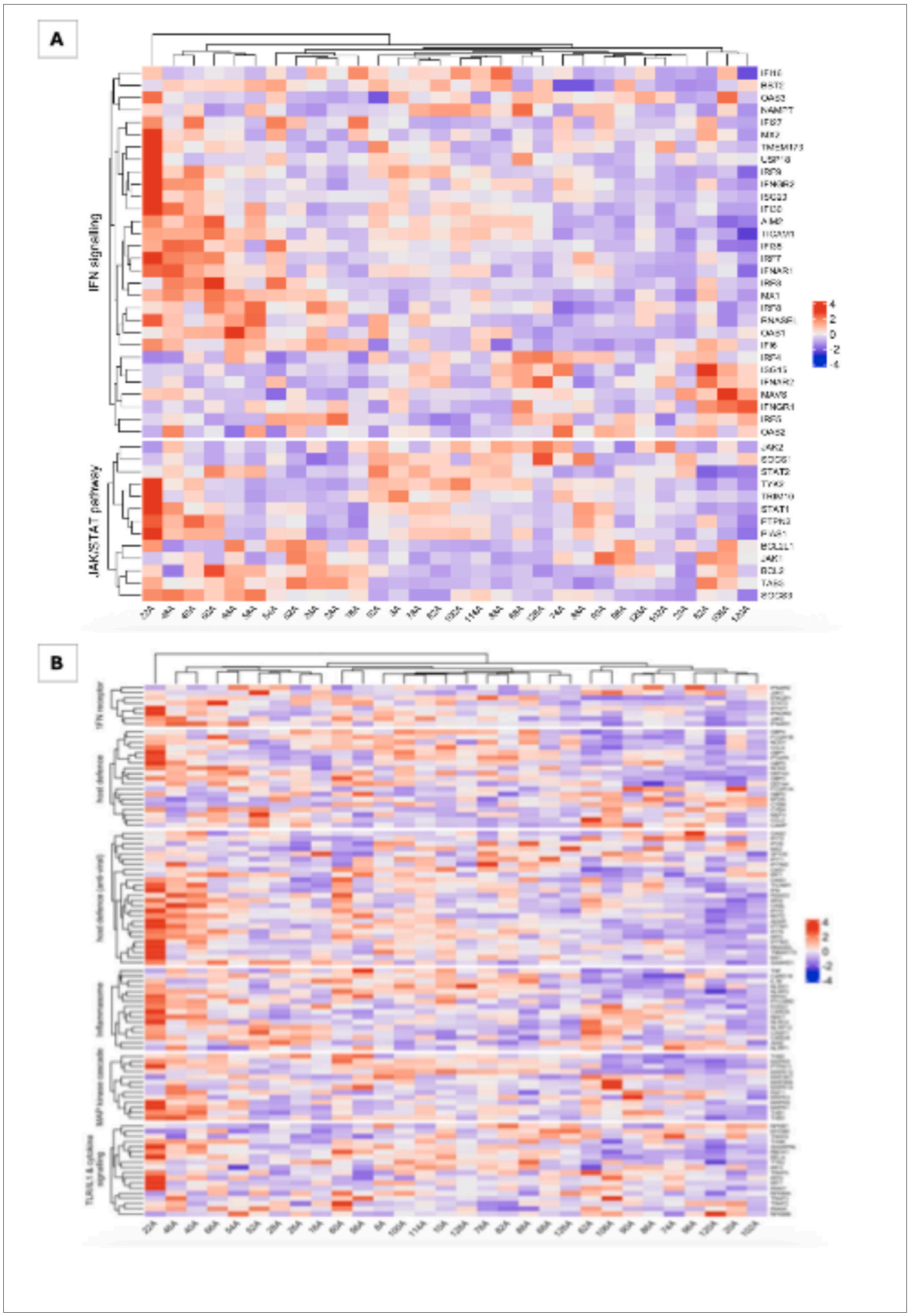

Fig. 5. Hierarchical clustering of immune-related gene expression following IFN-β administration. Heatmaps showing (A) expression patterns of genes from the Interferon signalling pathway (13 genes) and the JAK/STAT pathway (13 genes). (B) Expression patterns of 100 immune-related genes grouped into functional pathways: host defence (19 genes), host defence-antiviral (26 genes), IFN receptor signalling (8 genes), Inflammasome (16 genes), MAP kinase cascade (13 genes), and TLR/L1 & cytokine signalling (18 genes). The colour scale represents relative gene expression, with blue indicating lower and red indicating higher expression across samples.

