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LETTER TO THE EDITOR
Host-Microbe Biology

Different Biological Activities of Specific Interferon Alpha Subtypes

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On the basis of analysis of 25 transcripts from the hundreds of genes induced by interferons (IFN) (1), Schlaepfer et al. (2) conclude that "IFN-α subtypes do not induce different biological responses." Justification of such a broad conclusion would require whole-transcriptome and proteomic and posttranslational analyses, which were not done. Furthermore, the physiological relevance of their findings was not evaluated in a single *in vivo* therapeutics experiment, thereby ignoring the high complexity and interactive characteristics of *in vivo* systems compared to *in vitro* experiments. We also previously showed that IFN-α subtype 2 and subtype 14 had similar biological effects *in vitro* at the extremely high (1,000 pg/ml) concentrations that they used (3). However, the situation *in vivo* at therapeutic concentrations is not so simple. Since drugs and biologics cause adverse effects at high concentrations *in vivo*, it is critical to determine how well the maximal clinically tolerable dose of a drug or substance inhibits a targeted biological process such as virus production. We determined that the half-maximal inhibitory concentration (IC_{50}) of IFN-α14 for inhibition of HIV replication *in vitro* was 16.6 pg/ml compared to 179 pg/ml for IFN-α2. Thus, IFN-α14 inhibited HIV replication at a 10-fold-lower mass unit concentration than IFN-α2, suggesting that it might be a significantly better inhibitor *in vivo* as well. Dosages of biologics such as interferon are based on activity rather than mass units to account for the various fractions of inactive protein that are present in recombinant preparations (4). The same argument holds true for *in vitro* studies, but all of the experiments reported by Schlaepfer et al. were based on mass units and tested at very high mass concentrations. Schlaepfer et al. state that our studies "do not provide evidence that IFN-α subtypes intrinsically differ in their functional role." Our *in vivo* studies with interferon subtypes used equivalent units of activity at the maximal therapeutic dose shown to be clinically tolerable (5). We found that IFN-α14 treatment of human-immune-system mice not only suppressed HIV significantly better than IFN-α2 but also induced very different immune responses (3). The results were not attributable to donor effects because similar results were obtained with multiple human donors. Significantly better protection from HIV provided by IFN-α14 than by IFN-α2 in humanized mice was independently confirmed (6), and results from an *ex vivo* HIV study in human lamina propria explants (7), as well as from a study from Friend retrovirus infection of laboratory mice (8), convincingly demonstrate that different IFN-α subtypes, administered at clinically relevant and equivalent doses, induce distinct antiretroviral pathways with significantly different therapeutic efficacies. Finally, the conclusion that IFN-α subtypes do not induce different biological responses belies the strong purifying evolutionary selection of multiple interferon alpha subtypes in wide-ranging species, which strongly implies essential and nonredundant functions of different subtypes (9). *In vivo veritas*!

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