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Data in Brief

Gene expression profiling of Gram-negative bacteria-induced inflammation in human whole blood: The role of complement and CD14-mediated innate immune response

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ABSTRACT

Non-sterile pathogen-induced sepsis and sterile inflammation like in trauma or ischemia–reperfusion injury may both coincide with the life threatening systemic inflammatory response syndrome and multi-organ failure. Consequently, there is an urgent need for specific biomarkers in order to distinguish sepsis from sterile conditions. The overall aim of this study was to uncover putative sepsis biomarkers and biomarker pathways, as well as to test the efficacy of combined inhibition of innate immunity key players complement and Toll-like receptor co-receptor CD14 as a possible therapeutic regimen for sepsis. We performed whole blood gene expression analyses using microarray in order to profile Gram-negative bacteria-induced inflammatory responses in an *ex vivo* human whole blood model. The experiments were performed in the presence or absence of inhibitors of complement proteins (C3 and CD88 (C5a receptor 1)) and CD14, alone or in combination. In addition, we used blood from a C5-deficient donor. Anti-coagulated whole blood was challenged with heat-inactivated *Escherichia coli* for 2 h, total RNA was isolated and microarray analyses were performed on the Affymetrix GeneChip Gene 1.0 ST Array platform. The initial experiments were performed in duplicates using blood from two healthy donors. C5-deficiency is very rare, and only one donor could be recruited. In order to increase statistical power, a technical replicate of the C5-deficient samples was run. Subsequently, $\log_2$-transformed intensities were processed by robust multichip analysis and filtered using a threshold of four. In total, 73 microarray chips were run and analyzed. The normalized and filtered raw data have been deposited in NCBI's Gene Expression Omnibus (GEO) and are accessible with GEO Series accession number GSE55537. Linear models for microarray data were applied to estimate fold changes between data sets and the respective multiple testing adjusted *p*-values (FDR *q*-values). The interpretation of the data has been published by Lau et al. in an open access article entitled "CD14 and Complement Crosstalk and Largely Mediate the Transcriptional Response to *Escherichia coli* in Human Whole Blood as revealed by DNA Microarray" (Lau et al., 2015).

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Specifications	
Organism/cell line/tissue	Human whole blood (from healthy blood donors and a C5-deficient patient)
Sex	Male and female
Sequencer or array type	Affymetrix Human Gene 1.0 ST Array (GEO platform GPL6244)
Data format	Normalized $\log_2$-transformed signal intensities (CEL files)
Experimental factors	Stimulation (120 min, 37 °C) with heat-inactivated *Escherichia coli* strain LE392 versus PBS in presence or absence of inhibitors of C3, CD14, and C5aR1 (CD88)
Experimental features	Ex vivo human whole blood model of inflammation followed by whole blood RNA isolation and microarray analysis (including cDNA synthesis). Signal values (SV) from all chips were log-transformed ($\log_2$), normalized using Robust Multichip Analysis, and filtered using a threshold of $\log_2 SV = 4$.
Consent	A written informed consent was obtained from all blood donors before participation. The study protocol was approved by the Regional Ethical Committee (REC) of the Northern and South-Eastern Norway Regional Health Authorities.
Sample source location	Bodø, Norway

1. Direct link to deposited data

The normalized and filtered $\log_2$-transformed intensities are available here: http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE55537.

2. Experimental design, materials and methods

2.1. Ex vivo model of Gram-negative bacteria-induced inflammation in human whole blood

Venous blood from two healthy control donors (Ctrl, Ctrl2) and one complement factor 5 (C5)-deficient individual (C5D) was drawn into sterile 5 mL cryo tube vials (Nunc, Roskilde, Denmark) (4 mL blood per vial) containing the highly specific thrombin inhibitor lepirudin (Refludan®, Pharmion, Copenhagen, Denmark) at a final concentration of 50 μg/mL. Subsequently, the fresh anti-coagulated blood samples were divided into 1.8 mL Nunc cryo tube vials containing complement- and CD14 inhibitors or inhibitor controls at final concentrations of 25 μM compstatin (Ac-I[CV(1MeW) QDWGAHRC]T) (kindly provided by Prof. John Lambris), 10 μM CD88 (C5aR1)-specific C5a receptor antagonist (C5aR antagonist) AvF[OPdChaWR] (kindly provided by Prof. John Lambris) and 50 μg/mL anti-human CD14 $F(ab')_2$ antibody fragment (clone 18D11) (Diatec AS, Oslo, Norway). As inhibitor control, a cocktail consisting of a control peptide (25 μM; kindly provided by Prof. John Lambris) and a control $F(ab')_2$ (50 μg/mL; clone BH1) (Diatec AS, Oslo, Norway) was used. After preincubation for 7 min at 37 °C on a heating block, 1×10^6/mL (day 1, D1) or 5×10^6/mL (day 2, D2) heat-inactivated *Escherichia coli* strain LE392 (ATCC 33572, Manassas, VA) or phosphate buffered saline (PBS) containing calcium and magnesium (Sigma, St. Louis, MO) were added, and incubation was proceeded for 120 min at 37 °C on a rock'n'roller. The ratio between the volumes for inhibitor, blood and activator per sample was 1:5:1. The inflammatory reaction was stopped by the addition of 10 mM EDTA (pH 8.0). Experiments with C5D blood were set up twice, one set was performed with C5D blood and the other with C5D blood reconstituted with 80 μg/mL purified recombinant human C5 (Quidel, San Diego, CA) (C5DR) prior to preincubation with inhibitors or PBS. Importantly, the Nunc cryo tube vials used here are not entirely biocompatible, which results in a weak bacteria-independent inflammatory response during the two-hour incubation. As a reference for this background activation, we included an additional unstimulated sample for both healthy blood donors (Ctrl, Ctrl2) and C5D, which was terminated at time point zero after preincubation (initial state, T0).

The *ex vivo* model of inflammation, project-specific modifications and a detailed description of the blood donors have been published earlier [1–3]. The blood donors were adult male (Ctrl2) and female (Ctrl and C5D) Scandinavians, of whom two (Ctrl2 and C5D) displayed functionally equivalent genetic deficiencies in mannose binding lectin (MBL). MBL is involved in the lectin pathway of complement activation. Genetic variations in the MBL2 gene are very frequent, and their clinical manifestations are debated. In our model, MBL deficiency had no impact on the inflammatory responses tested earlier [2].

