Detection of drug-responsive T-lymphocytes in a case of fatal anti-tuberculosis drug-related liver injury

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ABSTRACT: Anti-tuberculosis (TB) drug exposure is associated with a mild elevation of liver enzymes that occasionally develops into severe liver injury. Herein, we identify ethambutol- and rifampicin-specific CD4+ and CD8+ T-cell clones in a patient with fatal anti TB-related liver injury. The clones were activated to proliferate and secrete IFN-γ, Il-13 and granzyme B following drug treatment. Drug-responsive T-cells may contribute to the pathogenesis of anti-tuberculosis-related liver failure.

A combination of isoniazid (INH), ethambutol (ETB), pyrazinamide (PZA) and/or rifampicin (RIF) is commonly used for the treatment of tuberculosis (TB). A mild elevation of liver enzymes that occasionally develops into severe liver injury represents a significant clinical problem.[1] The culprit drug involved in the pathogenesis of anti TD-related liver injury has not been fully defined and the mechanism of tissue injury has not been resolved. The delayed onset of clinical symptoms and the discovery of an HLA-risk allele[2] suggest that the adaptive immune system might contribute to the liver injury. Recently, Metushi et al. found anti-INH and anti-CYP auto antibodies in patients with INH-induced severe liver failure suggesting that INH hapten protein adducts activate humoral responses in patients with INH monotherapy-induced liver injury.[3] Moreover, the authors found an increased frequency of IL-10 secreting cells in patients with mild elevations in liver enzymes suggesting that anti-inflammatory IL-10 could be the reason that more serious liver injury did not occur.[4] Recently, we have characterized IL-10 secreting INH-specific CD4+ T-cell clones in patients with resolving forms of liver injury related to exposure to anti-TB drug combinations.[5] ETB, PZA and RIF did not activate T-cells in these patients. The objective of the current study was to characterize the specificity of drug-responsive T-cells in a case of fatal anti-TB drug-related liver injury.

The patient, a 50 year old Indian male, presented 29 days after start of Voractiv (PZA 2000mg, RIF 600mg, ETB 1100mg, INH 300mg/day) treatment for pulmonary and mediastinal TB. He was otherwise fit and well with no previous history of drug allergy. His baseline liver function was in the normal range (alanine aminotransferase [ALT] 23IU/L). After 3 weeks medication he reported feeling unwell with increased breathlessness, abdominal discomfort, nausea, and jaundice with elevation of ALT (262 IU/L). Blood counts (haemoglobin and total leukocytes and platelets) were in the normal range. Viral serologies (IgM anti-HAV, IgM anti-HEV, HBsAg, anti-HCV, hepatitis B virus DNA, hepatitis C virus RNA) and markers for autoimmune liver disease (anti-nuclear antibody, anti-smooth muscle antibody, anti-mitochondrial antibody, anti-liver kidney microsomal antibody) were negative. Medication was immediately stopped and the patient was diagnosed with a drug-induced liver reaction. Peripheral blood mononuclear cells (PBMC) were collected, with approval from the local ethics committee and informed consent, 16h later when ALT levels had increased to 514IU/L (bilirubin 32mg/dL). Over the next few days liver function deteriorated, with ALT peaking at 2373 IU/L, bilirubin at 459mg/dL and an INR of 4.4. He developed multi-organ failure and died 41 days after TB treatment commenced.
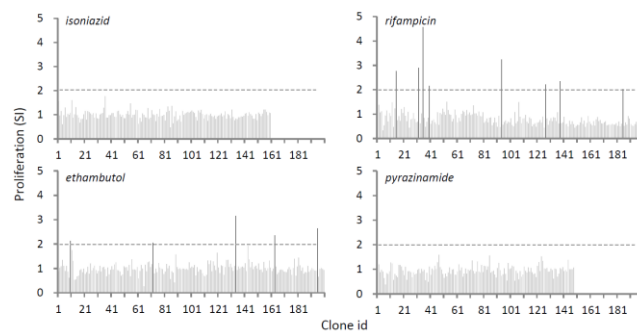

Figure 1. INH-, RIF-, ETB- and PZA-induced proliferation of T-cell clones. Clones were incubated with antigen presenting cells and drug and proliferation was measured 48h later through addition of [³H]thymidine. SI of 2 indicated by dashed line was considered positive and clones were expanded for additional studies.

Promising biomarkers have recently been identified that provide mechanistic insight into hepatic events associated with idiosyncratic and acetaminophen-induced liver injury.[6] Com-

pared with published data in healthy donors and acetaminophen overdose that does not cause liver injury,[7] all markers (total HMGB1 (35.6 ng/ml), mir-122 (1010.7 let-7 normalized), keratin-18 M65 (8352.5 U/l), GLDH (1078.9 U/l)) were elevated alongside peak ALT, with the exception of keratin-10 M30 (410.2 U/l).

A T-cell response to the suspect drugs was initially studied with the acute blood sample using diagnostic assays, the lymphocyte transformation test and IFN-γ PBMC ELIspot.[8,9] Briefly, PBMC were cultured with INH (0.1-4mM), RIF (0.01-0.4mM), ETB (0.01-4mM) and PZA (0.01-4mM) and proliferation and cytokine release were measured. PBMC were stimulated to proliferate and secrete IFN-γ in the presence of the positive control tetanus toxoid. However, a negative result (i.e., stimulation index less than 2) was observed using the 4 test compounds. These negative data during the acute reaction might be explained by pre-activation of the drug-specific T-cells in the patient. In fact, Pichler and Tilch,[10] suggest that T-cell assays for diagnosis should be conducted 4-8 weeks after a reaction for this reason.

