Human interferon-ε and interferon-κ exhibit low potency and low affinity for cell-surface IFNAR and the poxvirus antagonist B18R

Received for publication, May 10, 2018, and in revised form, August 16, 2018. Published, Papers in Press, August 31, 2018, DOI 10.1074/jbc.RA118.003617

Bethany D. Harris*, Jessica Schreiter*, Marc Chevrier‡, Jarrat L. Jordan§, and Mark R. Walter†

*From the Department of Microbiology, University of Alabama at Birmingham, Birmingham, Alabama 35243 and ‡Janssen Research & Development, LLC, Raritan, New Jersey 08869

Edited by Luke O’Neill

IFNε and IFNκ are interferons that induce microbial immunity at mucosal surfaces and in the skin. They are members of the type-I interferon (IFN) family, which consists of 16 different IFNs, that all signal through the common IFNAR1/IFNAR2 receptor complex. Although IFNε and IFNκ have unique expression and functional properties, their biophysical properties have not been extensively studied. In this report, we describe the expression, purification, and characterization of recombinant human IFNε and IFNκ. In cellular assays, IFNε and IFNκ exhibit ~1000-fold lower potency than IFNα2 and IFNα0. The reduced potency of IFNε and IFNκ are consistent with their weak affinity for the IFNAR2 receptor chain. Despite reduced IFNAR2-binding affinities, IFNε and IFNκ exhibit affinities for the IFNAR1 chain that are similar to other IFN subtypes. As observed for cellular IFNAR2 receptor, the poxvirus antagonist, B18R, also exhibits reduced affinity for IFNε and IFNκ, relative to the other IFNs. Taken together, our data suggest IFNε and IFNκ are specialized IFNs that have evolved to weakly bind to the IFNAR2 chain, which allows innate protection of the mucosa and skin and limits neutralization of IFNε and IFNκ biological activities by viral IFN antagonists.

IFNε and IFNκ are part of the human type-I interferon (IFN) family that consists of 16 different cytokines whose signaling properties are critical for the control and elimination of microbial pathogens (1–5). IFNs activate innate immunity through the induction of IFN-stimulated genes (ISGs) that exhibit antiviral activity (2, 6, 7). IFNs also promote adaptive immunity through up-regulation of major histocompatibility complex and the induction of chemokines (8–11). They also control cell growth and apoptosis (12, 13), B-cell lineage commitment (14), and induction of T regulatory cells (15, 16). Due to the importance of IFNs, medical applications of IFNs have been developed including the treatment of viral infections (17), cancer (18), and multiple sclerosis (19).

The biological activities of all 16 IFNs are initiated upon binding to the cell-surface receptors IFNAR1 and IFNAR2. IFN-IFNAR interactions activate JAK1 and TYK2 kinases and the transcription factors STAT1 and STAT2 (20–23). JAK/STAT signaling, and additional kinases and transcription factors (24), ultimately induce IFN gene expression programs that protect the host from virus, bacteria, and even fungi (5, 7, 25, 26). Due to the critical role IFNs play in protecting the host from infection, many pathogens produce proteins that block IFN activity at multiple steps in the IFN signaling pathway. For example, Dengue, West Nile, and Zika viruses disrupt IFN-mediated STAT2 signaling (27, 28). In contrast, poxviruses encode IFN-binding proteins that neutralize IFN activity by binding to secreted IFNs, which prevents them from engaging cell-surface IFNAR1 and IFNAR2 (29). The IFN-binding proteins, B18R and B19R, were identified in vaccinia virus strains Western Reserve and Copenhagen, respectively (30, 31). B18R/B19R encode the same secreted ~65-kDa glycoprotein that promiscuously binds to all of the type-I IFNs (32). Deletion of B18R from vaccinia virus resulted in an attenuated virus, emphasizing the importance of the type-I IFNs in controlling vaccinia virus infection (30, 31).

IFNε and IFNκ are unique from the other IFNs based on their amino acid sequences and limited expression in mucosa and skin. IFNε and IFNκ share 35% sequence identity with one another and the 14 other IFNs. The additional 14 IFNs consist of 12 IFNα subtypes, IFNα0, and IFNβ. The IFNα0 share 77–95% sequence identity with one another. IFNβ exhibits 35% sequence identity with all other IFNs, whereas IFNα0 shares the highest sequence identity (60%) with both IFNε/κ/β and the IFNα0. Based on sequence and structural comparisons, all IFNs exhibit an α-helical fold consisting of five helices, which are labeled from the N terminus as helix A, B, C, D, and E (33–35). A unique feature of IFNε is that it encodes an 18-amino acid C-terminal tail following helix F, whereas IFNκ has a 13-amino acid peptide insertion between helices D and E.

In addition to these novel sequence features, IFNε and IFNκ are expressed predominantly in the female reproductive track (FRT) and keratinocytes, respectively (26, 36). In fact, analysis of the IFNε gene identified putative progesterone-binding sites in the promoter, suggesting hormones regulate IFNε expression (37). Subsequent studies confirmed IFNε expression is...
Human IFNε and IFNκ exhibit low affinity for IFNARs and B18R

Induced by hormones but not by viral infection (e.g. TLRs) like other IFNs (26). Consistent with IFNε’s expression in the reproductive tract, IFNε is able to induce important restriction factors that prevent HIV-1 infection (38, 39). In fact, HIV-1 negative female sex workers express high levels of IFNε in their cervical tissues (40). IFNκ is constitutively expressed in keratinocytes, which are found in skin and in the mucosa of the FRT. Thus, IFNε and IFNκ are both found in the FRT, but IFNκ is inducible by virus and dsRNA, whereas IFNε is not (36). Although it appears that IFNκ appears to play an important role in immunity against HIV-1, IFNε expression is rapidly reduced in keratinocytes that are infected with human papilloma virus strains that induce cervical cancer (41). These data strongly argue that IFNε and IFNκ are essential components to the host response against pathogens in the FRT, whereas the specific role of IFNκ, produced by keratinocytes in the skin, remains to be determined.

Despite a critical role of IFNε and IFNκ signaling in mucosa and skin, their interactions with the IFNARs and their functional activities have not been extensively characterized. To address this issue, we have expressed human IFNε and IFNκ for comparative biophysical and functional studies with other IFN family members (often called IFN subtypes). Studies with purified IFNε and IFNκ reveal they induce ISGF3-mediated gene expression that is ~1000-fold weaker than IFNα2 or IFNω. The weaker potency of IFNε and IFNκ is consistent with their reduced affinities for the IFNAR2 receptor chain. However, IFNε and IFNκ exhibit IFNAR1 binding affinity that is similar to IFNα2 and IFNω.