2.2. Sample preparation for microarray analysis

Immediately after termination of the whole blood model experiments, 3 mL 1× nucleic acid purification lysis buffer (Life Technologies, Applied Biosystems™, Foster City, CA, USA; PN4305895) were added per 2 mL sample. Total RNA isolation was performed batch-wise with 16 to 18 samples per plate following the standard procedure for ABI PRISM™ 6100 Nucleic Acid PrepStation using Applied Biosystems AB6100™ total RNA chemistry. Total RNA was recovered in 150 μL nucleic acid purification elution solution (Applied Biosystems™; PN4305893) per well. For further purification, the RNA was precipitated overnight at −70 °C in the presence of 2.5 volumes ethanol (96%) and 10% 3 M sodium acetate, before it was washed in 70% ethanol, air-dried and recovered in 60 μL elution solution. Subsequently, RNA quality was approved using a 2100 Bioanalyzer (Agilent) and RNA concentration was determined using a Nanodrop system (Thermo). The average RNA integrity number was estimated to be 8.8, and the average RNA yield was 2.2 μg per mL venous blood.

2.3. DNA microarray analysis

The total experimental setup involved three independent main series (Ctrls, C5D, C5DR) consisting of four or two biological replicates per series (two Ctrls × two days; one C5D × two days; one C5DR × two days) and seven experimental conditions per replicate (PBS, *E. coli*, *E. coli* + inhibitors of C3 and CD14, alone or in combination, inhibitor of C5aR1, or inhibitor controls). In addition to three initial state controls (one for each individual: Ctrl, Ctrl2, C5D), this gave rise to a total of 59 samples. Due to repeated microarray analysis (technical replicate) of all D1 samples containing C5D or C5DR blood, a total of 73 arrays were run and data sets are available (for a summarized overview see Table 1).

For microarray analysis, 150 ng total RNA of each sample, in concentrations of minimum 50 ng/μL, was subjected to cDNA synthesis and amplification followed by in vitro transcription, clean-up and labeling using Affymetrix® GeneChip® Whole Transcript (WT) Sense Target Labelling Assay (Manual: P/N701880 Rev.4). cDNA was hybridized with arrays of the GeneChip Gene 1.0 ST Array (Affymetrix) platform (Gene Expression Omnibus platform ID: GPL6244). Staining and washing of the arrays was performed using the GeneChip Hybridization, Wash and Stain Kit (Affymetrix; P/N 900720) on the Fluidics Station 450 using protocol FS450_0007. For technical replicates of all day 1 samples of C5D and C5DR the here described procedure was performed in duplicates, starting with the same total RNA.

Scanned images of the arrays were processed using AGCC (Affymetrix GeneChip Command Console) software. For further analysis, the Affymetrix CEL files (containing probe intensities) were imported into the Partek Genomics Suite software (Partek, Inc. MO, USA). Expression data were normalized, background corrected and summarized yielding normalized, $\log_2$-transformed signal intensities using the Robust Multichip Analysis (RMA) algorithm implemented in Partek Genomic Suit software. Finally, the intensities were filtered using a threshold of $\log_2$ intensity = 4.

Table 1
List of microarray data sets generated by this study.

