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# Structural characterization and immunogenicity in wild-type and immune tolerant mice of degraded recombinant human interferon Alpha2b


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## Purpose

This study was conducted to study the influence of protein structure on the immunogenicity in wild-type and immune tolerant mice of well-characterized degradation products of recombinant human interferon alpha2b (rhIFNα2b).

## Methods

RhIFNα2b was degraded by metal-catalyzed oxidation (M), cross-linking with glutaraldehyde (G), oxidation with hydrogen peroxide (H), and incubation in a boiling water bath (B). The products were characterized with UV absorption, circular dichroism and fluorescence spectroscopy, gel permeation chromatography, reverse-phase high-pressure liquid chromatography, sodium dodecyl sulfate polyacrylamide gel electrophoresis, Western blotting, and mass spectrometry. The immunogenicity of the products was evaluated in wild-type mice and in transgenic mice immune tolerant for hIFNα2. Serum antibodies were detected by enzyme-linked immunosorbent assay or surface plasmon resonance.

## Results

M-rhIFNα2b contained covalently aggregated rhIFNα2b with three methionines partly oxidized to methionine sulfoxides. G-rhIFNα2b contained covalent aggregates and did not show changes in secondary structure. H-rhIFNα2b was only chemically changed with four partly oxidized methionines. B-rhIFNα2b was largely unfolded and heavily aggregated. Nontreated (N) rhIFNα2b was immunogenic in the wild-type mice but not in the transgenic mice, showing that the latter were immune tolerant for rhIFNα2b. The anti-rhIFNα2b antibody levels in the wild-type mice depended on the degradation

product: M-rhIFNα2b > H-rhIFNα2b ∼ N-rhIFNα2b ≫ B-rhIFNα2b; G-rhIFNα2b did not induce anti-rhIFNα2b antibodies. In the transgenic mice, only M-rhIFNα2b could break the immune tolerance.

### Conclusions

RhIFNα2b immunogenicity is related to its structural integrity. Moreover, the immunogenicity of aggregated rhIFNα2b depends on the structure and orientation of the constituent protein molecules and/or on the aggregate size.

## About this Article

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