Because poxviruses cause disseminated infections of the skin and mucosa and can block type-I IFN signaling, we evaluated the ability of B18R to neutralize IFNε and IFNκ cellular activity. Subsequent kinetic binding studies determined IFNε, IFNκ, as well as IFNα1, exhibit reduced binding to B18R, relative to the other IFNs. Sequence and structural models of IFNε/κ-IFNAR2 and IFNε/κ-B18R complexes identified residues responsible for the disrupted IFNAR2 and B18R binding phenotypes. Our data suggests IFNε and IFNκ have evolved to exhibit reduced IFNAR2 binding affinity and biological potency optimized for tissue-specific expression and escape from viral type-I IFN antagonists.

Results

Expression and characterization of IFNε and IFNκ proteins

Expression plasmids encoding human IFNε and IFNκ protein sequences, which also encode C-terminal histidine tags, were synthesized using optimized codons. Expression studies were performed in Escherichia coli where IFNε and IFNκ formed insoluble inclusion bodies. The guanidine-solubilized IFNs were refolded by rapid dilution into a refolding buffer. The refolded IFNs were purified using a 2-step strategy consisting of nickel affinity and cation exchange chromatography. Endotoxin levels for IFNε and IFNκ were less than 1 EU/μg, which is within the range of values observed in IFNω preparations obtained from commercial sources (42). SDS-PAGE gel analysis of the final purified IFNε and IFNκ protein preparations is shown in Fig. 1. The molecular weight of IFNε on SDS-PAGE gels matched its theoretical molecular weight of 23,249. However, IFNκ ran larger than expected (~25,000 kDa) in SDS-PAGE gels, suggesting the peptide could be a frameshift product (43) or exhibit aberrant gel migration. To resolve these possibilities, MS was performed on the samples, which demonstrated the molecular masses were consistent with full-length IFNε and IFNκ proteins without the N-terminal initiating methionine residues (Table 1).

The IFNε and IFNκ amino acid sequences include three and five cysteine residues, respectively. Based on prior structural and biophysical analysis of other IFNs (42, 44), IFNε and IFNκ are predicted to contain one free cysteine and form one and two disulfide bonds, respectively, in their folded forms. To confirm disulfide bond formation had occurred during the refolding process, IFNε and IFNκ were treated with iodoacetamide (IA) in the presence, or absence, of the reducing agent dithiothreitol (DTT, Table 1). IA selectively binds to free cysteines, resulting in an increase in mass of 57 Da. Upon IA treatment in the absence DTT, the mass of IFNε and IFNκ increased by 57 Da, which is consistent with one free cysteine in the folded proteins (Table 1). However, in the presence of DTT, the mass of IFNε and IFNκ increased by three IA and five IA mass units, respectively. This is consistent with the protection of two (IFNε) and four (IFNκ) cysteines, due to disulfide bond formation, in the folded proteins (Table 1). Thus, the MS data are consistent with the predicted IFNε and IFNκ disulfide bonding patterns.

IFNε and IFNκ have disrupted IFNAR2-binding properties

Figure 1. Final SDS-PAGE gel of purified IFNε, IFNκ, and two control IFNs, IFNα2a and IFNω.

Three preparations of IFNε and IFNκ were characterized for their ability to bind to soluble IFNARs (Fig. 2). The IFNs were injected over Biochip chips coupled with IFNAR1-FC, IFNAR2-FC, or an IFNAR1/IFNAR2-FC heterodimer, as previously described (45). Binding is reported as receptor occupancy (RO) for each IFN. For comparison with other IFNs, binding studies were also performed with IFN subtypes, IFNα2 and IFNω.

IFNα2 and IFNω bound to IFNAR2 with RO values of 53 and 69%, respectively (Fig. 2). In contrast, IFNε and IFNκ bound very poorly to IFNAR2, exhibiting IFNAR2 occupancies of 3 and 5%, respectively. Despite poor IFNAR2-binding properties, IFNε bound to IFNAR1 (RO = 26%) better than IFNα2 (RO = 19%), whereas IFNκ-IFNAR1 RO values were lower than IFNα2.
Human IFNε and IFNκ exhibit low affinity for IFNARs and B18R

Table 1

| Treatment | Observed mass | Expected mass | Delta | Interpretation |
|-----------|---------------|---------------|-------|----------------|
| IFNε      | 23,082        | 23,118        | 36    | +1 IA          |
| IA        | 23,145        | 23,139        | 6     | +1 IA          |
| IA + DTT  | 23,256        | 23,253        | 3     | +3 IA          |

IFNκ

| Treatment | Observed mass | Expected mass | Delta | Interpretation |
|-----------|---------------|---------------|-------|----------------|
| None      | 23,201        | 23,220        | 19    |                |
| IA        | 23,259        | 23,258        | 1     | +1 IA          |
| IA + DTT  | 23,496        | 23,486        | 10    | +5 IA          |

*Calculated mass without N-terminal methionine.

**Distinct neutralization of IFNε and IFNκ biological activity**

To further understand how IFNε and IFNκ engage cell-surface IFNARs, IFNε- and IFNκ-induced gene expression was monitored in reporter cells treated with a series of reagents that block IFN biological activity (Fig. 3). For comparative purposes, IFNα2a and IFNω were also included in the analysis. To validate that reporter activity was due to IFNε and IFNκ receptor binding, the pan-anti-IFNε neutralizing antibody (IFNα NAb) was added to the assay. Consistent with its specificity profile, the IFNα NAb efficiently blocked IFNα2 reporter activity, but not the activity of IFNω, IFNε, or IFNκ. To neutralize the activity of all four IFNs, the IFNs were incubated with the poxvirus antagonist B18R, which has been reported to block the activity of all IFN subtypes (31, 32). As expected, B18R efficiently neutralized IFNα2 and IFNω reporter activity. However, IFNε and IFNκ activity was only blocked at the higher concentration of B18R tested. Thus, IFNε and IFNκ are less sensitive to neutralization by B18R than IFNα2 and IFNω.