GEO sample ID	Sample title	CEL file	Protocol	Scan date
GSM1338877	C5DR_anti-CD14 + Compstatin_D1	10_C5_D1.CEL	C5 deficient, C5-reconstituted, 1 × E06/mL *E. coli*, combined C3 and CD14 inhibition, 120 min, day1, replicate 1	6/5/2010
GSM1338878	C5DR_anti-CD14 + Compstatin_D1_No2	10_C5_D1_Nr2.CEL	C5 deficient, C5-reconstituted, 1 × E06/mL *E. coli*, combined C3 and CD14 inhibition, 120 min, day 1, replicate 2	6/3/2010
GSM1338879	C5DR_anti-CD14 + Compstatin_D2	10_C5_D2.CEL	C5 deficient, C5-reconstituted, 5 × E06/mL *E. coli*, combined C3 and CD14 inhibition, 120 min, day 2	5/13/2010
GSM1338859	C5D_C5aR antagonist_D1	11_C5_D1.CEL	C5 deficient, 1 × E06/mL *E. coli*, C5aR-inhibition, 120 min, day 1, replicate 1	6/5/2010
GSM1338860	C5D_C5aR antagonist_D1_No2	11_C5_D1_Nr2.CEL	C5 deficient, 1 × E06/mL *E. coli*, C5aR-inhibition, 120 min, day 1, replicate 2	6/3/2010
GSM1338861	C5D_C5aR antagonist_D2	11_C5_D2.CEL	C5 deficient, 5 × E06/mL *E. coli*, C5aR-inhibition, 120 min, day 2	5/13/2010
GSM1338880	C5DR_C5aR antagonist_D1	12_C5_D1.CEL	C5 deficient, C5-reconstituted, 1 × E06/mL *E. coli*, C5aR-inhibition, 120 min, day 1, replicate 1	6/5/2010
GSM1338881	C5DR_C5aR antagonist_D1_No2	12_C5_D1_Nr2.CEL	C5 deficient, C5-reconstituted, 1 × E06/mL *E. coli*, C5aR-inhibition, 120 min, day 1, replicate 2	6/3/2010
GSM1338882	C5DR_C5aR antagonist_D2	12_C5_D2.CEL	C5 deficient, C5-reconstituted, 5 × E06/mL *E. coli*, C5aR-inhibition, 120 min, day 2	5/13/2010
GSM1338862	C5D_inhibitor ctrl_D1	13_C5_D1.CEL	C5 deficient, 1 × E06/mL *E. coli*, inhibitor controls, 120 min, day 1, replicate 1	6/5/2010
GSM1338883	C5DR_inhibitor ctrl_D1	14_C5_D1.CEL	C5 deficient, C5-reconstituted, 1 × E06/mL *E. coli*, inhibitor controls, 120 min, day 1, replicate 1	6/5/2010
GSM1338863	C5D_inhibitor ctrl_D1_No2	15_C5_D1_Nr2.CEL	C5 deficient, 1 × E06/mL *E. coli*, inhibitor controls, 120 min, day 1, replicate 2	6/3/2010
GSM1338864	C5D_inhibitor ctrl_D2	15_C5_D2.CEL	C5 deficient, 5 × E06/mL *E. coli*, inhibitor controls, 120 min, day 2	5/13/2010
GSM1338884	C5DR_inhibitor ctrl_D1_No2	16_C5_D1_Nr2.CEL	C5 deficient, C5-reconstituted, 1 × E06/mL *E. coli*, inhibitor controls, 120 min, day 1, replicate 2	6/3/2010
GSM1338885	C5DR_inhibitor ctrl_D2	16_C5_D2.CEL	C5 deficient, C5-reconstituted, 5 × E06/mL *E. coli*, inhibitor controls, 120 min, day 2	5/13/2010
GSM1338816	ctrl2_PBS_D1	17_C2_D1.CEL	Healthy donor 2, PBS, 120 min, day 1	5/20/2010
GSM1338817	ctrl2_PBS_D2	17_C2_D2.CEL	Healthy donor 2, PBS, 120 min, day 2	5/28/2010
GSM1338818	ctrl_PBS_D1	17_C5_D1.CEL	Healthy donor 1, PBS, 120 min, day 1	5/20/2010
GSM1338819	ctrl_PBS_D2	17_C5_D2.CEL	Healthy donor 1, PBS, 120 min, day 2	5/28/2010
GSM1338820	ctrl2_E.coli_D1	18_C2_D1.CEL	Healthy donor 2, 1 × E06/mL *E. coli*, uninhibited, 120 min, day 1	5/20/2010
GSM1338821	ctrl2_E.coli_D2	18_C2_D2.CEL	Healthy donor 2, 5 × E06/mL *E. coli*, uninhibited, 120 min, day 2	5/28/2010
GSM1338822	ctrl_E.coli_D1	18_C5_D1.CEL	Healthy donor 1, 1 × E06/mL *E. coli*, uninhibited, 120 min, day 1	5/20/2010
GSM1338823	ctrl_E.coli_D2	18_C5_D2.CEL	Healthy donor 1, 5 × E06/mL *E. coli*, uninhibited, 120 min, day 2	5/28/2010
GSM1338824	ctrl2_Compstatin_D1	19_C2_D1.CEL	Healthy donor 2, 1 × E06/mL *E. coli*, C3-inhibition, 120 min, day 1	5/20/2010
GSM1338825	ctrl2_Compstatin_D2	19_C2_D2.CEL	Healthy donor 2, 5 × E06/mL *E. coli*, C3-inhibition, 120 min, day 2	5/28/2010
GSM1338826	ctrl_Compstatin_D1	19_C5_D1.CEL	Healthy donor 1, 1 × E06/mL *E. coli*, C3-inhibition, 120 min, day 1	5/20/2010
GSM1338827	ctrl_Compstatin_D2	19_C5_D2.CEL	Healthy donor 1, 5 × E06/mL *E. coli*, C3-inhibition, 120 min, day 2	5/28/2010
GSM1338844	C5D_PBS_D1	1_C5_D1.CEL	C5 deficient, PBS, 120 min, day 1, replicate 1	6/5/2010
GSM1338845	C5D_PBS_D1_No2	1_C5_D1_Nr2.CEL	C5 deficient, PBS, 120 min, day 1, replicate 2	6/3/2010
GSM1338846	C5D_PBS_D2	1_C5_D2.CEL	C5 deficient, PBS, 120 min, day 2	5/13/2010
GSM1338828	ctrl2_anti-CD14_D1	20_C2_D1.CEL	Healthy donor 2, 1 × E06/mL *E. coli*, CD14-inhibition, 120 min, day 1	5/20/2010
GSM1338829	ctrl2_anti-CD14_D2	20_C2_D2.CEL	Healthy donor 2, 5 × E06/mL *E. coli*, CD14-inhibition, 120 min, day 2	5/28/2010
GSM1338830	ctrl_anti-CD14_D1	20_C5_D1.CEL	Healthy donor 1, 1 × E06/mL *E. coli*, CD14-inhibition, 120 min, day 1	5/20/2010
GSM1338831	ctrl_anti-CD14_D2	20_C5_D2.CEL	Healthy donor 1, 5 × E06/mL *E. coli*, CD14-inhibition, 120 min, day 2	5/28/2010
GSM1338832	ctrl2_anti-CD14 + Compstatin_D1	21_C2_D1.CEL	Healthy donor 2, 1 × E06/mL *E. coli*, combined C3 and CD14 inhibition, 120 min, day 1	5/20/2010
GSM1338833	ctrl2_anti-CD14 + Compstatin_D2	21_C2_D2.CEL	Healthy donor 2, 5 × E06/mL *E. coli*, combined C3 and CD14 inhibition, 120 min, day 2	5/28/2010
GSM1338834	ctrl_anti-CD14 + Compstatin_D1	21_C5_D1.CEL	Healthy donor 1, 1 × E06/mL *E. coli*, combined C3 and CD14 inhibition, 120 min, day 1	5/20/2010
GSM1338835	ctrl_anti-CD14 + Compstatin_D2	21_C5_D2.CEL	Healthy donor 1, 5 × E06/mL *E. coli*, combined C3 and CD14 inhibition, 120 min, day 2	5/28/2010
GSM1338836	ctrl2_C5aR antagonist_D1	22_C2_D1.CEL	Healthy donor 2, 1 × E06/mL *E. coli*, C5aR-inhibition, 120 min, day 1	5/20/2010
GSM1338837	ctrl2_C5aR antagonist_D2	22_C2_D2.CEL	Healthy donor 2, 5 × E06/mL *E. coli*, C5aR-inhibition, 120 min, day 2	5/28/2010
GSM1338838	ctrl_C5aR antagonist_D1	22_C5_D1.CEL	Healthy donor 1, 1 × E06/mL *E. coli*, C5aR-inhibition, 120 min, day 1	5/20/2010
GSM1338839	ctrl_C5aR antagonist_D2	22_C5_D2.CEL	Healthy donor 1, 5 × E06/mL *E. coli*, C5aR-inhibition, 120 min, day 2	5/28/2010
GSM1338840	ctrl2_inhibitor ctrl_D1	24_C2_D1.CEL	Healthy donor 2, 1 × E06/mL *E. coli*, inhibitor controls, 120 min, day 1	5/20/2010
GSM1338841	ctrl2_inhibitor ctrl_D2	24_C2_D2.CEL	Healthy donor 2, 5 × E06/mL *E. coli*, inhibitor controls, 120 min, day 2	5/28/2010
GSM1338842	ctrl_inhibitor ctrl_D1	24_C5_D1.CEL	Healthy donor 1, 1 × E06/mL *E. coli*, inhibitor controls, 120 min, day 1	5/20/2010
GSM1338843	ctrl_inhibitor ctrl_D2	24_C5_D2.CEL	Healthy donor 1, 5 × E06/mL *E. coli*, inhibitor controls, 120 min, day 2	5/28/2010
GSM1338865	C5DR_PBS_D1	2_C5_D1.CEL	C5 deficient, C5-reconstituted, PBS, 120 min, day 1, replicate 1	6/5/2010
GSM1338866	C5DR_PBS_D1_No2	2_C5_D1_Nr2.CEL	C5 deficient, C5-reconstituted, PBS, 120 min, day 1, replicate 2	6/3/2010
GSM1338867	C5DR_PBS_D2	2_C5_D2.CEL	C5 deficient, C5-reconstituted, PBS, 120 min, day 2	5/13/2010
GSM1338847	C5D_E.coli_D1	3_C5_D1.CEL	C5 deficient, 1 × E06/mL *E. coli*, uninhibited, 120 min, day 1, replicate 1	6/5/2010
GSM1338848	C5D_E.coli_D1_No2	3_C5_D1_Nr2.CEL	C5 deficient, 1 × E06/mL *E. coli*, uninhibited, 120 min, day 1, replicate 2	6/3/2010
GSM1338849	C5D_E.coli_D2	3_C5_D2.CEL	C5 deficient, 5 × E06/mL *E. coli*, uninhibited, 120 min, day 2	5/13/2010
GSM1338868	C5DR_E.coli_D1	4_C5_D1.CEL	C5 deficient, C5-reconstituted, 1 × E06/mL *E. coli*, uninhibited, 120 min, day 1, replicate 1	6/5/2010
GSM1338869	C5DR_E.coli_D1_No2	4_C5_D1_Nr2.CEL	C5 deficient, C5-reconstituted, 1 × E06/mL *E. coli*, uninhibited, 120 min, day 1, replicate 2	6/3/2010
GSM1338870	C5DR_E.coli_D2	4_C5_D2.CEL	C5 deficient, C5-reconstituted, 5 × E06/mL *E. coli*, uninhibited, 120 min, day 2	5/13/2010
GSM1338850	C5D_Compstatin_D1	5_C5_D1.CEL	C5 deficient, 1 × E06/mL *E. coli*, C3-inhibition, 120 min, day 1, replicate 1	6/5/2010
GSM1338851	C5D_Compstatin_D1_No2	5_C5_D1_Nr2.CEL	C5 deficient, 1 × E06/mL *E. coli*, C3-inhibition, 120 min, day 1, replicate 2	6/3/2010
GSM1338852	C5D_Compstatin_D2	5_C5_D2.CEL	C5 deficient, 5 × E06/mL *E. coli*, C3-inhibition, 120 min, day 2	5/13/2010
GSM1338871	C5DR_Compstatin_D1	6_C5_D1.CEL	C5 deficient, C5-reconstituted, 1 × E06/mL *E. coli*, C3-inhibition, 120 min, day 1, replicate 1	6/3/2010
GSM1338872	C5DR_Compstatin_D1_No2	6_C5_D1_Nr2.CEL	C5 deficient, C5-reconstituted, 1 × E06/mL *E. coli*, C3-inhibition, 120 min, day 1, replicate 2	6/3/2010
GSM1338873	C5DR_Compstatin_D2	6_C5_D2.CEL	C5 deficient, C5-reconstituted, 5 × E06/mL *E. coli*, C3-inhibition, 120 min, day 2	5/13/2010
GSM1338853	C5D_anti-CD14_D1	7_C5_D1.CEL	C5 deficient, 1 × E06/mL *E. coli*, CD14-inhibition, 120 min, day 1, replicate 1	6/5/2010

