ducted on the LightCycler system. The increasing numbers of these reports suggest the need for an online database for aberrant melting curves and their underlying variations.

References
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The recent tragedy, in which all active drug recipients developed life-threatening multiple organ failure during the phase I clinical trial of superagonistic anti-CD28 monoclonal antibody (TGN1412) (1), raised serious questions regarding the safety of human drug-trial participants. Results obtained in animal models do not always correspond to those observed in humans, and human cells placed in an in vitro culture environment may no longer exhibit physiologic functions that occur under natural conditions. An ideal precautionary method for investigational drugs designed to be administered intravenously is to mix each drug candidate with whole blood in test tubes and evaluate both desired function and unexpected adverse reactions. Such an ex vivo test is completely safe and applicable to drugs acting on leukocytes. The biggest technical challenge, however, is to identify drug actions in whole blood in a short period of time, because longer incubations might be associated with side effects. Gene expression analysis is an interesting candidate method, because mRNA transcription occurs earlier than protein synthesis and final biological outcomes.

In a recent issue of Clinical Chemistry (2), our group evaluated the effect of mouse antihuman CD28 monoclonal antibody because the humanized version used in the clinical trial (1) was not freely available. We obtained anonymized blood samples from 6 healthy adult volunteers (Apex Research), after receiving institutional review board approval. Triplicate 60-mL aliquots of each heparinized whole blood sample were incubated with either 1 mg/L of mouse antihuman CD28 monoclonal antibody IgG1x or control purified mouse IgG1x (BioLegend) at 37 °C for 2 h. The concentrations of various mRNAs were quantified with SYBR green real-time PCR as described previously (2). Increases were calculated by the difference from control IgG. As shown in Fig. 1, after addition of mouse antihuman CD28 monoclonal antibody and incubation at 37 °C for only 2 h, whole blood from 1 healthy individual showed significant induction of inflammation- and apoptosis-related mRNA, such as interleukin-2 (IL-2), tumor necrosis factor superfamily (TNFSF)-1 (lymphotoxin α), TNFSF-5 (CD40 ligand), CCL chemokine-20, and CXCL chemokine-10. The huge induction of IL-2 mRNA is reminiscent of the failure of systemic IL-2 therapy decades ago (3). In blood from other healthy individuals, we observed significant reductions in the concentrations of IL-6, TNFSF-2 (TNFα), CCL-8, CXCL-1, and CXCL-10 mRNA. The identification of these sensitive individuals serves as a warning of the potential toxicity of superagonistic anti-CD28 antibody as a drug candidate. The results also demonstrate that ex vivo mRNA analysis is an interesting assay model that can serve as a bridge from preclinical research to clinical trials.

Fig. 1. Drug-induced leukocyte mRNA in whole blood. * Address correspondence to this author at: Institute of Clinical Chemistry and Pathobiology, RWTH-University Hospital Aachen, Germany. Fax 49-241-80-82512; e-mail rweiskirchen@ukachen.de.

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Ex Vivo Simulation of Drug Action: Quantification of Drug-Induced mRNA as a Bridge Between Preclinical and Clinical Trials

To the Editor:

The recent tragedy, in which all active drug recipients developed life-threatening multiple organ failure during the phase I clinical trial of...
Antenatal Biochemical Expression of Cystinuria and Relation to Fetal Hyperechogenic Colon

To the Editor:

Cystinuria is an inherited disorder characterized by impaired apical transport of cystine and dibasic amino acids (e.g., ornithine, lysine, and arginine) in the renal proximal tubule and the small intestine epithelia. The overall estimated prevalence is 1/7000 neonates. Because of impaired renal cystine reabsorption, cystine precipitates and forms calculi that can produce urinary tract obstruction and may lead to renal insufficiency. Cystinuria is responsible for ~10% of all kidney stones observed in children; in ~50% of patients, stones form in the first decade of life. Diagnosis allows introduction of therapy to reduce stone formation and risk of renal impairment. Prenatal biochemical expression of the disease has not been described.

Fetal hyperechogenic small bowel (FHB), an infrequent ultrasound finding (0.1%–1.8% of pregnancies), is associated with severe fetal diseases such as cystic fibrosis and trisomy 21. Even less common is fetal hyperechogenic colon (FHC) in which the hyperechogenicity is strictly limited to the colon. Recently, it has been suggested that FHC could be associated with cystinuria.

We found biochemical prenatal evidence of cystinuria and confirmed the association of cystinuria with FHC.

We retrospectively studied the clinical records of 782 pregnancies for which amniotic fluid (AF) was sent to our laboratory between January 2003 and May 2006 for biochemical investigation, because of digestive tract abnormalities (hyperechogenic bowel, dilated loops, and peritonitis) detected by routine ultrasound scan. Of them, 6 presented with FHC and 12 with FHB. We defined 3 groups: the 6 FHC cases (group 1), 12 FHB cases randomly selected from 175 (group 2), and a 3rd-trimester control group (group 3) consisting of 12 randomly selected AF samples from pregnancies followed for unrelated malformations detected at routine ultrasound scan. Informed consent was obtained for AF sampling in all cases.

Samples were centrifuged (10 000 g, 5 min at 4 °C) and divided into 2 aliquots, 1 of which was immediately stored at −80 °C, while the other was assayed for digestive enzymes. The frozen aliquot was analyzed by ion-exchange amino acid chromatography using an Aminotac analyzer (Jeol) after deproteinization by 10-fold dilution in 200 g/L sulfosalicylic acid.

Concentrations of half-cystine, lysine, ornithine, and arginine in AF were within the previously