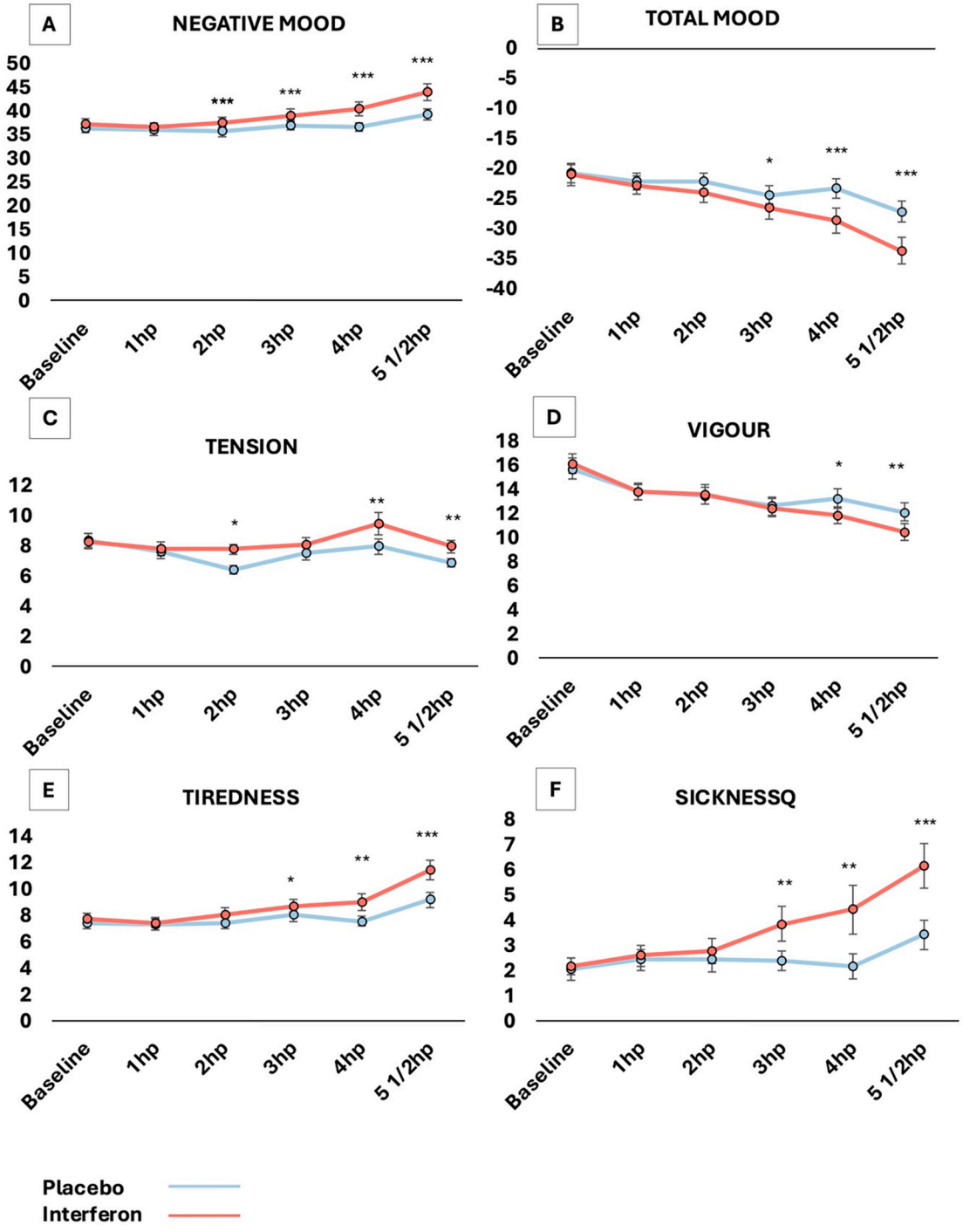

Fig. 6. Effects of IFN-β on subjective response. Panels A–E show significant changes in mood as measured by POMS subscales: (A) negative mood composite, (C) tension, (D) vigour, and (E) tiredness, along with (B) total mood disturbance score. Panel F shows responses on the Sickness Questionnaire (SicknessQ). Red lines represent IFN-β and blue lines placebo. Error bars represent SEM. Significant effects of IFN-β relative to placebo are indicated as *p < 0.05, **p < 0.01, ***p < 0.001.

screening, vital signs and clinical laboratory test results, including renal, liver, thyroid function and full blood count. In the younger cohort, the majority of participants identified as belonging to a white ethnic background (n = 13), with two participants identifying as of Asian origin. The older cohort was composed of participants who identified as being of white ethnicity. Participants were advised to avoid heavy exercise and the use of alcohol for 24 h before each session. The study was approved by the London-Camden & Kings Cross Research Ethics Committee (20/LO/0239).

