Nasal epithelial cells : effector cells in allergy
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General discussion
Chapter 7

**Concepts in allergy research.**

The cells of the immune system fulfill their roles in the defense against potential threats to the organism in the context of a local tissue that is exposed to the environment. This applies to both the initiation of an immune response where the potential threat is first detected and the elicitation of an immune response where immune effector cells try to eliminate this threat. Most of the research in the field of immunology has focused on the immune cells themselves, their specific contribution to the immune response, and how their function is affected by signals originating from the external environment. Many experimental techniques make use of *ex vivo* isolated or *in vitro* generated immune cells, leaving out many of the tissue factors that influence the response. In such an experimental design the potential influence from local tissue via the mediators they produce can not be studied. How different immune cells interact in the allergic cascade with each other and the local tissue is illustrated in figure 1.

Related to these design issues, the immune response as seen in allergy is often defined as an influx of immune competent cells into local tissues. However, little attention is paid to potential differences in immune competent cells or differences in local tissue cells between healthy and allergic individuals. When one considers allergy as a disease this can easily be understood from a clinical perspective where the allergic individual responds to a given trigger, while a healthy individual does not respond to the same trigger. From a mechanistic point of view however it should be highlighted that a healthy individual may have a cellular response to the same trigger as an allergic individual, but that this cellular response would be different from the response in an allergic individual so that in the healthy individual there is no expression of clinical symptoms.
Figure 1: Immune response to allergens. Schematic drawing of the processes that take place during sensitization and elicitation of an immune response. When an allergen comes into contact with the mucosa, the epithelial cells will activate and start producing cytokines and chemokines, which will attract dendritic cells which take up the allergen. These dendritic cells will then migrate to the lymph nodes where they present the allergen to T-cells that will skew towards T helper 2 phenotype. These Th2 cells will in turn instruct B-cells to produce specific antibodies that are loaded onto mast cells and eosinophils. When the allergens is encountered for the second time the epithelial cells will again produce cytokines and chemokines, that attract the now primed immune cells and allergic inflammation follows.
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In this thesis the focus is on nasal airway epithelial cells, how they respond to an environmental trigger (house dust mite allergen), and on identifying potential differences in the response to this trigger depending on the allergic status of the person these nasal epithelial cells were obtained from. The rationale of this approach is that epithelial cells could affect the local tissue environment in which immune competent cells are active, and that the epithelium can regulate an ongoing immune response through these immune competent cells. Three criteria need to be fulfilled in this concept. Firstly, nasal epithelial cells need to be able to detect changes in their environment and should be able to respond to these changes. Secondly, the response of nasal epithelial cells should to be able to affect the functions of immune competent cells. Thirdly, the epithelial responses in healthy and allergic individuals should either be different per se, or the tissue resident immune competent cells in healthy and allergic individuals should be differently affected by this epithelial response.

Detecting changes in the environment.

The first criterion is clearly fulfilled as airway epithelial cells have many mechanisms to detect changes in their environment. Through a collection of distinct receptors the epithelium is able to respond to structural components of micro-organisms like bacteria, viruses, and helminths, these are called Pattern Recognition Receptors (e.g. Toll Like and NOD Like Receptors), or to the enzymatic active components of potential allergens (Protease Activated Receptors) \(^1\)\(^-\)\(^3\). Although epithelial cells are able to respond to all these factors, research into the response to an environmental factor has necessarily focused on exposure to a single trigger. This does not reflect the normal situation where simultaneous exposure to a mixture of factors is more common. Moreover, although all the receptors have distinct signaling pathways they almost always seem to lead to activation of one particular
transcription factor (NF-κB), which is odd since it would seem to lead to a loss of the specificity achieved by the multitude of the receptors on airway epithelial cells. The data on the activation of epithelial cells by house dust mite allergen in this thesis clearly shows that NF-κB is not the only transcription factor affected, but that it is just one of a substantial number of transcription factors. It is highly relevant to note that a large portion of these transcription factors are able to physically interact with each other (NF-κB and AP-1 family members, together with ATF-3 and EGR-1) to form hybrid transcription factors that can have opposite effects than their normal counterparts.

The discovery of these transcription factors as downstream targets of the detection of house dust mite allergen exposure should perhaps not come as a surprise. The microarray approach followed throughout this thesis has allowed such an outcome, as such experiments are inherently unbiased.

At the effector side a similar picture emerges, with many cytokines, chemokines, and growth factors being secreted by nasal epithelial cells through which many downstream processes can be influenced. The microarray approach should also serve as a warning concerning generalizations of outcomes in experiments. When common outcome parameters like IL-6 and IL-8 would have been used, the responses of the bronchial cell line H292 and healthy primary epithelium look very similar. The microarray experiment shows that this similarity is only true for a limited number of mediators that represent only a small fraction of the overall response. However, this is not all bad as such common factors in the response between different cells, as limited as they may seem, could point to a conserved and thereby important mechanism that defines such a response. In the detailed analysis of the comparison of the responses in the different epithelial cells a conserved set of genes has been identified that could be seen to define the allergic response.
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Epithelial cells affecting the immune response.

