IgG3 is the Dominant Subtype of Anti-Isoniazid and Anti-P450 Antibodies in Patients with Isoniazid-Induced Liver Failure.

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IgG_3 Is the Dominant Subtype of Anti-isoniazid Antibodies in Patients with Isoniazid-Induced Liver Failure

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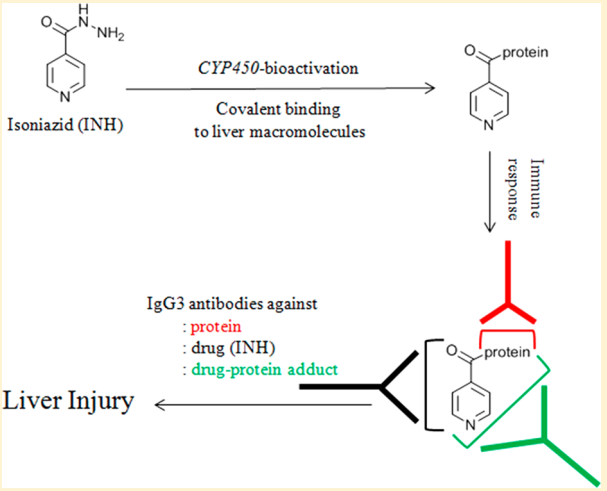
Supporting Information

ABSTRACT: Isoniazid (INH) therapy is associated with a significant incidence of idiosyncratic liver failure. We recently reported eight cases of INH-induced liver failure in which patients had antidrug and anticytochrome P450 antibodies. However, it was unclear what role these antibodies play in the mechanism of INH-induced liver injury. Here, we report that the dominant isotype of anti-INH antibodies was IgG, with IgG3 being the dominant subtype. IgG3 antibodies are associated with a Th1-type immune response and fix complement. IgG3 antibodies have been associated with other forms of liver injury and may play a pathogenic role in INH-induced liver injury.



Isoniazid (INH)-induced liver injury and liver failure remain a significant problem.[1] For decades, the mechanism of INH-induced liver failure was considered to represent "metabolic idiosyncrasy" based on the finding that the *N*-acetylhydrazine metabolite of INH was found to be hepatotoxic in rats[2] and the fact that rechallenge of patients with INH did not always lead to a prompt onset of liver injury.[2−4] However, the rat model represented acute hepatotoxicity with very different characteristics than the idiosyncratic liver injury in humans, and the metabolism of INH is significantly different in rats than in humans.[5,6]

Recently, we proposed that INH-induced liver injury is immune mediated.[7] We identified a new reactive metabolite, which involves bioactivation and covalent binding of the parent drug in the liver.[5,8] The observation that INH-modified proteins were recognized by T cells from patients with a history of INH-induced liver injury (i.e., a positive lymphocyte transformation test (LTT)) strongly supports an immune mechanism for INH-induced liver injury[9,10] and also that it is a reactive metabolite of INH rather than *N*-acetylhydrazine that induces the immune response. In addition, more severe cases of liver injury also had a positive LTT test to INH itself.[9,10] More recently, we have also identified anti-INH and anticytochrome P450 antibodies in patients with INH-induced liver failure[11] and a Th17 immune response in patients that develop mild liver injury due to INH.[12] An important question is whether these antibodies contribute to INH-induced liver injury. In this rapid report, we evaluated the isotype of anti-INH antibodies that were found in the serum of patients with INH-induced liver failure to gain a better understanding of the nature of the immune response that was induced by INH in these patients.

In all 8 serum samples, the dominant immunoglobulin isotype of these antibodies was IgG, although two patients (patients 1 and 6) also had a low titer of IgM antibodies (Figure 1). IgA and IgE antibodies were not detected in any of the serum samples.

These results were confirmed by ELISA, which showed a significant increase in absorbance when using secondary antibodies against human Ig or IgG to detect the anti-INH antibodies (Figure 2A). We had access to a liver biopsy sample from a patient who had been diagnosed with INH-induced liver failure, and we tested whether IgG antibodies were bound to hepatocytes in this patient. Immunohistochemical staining revealed that IgG was the predominant isotype of antibody present in the liver slide of this patient with INH-induced liver failure (Figure 3); no binding of IgM or IgA was detected. Additional slides showing IgG binding to the liver of this

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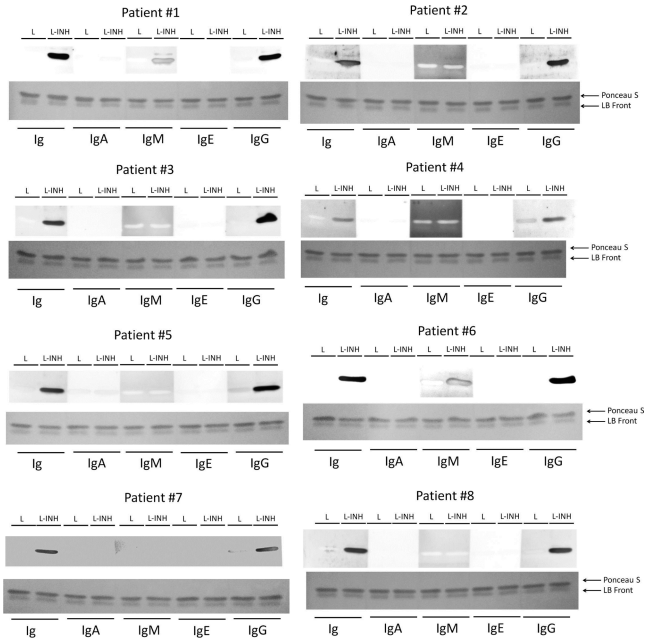

Figure 1. Phenotyping of the human anti-INH antibody by Western blotting. Lysozyme (L) or INH-modified lysozyme (L-INH) were used as described in Experimental Methods (Supporting Information). Secondary antibodies against Ig, IgA, IgM, IgE, or IgG were used to determine the antibody isotype. Ponceau S was used as the loading control. LB Front = loading buffer front.

