Universitätsmedizin GREIFSWALD

Klinik und Poliklinik für Frauenheilkunde und Geburtshilfe

Immune cells in crosstalk with the placental microbiome.

How *Fusobacterium nucleatum* affects macrophage function and its trophoblast interaction.

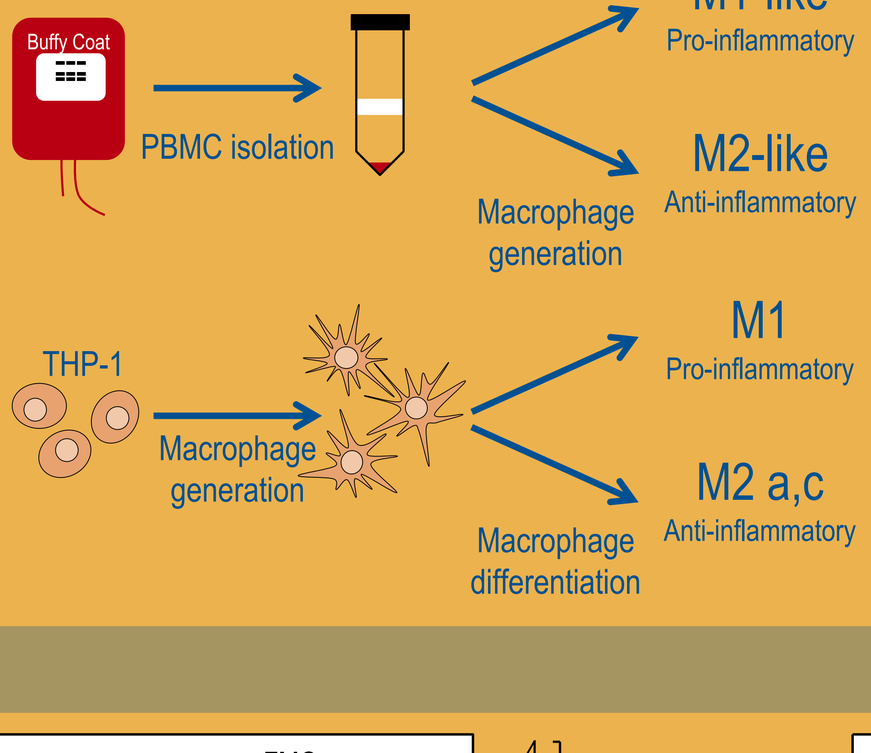
Einenkel R, Muzzio DO, Heusler M, Ehrhardt J, Zygmunt MT

Introduction

During early pregnancy leukocytes mediate trophoblast migration and invasion, thereby balancing between protection against infections and fetal rejection. The recent description of the placental microbiome raises new questions about their impact on the immune homeostasis at the fetal-maternal interface.

Fusobacterium (*F.*) *nucleatum* is one of the described species and shows immune-modulating effects in its association with colon carcinomas. Since macrophages are the second most common leukocytes at the fetal-maternal interface, we hypothesize that a regulatory effect of commensal *F. nucleatum* on macrophages may affect the immune balance in a beneficial way for pregnancy outcome.