2.2. Study design

We adopted a randomised, placebo-controlled, repeated-measures cross-over study. All participants were tested on two separate occasions between 2 and 6 weeks apart (Mean = 28.2 days). In one session, this was 0.4 mL of reconstituted IFN-β EXTAVIA® (100 μg), and in the other, 0.4 mL of 0.9 % saline. The order of intervention was randomised, with half the participants receiving IFN-β on their first study session and half a placebo.

Temperature, heart rate, systolic and diastolic blood pressure were measured at baseline and 6 further time-points in both study sessions (1

hr, 2 hr, 3 hr, 4 hr, 5½ and 6½ hours post-IFN-β/saline injection). Self-reported mood, fatigue and sickness questionnaires (Profile of Mood States (POMS) (McNair et al., 1971), fatigue Visual Analogue Scale (fVAS) (Gift, 1989) and Karolinska Sickness Questionnaire (SicknessQ) (Andreasson et al., 2018) were administered at baseline, and 5 additional time points. Blood samples were collected at baseline, 4 hr and 6½ hr post-challenge for full-blood count (FBC) and whole-blood mRNA analyses at baseline and 6 ½ hr post-injection. The study session timeline is illustrated in Fig. 1A.

2.3. Cytokine analysis

Blood samples for cytokine analysis were collected into BD Vacutainer plastic EDTA tubes with lavender hemogard closure (4 mL, 13x75mm) (Becton, Dickson and Company, Franklin Lakes, New Jersey, United States) at baseline, 4 h and 6 ½ hours post-injection. EDTA tubes were centrifuged immediately at 2000 rpm for 20 min. Plasma was removed, aliquoted and stored at −80 °C before analysis. IFN-β plasma concentration was measured using VeriKine-HS™ Human IFN Beta Serum High Sensitivity ELISA Kit (PBL Assay Science, NJ, USA). Detection limit was 2.3 pg/mL and intra and inter-essay coefficients of variation were 3.6 % and 7.9 % respectively.

Plasma levels of IL-6, TNF-α and IL-10 were quantified with Quantikine™ High Sensitivity ELISAs (R&D Systems inc., Minneapolis, USA). Detection limits were 0.156 pg/mL, 0.156 pg/mL and 0.78 pg/mL respectively and coefficients of variation were 3.6 % and 4.9 % (IL-6), 2.0 % and 6.7 % (TNF-α) and 5.8 % and 7.8 % (IL-10). Standards and samples were all tested in duplicate. Samples with measurements below the lowest standard were given a value of half the lower limit of detection for IL-10 and IFN-β cytokines (Breen et al., 2011).

2.4. Transcriptomics analysis

Blood samples were collected in PAxGene RNA tubes and stored at –80 °C. RNA was extracted from the 6½ hr post-injection samples following the PAXgene Blood RNA Kit, including a globin depletion step. RNA quality and concentration were assessed using Qubit and fragment analysis (https://www.thermofisher.com/uk/en/home/industrial/spectroscopy-elemental-isotope-analysis/molecular-spectroscopy/fluorometers/qubit/qubit-assays.html) before samples were shipped on dry ice to Lexogen for 3′ mRNA sequencing using the QuantSeq protocol (https://www.lexogen.com/quantseq-family/).

Differentially expressed genes (DEGs) in the IFN-β versus placebo (saline) condition were analysed using IPA (QIAGEN Inc., https://www.qiagenbioinformatics.com/products/ingenuity-pathway-analysis). To identify significant pathways, IPA uses the p-value of overlap, calculated using the right-tailed Fisher's exact Test. Differentially expressed genes were identified: ($|log2FC| > 1$ and adjusted p-value < 0.05) using edgeR/limma (Law et al., 2014; Smyth, 2004; Storey, 2002). Volcano plots were generated using the EnhancedVolcano R package and R 4.4.2.

Gene set enrichment analysis (GSEA) was performed using differential expression to evaluate the gene set significance data and assess whether predefined groups of genes showed coordinated up-or-down regulation. An enrichment score (ES) was calculated for each gene set, reflecting the degree to which its member genes are concentrated at the top or bottom of the ranked list of all genes.

Heatmaps were generated using the ComplexHeatmap package in R, with gene expression values scaled by row and hierarchical clustering performed using complete linkage.