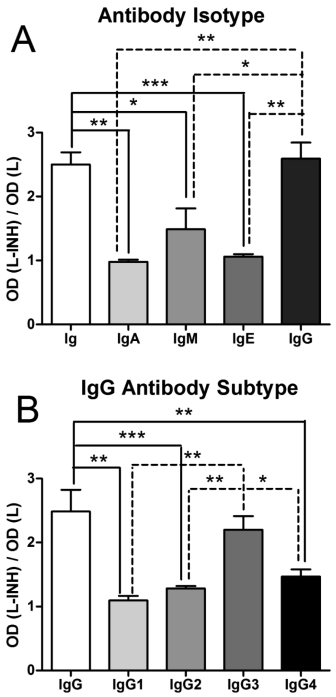

Figure 2. Phenotyping of the human anti-INH antibody by ELISA. Serum from eight patients that tested positive for anti-INH antibodies[11] were re-evaluated for the antibody subtype. The ELISA plate was coated either with lysozyme (L) or INH-modified lysozyme (L-INH); the ratio of OD absorbance from L-INH/L was used to determine the presence of antibodies as previously described.[11] The secondary antibody against Ig, IgA, IgM, IgE, IgG, and IgG1−4 was used to determine the antibody subtype. Values represent the mean ± SE; *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$.

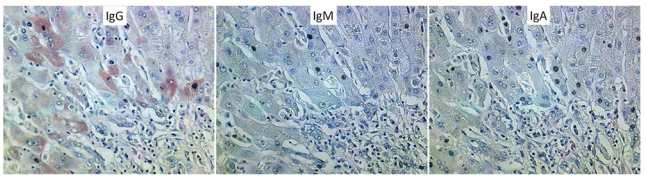

Figure 3. Immunohistochemical staining of the liver from a patient with INH-induced liver failure. A liver slide from patient number 2 was blotted with secondary antibodies against IgG, IgM, and IgA. Only blotting with the secondary antibody against human IgG resulted in a signal, which is a reddish-brown stain.

patient are shown in Figure S1 (Supporting Information). There are four main subtypes of IgG antibodies (IgG1−4), and we set out to determine the subtype of this IgG antibody. Our results indicated that the predominant subtype was IgG3 as shown in Figure 2B. The presence of IgG3 anti-INH antibodies was also confirmed by Western blotting by using additional leftover serum from four patients. Specifically, a band was only observed when transferring lysozyme modified by INH on the membrane and using antihuman IgG3-peroxidase secondary antibody to detect binding (Supporting Information, Figure S2).

The dominance of IgG antibodies in these patients is consistent with other studies that found a predominance of IgG antibodies in idiosyncratic drug reactions caused by other drugs such as sulfomethoxasole, trimethoprim,[13] and amodiaquine.[14] Both IgM and IgG3 activate the complement leading to the recruitment of cells that carry the Fc receptor, and they are capable of causing antibody-dependent cell-mediated cytotoxicity.[15,16] IgG3 antibodies have also been implicated in the mechanism of cell injury because of their ability to form cryoglobulins,[17,18] and they have been shown to be able to bind to hepatocytes and induce cell necrosis through complement activation.[19,20] IgG3 antibodies have been implicated in the induction of a lupus-like autoimmune reaction,[17] and although there is no evidence that INH-induced liver injury is an autoimmune reaction, INH treatment is well known to cause a lupus like syndrome.[21]

The basis for the idiosyncratic nature of INH-induced liver injury is unknown, but an immune mechanism is one possible explanation. Furthermore, although we cannot be certain that these antibodies play an important role in the mechanism of INH-induced liver injury, these data also support an immune mechanism for INH-induced liver injury, and it is also consistent with other evidence that suggests that INH can activate an adaptive immune response.[12] It will be important to investigate the role of T cells in the formation of these IgG3 anti-INH antibodies. T cells play an important role in the production of antibodies.[22] Class switching to IgG3 is promoted by the Th1 cytokine, IFN-γ,[17] and this suggests that INH-induced liver injury involves a Th1 immune response.

■ ASSOCIATED CONTENT

Supporting Information

Experimental methods; members and institutions participating in the Acute Liver Failure Study Group; additional immunohistochemical staining of the liver slide from patient number 2 who had INH-induced liver failure; and phenotyping of the human anti-INH IgG antibody subtype. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS

ALFSG, Acute Liver Failure Study Group; ELISA, enzyme-linked immunosorbent assay; HBV, hepatitis B virus; HLM, human liver microsomes; IHC, immunohistochemistry; INA-NHS, *N*-hydroxysuccinimide ester of isonicotinic acid; INH, isoniazid; L, lysozyme; L-INH, lysozyme chemically modified to mimic binding by the reactive metabolite of INH; LTT, lymphocyte transformation test; TBST, tris-buffered saline with Tween

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