Despite these initial negative results, drug-reactive T-cells were enriched by stimulation of PBMC with PZA, RIF, ETB and INH for two weeks. T-cell clones were generated by serial dilution and repetitive mitogen-driven expansion.[11] An Epstein-Barr virus-transformed B-cell line was generated and used as antigen-presenting cells. Reactivity of the T-cell clones (5×10^4 T-cells, 1×10^4 antigen presenting cells; 200μl total volume, 96 well cell culture plates) to the compounds was tested by measurement of proliferation using [^{3}H]-thymidine after a 48h culture period. A total of 710 clones were generated; 5 displayed reactivity against ethambutol and 8 displayed reactivity against rifampicin. PZA and INH responsive clones were not detected. The ETB clones expressed the CD4+ co-receptor. Four RIF-responsive clones were available for phenotyping; 3 were CD4+, 1 was CD8+.
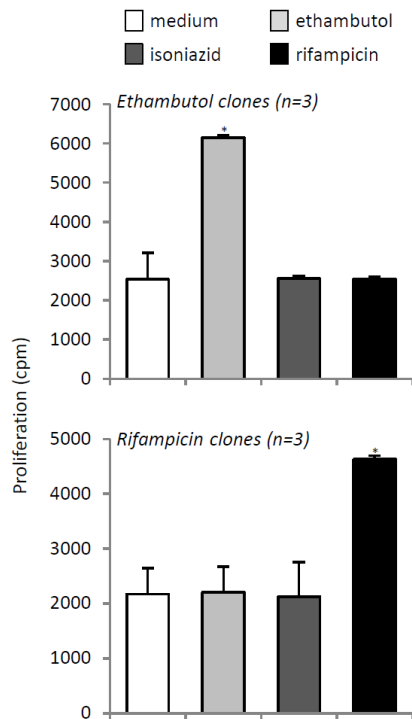

Figure 2. Specificity of ETB and RIF-responsive T-cell clones. Clones were cultured with antigen presenting cells ± drugs (PZA was not included as all previous studied yielded negative results) and proliferation was measured using [^{3}H]thymidine (*P<0.05; Mann-Whitney test).

All of the ETB and RIF-responsive clones were expanded for dose-response studies, to assess drug specificity and to characterize the secretion of cytokines and cytolytic molecules. Clones were stimulated to proliferate in a dose-dependent manner in the presence of either ETB or RIF (results not shown). The other drugs the patient was exposed to at the time of tissue injury did not activate the clones (Figure 2). These data show that 2 structurally divergent drugs interact with and activate the patients' adaptive immune system prior to the development of liver failure. These data are in stark contrast to our earlier study utilizing PBMC from patients with reversible anti TB drug-related liver injury, where T-cells were selectively activated with isoniazid.

The final component of the project was to assess whether the clones secreted cytokines and cytolytic molecules following drug stimulation. Th1 and Th2 cytokines IFN-γ and IL-13, respectively, were selected for the ELIspot analysis. All ETB and RIF-responsive clones (including the single CD8+ clone) secreted IFN-γ and IL-13. Interestingly, the clones also secreted the pro-apoptotic molecules granzyme B, which indicates that the clones have the ability to damage target tissue.
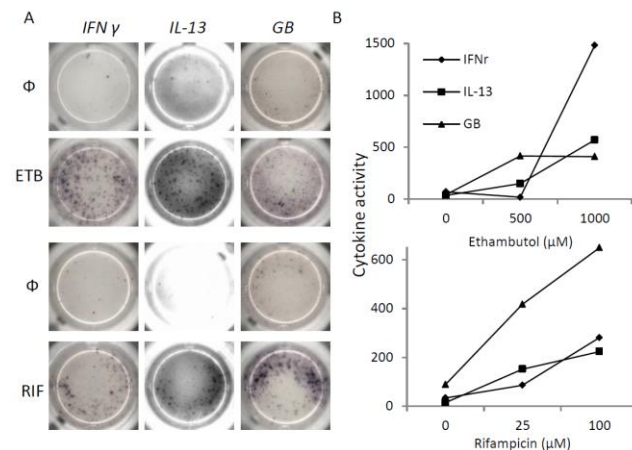

Figure 3. Cytokine/cytolytic molecule secretion from T-cell clones. Clones were cultured with antigen presenting cells ± drugs and cytokine secretion was visualized by ELIspot. A) Representative ELIspot images from 2 clones (1 ETB & 1 RIF-specific). B) Mean dose-dependent cytokine release from the ETB and RIF clones.

To summarize, ETB and RIF-specific CD4+ and CD8+ clones were generated from a patient with anti-TB drug-related liver failure. Our investigation of patients with resolving and this fatal form of liver injury show that drug-specific T-cell activation is a common feature. Although further studies with a larger patient cohort are required, our data suggests that isoniazid activates T-cells in patients with resolving forms of liver injury, whereas in fatal liver injury the T-cells are activated by other drugs such as ETB and RIF in this case. Future studies are on-going to characterize the risk and protective factors that influence individual susceptibility.

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ABBREVIATIONS

INH, isoniazid; ETB, ethambutol; PZA, pyrazinamide; RIF, rifampicin; PBMC, peripheral blood mononuclear cells; TB, tuberculosis; ALT, alanine aminotransferase; .

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anti-TB
drugs → Liver failure → T-cells

ethambutol

rifampicin