2.5. Statistical analysis

Main effects of IFN-β, age, and their interaction on physiological, cellular, immune, and behavioural data were analysed using repeated measures mixed factorial ANOVAs. The within-subject factors were condition (IFN-β/placebo) and time (pre- and post-injection, as described), and the between-subject factor was age (young/old). Statistical significance was set at $p < 0.05$. Significant interactions were followed up with post-hoc paired t-tests comparing conditions at each time point with Bonferroni correction applied to control for multiple comparisons. Pearson's correlations were used to assess associations between cytokines and total and differential cell counts (computed as peak change minus baseline for the IFN-β condition). Exploratory analyses including sex as a between-subjects factor were also conducted across physiological, immune, and cytokine outcomes. Data analyses were completed using the SPSS 27 statistical package.

3. Results

3.1. Physiological response

3.1.1. Temperature

Repeated measures ANOVA revealed a significant main effect of condition (placebo/IFN-β) ($F(1,28) = 45.5$, $p < 0.001$) and a significant condition × time interaction ($F(3.3,94.48) = 30.88$, $p < 0.001$). IFN-β increased temperature compared to placebo from 3 h post-injection ($t(28) = -2.87$, $p = 0.008$), peaking at the final 6½-hour post-injection observation (+1.1 °C in the IFN-β condition, $t(28) = -8.32$, $p < 0.001$). After Bonferroni correction, the increase remained significant from 4 to 6½ hours, while the 3-hour effect was marginal and did not survive correction (Fig. 2A1). A significant main effect of age was also observed ($F(1,28) = 6.79$, $p = 0.014$), with older individuals showing a lower mean temperature (mean difference = 0.27 °C, SE = 0.10) compared to younger participants, regardless of condition or time (Fig. 2A2). No significant condition x age or condition x age x time interactions were found.

3.1.2. Heart rate

Repeated measures ANOVA indicated a significant main effect of condition on heart rate ($F(1,28) = 21.34$, $p < 0.001$) and a significant condition × time interaction ($F(4.14,115.93) = 16.62$, $p < 0.001$). IFN-β increased heart rate relative to placebo from 3 h post-challenge ($t(28) = -5.6$, $p = 0.005$), peaking at 6½ hours post-injection (+11 bpm in the IFN-β condition, $t(28) = -10.33$, $p < 0.001$). Similar to temperature, IFN-β increased heart rate relative to placebo from 3 h post-challenge ($t(28) = -5.6$, $p = 0.005$), peaking at 6½ hours post-injection (+11 bpm in the IFN-β condition, $t(28) = -10.33$, $p < 0.001$). After Bonferroni correction, the increase remained significant from 3 to 6½ hours, while the (Fig. 2B1). There were no significant condition x age or condition x age x time interactions. A significant main effect of age on heart rate was found ($F(1,28) = 6.27$, $p = 0.018$), with older individuals having a lower resting heart rate than younger participants (mean difference = -7.66 bpm, SE = 3.05) (Fig. 2*B2)*.

3.2. Blood pressure

For systolic blood pressure, repeated measures ANOVAs showed a trend-level main effect of condition ($F(1,28) = 4.15$, $p = 0.051$) but no significant condition x time or condition x age x time interactions. No significant differences were observed in diastolic blood pressure or its interactions (Fig. 2*C1)*. Between-subject effects revealed a significant age-associated difference in systolic BP ($F(1,28) = 14.58$, $p < 0.001$), with older individuals exhibiting higher systolic BP compared to younger participants (mean difference = 19.086 mmHg, SE = 4.9) (Fig. 2*C2)*. No age-associated effects were found for diastolic BP.

3.3. Cellular immune response

Repeated measures ANOVA indicated significant condition x time interactions for total WBC ($F(1.49,40.47) = 10.92$, $p < 0.001$) and differential counts: monocytes ($F(1.52,42.79) = 7.22$, $p < 0.001$), neutrophils ($F(1.52,42.43) = 15.9$, $p < 0.001$), and lymphocytes ($F(1.33,37.32) = 72.46$, $p < 0.001$). A main effect of condition was

observed for lymphocytes ($F(1,28) = 112.26$, $p < 0.001$) and for neutrophil to lymphocyte ratio (NLR) ($F(1,28) = 35.95$, $p < 0.001$), with a significant condition x time interaction for NLR ($F(1.06,29.7) = 45.78$, $p < 0.001$) (Fig. 3*A-E*). Post-IFN-β, cell counts (expressed as relative delta percentage ± CI) increased for neutrophils (59.2 ± 20.4 %) at 6½ hr post-injection. NLR increased at both 4 hr (43.8 ± 11.6 %) and 6½ hr (245.4 ± 67.2 %) post-IFN-β. Lymphocytes decreased by 21.2 ± 4.8 % and 49.3 ± 4.8 % at 4 and 6½ hr, respectively. Monocytes decreased by 29.6 ± 8.5 % at 4 hr post-IFN-β, with WBC also showing a decrease of 3.5 ± 5.8 % at 4 hr relative to baseline. All reported effects survived multiple comparisons. Full blood count data are provided in the supplementary material (Supplementary Table 1).

