EAACI Fellowship Awards

Final Report

Long-term EAACI Research Fellowship
Research Fellow: MSc. Dries Van Elst
Host supervisor: Cezmi A. Akdis, MD, Prof. Dr. med.
Duration: from 2018/07/01 till 2018/06/30, 1 year
Location: Swiss Institute of Allergy and Asthma Research (SIAF), Davos, Switzerland

Original Research Title:
Innate Immune Receptors (NLRs, RLRs, ALRs) Regulate Epithelial Barrier Integrity In Asthma

Adapted Research Title:
The effect of residue detergent compounds on the esophageal epithelium.

Reason for adaptations from the original project

In agreement with Prof. Akdis we decided to change the scope of the research plan from asthma to gastrointestinal (GI) related diseases. Given the rise in the prevalence of allergic and barrier-dysfunctional diseases of the GI tract and skin, it seemed prudent to investigate what might be the cause of this increase. We were able to start a cooperation with Dr. Straumann which enabled us to obtain fresh esophageal epithelial biopsy samples, which provided us with an excellent starting point for our research, namely the isolation and growth of esophageal epithelium in monolayer and air-liquid interface (ALI) culture. With these tools we have started investigating the effect of detergents, namely dishwasher and industrial detergents as well as rinsing detergents used in dairy farms, on the epithelial barrier integrity of the esophagus. These detergents contain numerous compounds hazardous to the human epithelial barriers, such as oxygen based bleaching agents, enzymes, surfactants and preservatives. As the constant exposure to detergents could have a pernicious effect on the development of epithelial barriers in humans, not to mention young children, it seemed prudent to perform research into this subject. We hope this will shed a light on any health risks that might be caused by the constant exposure to these compounds.

Background and rationale

Prevalence of atopic dermatitis, food allergy as well as other GI diseases associated with epithelial barrier dysfunction (eg. eosinophilic esophagitis, EoE) has been increasing during the last decennia[1,2]. This presents a big burden on the patients' quality of life as well as the health care systems worldwide. Finding the root cause of these diseases would aid us significantly in finding a cure, or better primary prevention of the disease. Coinciding with this increase in barrier-related diseases is the universalization of dishwashers and their detergents in modern society. This same trend is visible in industry and agriculture. Though now ubiquitous around the world, it has already been established

that detergents can have detrimental effects on the tight junction barrier integrity in the lungs as well as the skin, as they contain hazardous components such as surfactants, enzymes, preservatives and, bacterial toxins[3]. Preliminary data gathered at SIAF demonstrated a clear toxic effect of laundry rinse water and its content of sodium dodecyl bisulphate still at toxic levels to air-liquid interface (ALI) cell cultures of bronchial epithelial cells and skin keratinocytes[3].

Hypothesis

Human society is being exposed to a hazardous concentration of detergent residue on a daily basis, from tableware washed in a dishwasher, to milk and other food products which were exposed to detergents during processing. This exposure is at least in part the cause of the increase in barrier-dysfunctional diseases, posing a health risk to society.

Objective

This study aims to set up a protocol for the isolation and growth of esophageal epithelial cells obtained from patients with eosinophilic esophagitis (EoE) as well as from healthy esophageal epithelial tissue. We will establish the concentration of dishwasher detergent ingredients present in common house hold products, namely dishwasher-washed tableware, detergent rinse residue, water and milk, with potential for other products. Esophageal ALI- and monolayer cultures will be exposed to the dishwasher detergent ingredients.

Materials and methods

Subjects

Esophageal epithelial tissue biopsies were collected from dr. Alex Straumann in Olten, an expert in the field, providing both inflammed epithelial tissue from EoE patients and non-inflammed healthy epithelial tissue from patients without a diagnosis for EoE. All biopsies were obtained in accordance with the CER-VD ethical regulations. Both fresh tissue and frozen tissue samples preserved in tissue-TEK were obtained. A full clinical report of each patient was collected by dr. Straumann and provided to us in an anonymized worksheet to ensure confidentiality.
Additional human esophageal epithelial cells were purchased from ScienCell and used to test growth medium and work as an additional control.