In this thesis we have not tried to show that epithelial cells can affect downstream processes, but available data in literature and our own expression profiling clearly identifies many processes they could influence. One of the drawbacks of a traditional microarray experiment is that so many genes change and that it would be easy to pick a few genes of interest and show that any given process could be affected by epithelial cells. However, if we would focus on the data in chapter 4, where a core response of epithelial cells to the exposure to house dust mite has been defined, we can attribute a role for all secreted mediators in controlling certain aspects of the immune response. When we briefly summarize our observations of chapter 2 we see that epithelial cells are actively involved in the recruitment of neutrophils (GRO-α, GRO-β, GRO-γ, and IL-8) and dendritic cells (MIP-3α). Moreover, epithelial cells produce mediators that can both inhibit activation of dendritic cells (IL-6) and stimulate their migration of out of the tissue (IL-1β). The epithelial expression of IL-6 could be related to our unpublished observations that epithelium culture supernatant can suppress the spontaneous maturation of monocyte-derived dendritic cells.

Epithelial cells and the allergic condition.

The allergic condition is often defined as an influx of inflammatory cells into local tissues. At the effector side of the immune response such a definition would be fine as these cells are only present in an ongoing immune response. However, in the same overview one would also include dendritic cells, not only when actively surveying local tissues prior to the detection of a potential threat, but also when contributing to on ongoing response after they have been recruited to a site of ongoing inflammation. This might represent a generalization and could introduce a potential blind spot as it
does not consider that the diseased state itself may affect the function of these cells. Some activities of dendritic cells have indeed been reported to be affected by the different immunological states found in healthy or allergic individuals. However, it remains unexplored whether the differences between the dendritic cells in healthy and allergic individuals are a consequence of the allergic condition, if they contribute to the allergic condition, or even if they are the cause of this condition.

The issue of a disease state affecting cells is particularly relevant for cells making up local tissues as they will always be present independent of the disease state. The data presented in this thesis show that the allergic condition has a profound effect on nasal epithelial cells. When epithelial cells are isolated from healthy or allergic individuals and cultured for two weeks, the expression profiles of these epithelia are very different between healthy and allergic. A substantial part of the differences between the epithelial cells from healthy and allergic individuals can best be described as an activated state at baseline in allergic individuals that is only induced in epithelial cells of healthy individuals after in vitro exposure to house dust mite. If one considers that the epithelial cells have been exposed in both healthy and allergic individuals when the cells were still in situ, one must conclude that the effect of this in situ exposure is still visible after two weeks in culture. This indicates a mechanism of imprint of an epigenetic or autocrine origin. The exact mechanism has not been explored, but could involve TNF-α, as network analysis has identified this player as a linking factor in many of the genes that are affected in the allergic condition. Epithelial cells are able to respond to exogenously added TNF-α, but when considering the role of autocrine TNF-α we should note that TNF-α is initially produced in a membrane associated form that does not only serve a precursor for soluble TNF-α, but that it is able to activate an independent TNF-α-receptor too. It is interesting that TNF-α has received a lot of attention in autoimmunity and that effective treatment regimes in rheumatoid arthritis have been developed that target TNF-α.
signaling. The role of TNF-α in PAR mediated allergy was also confirmed in a study in which it was shown that activation of PAR-2 with an activating peptide leads to immune activation to simultaneous administered OVA, and that this process could be inhibited by blocking TNF-α signaling \(^{14}\).

In our results we also observed changes in TLR-3 (a pattern recognition receptor specific for viruses \(^{16}\)), and TIRAP (an important downstream signaling molecule for TLR-3 \(^{17}\)) indicating that there could also be interaction between the signaling cascade that is initiated upon allergen exposure and viral infection. In a recent review on viral exacerbations in allergic disease \(^{15}\) it was remarked that even at the epithelial mediator level it can be difficult to discriminate a viral infection from allergy, with many of the mediators induced by viruses after infection of epithelial cells also being reported to be elevated in allergy.

**Future opportunities for research.**

This thesis has taught us the extent of the response of airway epithelial cells to house dust mite extract and how this response differs between the nasal epithelial cells from healthy and allergic individuals. The identification of a viral detection signaling cascade could indicate similarities between the two responses. Moreover, part of the identified response could be a normal response triggered by stress of some description in airway epithelia and that this response would be shared by many environmental triggers. To resolve these questions it would be important to compare more epithelial responses to different triggers.

A first choice would be to study whether different allergens trigger a similar response to house dust mite in nasal epithelial cells. Both similarities and differences in the response would help us to understand the allergic response better. The similarities would point to a common mechanism by which epithelial cells could contribute to the allergic response, while the
differences might help us to understand why some individuals have become allergic to, for instance, house dust mite and others to grass or tree pollen. Given that viral infections can trigger asthma exacerbations in allergic individuals independent of what allergen they are sensitized for, it could perhaps be expected that viral and allergen induced signaling both include similar genes.