A significant between-subject effect was found for monocytes ($F(1,28) = 7.93$, $p = 0.026$), with older individuals showing lower monocyte counts regardless of time and condition (mean difference = 0.126 (10^9/L), SE = 0.045) (Fig. 3G). A trend-level condition x age interaction was observed for lymphocytes ($F(1,28) = 3.13$, $p = 0.088$) (Fig. 3F). No other interactions or age-associated differences were observed for other WBC counts.

3.4. Cytokine response

As anticipated, following IFN-β challenge, plasma concentrations of IFN-β (pg/mL) significantly increased, as indicated by repeated measures ANOVA ($F(1.19,32.19) = 10.14$, $p < 0.001$). Concentrations peaked at 4 hr ($t(29) = -8.74$, $p < 0.001$) then decreased at 6½ hr post-challenge ($t(29) = -8.55$, $p < 0.001$), with significant differences in concentration observed between the 4 and 6½ −hour timepoints ($t(29) = 6.98$, $p < 0.001$) consistent with its known short (<5 hr) half-life. From baseline, IFN-β levels increased approximately 15-fold at 4 hr and 9-fold at 6 ½ hr (Fig. 1B). These post-hoc comparisons remained significant after Bonferroni correction. Although no significant age effects were observed, there was a trend towards a time x age interaction, with higher IFN-β levels observed in younger individuals ($F(1.18,33) = 2.8$, $p = 0.098$) (Fig. 4A).

IFN-β also increased circulating IL-6 levels (approximately 6-fold), as shown by significant main effect of condition ($F(1.28) = 10.14$, $p = 0.004$) and condition x time interactions ($F(1.45,40.78) = 4.49$, $p = 0.027$). A time x age interaction was noted ($F(1.85,51.83) = 4.16$, $p = 0.024$), with mean ± SEM IL-6 concentrations of 6.98 ± 1.1 (young) and 8.25 ± 1.1 (old) at 4 hr, and 8.38 ± 1.4 (young) and 13.24 ± 1.4 (old) at 6½ hr post-injection. Post-hoc analyses indicated higher IL-6 levels in the IFN-β condition at 4 and 6½ hr ($p < 0.05$), but these effects did not survive Bonferroni correction. Additionally, there was a trend towards a condition x time x age interaction, with older individuals showing higher IL-6 concentrations at 6½ hr post-injection, raising the possibility of a more prolonged effect in this age group ($F(1.45,40.78) = 2.65$, $p = 0.097$). IL-6 distribution of plasma concentrations is provided in Fig. 4B1-2.

Condition x time interaction effects were observed for TNF-α ($F(1.56,43.94) = 22.07$, $p < 0.001$) with an average increase of approximately 1.3-fold following IFN-β (Fig. 4C). Levels were significantly higher at 6½ hr post-injection ($p = 0.001$), and this effect survived Bonferroni correction.

No condition x age interactions or age-associated differences were found. There was no significant main effect of IFN-β on IL-10 concentrations ($p > 0.1$). However, between-subjects effects revealed a significant age-associated difference ($F(1.28) = 6.48$, $p = 0.017$), with older individuals showing lower IL-10 levels compared to younger participants (mean difference = 0.889 pg/mL, SE = 0.349) (Fig. 4D1-2). Cytokine values are provided in Supplementary Table 2.

Associations between immune modulators and cellular immune responses were examined, revealing a significant correlation between IFN-β concentration (ΔIFN-β: 4 h – baseline) and induced changes in neutrophil-to-lymphocyte ratio (ΔNLR: 4 h – baseline) ($r^2 = 0.15$, $p = 0.033$, 95 % CI: 0.002–0.035). No other significant correlations were found between cytokine levels and cellular immune responses. Exploratory analyses including sex as a between-subjects factor revealed no significant sex × condition × time or lower order interactions across physiological, immune, or cytokine outcomes (all $p > 0.05$).

3.5. Transcriptomic response

Analysis of DEGs identified 2209 differentially expressed genes including upregulation of genes involved in the interferon pathways such as ISG15, IFIT3, IFIH1, IFI35, DDX58, IRF7, IFI16, IFITM1, and IFNGR2 (Fig. 1*C1*). This included several genes of the inflammasome pathway such as IL1B, IL18R1, NOD1, NOD2, NLRC4 and NEK7, which regulate the NLRP3 inflammasome (He et al., 2016) (Fig. 1*C2*). Of note here, NEK7 is an essential mediator of NLRP3 activation downstream of potassium efflux. Interestingly, there was also upregulation of CASP1, CASP4 and CASP5 genes, suggesting canonical (CASP1) as well as non-canonical (CASP4, CASP5) inflammasome activation.