Macrophage generation

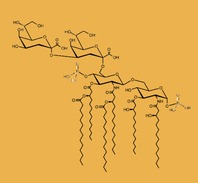


Activation

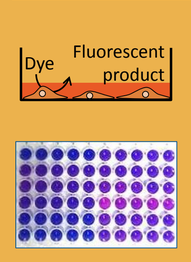
+ oxygen-inactivated *F. nucleatum*

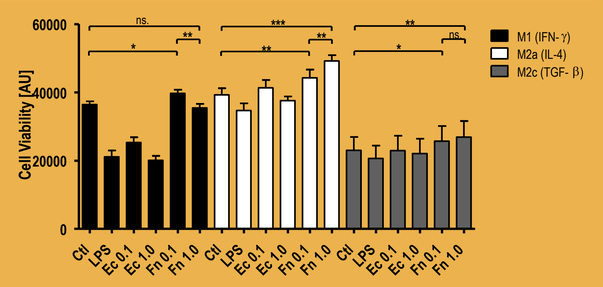
+ ethanol-inactivated *E. coli*

+ LPS

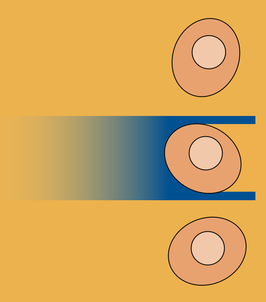


Cell viability assay

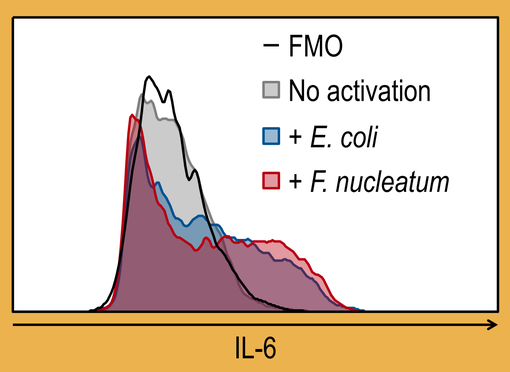


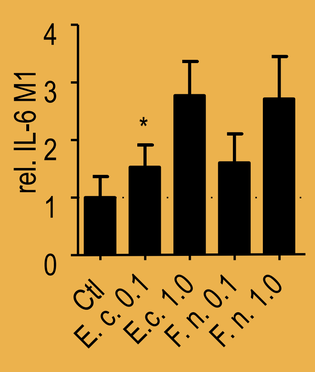


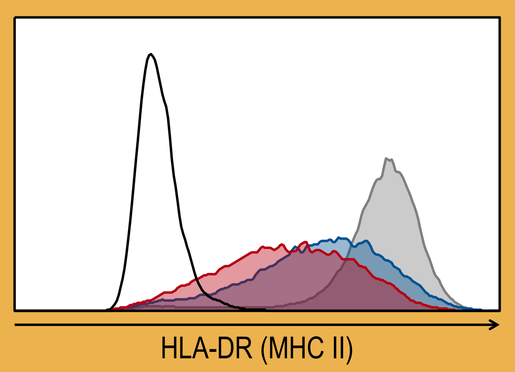
Flow cytometry

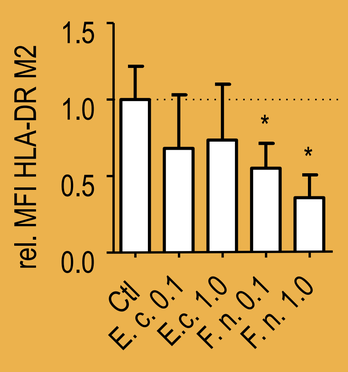


→ Size & granularity
→ Surface marker
→ Identification
→ Activation status
→ Intracellular staining
→ Expression products
→ Compartments