Table 1 (*continued*)

GEO sample ID	Sample title	CEL file	Protocol	Scan date
GSM1338854	C5D_anti-CD14_D1_No2	7_C5_D1_Nr2.CEL	C5 deficient, 1 × E06/mL *E. coli*, CD14-inhibition, 120 min, day 1, replicate 2	6/3/2010
GSM1338855	C5D_anti-CD14_D2	7_C5_D2.CEL	C5 deficient, 5 × E06/mL *E. coli*, CD14-inhibition, 120 min, day 2	5/13/2010
GSM1338874	C5DR_anti-CD14_D1	8_C5_D1.CEL	C5 deficient, C5-reconstituted, 1 × E06/mL *E. coli*, CD14-inhibition, 120 min, day 1, replicate 1	6/5/2010
GSM1338875	C5DR_anti-CD14_D1_No2	8_C5_D1_Nr2.CEL	C5 deficient, C5-reconstituted, 1 × E06/mL *E. coli*, CD14-inhibition, 120 min, day 1, replicate 2	6/3/2010
GSM1338876	C5DR_anti-CD14_D2	8_C5_D2.CEL	C5 deficient, C5-reconstituted, 5 × E06/mL *E. coli*, CD14-inhibition, 120 min, day 2	5/13/2010
GSM1338856	C5D_anti-CD14 + Compstatin_D1	9_C5_D1.CEL	C5 deficient, 1 × E06/mL *E. coli*, combined C3 and CD14 inhibition, 120 min, day 1, replicate 1	6/5/2010
GSM1338857	C5D_anti-CD14 + Compstatin_D1_No2	9_C5_D1_Nr2.CEL	C5 deficient, 1 × E06/mL *E. coli*, combined C3 and CD14 inhibition, 120 min, day 1, replicate 2	6/3/2010
GSM1338858	C5D_anti-CD14 + Compstatin_D2	9_C5_D2.CEL	C5 deficient, 5 × E06/mL *E. coli*, combined C3 and CD14 inhibition, 120 min, day 2	5/13/2010
GSM1338886	ctrl2_T0_D2	K2_T0_Dag2.CEL	Healthy donor 2, PBS, 0 min, day 2	5/28/2010
GSM1338887	ctrl_T0_D2	K5_T0_Dag2.CEL	Healthy donor 1, PBS, 0 min, day 2	5/28/2010
GSM1338888	C5D_T0_D2	T0_Dag1_C5_D2.CEL	C5 deficient, PBS, 0 min, day 2	5/13/2010

The normalized and filtered $\log_2$ intensities of all passed transcripts ($n = 19{,}695$) have been used for downstream statistical analyses and deposited in NCBI's Gene Expression Omnibus (GEO) and are accessible with GEO Series accession number GSE55537 (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE55537).

2.4. Microarray quality control analyses

RMA normalized, unfiltered $\log_2$ intensities of all individual arrays ($n = 73$) were displayed in an intensity graph, which indicated consistency within the entire data set as well as limited technical variation between scan dates (Fig. 1). The same data were subjected to principle component analysis (PCA) using Partek Genomics Suite software. Most of the experimental and technical replicate arrays for the different experimental conditions clustered together in the PCA 3D scatter plots (Fig. 2A–C), indicating high reproducibility of (i) the samples, despite of the use of unmatched control donors and different *E. coli* concentrations, and (ii) the hybridization protocol. PCA plots were generated for the three series independently. Further, Pearson's correlation analyses were performed using normalized and filtered $\log_2$ intensities. The Pearson correlation coefficients (r) for each pair of arrays ranged from 0.88 to 0.99 and reflected the overall consistency, also of the filtered data set (Fig. 3).