Together, this demonstrated that in addition to triggering of IFN-α/β signalling pathways, IFN-β administration also activated multiple innate immune pathways that lead to inflammation, such as the Pathogen-induced cytokine storm signalling pathways, pattern recognition pathways, inflammasome, Toll-like receptor signalling pathways, cGAS-STING as well as RIG-I and TLR3 pathways. Fig. 5*A-B* provides an overview of how these main biological pathways relate to one another. In addition, it shows how these interferon signalling pathways are linked to both anti-viral immune responses and innate immune pathways that lead to inflammation.

To further validate the involvement of interferon-related pathways, we performed gene set enrichment analysis (GSEA) using the ranked list of differentially expressed genes. This analysis confirmed significant enrichment of gene sets related to Type I interferon signalling and JAK/STAT pathway (Fig. 1D).

3.6. Subjective sickness response

Repeated measures ANOVA revealed IFN-β-induced significant mood shifts that were observed in five POMS subscales, with the most pronounced changes at 5½ hours post-injection. IFN-β significantly decreased total mood score (Condition x Time $F(3.13,87.82) = 5.93$, $p < 0.001$) and vigour ($F(3.5,98.58) = 3.19$, $p = 0.021$), and increased negative mood ($F(3.28,91.98) = 5.61$, $p < 0.001$), tiredness ($F(2.44,68.5) = 4.97$, $p = 0.006$), and tension ($F(3.89,109.13) = 2.73$, $p = 0.034$). Post-hoc analyses showed that negative mood was consistently higher from 2 to 5½ hours, all of which survived Bonferroni correction. Total mood score reductions were significant at 4 and 5½ hours, and tiredness increased at 5½ hours, both remaining significant after correction. Effects on vigour and tension were weaker and did not survive correction.

Sickness symptoms increased significantly, as shown by the SicknessQ, with a significant main effect of condition ($F(1,28) = 7.64$, $p = 0.01$) and condition x time interaction ($F(2.88,80.87) = 7.39$, $p = 0.001$), peaking at 5½ hours post-injection (Fig. 6*A-F*). Post-hoc analyses indicated significant increases from 3 h onwards, but only the 5½-hour peak survived Bonferroni correction. No significant effects were found on the fatigue scale (fVAS), and no condition x time x age interactions or age-related effects were observed for any scale ($p > 0.1$).

3.7. Side effects

Injection site reactions are also commonly reported; however, in our study, only one participant experienced mild itchiness and redness in the injection area, which resolved within 24 hr. This reaction was likely due to the reconstituted interferon solution being refrigerated briefly prior to administration rather than being injected immediately after preparation. Overall, symptoms were mild and resolved by the evening of the intervention for most participants. A few older individuals reported

experiencing more prolonged symptoms that persisted overnight and into the following morning. However, these symptoms were again mild, not associated with significant functional impairment and were not considered unexpected or adverse in any of the participants.

4. Discussion

Here, we establish IFN-β as a safe and reliable experimental model of acute inflammation in adults aged from 18 to 75 years. Our findings demonstrate that IFN-β elicits a robust systemic immune response, characterised by changes in physiological, immunological and behavioural measures. This includes elevated temperature and heart rate, immune cell activation (lymphocytes, monocytes and neutrophils), and heightened cytokine levels (IFN-β, IL-6 and TNF-α). These physiological changes were accompanied by pronounced subjective responses, such as increased negative mood, fatigue and sickness symptoms. Additionally, we observed a significant upregulation of Type-I interferon-related genes, further suggesting a role for IFN-β in driving a coordinated immune and behavioural reaction.

IFN-β induced a significant systemic inflammatory response, with notable increases in body temperature and heart rate peaking at 6½ hr post-injection, consistent with previous studies (Exton et al., 2002; Kümpfel et al., 2000; Salmon et al., 1996). The transient changes in temperature (+1.1 °C) and heart rate (+11 bpm) were similar in magnitude to those reported with IFN-α but milder than those typically observed with low dose (0.8–1.0 ng/Kg) LPS, and they were not associated with any periods of bradycardia or postural hypotension that can sometimes be observed with the LPS model. Conversely, they elicited a more robust temperature and heart rate response to that reported with the typhoid (Typhim) vaccination model (Fukuhara et al., 1999; Glue et al., 2000; Han et al., 2013; Harrison, Brydon, Walker, Gray, Steptoe, Dolan, et al., 2009; Harrison et al., 2014; Hijma et al., 2020; Lasselin et al., 2017). IFN-β also induced an approximately sixfold increase in circulating IL-6, consistent with patterns seen in IFN-based therapies for Hepatitis-C (Capuron & Miller, 2004; Davi es et al., 2020), less potent that LPS (Lasselin et al., 2017; Peters van Ton et al., 2021; Sandiego et al., 2015) but more potent than typhoid vaccination models (Harrison et al., 2014, 2016).

