EDI-Co gut, a human co-culture from intestinal epithelia and whole-blood used as test model to characterize the effects of microbial metabolites on immune-cell function

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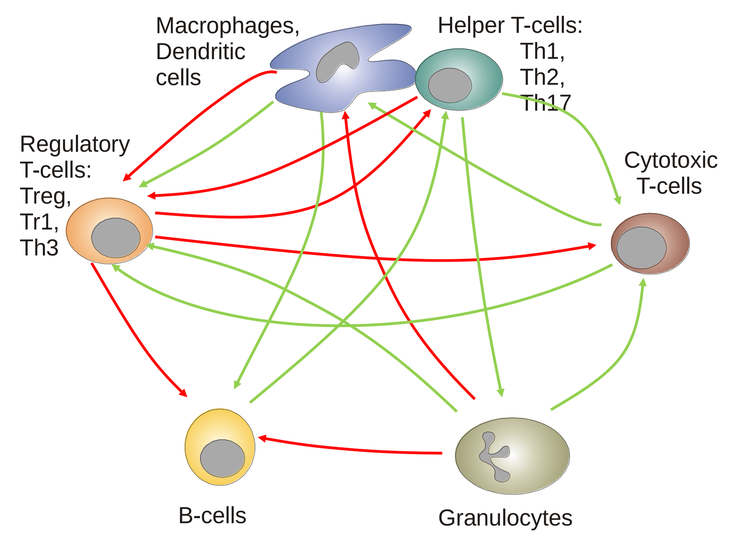
Background

Complex interaction of immunocompetent cells



The dialogue between cells of the immune system and cells of various tissues controls immune reactions and is in part mediated by a large variety of cytokines, chemokines, etc. This network may be strongly influenced by environmental factors, especially in the gut.

Microbial metabolites were tested for effects on cells of the human immune system in an organo-typical co-culture model of whole-blood and intestinal epithelial cells, normally used to examine drug effects. A differentiated gut epithelium (CaCo-2) in the upper compartment of a two-chamber system, was placed above fresh blood from healthy donors. Different short-chain fatty acids (SCFA: C2, C3, C4, C5, C6) were applied onto the "luminal" surface of the epithelia before the blood cells were activated to mimic inflammation.

Ca. 90 mediators and other parameters were tested in the supernatants of these cell cultures by means of a multiplexed bead assays (RBM MAP analysis).

Multi-Analyte Profiles (MAPs)

Cytokines

- **Th1-related:** Interferon-γ, Interleukin-12p40, Interleukin-12p70
- **Th2-related:** Interleukin-4, Interleukin-5, Interleukin-13
- **Treg-related:** Interleukin-10
- **Monocyte/MØ-related:** G-CSF, Interleukin-1 ra, Interleukin-1 α, Interleukin-1 β, Interleukin-6, Tumor Necrosis Factor-α
- **Others:** Epidermal Growth Factor, FGF-basic, GM-CSF, IGF-1, Stem Cell Factor, Tumor Necrosis Factor-β, Interleukin-2, Interleukin-3, Interleukin-7, Interleukin-15, Interleukin-16

Chemokines

ENA-78, Eotaxin, Interleukin-8, Lymphotactin, MCP-1, MDC, MIP-1 α, MIP-1 β, RANTES

Cancer antigens

Alpha-Fetoprotein, Cancer Antigen 19-9, Cancer Antigen 125, Carcinoembryonic Antigen, Prostatic Acid Phosphatase, PSA, Free

Cardio-/vascular disease related proteins

Apolipoprotein A-1, Apolipoprotein C-III, CK-MB, Endothelin-1, Fatty Acid BP, Lipoprotein (a), Myoglobin, PAPP-A

Enzymes

GST, MMP-2, MMP-3, MMP-9, Myeloperoxidase, SGOT

Hormones/BP

Adiponectin, Calcitonin, Erythropoietin, Growth Hormone, Insulin, Leptin, Sex Hormone Binding Globulin, Thrombopoietin, Thyroid Binding Globulin, Thyroid Stimulating Hormone