IFNε- and IFNκ-mediated bioactivity was also studied in the presence of NAbs against cell-surface IFNAR1 and IFNAR2. The IFNAR1 NAb was equally effective in blocking IFNα2, IFNε, and IFNκ activity, but was much less effective in blocking IFNω activity. The anti-IFNAR2 NAb exhibited a different blocking profile than the IFNAR1 NAb. The IFNAR2 NAb most efficiently blocked IFNα2 activity at both high and low concentrations tested. Neutralization of IFNε and IFNω activity was intermediate, relative to IFNα2 neutralization, whereas IFNκ activity was largely insensitive to IFNAR2 NAb inhibition. These data suggest neutralization by the IFNAR2 NAb is sensitive to differences between the IFN subtypes that go beyond receptor affinity. Soluble IFNAR1-FC and IFNAR2-FC proteins were also used to block IFNε and IFNκ activity. As expected based on its low affinity, IFNAR1-FC was unable to block the activity of any of the IFNs. In contrast, soluble IFNAR2-FC blocked IFNα2 and IFNω activity, but was unable to block IFNε or IFNκ activity. Thus, IFNAR1-FC and IFNAR2-FC-mediated neutralization was consistent with IFN-IFNAR receptor affinities.

**Surface plasmon resonance of IFNε and IFNκ receptor binding**

IFNε/κ-IFNAR interactions were studied using surface plasmon resonance (SPR) (Fig. 4, Table 2). SPR analysis demonstrated IFNε (KD = 2.2 μM) and IFNα2a (KD = 2.3 μM), as well as IFNκ (KD = 0.3 μM) and IFNω (KD = 0.5 μM), share similar IFNAR1 affinities. Despite similar IFNAR1 affinities, IFNε’s affinity for IFNAR2 (KD = 70 nM) is 14-fold lower than IFNα2. Analysis of the rate constants for the interactions reveals the IFNε/IFNAR2 association rate constant (k+a) is 10-fold lower than for IFNα2/IFNAR2. Despite a slightly higher affinity (IFNε/IFNAR2 KD = 21 nM) IFNκ also exhibits a very slow k+a value (1 × 107 M−1 s−1) relative to IFNα2a (5.9 × 108 M−1 s−1) or IFNω (7 × 107 M−1 s−1). Thus, IFNε and IFNκ exhibit poor IFNAR2 affinities due to reduced association rate constants.

IFNε and IFNκ binding to the IFNAR1/IFNAR2-FC was also determined (Table 2). The IFNAR1/IFNAR2-FC provides a biochemical mimic of the cell-surface IFNAR1/IFNAR2 heterodimer and the resulting affinities are due to IFN binding to both IFNAR1 and IFNAR2 (45). As a control, IFNα2 bound to the IFNAR1/IFNAR2-FC with a KD of 54 pm. Notably, this affinity constant is on the same order of magnitude of IFNα2’s EC50 (11 pm) in the ISGF3 reporter assays (Fig. 2B, Table 2). Similarly, IFNω exhibits a KD of 25 pm for IFNAR1/IFNAR2-FC and an EC50 value of 6 pm in the reporter assay. In contrast to the picomolar affinities observed for IFNα2 and IFNω, IFNε and IFNκ exhibit nanomolar KD values for the IFNAR1/IFNAR2-FC (IFNε KD = 3.5 × 10−9 and IFNκ KD = 2 × 10−9). These affinity values are consistent with the reduced activity of IFNε and IFNκ in the reporter assays. The results obtained with the IFNAR1/IFNAR2-FC also show a 24–66-fold reduction in the k+a values of the interactions. Thus, IFNε and IFNκ bind with reduced affinity to IFNAR2, relative to other IFN subtypes, such as IFNα2 and IFNω.

**Distinct binding of IFNε and IFNκ to the poxvirus antagonist B18R**

IFNε and IFNκ exhibit unique B18R neutralization profiles, relative to IFNα2 and IFNω (Fig. 3). To study this further, IFNε/ B18R and IFNκ/B18R interactions were compared with the other 14 IFNs using SPR (Fig. 5). The 16 IFNs were injected at two concentrations (high, 50 nM; low, 5 nM), over a Biacore chip coupled with B18R from poxvirus strain Copenhagen (Fig. 5).
Human IFNε and IFNκ exhibit low affinity for IFNARs and B18R

Consistent with the reduced ability of B18R to neutralize IFNε and IFNκ activity in the reporter assay (Fig. 3), B18R bound 80 and 64% of IFNε and IFNκ, respectively, when injected at the high concentration. However, the percentage dropped to 14% for IFNε and 7% for IFNκ when the IFNs were injected at the low concentration (Fig. 5B). Interestingly, the B18R binding profile of IFNε1 mimicked IFNε and IFNκ yielding 52% binding at high concentration and 11% at the low concentration.

Based on the results of the binding screen (Fig. 5, A and B), four IFNs that span the strong B18R binder group (IFNε14 and IFNκ4), and the weak binders (IFNε1 and IFNκ), were subjected to further kinetic analysis (Fig. 5). Elucidating their binding parameters revealed the strong and weak binders were distinguished by their association rate constants ($k_a$, Table 2). Specifically, the poor binders had small $k_a$ values ($k_a = 1.5 \times 10^5$ to $5.5 \times 10^5$ M$^{-1}$ s$^{-1}$), whereas the $k_a$ values of the strong binders were 10- to 73-fold faster ($k_a = 5.8 \times 10^6$ to $1 \times 10^7$ M$^{-1}$ s$^{-1}$). Importantly, all four IFNs tested exhibited very slow ($t_{1/2} \sim 10$ h) dissociation rates that differ from one another by no more than 2-fold. Thus, as observed for IFNAR2 interactions, the B18R neutralization profiles of IFNε and IFNκ correlate with reduced association rates, which reduce the ability of B18R to bind and neutralize their biological activity (Fig. 3). These observations suggest IFNε, IFNκ, and even IFNε1, may have a selective advantage during poxvirus infections by partially escaping B18R-mediated IFN neutralization.

A molecular model to explain IFNε/κ binding to IFNAR2 and B18R

SPR analysis revealed IFNε, IFNκ, as well as IFNε1, have reduced association rate constants for B18R and IFNAR2, relative to the other type-1 IFNs. To identify regions of IFNε, IFNκ,
and IFNα1 that could explain their unique binding properties, we aligned the amino acid sequences of all of the IFNs. This analysis revealed IFNα2 is the only IFN that has replaced the conserved IFNAR2-binding residue, Arg-56, with an asparagine (Fig. 6E). Structural and biophysical studies confirm Arg-56 makes extensive interactions with IFNAR2 and an R56A mutant drastically disrupts the IFN-IFNAR2 interaction (46, 47). These data suggest the change of Arg-56 to an asparagine in IFNα2 could be sufficient to explain its reduced affinity for IFNAR2.