Isolating and culturing esophageal epithelium

Esophageal epithelial cells were isolated from biopsy tissue maximum 6 hours after surgery. A previously established isolation protocol used at SIAF for bronchial and nasal epithelial cells was used for the isolation of the esophageal epithelial cells. I obtained a esophageal epithelial growth protocol from pr. Farre[4], which was augmented by experts at SIAF to optimize proliferation of the cells. Growth of the cells was subsequently tested in different media, namely ScienCell Epithelial Cell Medium-2 EpiCM-2 (ScienCell), PromoCell Airway Epithelial Growth medium (PromoCell), Keratinocyte-SFM medium with L-glutamine, EGF and BPE (Gibco), Lonza Bronchial epithelial cell growth medium (Lonza), Epithelix hAEC culture medium (Epithelix) and CellBiologics complete epithelial cell medium (CellBiologics).
ALI cultures were established using an in house protocol for bronchial and nasal epithelial cells, optimized for esophageal epithelial cells. The ALI medium used for these cultures consisted of 50%

Dulbecco's Modified Eagle's medium (DMEM, Sigma Aldrich) and 50% epithelial medium with adjusted supplements. Cells were cultured until passage 3 and subsequently used in experiments. Transepithelial electrical resistance (TER) and confocal stainings for claudin-1, zonula occludens 1 (ZO-1) and occludin, three tight junction (TJ) proteins, were used to establish the differentiation of the epithelial layers in ALI.

Determining dishwasher detergent compounds

I selected a standard dishwasher detergent of known composition. A comprehensive list was formulated of the most hazardous compounds present in this detergent, providing us with 11 targets to investigate.

I started a cooperation with local dairy farms in Davos, Switzerland, in order to obtain data concerning a list of detergents used throughout the milking process together with the milking protocol. These were used to gain an understanding in the level of exposure to detergents which occurs from consuming household products such as milk. After investigating the contents of the detergents used in these dairy farms, 4 more targets were added to the list.
The company Intertek was contacted in order to perform mass spectrometry analysis of rinse water, dishwasher detergent samples, milking equipment detergent rinse water, as well as numerous other samples, in order to gain insight into the concentrations of the most hazardous detergent ingredients to be investigated.

Exposure of esophageal cells to detergent compounds

Monolayers of esophageal epithelial cells were exposed to levels of different detergent compounds present in bodily fluids. The cells were exposed to increasing concentrations in order to establish the level of cytotoxicity of each compound. Cytotoxicity was measured using Pierce LDH cytotoxicity assay kit (Thermo Fisher Scientific). RNA was collected for further analysis.

Current results

In total 51 biopsies were collected from dr. Straumann, of which 43 were EoE patients and 8 were non-EoE patients (controls). 20 of the EoE samples as well as 3 of the control patient samples were successfully isolated and capable of proliferating past passage 0. Esophageal epithelial cells unfortunately showed suboptimal growth when first cultured in the ScienCell medium. TER measurements were below the threshold value of 400 Ohm necessary to successfully start ALI experiments. Different parameters were modified in order to optimize the protocol, namely the medium used, the addition of all-trans retinoic acid (ATRA) to the ALI medium and the duration of growth in ALI.

The different media tested were ScienCell, PromoCell, Epithelix, CellBiologics, Gibco and Lonza (fig. 1). Cell yields were highest in ScienCell and PromoCell, with CellBiologics medium performing the worst, as all cells detached and died.

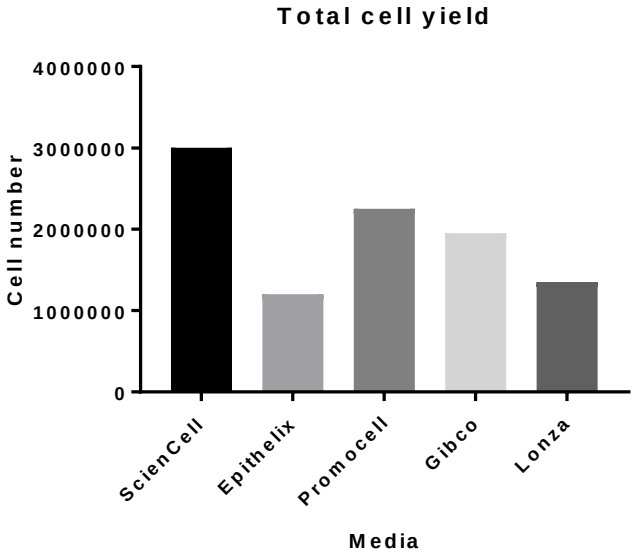

figure 1. Cell number of the same donor (50F passage 3) grown in 5 different media .