IFN-β raised TNF-α levels by approximately 1.3-fold, this broader cytokine response is also seen in endotoxin studies but is not typically a feature of typhoid vaccine or IFN-α models (Davies et al., 2020; Harrison, Brydon, Walker, Gray, Steptoe, Dolan, et al., 2009). In this regard, acute IFN-β has also been shown to contribute to the inflammatory response by increasing TNF-α and IL-6 in MS patients (Kümpfel et al., 2000). Although IFN-α and IFN-β are both Type-I interferons, their distinct binding affinities and subsequent effects on cellular signalling pathways may explain their differing impacts on TNF-α production (de Weerd et al., 2013; Ivashkiv & Donlin, 2014).

IL-10 levels, which typically increase in response to IFN-α and LPS, did not significantly change after IFN-β in our study. While IFN-β is known to boost IL-10 in MS, contributing to its anti-inflammatory effects (Kvarnström et al., 2013; Özenci et al., 2000), the absence of a significant IL-10 response in our study may relate to one of two reasons: 1) The lower IFN-β dose we used (100 μg) compared to MS studies, where maintenance doses are typically higher (250 μg); 2) timing of response: In MS IL-10 increases are typically noted between 6 and 12 hr post-injection, while our last blood collection was at 6½ hours, therefore we may have missed this later IL-10 rise (Williams & Witt, 1998).

The cellular response to IFN-β was also similar to that observed with IFN-α, with notable increases in Neutrophil counts (~50 %) and decreases in Lymphocytes (~50 %) at 6½ hours post dose and a biphasic Monocytes response which decreased (~30 %) at 4 h then increased (~35 %) by 6 ½ hours. All of these cellular responses were milder than those seen with 0.8–1 ng/Kg Endotoxin but followed a similar temporal profile and trend. Overall, IFN-β induced a transient inflammatory state with consistent symptom development, comparable to IFN-α but less intense than Endotoxin, and more pronounced than the Typhoid vaccination model. A comparison table for the physiological, immune and transcriptomics response can be found in Supplementary Table 3.

The pharmacokinetics of IFN-β may display some variations that will depend on the administration route and dosing. Overall, in healthy individuals, IFN-β has a half-life of 4–5 hr and bioavailability of 30–50 %, reaching peak concentrations within 1–8 hr post-injection (Hu et al., 2016; Salmon et al., 1996). Our results show that Plasma IFN-β concentrations increased dramatically by ~ 15-fold at 4 hr and ~ 9-fold at 6 ½ hr post-injection. Participants also reported a significant rise in side effects starting from 3 hr after administration.

Our findings demonstrate that IFN-β administration as an in-vivo experimental medicine model induces strong interferon- and inflammasome-related gene signatures, recapitulating the host immune response to viral infection. Differential expression analysis revealed significant upregulation of genes involved in the interferon pathway, including ISG15, IFIT3, IFIH1, IFI35, DDX58, IRF7, IFI16, IFITM1, and IFNGR2. Additionally, cytosolic nucleic acid sensors such as TLR7 and cGAS, known to trigger IFN-β upon activation, were also upregulated (Costa Franco et al., 2018; Saitoh et al., 2017). Alongside this interferon response, genes regulating the inflammasome pathway, including IL1B, IL18R1, NOD1, NOD2, NLRC4, and NEK7, were upregulated, highlighting activation of the NLRP3 inflammasome. The upregulation of CASP1, CASP4, and CASP5 further suggests both canonical (CASP1) and non-canonical (CASP4, CASP5) inflammasome activation. These results provide new insights into how IFN-β administration mimics the host response to viral infection and triggers an immune response, reinforcing its relevance as a model for studying inflammation and inter-individual differences in host responses to viral infection. Notably, this included upregulation of cytosolic nucleic acid sensors such as TLR7 and cGAS, which, once activated, can trigger IFN-β release, suggesting a potential positive feedback loop amplifying interferon signalling. Taken together, these findings provide the first illustration that IFN-β administration in humans induces a robust IFN-related and inflammasome-related gene signature resembling the coordinated host response to infection.