To define the impact of the IFNα2 Arg to Asn change on B18R binding, we generated a three-dimensional model of the human IFNα2/B18R complex based on the structure of the murine ectromelia virus type-I IFN antagonist, C12R (PDB 3OQ3). The B18R model is highly reliable because C12R shares 89% sequence identity with B18R (Fig. 6). Our analysis focused on the C-terminal domain of B18R (D3 domain 91% sequence identity with C12R), which forms contacts with the IFNAR2-binding site of the IFNs (Fig. 6B). Consistent with our hypothesis, Arg-56 in the IFNα2/B18R complex forms a salt bridge with glutamate 297 (C12R numbering) that would be significantly disrupted by replacing it with an asparagine as found in IFNα. Because electrostatic interactions drive association rate constants, we looked for additional salt bridge interactions that are conserved in the high affinity IFNα2/IFNAR2 and IFNα2/B18R complexes. This analysis identified two structurally conserved salt bridge interactions, formed with IFNα2 residues Arg-35 and Arg-36 (to IFNAR2 Glu-190 and B18R Asp-260) and Arg-172 (to IFNAR2 Glu-77 and B18R Asp-276). Arg-172, located in helix F, is conserved in all 16 IFNs. In contrast, Arg-35 and Arg-36 are not conserved in either IFNε (Gln-35/Glu-36) or IFNκ (Trp-35/Gln-36), suggesting these residues contribute to the reduced IFNAR2 and B18R association rates observed for IFNα2 and IFNα.

This analysis suggests that IFNε and IFNκ have reduced affinities for IFNAR2 and B18R because they have replaced Arg-35, Arg-36, and Arg-56 (IFNκ) with alternative residues that are suboptimal for IFNAR2 and B18R binding. Although this provides a possible explanation for disruption of IFNε/κ binding, all three arginine residues are conserved in the sequence of IFNα1. Thus, other residues must be responsible for the reduced affinity of IFNα1 for IFNAR2 and B18R. Further sequence analysis identified three residues that are unique to IFNα1, relative to the high affinity B18R binders (Fig. 6). The three residues in the high affinity IFNs (Arg-45, Phe-50, and Lys-54) are replaced with Ser-45, Ser-50, and Met-54 in IFNα1. Interestingly, these residues are all located adjacent to Arg-56 in the AB loop of IFNα1. This suggests IFNα1 alters IFNAR2

Figure 3. Neutralization of IFN biological activity, by antibodies, soluble IFNARs, and the viral antagonist B18R. IFNs and antagonists were incubated with reporter cells for 18 h, followed by measurement of ISG54 reporter activity. All measurements are normalized relative to the IFN control, which is the average of 4 measurements corresponding to IFN alone and IFN + three isotype antibody controls.
affinity by indirectly changing the conformation of Arg-56, and/or stability of the entire AB loop, rather than replacing Arg-56 with an Asn, as observed for IFN\(\alpha\)/H9260. Notably, the same three residues are also different in the sequences of IFN\(\alpha\)/H9280 and IFN\(\alpha\)/H9260, relative to the high affinity IFNs (Fig. 6). This suggests IFN\(\alpha\)1, IFN\(\alpha\)2, and IFN\(\kappa\) modulate IFNAR2 and B18R affinity through salt bridge/hydrogen bond interactions that particularly manipulate Arg-56-mediated interactions.

**Analysis of IFN\(\alpha\)1 and IFN\(\kappa\) mutants**

To test the structural model, mutants of IFN\(\alpha\)1 and IFN\(\kappa\) were made that replaced putative “poor binding” residues with residues found in high affinity IFNs (Fig. 6). First, an IFN\(\alpha\)1 double mutant (S45R/V50F) was evaluated (IFN\(\alpha\)1-M1). Consistent with the modeling study, IFN\(\alpha\)1-M1 exhibited 10-fold higher activity (26 ± 10 pM) in the ISGF3 reporter assay than IFN\(\alpha\)1 (Fig. 7A, Table 2). IFN\(\alpha\)1-M1 also exhibited 14-fold higher affinity for IFNAR2, 2-fold higher affinity for IFNAR1, and 3-fold higher affinity for the IFNAR1/2 heterodimer, relative to IFN\(\alpha\)1 (Table 2). IFN\(\alpha\)1-M1 also exhibited 4-fold higher affinity for B18R than IFN\(\alpha\)1, which was almost entirely due to a faster association rate constant (\(k_a\)) for the IFN\(\alpha\)1-M1/B18R interaction (Fig. 7C, Table 2).

The activity profiles of three IFN\(\kappa\) mutants (IFN\(\kappa\)-M1, -M2, and -M3, Fig. 6) confirmed the importance of the N56R substitution for increasing IFN\(\kappa\) biological potency (Fig. 7B, Table 2). However, the mutants also demonstrated that the conformation/biophysical properties of the IFN\(\kappa\) N-terminal region (e.g. helix A, AB loop, and helix B) are distinct from other IFNs. For example, making the S45R/V50F double mutant in IFN\(\kappa\) (IFN\(\kappa\)-M1), the same mutations that significantly increased the bioactivity and receptor binding affinity of IFN\(\alpha\)1, resulted in decreased bioactivity (Fig. 7B). In contrast, the IFN\(\kappa\)-M2 triple mutant (S45R/V50F/N56R) increased ISGF3 bioactivity (6-fold) and IFNAR1/2 binding affinity (5-fold), relative to IFN\(\kappa\). This demonstrates IFN\(\kappa\) is not optimized to induce maximal bioactivity, or to bind with the highest affinity possible to the IFNAR receptors. Although accurate rate constants could

![Figure 4. SPR analysis of IFN\(\alpha\), IFN\(\kappa\), IFN\(\alpha\)2, and IFN\(\omega\) binding to IFNAR1-FC, IFNAR2-FC, and the IFNAR1/IFNAR2-FC heterodimer. SPR sensorgrams for each IFN-IFNAR interaction are shown in black. The calculated sensorgrams, derived from fitting the data to a 1:1 binding model, are shown in black (IFN\(\alpha\)2), red (IFN\(\kappa\)), blue (IFN\(\alpha\)), and green (IFN\(\omega\)). Kinetic and equilibrium constants derived from the data are shown in Table 2.](https://example.com/thumbnail.png)
not be derived from IFNω/B18R or IFNω-M2/B18R sensors, B18R bound more IFNω-M2 (53%) than IFNω (7%) when equal concentrations (5 nM) of the IFNs were injected over B18R surfaces (Fig. 7D).