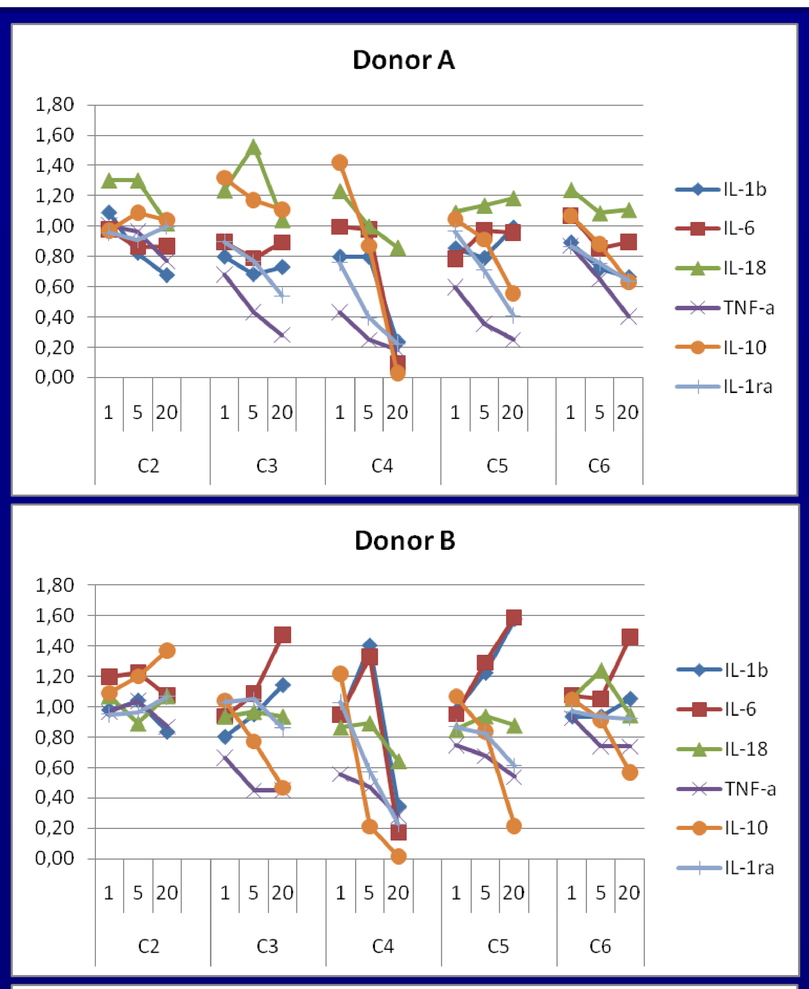
Receptors/Ligands

CD40, CD40Ligand, ICAM-1, Tissue Factor, Tumor Necrosis Factor RII, VCAM-1

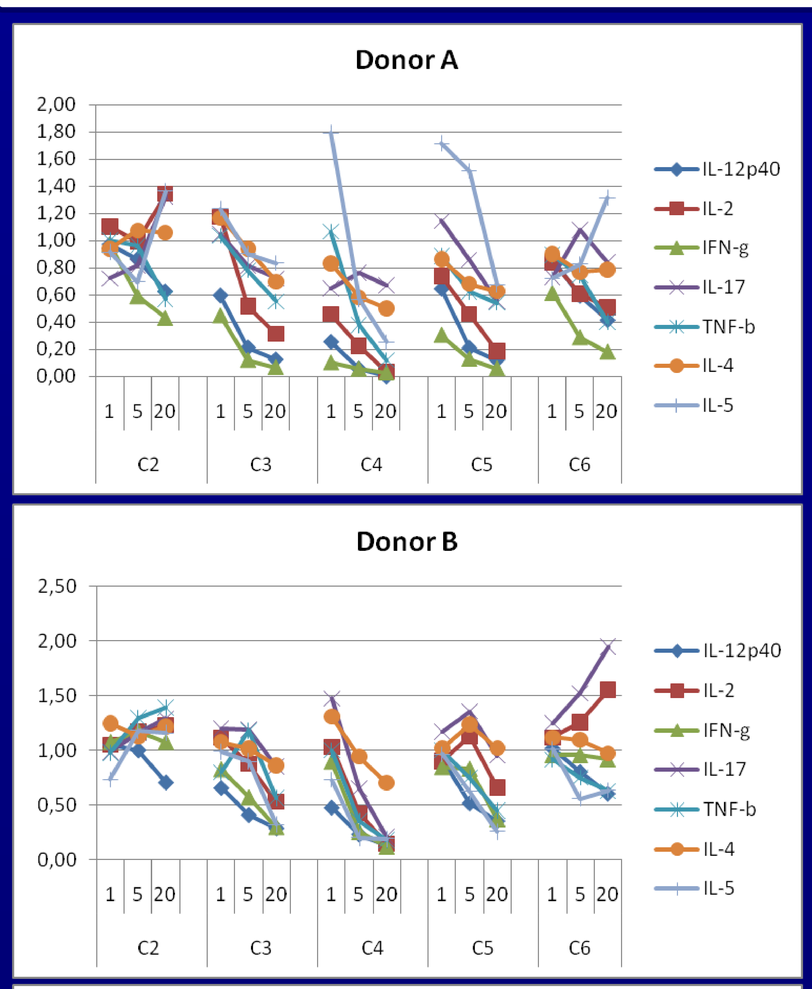
Others

Alpha-1 Antitrypsin, Alpha-2 Macroglobulin, Apolipoprotein H, BDNF, Beta-2 Microglobulin, Complement 3, C-Reactive Protein, ENRAGE, Ferritin, Haptoglobin, Serum Amyloid P, Immunoglobulin A, Immunoglobulin M, Immunoglobulin E, VEGF, Factor VII, Fibrinogen, von Willebrand Factor, PAI-1, TIMP-1

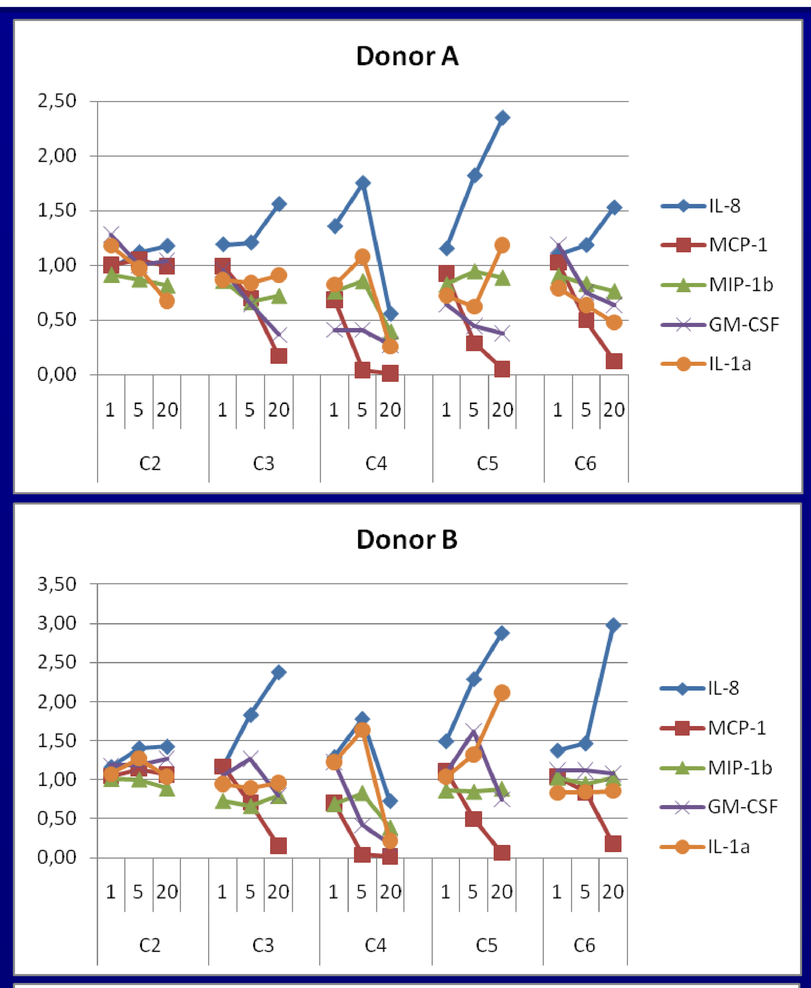
Monocyte/MΦ-related mediators



Th1/Th2-related mediators



Growth factors/epithelial cytokines



Results and Conclusions

SCFAs dose-dependently modulated a whole variety of pro-inflammatory mediators from either Th1 (T helper cell type 1) or Th2 cells, such as interferon (IFN)-gamma, interleukin (IL)-2, IL-12p40, IL-4, -5, but also tumour necrosis factor (TNF)-alpha, GM-CSF, or IL-18, respectively. Both, IL-10 and IL-1ra, as anti-inflammatory mediators were also down-regulated after 24h of co-culture. The strongest inhibition was seen with C4 SCFA (butyrate), one of the most abundant SCFA in the gut.

Only a few mediators were found to be upregulated, such as IL-6 in the presence of C5, or IL-8 in the presence of C3, C4, C5, and C6. Even IL-17 was found to be stimulated in the cultures of at least donors B and C with several SCFAs.

These regulatory properties of SCFAs are likely important to maintain the relative quiescence of the intestinal immune system despite the local microbial overload.

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