Supplemental material

Toxicity assays

HSCs from three mice were used for the toxicity assays (from two mice, eight samples per dose-level; from one mouse, five samples per dose level were used). Toxicity was examined using an ANOVA-model in which the FDH assay values as a measure of toxicity were the criterion variable. The Anakinra doses were considered fixed factors, and the animals were considered random factors.

HSC proliferation

HSCs from six mice were used for the examination of HSC-proliferation, 4-times for each combination of the three IL-1β stimulation levels (control, 0.1 ng/ml and 1 ng/ml) and the two Anakinra dose levels (no Anakinra and 2.5 µg/ml Anakinra), resulting in a total of 144 measurements (of which two were not evaluable).

Effect of IL-1β on HSC proliferation

The examination of the effect of IL-1β on HSC proliferation was based on all measurements without Anakinra. An ANOVA model was used where HSC proliferation as a measure of fibrogenesis was the criterion for variability. The IL-1β level (control vs. 0.1 ng/ml IL-1β vs. 1.0 ng/ml IL-1β) was considered a fixed factor, and animals as a random factor. Furthermore, the IL-1β-levels were compared pairwise—i.e., non IL-1β application was compared first with 1.0 ng/ml IL-1β and second with 0.1 ng/ml IL-1β, by similar ANOVA models as described above. These ANOVA models included only the IL-1β levels of the respective pair. The predefined sequence of these pairwise comparisons represents a hierarchical order of the underlying hypotheses. This hierarchical testing procedure provides control of Type I error in this multiple testing situation.

Effect of Anakinra and IL-1β on HSC proliferation

The examination of the effect of Anakinra and IL-1β on HSC proliferation was based on all measurements. An ANOVA model was used where HSC proliferation as a measure of fibrogenesis was the criterion variable. The Anakinra dose (no Anakinra vs. 2.5 µg/ml Anakinra) and IL-1β level (no IL-1β vs. 0.1 ng/ml IL-1β vs. 1.0 ng/ml IL-1β) were considered as fixed factors and animals as a random factor. Furthermore, the interaction between the factors of the Anakinra dose and IL-1β level were considered in the model.

Pairwise comparison between the Anakinra dose (no Anakinra vs. 2.5 µg/ml Anakinra) within each IL-1β level was performed for explorative examination of the interactive effects between Anakinra and IL-1β. ANOVA models were used where the Anakinra dose (no Anakinra vs. 2.5 µg/ml Anakinra) was considered a fixed factor and the animals as a random factor. The sample for each pairwise comparison was limited to measurements from the respective IL-1β level.