We also tested if additional amino acid changes (W35R and Q36R) in IFNω-M2 (e.g. IFNω-M3) would further increase IFNω bioactivity. However, IFNω-M3 exhibited the poorest bioactivity of the three IFNω mutants tested (Fig. 7B), suggesting these mutations may significantly disrupt the structure of IFNω’s IFNAR-binding sites.

Discussion

Genes encoding IFNɛ and IFNκ protein sequences were identified over a decade ago (36, 37), yet only recently has their importance in host defense, especially in the FRT, come to light (26, 38, 41). Although animal studies are progressing, studies that directly characterize the purified human proteins are lacking. At least one reason for the paucity of information about IFNɛ and IFNκ is that they cannot be expressed using protocols suitable for other IFNs (42, 47).

A major question we sought to address in this report is how IFNɛ and IFNκ bind to the IFNAR1 and IFNAR2 receptors, compared with previously characterized IFNs. These analyses demonstrated both IFNɛ and IFNκ have greatly reduced affinity for the IFNAR2 chain, yet retain IFNAR1 affinities similar to other IFNs. In addition to evaluating binding to the single IFNAR chains, we also characterized IFNɛ/κ binding to an IFNAR1/IFNAR2-FC, which provides a soluble mimic of the cell-surface receptor heterodimer. All three analyses support the conclusion that the reduced potency of IFNɛ and IFNκ is due to reduced receptor binding, predominantly IFNAR2. We followed up these studies by blocking IFNɛ/IFNκ functional
activity with a variety of antagonists against the cell-surface receptors, soluble receptors, or using the poxvirus antagonist B18R. The main finding of this analysis was IFNε and IFNκ exhibited a different neutralization profile against B18R, compared with IFNα2 or IFNω. To explore this in greater detail, the interaction of all 16 IFNs with B18R was evaluated by SPR. As suggested in the cell-based neutralization studies, B18R bound weakly to IFNε and IFNκ. Surprisingly, IFNα1 also exhibited a B18R binding profile that matched IFNε and IFNκ. The common biophysical property between IFNε, IFNκ, and IFNα1 is that they all interact with the IFNAR2 chain with much lower affinity than IFNα2, IFNω (this study), or other IFNs (49–51). Rate constant analysis revealed the three low affinity IFNs, IFNε, IFNκ, and IFNα1, all share reduced association rates for IFNAR2, but different dissociation rates. In particular, IFNα1 dissociates very fast ($k_d = 0.18 \text{ s}^{-1}$) from IFNAR2, whereas IFNε dissociates from IFNAR2 similar to IFNα2 and IFNω ($k_d = 0.01 \text{ s}^{-1}$), and IFNκ dissociates very slowly ($k_d = 0.002 \text{ s}^{-1}$) from IFNAR2, as observed for IFNβ (51).

The amino acid sequences of IFNε, IFNκ, and IFNα1 share ~30% identity with one another. Thus, global analysis of the sequences do not explain how these three IFNs bind weakly to IFNAR2 and B18R, relative to other IFNs. However, analysis of the sequences that form the IFNAR2-binding site, which were identified from the IFN/IFNAR1/IFNAR2 crystal structure (46), and a structural model of B18R derived from the ectromelia virus C12R structure (PDB 3OQ3), revealed a series of conserved contacts in both interfaces. Overall, the modeling and mutagenesis data suggest the three low affinity IFNs have evolved a strategy to modulate IFNAR2 and B18R binding that is beneficial in protecting individuals from pathogen challenge. These data support a unified molecular mechanism of manipulating the AB loop to reduce the potency of IFNε, IFNκ, and IFNα1, relative to the other IFNs. These molecular changes may also allow IFNε, IFNκ, and IFNα1 to partially escape neutralization by poxvirus. In the case of IFNκ, it is interesting that cancer-inducing strains of human papilloma virus have found an alternative strategy, inhibition of IFNκ expression, to evade IFNκ antiviral activity (41).

The biochemical properties of murine IFNε (murIFNε) were recently characterized (52). Interestingly, murine and human IFNκ share 59% sequence identity, which is higher than human IFNε shares with any other human type-I IFN. The similarity of the murine and human IFNκ sequences, at least partially, explains why murIFNκ is active on human cells (52). As reported in our study for human IFNκ, murIFNκ also exhibits higher affinity for the murine IFNAR1 chain than for the murine IFNAR2 chain. However, in the context of the entire murine type-I IFN family, other murine IFN–IFNAR interactions appear to be very different from what is observed for the human IFNs. For example, the $K_D$ for murIFNα1/IFNAR2 interaction is 2.2 nM (52), whereas our analysis of the human IFNα1-IFNAR2 interaction results in a $K_D$ of 353 nm, which is similar to the value reported by Jaks et al. (49). Interestingly, the high affinity of murine IFNα1 for IFNAR2 is consistent with murine IFNα1 exhibiting the same residue combinations identified for the high affinity (strong binding) human IFNs (Fig. 6E). Thus, there does not appear to be a direct correspondence in binding or function between human IFNα1 and murine IFNα1. However, both humans and mice have evolved IFNs that exhibit very different affinities for the IFNAR1 and IFNAR2 chains.

There is some evidence to suggest weak IFN–IFNAR2 interactions, as observed for IFNε, IFNκ, and IFNα1, may result in distinct signaling properties of the IFNs, beyond simply a weaker response. In an extreme case, cells from IFNAR2 knockout mice, when treated with IFNB, could still induce inflammatory signals through the IFNAR1 chain (53). However, murine IFNβ exhibits very high affinity for the IFNAR1 chain (49), sug-
gesting a possible mechanism for IFNβ-mediated activation of IFNAR1 that is not conserved by IFNα, IFNγ, or IFNλ. Thus, if these IFNs induce IFNAR1-specific signals, it must occur by a distinct mechanism relative to a previously described mechanism for murine IFNβ. Although differences in signaling outputs by IFNα and IFNγ require further studies, it is clear that human IFNα can inhibit HIV-1 at several steps in its replication cycle (38, 39). Furthermore, studies in mice demonstrate IFNγ controls Chlamydia and herpes simplex virus 2 (26). These data suggest that, just as IFNα2 was formulated as an anti-hepatitis C therapy, the unique biochemical properties of IFNα and IFNγ may be useful to protect women from a variety of pathogens that colonize surfaces of the reproductive tract (5).