The PCA plots revealed donor-specific clustering of arrays for Ctrls (Fig. 2A), which needed to be considered for downstream statistical analyses. Arrays for C5D (Fig. 2B) and C5DR (Fig. 2C) could be found in two separated clusters, where one of them contained the arrays of the CD14-inhibited response (anti-CD14), combined inhibited response (anti-CD14 + compstatin) and background activation (PBS), only. The same clustering was observed for Ctrls, when only a limited number of genes ($n = 7786$) was included according to an ANOVA, which removed the noise from non-significant differences (FDR q-value ≥0.1) between experimental conditions (not shown). This observation is in agreement with the high efficacy of the combined inhibitory strategy in our model and the increased importance of CD14 in C5D compared to Ctrls [1].

Both, PCA and Pearson's correlation analysis revealed that the three initial state control samples taken at time point zero (T0; GSM1338886, GSM1338887, GSM1338888) as well as one single array of Ctrl2 (*ctrl2_C5aR antagonist_D1*; GSM1338836) correlated the least with any other array. However, all correlation coefficients were still high ($r \geq 0.88$), and none of the data sets were excluded. The low correlation of the initial state controls is likely due to (i) the lack of intrinsic activation

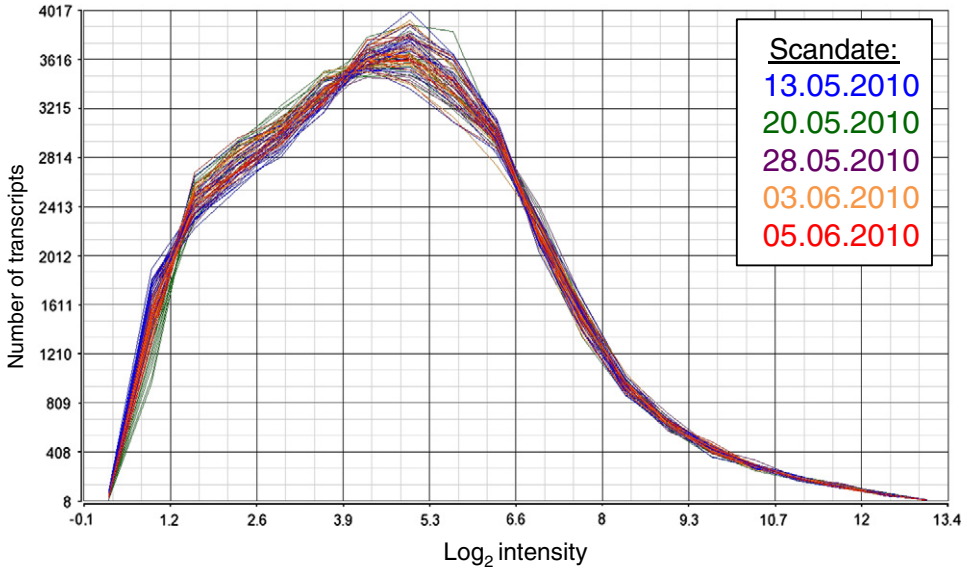

Fig. 1. The intensity graph shows RMA normalized, unfiltered $\log_2$ intensities for all 73 arrays. The arrays were scanned in batches on five different days. Arrays which were scanned at the same day are highlighted in the same color. See Table 1 for a detailed overview of the arrays and their scan dates.

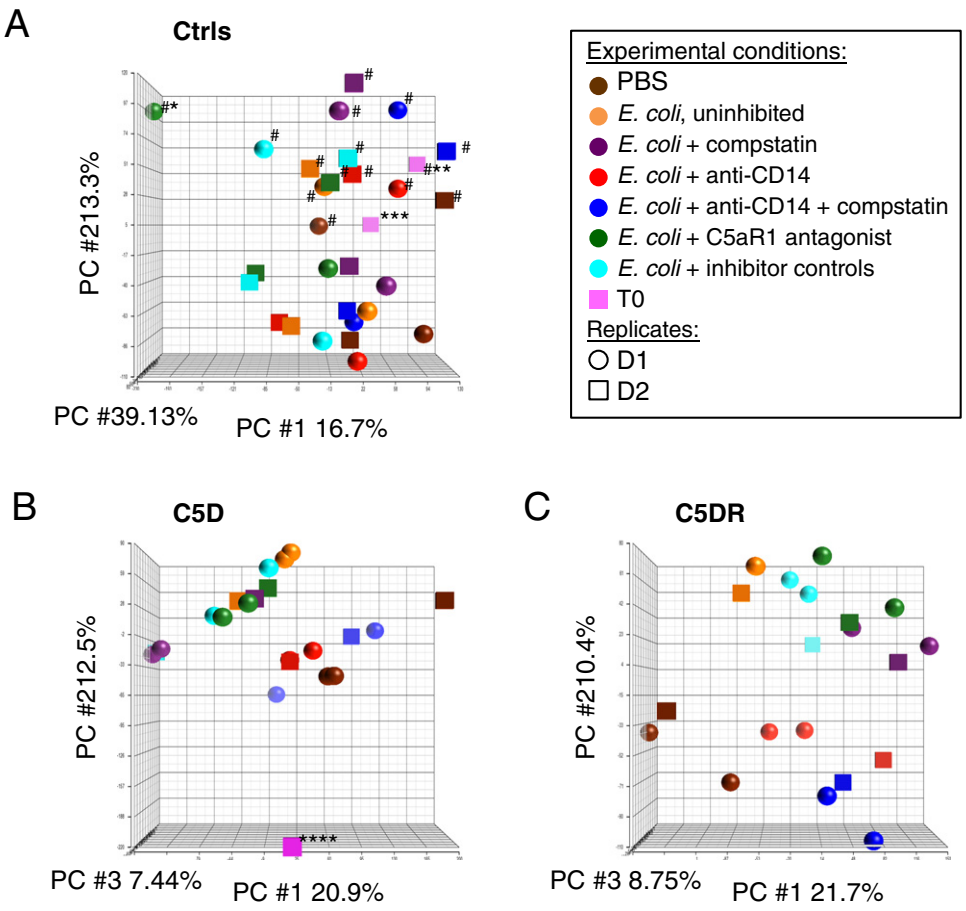

Fig. 2. Principal component analysis (PCA) 3D scatter plots were generated for all 73 arrays contained by the three series healthy blood donors (Ctrls) (A), C5-deficient (C5D) blood (B), and C5-reconstituted C5-deficient (C5DR) blood (C). Colors indicate experimental conditions, while symbols indicate replicates from day 1 (D1, circle) and day 2 (D2, rectangle). The replicates are either biological (two healthy blood donors, Ctrl and Ctrl2) (A) or technical (duplicate microarray analyses of D1 samples) (B and C). See Table 1 for a detailed overview of the arrays. #Ctrl2 samples, *potential outlier array (GSM1338836; *ctrl2_C5aR antagonist_D1*), **T0 sample of Ctrl 2 (GSM1338886), ***T0 sample of Ctrl (GSM1338887), ****T0 sample of C5D (GSM1338888).