Cells were put on ALI with and without the addition of ATRA (15 ng/mL) in the medium. Comparison between the TER of the different media (Gibco experiments are still in progress) showed that the TER overall deteriorates over time to a minimum of 200 Ohm. Additionally, it is clear that adding ATRA to the ALI medium lowers the TER measured in the ALI cultures. The highest TER's were served in donors grown in PromoCell ALI medium without ATRA (fig. 2). Confocal stainings showed no sign of TJ barrier structures at day 10 in any of the different media tested, indicating the epithelial cells have not yet differentiated at this stage. The expected honeycomb structures were visible from day 17, most prominently in epithelium grown in PromoCell medium with ATRA. Without ATRA present no well-defined honeycomb structure was visible (fig. 3). This showcases the necessity of ATRA in order for the cells to differentiate in ALI.

These results, together with the high cell yield from the monolayer cultures as well as the tight junction (TJ) confocal stainings prompted us to switch from ScienCell to PromoCell as the primary growth medium.

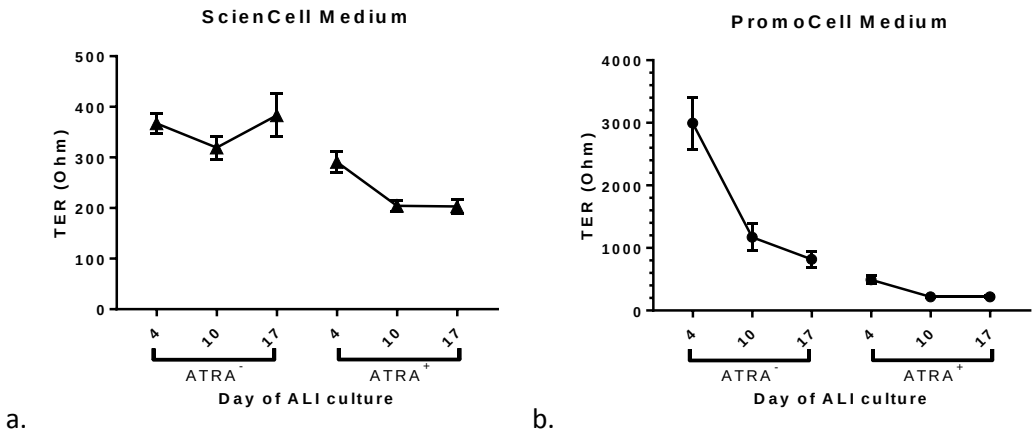

figure 2. TER measurements in a. ScienCell and b. PromoCell medium over the course of 17 days after seeding in the inserts, both with and without the addition of ATRA to the ALI-media. Medium in the inserts was removed at day 4. Measurements were performed on the same donor, 50F passage 3.

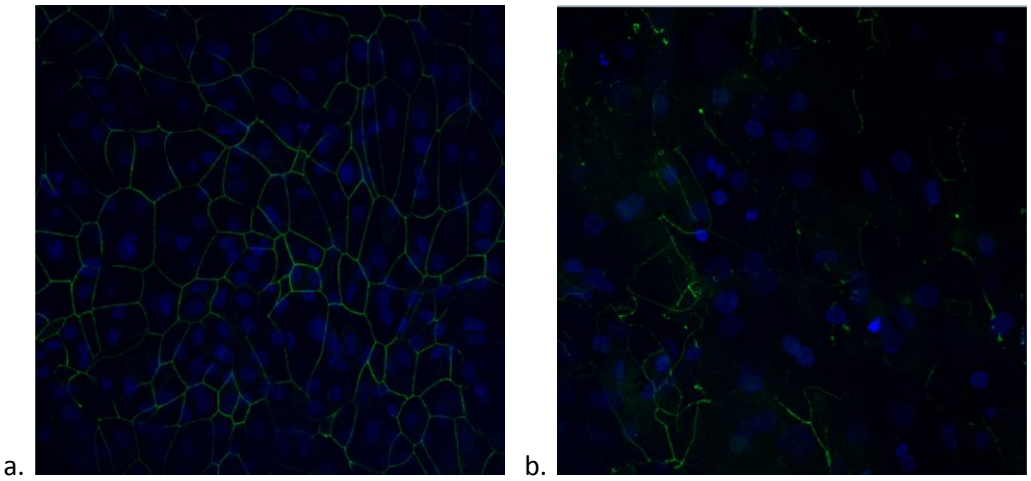

figure 3. Confocal staining for ZO-1 (green) and DAPI (blue) in PromoCell medium grown esophageal epithelial ALI cultures (donor 21731, passage 3) at day 17. a. ATRA added to the medium (15 ng/mL), the honeycomb structure is clearly visible and shows the barriers clearly. b. no ATRA added to the medium, no clear honeycomb structure visible, cells poorly differentiated.