**Experimental procedures**

**Protein expression and refolding**

DNA sequences encoding the mature IFNα and IFNγ protein sequences were synthesized with optimized codons for expression in *E. coli* (ATUM). The codon-optimized cDNAs were subcloned into the PET21b plasmid. All mutagenesis was performed using the QuikChange site-directed mutagenesis kit (Stratagene). The plasmids were transformed into *E. coli* BL21-CodonPlus (DE3)-RIPL cells (Agilent) for expression. Cultures were grown at 37 °C for 3.5 to A600 values of 0.6–0.8, before induction with 1 mM isopropyl 1-thio-D-galactopyranoside. After induction, cultures were grown for an additional 3 h at 37 °C before they were harvested by centrifugation. Protein expression of IFNα1 was as previously described (42).

Expression of IFNα and IFNγ resulted in the formation of insoluble inclusion bodies. The inclusion bodies were solubilized in 6 M guanidine HCl and full-length denatured IFNα and IFNγ were purified by nickel affinity chromatography (Takara). The denatured proteins were subsequently refolded by a rapid 1:10 dilution into a refolding buffer consisting of 0.1 M Tris-HCl, pH 8, 50 mM NaCl, 2.5 mM EDTA, 0.2 mM oxidized GSH, 2 mM reduced GSH, and 0.8 M arginine. The refolding mixture was incubated for 18 h at 10 °C and then dialyzed into 20 mM Tris, pH 7.5, and 20 mM NaCl. Refolded IFNα and IFNγ were subsequently purified using SP cation exchange chromatography (GE Healthcare), which resulted in highly purified IFNα and IFNγ preparations. Residual endotoxin was removed from the samples using the High Capacity Endotoxin Removal Spin Columns (Pierce). Endotoxin levels were determined using a Limulus Amoebocyte Lysate Endotoxin Quantitation Kit (Pierce).

**Mass spectrometry**

Mass analyses were performed using MALDI-TOF MS. Briefly, samples were analyzed in the positive mode on a Voyager Elite mass spectrometer with delayed extraction technology (PerSeptive Biosystems, Framingham MA). The acceleration voltage was set at 25 kV and 100 laser shots were summed. Experiments were performed using sinapinic acid (Sigma) at 5 mg/ml dissolved in acetonitrile, 0.1% TFA (1:1) as the matrix. The mass spectrometer was calibrated using apomyoglobin (Sigma). Samples were diluted 1:10 with matrix, and 1 µl was
plates were moved to room temperature for 10 min, followed by incubation for 5 h at 37 °C. Following the 5-h incubation, the plates were moved to room temperature for 10 min, followed by the addition of 50 μl of luciferase assay reagent (Steady-Glo, Promega) to each well. Luminescence was measured on a Biotek Synergy 2 plate reader and the dose-response curves were analyzed using PRISM with a three-parameter fit (GraphPad Inc.).

**ISG54 reporter assay**

HEK-Blue IFN-α/β cells (Invivogen) were used to characterize IFN-mediated stimulation of the ISG54 promoter. Activation of the ISG54 promoter results in dose-dependent secretion of embryonic alkaline phosphatase (SEAP). For the assay, plates were seeded with 50,000 cells/well and incubated overnight. The following day, the cells were stimulated with IFNs (IFNα2 and IFNγ from PBL Assay Science) in a total volume of 100 μl and incubated for 18 h. Following the 18-h incubation, SEAP levels are quantified by mixing 40 μl of cell supernatant with 160 μl of quanti-blue substrate (Invivogen), followed by an additional 5-min incubation. The plates were then read using a Spectramax plate reader at 650 nm.

**Neutralization assays**

Neutralization assays were performed using the ISG54 reporter assay with HEK-Blue IFNα/β cells. Reagents used in the assays included anti-IFNα (MMHA-2, PBL Assay Science), anti-IFNAR1 (ab10739, Abcam), and anti-IFNAR2 (MMHAR-2, PBL Assay Science) neutralizing antibodies, soluble receptors IFNAR1-FC (245-AB-050, R&D Systems) and IFNAR2-FC (4015-AB-050, R&D Systems), and viral type-I antagonist B18R (34-8185-85, eBioscience). The neutralization reagents were added to cells at low (3.5 nM) and high (67 nM) concentrations with each IFN. After 18 h, SEAP levels were determined. The data are reported as the mean ± S.D. of three experiments using Prism.

**Surface plasmon resonance**

SPR experiments were performed using a Biacore T200 (GE Healthcare) at 25 °C using a running buffer consisting of 10 mM HEPES, pH 7.4, 150 mM NaCl, 0.0125% P20, and 125 μg/ml of bovine serum albumin (BSA). Monomeric IFNAR1-FChk, IFNAR2-FChk, and the IFNAR1/IFNAR2-FChk heterodimer proteins (as described in Ref. 45) were captured onto CM-5 sensor chips using the anti-murine FC Ab. IFN subtypes, expressed as described in Ref. 42, were injected over the B18R-FC surface. IFN-B18R-FC interaction screening was performed at IFN concentrations of 50 and 5 nM using a flow rate of 40 μl/min. For kinetic analyses, IFNs were injected over the B18R-FC surface for 2 min and dissociation was monitored for 20 min. The resulting sensorgrams were globally fit to a 1:1 binding model using Biacore T-200 evaluation software version 1.0.

Author contributions—B. D. H. and M. C. formal analysis; B. D. H. and M. R. W. validation; B. D. H., J. S., and M. C. investigation; B. D. H. and M. R. W. methodology; B. D. H., J. S., M. C., J. L. J., and M. R. W. writing-review and editing; M. C., J. L. J., and M. R. W. conceptualization; M. C., J. L. J., and M. R. W. resources; M. C., J. L. J., and M. R. W. supervision; M. R. W. funding acquisition; M. R. W. writing-original draft; M. R. W. project administration.

Acknowledgments—We thank Ashlesha Deshpande for helpful discussions on protein purification. We thank Gilles Uzé for HL116 cells.

