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Interleukin-4 and Acetaminophen Hepatotoxicity: A Story of Conflicting Results and Conclusions

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To the Editors

Pires and coworkers reported on the role of interleukin-4 (IL-4) in acetaminophen (APAP)-induced liver injury using IL-4-deficient mice [1]. The authors concluded that IL-4-deficiency protected due to reduced depletion of GSH [1]. Interestingly, these results are the exact opposite compared to what was shown recently by Lance Pohl's group [2]. These authors reported that IL-4-deficient mice are much more susceptible to an APAP overdose mainly due to a delay in GSH re-synthesis after the initial massive GSH depletion [2]. The recovery of GSH in hepatocytes, especially in mitochondria, is critical for scavenging reactive oxygen species and peroxynitrite, which are determining factors for cell necrosis after APAP overdose [3,4]. Thus, the fundamental question that remains is IL-4 beneficial by promoting GSH synthesis as suggested by Ryan et al. [2] or detrimental by promoting metabolic activation of APAP as concluded by Pires et al [1].

In order to answer this question, a closer look at the data supporting the conclusion in both papers is necessary. The increased injury in IL-4-deficient mice as reported by Ryan et al. was preceded by lower protein adducts formation in these animals [2]. The increased injury as observed in IL-4-deficient mice was confirmed by using an anti-IL-4 antibody in wild type animals [2]. In addition, the authors treated mice 3 h after APAP with recombinant IL-4, which enhanced glutamate-cysteine ligase expression and GSH synthesis resulting in attenuated APAP-induced liver injury compared to control animals [2]. Together these studies support the conclusions that IL-4 does not inhibit metabolic activation of APAP but that the protective effect of IL-4 appears to be related to the faster recovery of hepatic GSH with the enhanced capacity to scavenge reactive oxygen and peroxynitrite. In contrast, the study by Pires et al. relied exclusively on measurement of GSH at 2 h after 350 mg/kg APAP. At that time, GSH levels actually showed a modest increase, which was followed by a gradual decline to about 50% of the baseline [2]. Given that every dose of APAP between 75 mg/kg and 600 mg/kg causes a 90% loss of GSH in wild type animals within 30 min [5], this drastic delay and overall inhibition of GSH depletion with 350 mg/kg APAP can only be interpreted as indication for inhibition of reactive metabolite formation. As the metabolic activation of APAP and subsequent protein binding is the key initiating event of the entire pathophysiology including mitochondrial dysfunction, oxidant stress, MAPkinase activation, DNA fragmentation and eventually an inflammatory response [6], it is not surprising that there is reduced injury when the most upstream event is blocked. The critical question is what is the role of IL-4 in this mechanism? It has been shown that IL-4 promotes Cyp2e1 induction in hepatocytes and thus could enhance toxicity [7,8]. However, despite the capacity of IL-4 to induce the key P450 enzyme for APAP metabolism, this effect may not explain the reduced metabolic activation in the study by Pires et al. [1] because IL-4 formation occurs rather late during APAP toxicity well past the drug metabolism phase [2].

In addition, the opposite response to APAP overdose observed in 2 different confirmed IL-4-deficient mice suggests that the results may have been an off-target effect unrelated to IL-4 in one of the mouse models, most likely in the Pires study.

Off-target effects are increasingly recognized as problems of gene-deficient mice. Some of the off-target effects are relatively easy to detect if the gene-deletion causes a severe cellular stress with compensatory responses. An example is the conditional deletion of the essential autophagy gene Atg5 in hepatocytes, which triggers Nrf2 activation, chronic apoptosis and regeneration [9]. As a consequence, Atg5-deficient mice are completely resistant to APAP hepatotoxicity [9]. Other off-target effects are less obvious. Recent studies using V α 14iNKT cell-deficient (J α 18 $^{-/-}$) mice, ultimately derived from the same source, reported opposite effects [10,11]. One study showed protection against APAP toxicity in J α 18 $^{-/-}$ mice due to higher baseline GSH levels and more effective GSH conjugation of the reactive metabolite [10]. In contrast, the same mice were shown to be more susceptible to APAP toxicity because the lack of IL-4 produced by NKT cells is responsible for more ketone body formation leading to Cyp2e1 induction and higher injury [11]. In both cases, IL-4- and J α 18-deficient mice, the different results may be caused by unknown off-target effects. This could include infections in the animal facilities, different diets, etc. It is clearly necessary to evaluate these models further before any reliable conclusions regarding the role of IL-4 in APAP toxicity can be drawn. These examples also illustrate that any results in transgenic or gene-deficient mice should be supported by independent experiments as was done in 2 of these studies [2,11]. Relying only on results of a single mouse model may be misleading if off-target effects can affect the outcome of the experiment [1,10].

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