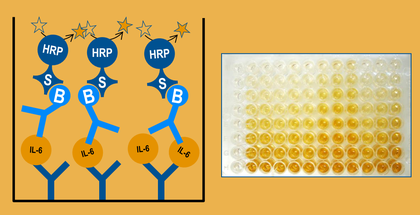


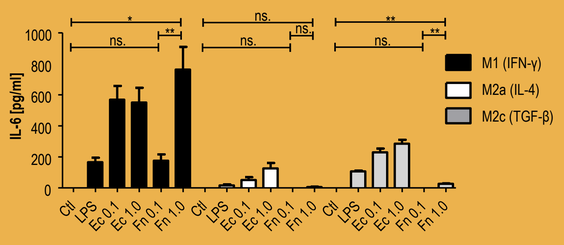




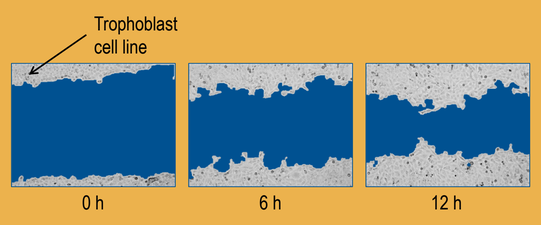


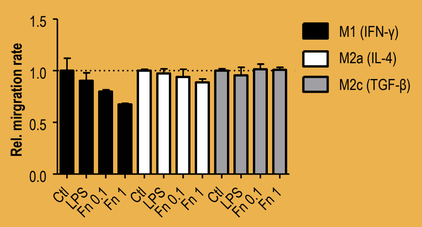
ELISA



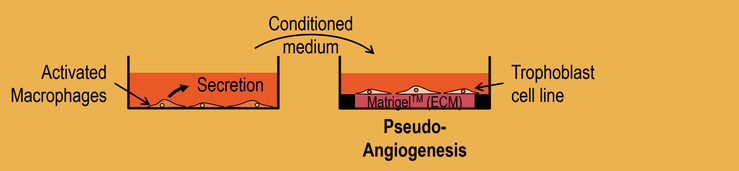


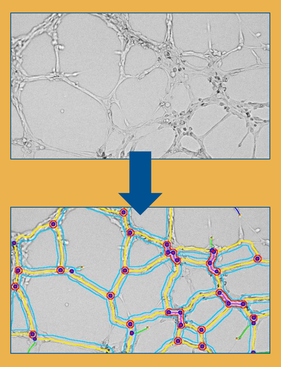
Migration assay (Scratch/Wound healing assay)

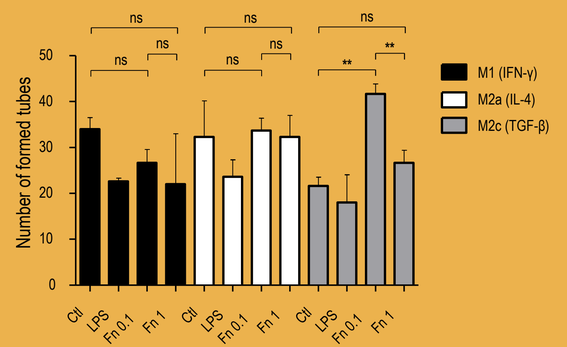




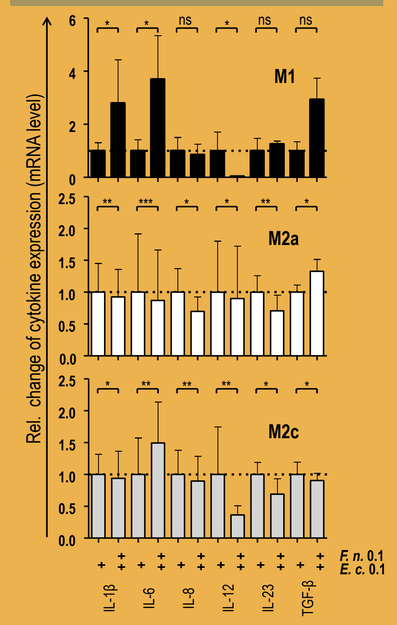
Tube formation assay







qPCR



Results

The treatment of macrophages with *F. nucleatum* resulted in higher viability/metabolic activity and less pro-inflammatory reactions compared to *E. coli* and LPS. A subsequent experiment could show that *F. nucleatum* pre-treatment regulated the reaction to *E. coli* regarding cytokine expression.

One step closer to the functional relevance, migration and angiogenetic behavior of trophoblast cells in the presence of conditioned medium obtained from treated macrophages were analyzed. Secreted factors of *F. n.*-treated M1 macrophages decreased trophoblast migration rate. Macrophages stimulated with LPS resulted in an impeded tube formation. However, low-dose stimulation of M2c macrophages with *F. n.* led to a increased tube formation compared to non-treated and high-dose-treated M2c macrophages.

Conclusion

For obvious reasons bacteria affect macrophages changing surface molecule expression (e.g. antigen presentation and costimulation) and cytokine expression. Thereby, *F. n.* caught attention by leading mostly to an activation of an less stronger extend compared to *E. coli*. Moreover, *F. n.* could change the reaction of the macrophages to *E. coli* pointing to the regulatory capacity of *F. n.* even in complex microbial communities. Since the placenta is reported as low-abundance site, differences as shown for tube formation underline the importance to consider low bacterial concentrations and especially to differentiate between physiology (low abundance) and infection (high abundance).

Promotionsbörse - Tag der Wissenschaft der Medizin

Universitätsmedizin Greifswald
Direktor der Klinik und Poliklinik für Frauenheilkunde und Geburtshilfe

Prof. Dr. med. Marek T. Zygmunt

Universitätsmedizin Greifswald
Forschungslabor Frauenheilkunde

Dr. Damián O. Muzzio
Telefon: 03834/ 86 6879
E-mail: damian.muzzio@uni-greifswald.de

Universitätsmedizin Greifswald
Forschungslabor Frauenheilkunde

Rebekka Einenkel (M. Sc.)
Telefon: 03834/86 6859
E-Mail: rebekka.einenkel@uni-greifswald.de