I also investigated the application of different ATRA concentrations and their effect on the integrity of the esophageal epithelial barrier in ALI. This unfortunately yielded no positive results, as the TERs did not improve over time for any concentration of ATRA (ranging from 0 to 15 ng/mL) used in the medium.

Additional experiments were performed to test the effect of keeping the cells submerged in growth medium in the inserts. However, none of the inserts treated this way yielded satisfactory results.

As of this moment esophageal epithelial cells in monolayers (PromoCell) were exposed to various concentrations of dishwasher detergent ingredients. Three target compounds were already tested in order to acquire a better understanding of their effect on human esophageal cells. The Pierce cytotoxicity assay showed low cytotoxicity for these compounds in concentrations ranging from 10 ng/mL to 10000 ng/mL, with a maximum of 7.8% of cell death occuring when cells were treated with

the highest dosage. RNA samples were collected for all concentrations and are now being analysed for inflammatory and non-inflammatory cytokines to gain a better understanding of the cells reaction to these compounds. It should also be noted that I was only able to check the acute toxicity using the Pierce LDH cytotoxicity assay kit.

Future experiments

More research is necessary to establish a fail proof method for an esophageal ALI model for primary esophageal epithelial cells. Currently the Gibco medium is still being tested with various donors to check its potential in generating a successful ALI model.
Using the data which will be acquired via mass spectrometry by a private company, I will be able to pinpoint the exact level of exposure to dishwasher as well as other detergents on a daily basis. Application of these concentrations to the esophageal cells will give us a better understanding of the acute effect of detergents on the esophageal epithelium, while also providing a solid basis for research into chronic toxicity using mouse models.

Conclusions

During this study our main focus was the effect of detergents on esophageal epithelium. I was able to successfully isolate esophageal epithelial cells from biopsies, and ascertained that ATRA is a necessary addition to the ALI growth medium in order to stimulate differentiation. I can also conclude that the growth protocol for company-bought human esophageal epithelial cells does not necessarily provide the optimal growing conditions for primary esophageal epithelial cells. The ALI protocol for primary esophageal epithelial cells needs to be further optimized before usage in experiments.
From our pilot studies into the acute cytotoxicity of various dishwasher detergent ingredients it appears that the compounds investigated so far yield a relatively low cytotoxicity. Testing of the other detergent ingredients will certainly provide us with important information regarding the safety of daily detergent exposure. However, more research is necessary to investigate the effect of chronic exposure to these compounds.

Impact and benefit of this research

With this research I aimed to test the level of exposure to detergents and what effect this might have on a daily basis. This study can be used to raise awareness in the scientific community as well as in society by pointing the ubiquity of hazardous compounds in their daily life. The greatest benefit of the study might be unraveling the mechanisms with which these detergents interact with the barrier epithelium.

Additional EAACI Fellowship activities

Organization committee (IT group) of the World Immune Regulatory Meeting (WIRM) in Davos, Switzerland on 14-17 March 2018

Acknowledgement and personal reflections

I am very grateful to have been given the fantastic opportunity by EAACI to perform research at a top of the bill institute such as SIAF, and develop my skills as a scientist. The fellowship provided me with huge learning opportunities concerning new methodologies as well as successfully planning research and cooperating in the laboratory with fellow scientists. I would also like to thank my host supervisor, Professor Cezmi Akdis, as well as Dr. Milena Sokolowska for mentoring and guiding me throughout the year, and for all the support, help and answers they provided me with whenever I had a problem. Neither of them would every shy away from instigating a scientific discussion or challenging my point of view, which is a tool I will make use of for the rest of my life. Furthermore I would like to thank the entirety of the SIAF scientific and support team for creating the great work environment I had the pleasure of being a part of.

I strongly recommend any young scientists with an interest in the field of clinical immunology and allergy to participate in the EAACI fellowship awards.

Bibliography

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