Consistent with previous findings using LPS and typhoid vaccine immune challenges (De Marco et al., 2022, 2023; Eisenberger et al., 2010; Harrison et al., 2015), IFN-β induced similar transient changes in mood, fatigue, and sickness symptoms. These changes were measured using self-rating questionnaires POMS, fVAS and SicknessQ, commonly used to assess the impact of systemic inflammation on mood and fatigue. This provides further support for using IFN-β as a novel acute inflammatory challenge (Supplementary Table 4). Animal studies also suggest that sickness behaviours such as reduced voluntary activity can occur independently of IFN-β. For example, poly I:C administration has been shown to reduce wheel running in mice regardless of IFN-β signalling (Matsumoto & Seya, 2008). This highlights that while IFN-β is sufficient to induce behavioural and physiological changes in humans, it may not be the sole pathway mediating infection-related reductions in activity across species.

A major goal of the current study was to assess the safety and tolerability of IFN-β in an older population and determine whether age significantly influences the effects of IFN-β on physiological, behavioural, and cognitive responses. Given that older individuals often experience more pronounced behavioural responses to infections, and severe infections can lead to lasting cognitive decline (Iwashyna et al., 2010), we hypothesized that inflammation would differentially impact older individuals. Specifically, we expected to observe notable differences in the progression of physiological and immune responses. However, no significant condition × age or condition x age x time interactions were observed for physiological, behavioural or most immune markers. However, we did observe a significant time × age interaction for IL-6 and a trend towards a condition x age interaction on lymphocyte count, suggesting that any age-related differences in response to IFN-β are likely of small to medium effect size. Further studies incorporating larger samples might enhance the power to detect

more of these more subtle age-related effects.

Immune senescence and inflammageing are often used to explain why older individuals often exhibit less efficient immune responses (Franceschi et al., 2018; Pawelec, 2012) Although older age is linked to higher inflammation markers (Cohen et al., 2003; Walston et al., 2002), the exact impact of age on inflammation-induced changes remains unclear. Evidence from sepsis studies indicates a delayed resolution of inflammation in older adults (Bruunsgaard et al., 1999; Kale et al., 2010). In this study, some older participants anecdotally described more intense symptoms post-session. Although no formal quantitative analysis was conducted, these subjective reports underscore the importance of considering age-related differences in symptom experience. However, practical challenges such as participant burden and logistical constraints limited the feasibility of extended follow-up periods. Further research will be needed to elucidate the precise mechanisms underlying age-related differences in inflammation and immune responses. Here, longitudinal studies with more extensive follow-up periods could offer deeper insights into the temporal dynamics of host anti-viral responses in older adults and explore how these relate to more prolonged subjective responses.

Differences in IFN-β absorption and metabolism between age groups may also have accounted for the observed results. At 4 hr post-challenge, young participants had ~ 30 % higher serum IFN-β concentrations compared to older individuals, coupled with a faster elimination rate. This indicates that older participants may absorb IFN-β more slowly but consequently retain it for longer, potentially affecting cytokine responses and the temporal evolution of host inflammatory responses. Such differential metabolism may influence age-related variations in inflammatory responses. Overall, while the study did not find significant age-related differences in IFN-β responses, the observed trends and potential metabolic differences warrant further investigation to fully understand how age affects inflammatory responses.

In conclusion, by characterising the physiological, behavioural and immunological responses to IFN-β across young and older participants, this study demonstrates that IFN-β provides a promising, minimally invasive experimental model of inflammation that can be used across the age range. Our findings suggest that IFN-β elicits a mild but robust response (more effective than the typhoid model but not as potent yet simpler to use than endotoxin challenges), supporting its suitability for wider experimental applications, including in more vulnerable populations. While the present results indicate that IFN-β is reasonably safe and well tolerated, we recognise that our sample size was modest and that larger, independent studies will be important to confirm safety and reliability more definitively. Future work should also explore dose–response relationships and replicate findings in different populations to strengthen the evidence base for this model.

5. Data access

Data will be made available on request.

CRediT authorship contribution statement

Eva Periche-Tomas: Writing – original draft, Methodology, Investigation, Conceptualization. **Jonathan Underwood:** Investigation. **Claire MacIver:** Investigation. **Helena Leach:** Investigation. **Claudia Bone:** Investigation. **Carol A. Guy:** Investigation. **Kathy Triantafilou:** Methodology, Conceptualization. **Barbara Szomolay:** Methodology, Conceptualization. **Simon Jones:** Methodology, Conceptualization. **Neil A. Harrison:** Writing – review & editing, Methodology, Investigation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbi.2025.106173.

Data availability

Data will be made available on request.

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