References

1. Samuel, C. E. (2001) Antiviral actions of interferons. Clin. Microbiol. Rev. 14, 778–809, table of contents CrossRef Medline
2. Ivashkiv, L. B., and Donlin, L. T. (2014) Regulation of type I interferon responses. Nat. Rev. Immunol. 14, 36–49 CrossRef Medline
3. Pestka, S., Langer, J. A., Zoon, K. C., and Samuel, C. E. (1987) Interferons and their actions. Annu. Rev. Biochem. 56, 727–777 CrossRef Medline
4. Pfeffer, L. M., Dinarello, C. A., Herberman, R. B., Williams, B. R., Borden, E. C., Borden, R., Walter, M. R., Nagabhushan, T. L., Trotta, P. P., and Pestka, S. (1998) Biological properties of recombinant α-interferons: 40th anniversary of the discovery of interferons. Cancer Res. 58, 2489–2499 CrossRef Medline
5. Borden, E. C., Sen, G. C., Uze, G., Silverman, R. H., Ransohoff, R. M., Foster, G. R., and Stark, G. R. (2007) Interferons at age 50: past, current and future impact on biomedicine. Nat. Rev. Drug Discov. 6, 975–990 CrossRef Medline
6. Der, S. D., Zhou, A., Williams, B. R., and Silverman, R. H. (1998) Identification of genes differentially regulated by interferon α, β, or γ using oligonucleotide arrays. Proc. Natl. Acad. Sci. U.S.A. 95, 15623–15628 CrossRef Medline
7. Schoggins, J. W., Wilson, S. J., Panis, M., Murphy, M. Y., Jones, C. T., Bieniasz, P., and Rice, C. M. (2011) A diverse range of gene products are effectors of the type I interferon antiviral response. Nature 472, 481–485 CrossRef Medline
8. Yang, C. H., Wei, L., Pfeffer, S. R., Du, Z., Murti, A., Valentine, W. I., Zheng, Y., and Pfeffer, L. M. (2007) Identification of CXCL11 as a STAT3-dependent gene induced by IFN. *J. Immunol.* **178**, 986–992

9. Gray, R. C., Kuchtey, J., and Harding, C. V. (2007) CpG-B ODNs potently induce low levels of IFN-αβ and induce IFN-αβ-dependent MHC-1 presentation in DCs as effectively as CpG-A and CpG-C ODNs. *J. Leukoc. Biol.* **81**, 1075–1085

10. Loh, J. E., Chang, C. H., Fodor, W. L., and Flavell, R. A. (1992) Dissection of the interferon γ-MHC class II signal transduction pathway reveals that type I and type II interferon systems share common signalling component(s). *EMBO J.* **11**, 1351–1363

11. Zhao, W., Cha, E. N., Lee, C., Park, C. Y., and Schindler, C. (2007) Stat2-dependent regulation of MHC class II expression. *J. Immunol.* **179**, 463–471

12. Chawla-Sarkar, M., Lindner, D. J., Liu, Y. F., Williams, B. R., Sen, G. C., Silverman, R. H., and Borden, E. C. (2003) Apoptosis and interferons: roles of interferon-stimulated genes as mediators of apoptosis. *Apoptosis* **8**, 237–249

13. Herzer, K., Hofmann, T. G., Teufel, A., Schimanski, C. C., Moehler, M., Kanzler, S., Schulze-Bergkamen, H., and Galle, P. P. (2009) IFN-α-induced apoptosis in hepatocellular carcinoma involves promyelocytic leukemia protein and TRAIL independently of p53. *Cancer Res.* **69**, 855–862

14. de Goër de Herve, M. G., Durali, D., Dembele, B., Giulian, M., Tran, T. A., Azzarone, B., Eial, P., Tardieu, M., Delfrassay, J. F., and Taoufik, Y. (2011) Interferon-α triggers B cell effector 1 (Be1) commitment. *PLoS ONE* **6**, e19366

15. Liu, Y., Carlson, R., Comabella, M., Wang, J., Kosicki, M., Carrion, B., Hasan, M., Wu, X., Montalban, X., Dziejmel, M. H., Sellebjerg, F., Sorensen, P. S., Helin, K., and Issazadeh-Navikas, S. (2014) FoxA1 directs the lineage commitment of novel genes of a novel regulatory T cell population in EAE and MS. *Nat. Med.* **20**, 272–282

16. Delgoffe, G. M., and Vignali, D. A. (2014) A Fox of a different color: FoxA1 mediates signalling. *Nat. Rev. Immunol.* **14**, 377–423

17. Foster, G. R. (2010) Pegylated interferons for the treatment of chronic hepatitis C: pharmacological and clinical differences between peginterferon-α-2a and peginterferon-α-2b. *Drugs* **70**, 147–165

18. Kirkwood, J. (2002) Cancer immunotherapy: the interferon-alpha experience. *Semin. Oncol.* **29**, 18–26

19. Jacobs, L. D., Cookfair, D. L., Rudick, R. A., Herndon, R. M., Richert, J. R., Jarmulis, S. W., Krier, C., Streb, M., and VerMilyea, M., Coutifaris, C., Kossenkov, A. V., Matthews, A. Y., de Weerd, N. A., Hertzog, P. J., and Mak, J. (2017) Interferon-α promotes HCV restriction at multiple steps of viral replication. *Immunol. Cell Biol.* **95**, 478–483

20. Tasker, C., Subbian, S., Gao, P., Couret, J., Levine, C., Ghanny, S., Sotero-poulos, P., Zhao, X., Landau, N., Lu, W., and Chang, T. L. (2016) IFN-ε protects primary macrophages against HIV infection. *JCI insight* **1**, e88255

21. Tasker, C., Subbian, S., Gao, P., Couret, J., Levine, C., Ghanny, S., Sotero-poulos, P., Zhao, X., Landau, N., Lu, W., and Chang, T. L. (2016) IFN-ε protects primary macrophages against HIV infection. *JCI insight* **1**, e88255

22. Liu, T., Niu, X., Zhang, X., Wang, S., and Liu, Z. (2017) Recombinant human IFNa-2b response promotes vaginal epithelial cells defense against *Candida albicans*. *Front. Microbiol.* **8**, 697

23. Li, T. Y., Mangan, N. E., Cumming, H., Horvat, J. C., Mayall, J. R., Stiffer, S. A., De Woerd, N., Roisman, L. C., Rossjohn, J., Robertson, S. A., Schjeken, J. E., Parker, B., Gargett, C. E., Nguyen, H. P., Carr, D. J., Hansbro, P. M., and Hertzog, P. J. (2013) Interferon-ε protects the female reproductive tract from viral and bacterial infection. *Science* **339**, 1088–1092

24. Jones, M., Davidson, A., Hibbert, L., Gruenwald, P., Schaak, J., Ball, S., Foster, G. R., and Jacobs, M. (2005) Dengue virus inhibits α interferon signaling by reducing STAT2 expression. *J. Virol.* **79**, 5414–5420

25. Fung, K. Y., Mangan, N. E., Cumming, H., Horvat, J. C., Mayall, J. R., Stiffer, S. A., De Woerd, N., Roisman, L. C., Rossjohn, J., Robertson, S. A., Schjeken, J. E., Parker, B., Gargett, C. E., Nguyen, H. P., Carr, D. J., Hansbro, P. M., and Hertzog, P. J. (2013) Interferon-ε protects the female reproductive tract from viral and bacterial infection. *Science* **339**, 1088–1092