With the allergic condition defined it would be relevant to understand how this allergic condition state is maintained and what can be done to convert the allergic response in epithelium back to a normal response. A detailed analysis for the transcription factors in the allergic model and the way their interactions affect gene transcription is necessary. In particular it would be important to understand if the different expression clusters we have identified in chapter 3 are related to the function of transcription factors. It has been shown that NF-κB transcription can be inhibited by AP-1. Given that our data show that AP-1 is down-regulated in allergic individuals, while it is up-regulated in healthy individuals. This could partly explain why the house dust mite response is maintained in allergic individuals. Another factor that is known to inhibit NF-κB transcription is EGR-1 of which we see up-regulation in healthy individuals and down-regulation in allergic individuals. Further research on these interactions can provide further indications towards understanding how the allergic condition is maintained.

When we investigate the clusters in more detail we start to see aspects of concomitant regulation of genes related to a similar process. For instance cell-cell contact which is maintained by the tight junctions located between adjacent epithelial cells. Maintenance of these junctions is vital to the organism, and since house dust mite extract is capable of cleaving tight junctions, exposure to such allergen should result in a response where tight junctions are reinstated. Such a response can be observed and includes genes like Claudin-1, Claudin-4, Tight Junction Protein-1, Tight Junction Protein-2, and Occludin, which are regulated in an identical fashion and all
can be found in the same cluster \(^{18-20}\). However, it would go too far to assume that all genes within one expression cluster are involved in the same function or process. Conversely, not all genes corresponding to the same process should be expected to belong to the same cluster. Rab-7, for instance, is required to bring Claudin molecules to the cell surface, yet belongs to a different cluster than the other proteins related to the tight junctions belong to. Although these are two different clusters they both share the “activated in allergic” phenotype described in chapter 3. What is important is that clusters contain genes with similar behavior and could thereby be a target for treatment. When considering target genes in the allergic condition for treatment it could be important to influence sufficient genes that contribute to this state as it is unlikely, that a single factor can be held responsible for allergy or that targeting a single factor would resolve the disease. Moreover down-regulating genes without knowing if their expression is orchestrated by the allergic condition could present problems with other cellular defenses or autoimmunity. Although the field of microRNAs is relative unexplored in allergy it could be very relevant to study deregulation of these small regulatory RNAs as they have been shown to play an important role in maintaining messenger RNA stability and inducing degradation. Through binding to the 3’-end of messenger RNA they are able to regulate gene expression, just as binding sites for specific transcription factors can do in the promoter regions of genes.

An exciting application of the knowledge we have gathered and the analysis tools we have used could be in the diagnosis of allergic disease in young children. The diagnosis in children under the age of two is inherently difficult. Skin prick testing and serum levels of IgE are not yet reliable objective outcome measures and the interpretation of clinical symptoms like rhinorrhea and sneezing for allergic rhinitis or cough and wheeze for allergic asthma are hard to tell apart from similar symptoms caused by common viral and bacterial respiratory tract infections that are so prevalent.
in young children. Since principal component analysis in our experiments was capable of separating allergic and healthy individuals, we could also use such an approach for young children, where we now have to depend on clinical symptoms which are sometimes hard to distinguish. A major advantage of principal component analysis is that every individual is a single data point in multidimensional space so that a conclusion can be reached for each individual, this in contrast to more common analysis methods that require some characteristic of a group of patients making it hard to draw conclusions for a single individual. Some issues need to be resolved before this application can be used as a diagnostic tool. As already indicated the allergic model does include signaling pathways for viral infections so that we need to determine which genes are specific for the allergen exposure and which are shared with viral infections. If we were able to define established allergy in children we would still need to define the allergic condition in children as the immune system of children under the age of three years is still under development and may therefore yield a different outcome. Although a definition for allergic rhinitis would be a great step forward in the early detection and treatment, it would be an added bonus if a similar approach could be used to define allergic asthma in young children. In adults it could be conceived that similar analysis methods like the ones described in this thesis could also be used for primary bronchial epithelial cells, however, in young children bronchial epithelial cells are not easily obtainable due to invasive nature of a bronchoscopy. However, there is some tentative data suggesting that lower airway disease may also be reflected in the upper airways. Data by our group has shown that in nasal samples taken during a rhinosyncytial virus infection, IL-18 can only be detected when the infection is not restricted to the upper airways but also includes the lower airways. It would be interesting to see if similar observations can be made for allergic asthma, and if some aspects of the allergic condition in the lower airways are reflected by the nasal epithelium. A final application of expression profiling
could be in following a cohort of children at risk for the development of allergic disease in order to find an early predictor for children that will develop allergic disease and that will proceed to develop asthma later in life.

Allergic disease will be with us for some years to come, but the data described in this thesis sheds some new light on established allergic rhinitis, identifying potential mechanisms that could be targeted for treatment, and opening avenues for the development of new diagnostic tools in the allergy field.

Reference List