in the samples taken at time point zero as compared to background levels at the end of the experiment, and (ii) the fact that those samples were kept for 2 h on ice prior to lysis, compared to immediate lysis of the remaining samples. Notably, good correlation was observed between the three T0 data sets (Fig. 3). No replicates were performed, here. The transcript intensities of the single outlier array (GSM1338836) correlated rather well with those of the three replicate samples (GSM1338837, GSM1338838, GSM1338839) as well as of the six samples from C5D and C5DR containing the same inhibitor (GSM1338859, GSM1338860, GSM1338861, GSM1338880, GSM1338881, GSM1338882) (Fig. 3).

The validity of the microarray data was proven by qPCR performed on selected genes using the same RNA material [1].

2.5. Downstream statistical analyses

For interpretation of the results, which have been published elsewhere [1], the normalized and filtered $\log_2$ intensities of the 19,695 passed transcripts and for all 73 arrays were subjected to downstream statistical analyses using linear models for microarray data (Limma Bioconductor) [4,5]. To correct for multiple testing, FDR q-values were computed from the p-values using the Benjamini–Hochberg method for controlling the false discovery rate (FDR) [6]. Genes with FDR q-values below 0.05 were considered to be significantly differentially expressed.

Statistical significance was determined for differential expression in (i) uninhibited (presence of *E. coli*) vs. background activation (absence of *E. coli*), and (ii) inhibited (presence of *E. coli* and inhibitors of C3 and CD14, alone or in combination, inhibitor of C5aR1, or inhibitor controls) vs. uninhibited activation for each of the three series (Ctrls, C5D, C5DR). Also, these data were compared across series (Ctrls vs. C5D, Ctrls vs. C5DR, and C5D vs. C5DR).

For the two healthy donors (Ctrl and Ctrl2), fold change expression estimates ($\log_2$FC) for each replicate were combined as follows. The estimates of day 1 (D1) and day 2 (D2) were pooled for each donor before the mean of both pools was calculated. For C5D or C5DR, fold change estimates were calculated from the pooled data of the technical replicates of D1 and from the data of D2, before the mean of both estimates was calculated. The technical replicate, which substituted for the lack of a biological one for C5D and C5DR, contributed to higher correlation between data sets compared to data sets from healthy donors, and thus to a higher statistical significance.

Of the 19,695 transcripts included in the analysis, 2335 had an FDR q-value below 0.05 for the uninhibited *E. coli* response vs. background activation in healthy controls, and were designated *E. coli*-responsive genes (*ERGs*). For all final analyses only the data sets for *ERGs* were tested for statistical significance.

For detailed tests of combined inhibitory versus single inhibitory effects, analysis of variance (ANOVA) was applied [1].