26. Reiser, J., Hurst, J., Voges, M., Krauss, P., Münch, P., Iftner, T., and Stubenrauch, F. (2011) High-risk human papillomaviruses repress constitutive interferon transcription via E6 to prevent pathogen recognition receptor and antiviral-gene expression. *J. Virol.* **85**, 11372–11380
42. Kuruganti, S., Accavitti-Loper, M. A., and Walter, M. R. (2014) Production and characterization of thirteen human type-I interferon-α subtypes. *Protein Expr. Purif.* **103**, 75–83 CrossRef Medline
43. Yoon, S. I., and Walter, M. R. (2007) Identification and characterization of a +1 frameshift observed during the expression of Epstein-Barr virus IL-10 in *Escherichia coli*. *Protein Expr. Purif.* **53**, 132–137 CrossRef Medline
44. Radhakrishnan, R., Walter, L. J., Hruza, A., Reichert, P., Trotta, P. P., Nagabhushan, T. L., and Walter, M. R. (1996) Zinc mediated dimer of human interferon-α2b revealed by X-ray crystallography. *Structure* **4**, 1453–1463 CrossRef Medline
45. Deshpande, A., Putcha, B. D., Kuruganti, S., and Walter, M. R. (2013) Kinetic analysis of cytokine-mediated receptor assembly using engineered FC heterodimers. *Protein Sci.* **22**, 1100–1108 CrossRef Medline
46. Thomas, C., Moraga, I., Levin, D., Krutzik, P. O., Podoplelova, Y., Trejo, A., Lee, C., Yarden, G., Vleck, S. E., Glenn, J. S., Nolan, G. P., Pиеhler, J., Schreiber, G., and Garcia, K. C. (2011) Structural linkage between ligand discrimination and receptor activation by type I interferons. *Cell* **146**, 621–632 CrossRef Medline
47. Pиеhler, J., and Schreiber, G. (1999) Biophysical analysis of the interaction of human ifnar2 expressed in *E. coli* with IFNa2. *J. Mol. Biol.* **289**, 57–67 CrossRef Medline
48. Couret, J., Tasker, C., Kim, J., Sihvonen, T., Fruitwala, S., Quayle, A. J., Lespinasse, P., Heller, D. S., and Chang, T. L. (2017) Differential regulation of IFNa, IFNb, and IFNε gene expression in human cervical epithelial cells. *Cell Biosci.* **7**, 57 Medline
49. Jaks, E., Gavutis, M., Uze, G., Martal, J., and Pиеhler, J. (2007) Differential receptor subunit affinities of type I interferons govern differential signal activation. *J. Mol. Biol.* **366**, 525–539 CrossRef Medline
50. Lavoie, T. B., Kalie, E., Crisafulli-Cabatu, S., Abramovich, R., DiGioia, G., Moolchan, K., Pestka, S., and Schreiber, G. (2011) Binding and activity of all human α interferon subtypes. *Cytokine* **56**, 282–289 CrossRef Medline
51. Jaitin, D. A., Roisman, L. C., Jaks, E., Gavutis, M., Pиеhler, J., Van der Heyden, J., Uze, G., and Schreiber, G. (2006) Inquiring into the differential action of interferons (IFNs): an IFN-α2 mutant with enhanced affinity to IFNAR1 is functionally similar to IFN-β. *Mol. Cell. Biol.* **26**, 1888–1897 CrossRef Medline
52. Stifter, S. A., Matthews, A. Y., Mangan, N. E., Fung, K. Y., Drew, A., Tate, M. D., Soares da Costa, T. P., Hampsey, D., Mayall, J., Hansbro, P. M., Garcia Minambres, A., Eid, S. G., Mak, J., Scoble, J., Lovrecz, G., deWeerd, N. A., and Hertzog, P. J. (2018) Defining the distinct, intrinsic properties of the novel type I interferon, IFN. *J. Biol. Chem.* **293**, 3168–3179 CrossRef Medline
53. Samarajiwa, S. A., Mangan, N. E., Hardy, M. P., Najdovska, M., Dubach, D., Braniff, S. J., Owczarek, C. M., and Hertzog, P. J. (2014) Soluble IFN receptor potentiates in vivo type I IFN signaling and exacerbates TLR4-mediated septic shock. *J. Immunol.* **192**, 4425–4435 CrossRef Medline
54. Myszka, D. G. (1999) Improving biosensor analysis. *J. Mol. Recognit.* **12**, 279–284 CrossRef Medline

---

**Human IFNε and IFNκ exhibit low affinity for IFNARs and B18R**

49. Jaks, E., Gavutis, M., Uze, G., Martal, J., and Pielehler, J. (2007) Differential receptor subunit affinities of type I interferons govern differential signal activation. *J. Mol. Biol.* **366**, 525–539 CrossRef Medline
50. Lavoie, T. B., Kalie, E., Crisafulli-Cabatu, S., Abramovich, R., DiGioia, G., Moolchan, K., Pestka, S., and Schreiber, G. (2011) Binding and activity of all human α interferon subtypes. *Cytokine* **56**, 282–289 CrossRef Medline
51. Jaitin, D. A., Roisman, L. C., Jaks, E., Gavutis, M., Pielehler, J., Van der Heyden, J., Uze, G., and Schreiber, G. (2006) Inquiring into the differential action of interferons (IFNs): an IFN-α2 mutant with enhanced affinity to IFNAR1 is functionally similar to IFN-β. *Mol. Cell. Biol.* **26**, 1888–1897 CrossRef Medline
52. Stifter, S. A., Matthews, A. Y., Mangan, N. E., Fung, K. Y., Drew, A., Tate, M. D., Soares da Costa, T. P., Hampsey, D., Mayall, J., Hansbro, P. M., Garcia Minambres, A., Eid, S. G., Mak, J., Scoble, J., Lovrecz, G., deWeerd, N. A., and Hertzog, P. J. (2018) Defining the distinct, intrinsic properties of the novel type I interferon, IFN. *J. Biol. Chem.* **293**, 3168–3179 CrossRef Medline
53. Samarajiwa, S. A., Mangan, N. E., Hardy, M. P., Najdovska, M., Dubach, D., Braniff, S. J., Owczarek, C. M., and Hertzog, P. J. (2014) Soluble IFN receptor potentiates in vivo type I IFN signaling and exacerbates TLR4-mediated septic shock. *J. Immunol.* **192**, 4425–4435 CrossRef Medline
54. Myszka, D. G. (1999) Improving biosensor analysis. *J. Mol. Recognit.* **12**, 279–284 CrossRef Medline