**Involvement of Pattern Recognition Receptors in the Direct Influence of Bacterial Components and Standard Anti-Acne Compounds on Human Sebaceous Gland Cells**

Christos C. Zouboulis1,2\*, Marina K. Oeff2,3†\*, Holger Seltmann1,2, Naoki Hiroi3⁋, Evgenia Makrantonaki1,2‡, Stefan R. Bornstein3

1Departments of Dermatology, Venereology, Allergology and Immunology, Dessau Medical Center, Brandenburg Medical School Theodor Fontane, Dessau, Germany

2Laboratory of Biogerontology, Dermato-Pharmacology and Dermato-Endocrinology, Dessau Medical Center, Dessau, Germany

3Department of Internal Medicine III, University Hospital Carl Gustav Carus, University of Dresden, Germany

**Supplementary data**

**Materials and Methods**

Cell culture conditions and art of SZ95 sebocyte challenge have been selected in preliminary experiments in order to study the immunological effects of LPS, LTA, retinoids and HC at concentrations at which these compounds do not affect proliferation, differentiation or viability of SZ95 sebocytes.

*Cell Proliferation*

Cells were cultured in 96-well tissue culture plates at a density of 2,000 cells per well for two days. The wells were then washed in phosphate-buffered saline without Ca2+, and Mg2+ (PBS) and medium with or without active compounds were added. Cell proliferation was assessed by 4-methylumbelliferyl heptanoate (MUH) fluorescence assay and measured automatically as previously described [25]. Briefly, the medium was removed on the day of evaluation, the cells were washed twice in PBS, and 100 µl of a 100 µg/ml MUH solution in PBS was added to each well. The plates were then incubated at 37°C for 30 min, and the fluorescence released, which is representative of cell numbers, was read on a Molecular Devices SPECTRAmax Gemini spectrofluorometer using 355 nm excitation and 460 nm emission filters. Experiments were performed in quadruplicate, with 10 wells evaluated for each data point in each experiment.

*Detection of Lipid Content*

The cells were cultured in 96-well tissue culture plates at a density of 2,500 cells per well for two days. The wells were then washed in PBS, and Sebomed® Complete Medium was added. The medium was harvested after two days, and fresh medium with or without active compounds was added. The supernatants were harvested 72 h later; the wells were washed twice in PBS, and 100 µl of 10 µg/ml Nile red solution in PBS was added to each well. The plates were then incubated at 37°C for 30 min, and the released fluorescence was read on a Molecular Devices SPECTRAmax Gemini spectrofluorometer. The results are presented as percentages of the absolute fluorescence units in compared against controls using 485 nm excitation and 565 nm emission filters for neutral lipids and 540 nm excitation and 620 nm emission filters for polar lipids. Experiments were performed in triplicate, with ten wells evaluated for each data point in each experiment.

*Detection of Cell Viability*

Cells were cultured in 96-well tissue culture plates at a density of 20,000 cells per well for 24 h. The wells were then washed in PBS and medium was added. After two days, the medium was harvested and fresh medium either with or without active compounds was added to the cells. Supernatants were collected 24 h later and centrifuged to remove cell detritus, and 100 µl was taken for measurement of lactate dehydrogenase (LDH) release with a LDH Assay kit (Boehringer, Mannheim, Germany) according to the manufacturer’s instructions. Experiments were performed in triplicate, with ten wells evaluated for each data point in each experiment.

**Results**

*Proliferation, Viabilility and Lipid Content of Challenged SZ95 Sebocytes*

Cell culture conditions and art of SZ95 sebocyte challenge have been selected in preliminary experiments in order to study the immunological effects of LPS, LTA, retinoids and HC at concentrations at which these compounds do not affect proliferation, differentiation or viability of SZ95 sebocytes. Therefore, we did not detect any effect of the compounds tested on proliferation, lipid content and viability of challenged SZ95 sebocytes at the conditions applied.