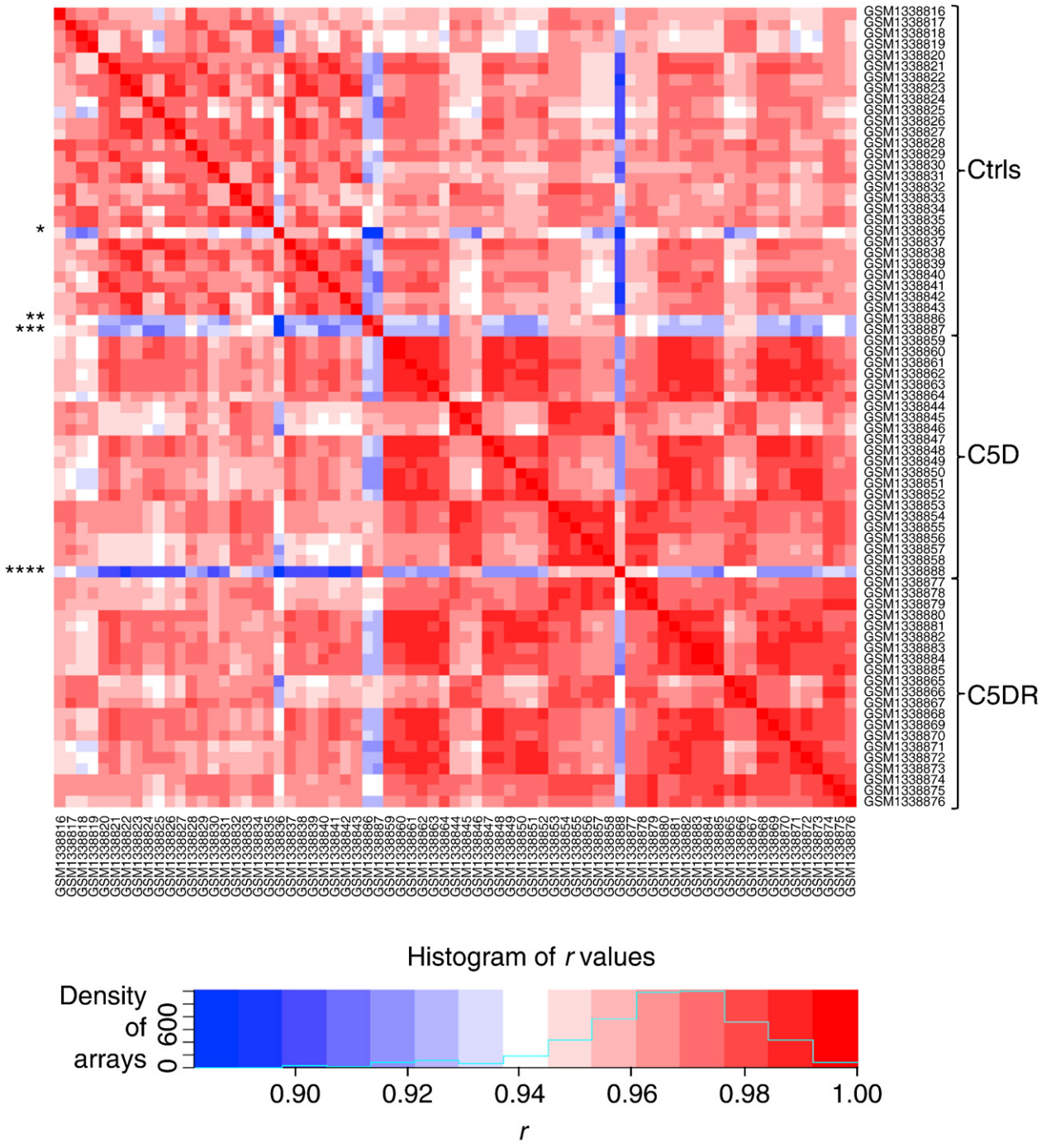

Fig. 3. Pearson correlation coefficients (r) were estimated for each pair of arrays using the RMA normalized, filtered $\log_2$ intensities of the 19,695 passed transcripts and all 73 arrays. The arrays are arranged according to the three series healthy donors (Ctrls), C5-deficient (C5D) and C5-reconstituted C5-deficient (C5DR). Arrays are indicated with their GEO depository sample IDs. See Table 1 for a detailed overview of the arrays. The heat map was generated using the heatmap.2 function in library gplots (http://cran.r-project.org/package=gplots). A histogram of r values is shown, with the density of arrays with respective r values indicated as a blue line. *Potential outlier array (GSM1338836; *ctrl2_C5aR antagonist_D1*), **T0 sample of Ctrl 2 (GSM1338886), ***T0 sample of Ctrl (GSM1338887), ****T0 sample of C5D (GSM1338888).

2.6. Study design control analyses

The replicate combination strategy for healthy controls was based on the observation that the inflammatory response was more dependent on the donor (male MBL defect versus female MBL sufficient) than on variations in *E. coli* concentrations used here. No significant *E. coli* concentration-dependent changes were found for healthy controls (FDR q-value <0.05), when pooled fold change data for uninhibited vs. background activation of D1 (1×10^6/mL) were compared to those of D2 (5×10^6/mL). In contrast, a reasonable but low number of responses were significantly donor-dependent, when uninhibited ($n = 94$) or combined C3/CD14 inhibited ($n = 96$) *E. coli* responses were compared between healthy donors (Fig. 4A). Background activation was more donor-dependent, with roughly 42% ($n = 992$) of all *ERGs* being differentially affected between the two healthy controls (Fig. 4A). However, only 10% ($n = 95 = 41 + 15 + 39$) of these genes were also donor-dependent in the presence of *E. coli*, either with or without combined inhibition, which were the most important experimental conditions

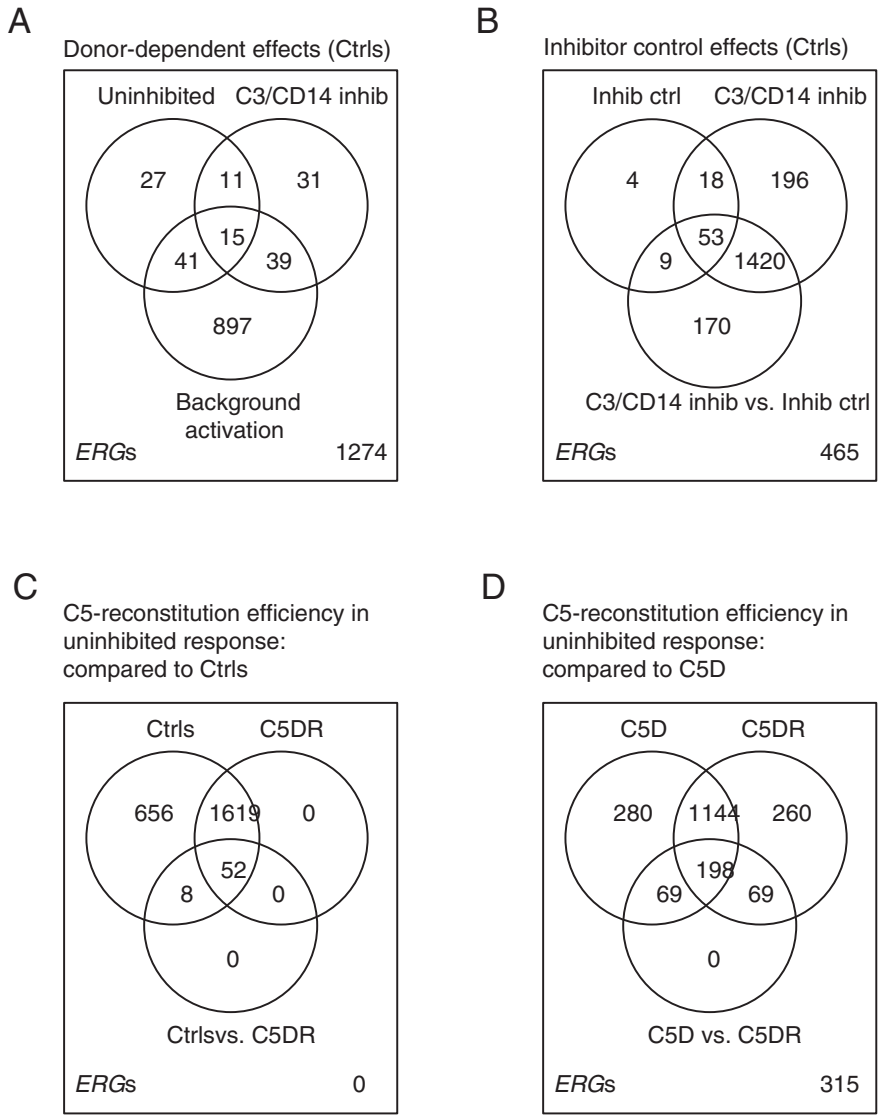

Fig. 4. Venn diagrams based on Limma-derived gene lists containing statistical significant fold changes (FDR q-value <0.05) for the indicated comparisons for the 2335 *E. coli*-responsive genes (*ERGs*). Numbers of *ERGs* with significant changes are displayed within circles while numbers of the remaining *ERGs* with non-significant changes are displayed in the lower right corner. A, Log_2 intensities of the 2335 *ERGs* contained by healthy control-specific (Ctrl or Ctrl2) CEL files for uninhibited *E. coli* response (*E. coli*) or initial state time point zero (T0) were compared to those for background activation in absence of *E. coli* (PBS) for each healthy control alone. In the same way, combined C3 and CD14 inhibition was compared to uninhibited response. Afterwards, significant differences were established between the two control donors for the three comparisons entitled *uninhibited*, *background activation*, and *C3/CD14 inhib*. B, *ERG* log_2 intensities contained by all healthy control (Ctrls) CEL files for inhibitor controls (inhibitor ctrl) and combined C3 and CD14 inhibition (anti-CD14 + Compstatin) were compared to those for the uninhibited response (*E. coli*). Afterwards, significant differences were established between the results of these analyses (*C3/CD14 Inhib* vs. *Inhib ctrl*). C and D, *ERG* log_2 intensities contained by CEL files for the uninhibited response (*E. coli*) were compared to those for background activation (PBS) for healthy donors (Ctrls), C5-deficient (C5D) and C5-reconstituted C5-deficient (C5DR), separately. Afterwards, significant differences were established between Ctrls and C5DR (C; *Ctrls* vs. *C5DR*) and C5D and C5DR (D; *C5D* vs. *C5DR*). See Table 1 for a detailed overview of the arrays and their CEL files.

tested in this study. In our study, these observed donor-dependent differences could be due to various factors including age and sex. Also, we cannot exclude that MBL deficiency affects the inflammatory response on the transcriptional level, although we have not seen alterations in functional read-outs [2].

Further, we controlled for non-specific effects induced by the administration of inhibitor molecules per se. Combined inhibition of C3 and CD14 led to significant differential expression of 1687 *ERGs* compared to the uninhibited response (Fig. 4B). Administration of the inhibitor control cocktail affected only 84 *ERGs*, most of which ($n = 53$) had significantly different effects than combined inhibition. Care must be taken when interpreting the results for the remaining 18 transcripts. Here, combined inhibition and control inhibition had almost indistinguishable effects, which were, however, rather minor (below two-fold compared to uninhibited) (not shown).

C5-reconstitution of C5D had earlier been shown by Lappegård et al. to fully restore C5-dependent functions like monocyte tissue factor and CD11b expression, phagocytosis and oxidative burst [2]. Importantly, C5-reconstitution of C5-deficient blood (C5DR) was functionally sufficient also on the gene expression level. C5DR resembled healthy donor samples (Ctrls) more than C5D when significant differences in gene expression between C5DR and Ctrls or C5D were estimated. For example, for the uninhibited response to *E. coli*, 60 and 336 *ERGs* responded significantly different in Ctrls compared to C5DR and C5D compared to C5DR, respectively (Fig. 4C and D).

3. Discussion

The here described comprehensive set of microarray data allows for (i) studying gene expression in the inflammatory response to Gram-

negative bacteria induced in an *ex vivo* human whole blood model, (ii) characterization of the role of innate immunity key components, i.e., complement and CD14, and (iii) identification of potential sepsis markers. Applying statistical analyses with multiple testing, we detected a reasonable large number of significant differences with interpretable fold changes. We found 2335 (12%) of all included 19,695 transcripts to respond significantly to treatment with *E. coli* in healthy donor blood, with 362 responding more than two-fold compared to untreated. Of the 2335 transcripts, 72% were affected by combined inhibition of C3 and CD14, which was the most efficient inhibition strategy tested. Using blood of a C5-deficient individual, either C5-reconstituted (C5DR) or not (C5D), shed light on the C5-dependency of the transcriptional response and the role of CD14 in another innate immunity key component-deficient background. Importantly, C5 deficiency is very rare. At the time point of the present study, the here involved C5-deficient individual was the only one known in Norway.

Despite a limited number of donors, three to four replicate data sets of each experimental condition were generated for three independent series (healthy donor, C5D, and C5DR) in order to strengthen the downstream statistical analyses. We suggest our general observations to be representative and indicative for subsequent studies. However, since transcriptional responses can in fact be donor-dependent, the general value for effects on single genes should be further tested by including more healthy donors. Microarray analysis is a high throughput approach, allowing for the study of more than 20,000 transcripts in various conditions, as long as high quality RNA can be retrieved. The here presented RNA profile reflects the reprogramming of blood cells in response to bacterial challenge. However, the response to such acute insults occurs not only on the level of transcription. On the contrary, the extension of the present study into the fields of more instant posttranscriptional and, most importantly, epigenetic regulations would further contribute to the overall understanding of innate immunity in systemic inflammation, either sterile or bacteria-induced.

Acknowledgments

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References

[1] C. Lau, S. Nygard, H. Fure, O.K. Olstad, M. Holden, K.T. Lappegard, O.L. Brekke, T. Espevik, E. Hovig, T.E. Mollnes, CD14 and complement crosstalk and largely mediate the transcriptional response to *Escherichia coli* in human whole blood as revealed by DNA microarray. PLoS ONE 10 (2015) e0117261.
[2] K.T. Lappegard, D. Christiansen, A. Pharo, E.B. Thorgersen, B.C. Hellerud, J. Lindstad, E.W. Nielsen, G. Bergseth, D. Fadnes, T.G. Abrahamsen, E.A. Hoiby, L. Schejbel, P. Garred, J.D. Lambris, M. Harboe, T.E. Mollnes, Human genetic deficiencies reveal the roles of complement in the inflammatory network: lessons from nature. Proc. Natl. Acad. Sci. U. S. A. 106 (2009) 15861–15866.
[3] T.E. Mollnes, O.L. Brekke, M. Fung, H. Fure, D. Christiansen, G. Bergseth, V. Videm, K.T. Lappegard, J. Kohl, J.D. Lambris, Essential role of the C5a receptor in E coli-induced oxidative burst and phagocytosis revealed by a novel lepirudin-based human whole blood model of inflammation. Blood 100 (2002) 1869–1877.
[4] R.C. Gentleman, V.J. Carey, D.M. Bates, B. Bolstad, M. Dettling, S. Dudoit, B. Ellis, L. Gautier, Y. Ge, J. Gentry, K. Hornik, T. Hothorn, W. Huber, S. Iacus, R. Irizarry, F. Leisch, C. Li, M. Maechler, A.J. Rossini, G. Sawitzki, C. Smith, G. Smyth, L. Tierney, J.Y. Yang, J. Zhang, Bioconductor: open software development for computational biology and bioinformatics. Genome Biol. 5 (2004) R80.
[5] G.K. Smyth, Linear models and empirical bayes methods for assessing differential expression in microarray experiments. Stat. Appl. Genet. Mol. Biol. 3 (2004) (Article3).
[6] Y. Benjamini, Y. Hochberg, Controlling the false discovery rate: a practical and powerful approach to multiple testing. J. R. Stat. Soc. Ser. B 57 (1995) 289–300.