

Original article

Comparison of expression profiles induced by dust mite in airway epithelia reveals a common pathway

Background: Airway epithelial cells have shown to be active participants in the defense against pathogens by producing signaling and other regulatory molecules in response to the encounter.

Methods: In previous manuscripts, we have studied the effect of house dust mite (HDM) extract on both an epithelial cell-line (H292) and primary nasal epithelial cell. When we compare these responses we conclude that the H292 cells more closely resemble nasal epithelium of healthy controls (share 107 probe-sets) than of allergic individuals (share 17 probe-sets).

Results: Interestingly, probably because of an absent intraindividual variation between samples, more probe-sets (8280) change expression significantly in H292 than in either healthy (555) or allergic (401) epithelium.

Conclusions: A direct comparison of all the responses in these epithelial cells reveals a core-response to HDM of just 29 genes. These genes (CCL20, IL-8, CXCL2, CXCL1, IL-1B, AREG, TNFAIP3, HBEGF, PTGS2, BMP2, LDLR, PLAUR, PLAU, NFKB2, NFKB1, JUN, ATF3, EGR1, NPC1, TICAM1, EPHA2, CTGF, DUSP1, SPRY1, TLR-3, complement factor C3, IVNS1ABP, SerpinB3, and PSAT1) have described links with allergy or inflammation and may even describe the well-established relationship between viral infections and allergic exacerbations or allergy development.

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Airway epithelial cells have shown to be active participants in the defense against pathogens by producing signaling and other regulatory molecules in response to the encounter (1, 2). In this way, the airway epithelium contributes to the tissue microenvironment where cells of the immune system reside and are activated as part of the defense mechanism. A detailed analysis on the protein and mRNA level of such a response in primary nasal epithelium and in a bronchial cell-line (3, 4) by our group has identified a very diverse and seemingly well-coordinated response. In our experiments, we have investigated the effect of house dust mite (HDM) extract on airway epithelium.

Both in primary epithelial cells and in a bronchial epithelial cell-line, a complex but defined, network was detected that at least shared the involvement of epithelial cells that produced and secreted TNF- α . More recently, it has been shown that activation of protease-activated receptor (which can occur on account of proteolytic allergens) promotes ovalbumine (OVA) sensitization in BALB/c mice, and that this process can be blocked by adding anti-TNF- α (5). In the analysis of the primary nasal epithelial cells and their response to allergen, we showed an activated state in the epithelial cells of the allergic individuals. This activated state is reflected in a

limited number of genes in allergic epithelium that at baseline already display a high level of expression that, in primary epithelium from healthy individuals, is only reached after HDM extract exposure. Moreover, we saw increased expression of two transcription factors (NF- κ B and AP-1) and their regulatory members in healthy epithelium upon allergen exposure. Most striking is that, while the already activated NF- κ B regulatory pathway remained unchanged in allergic epithelium, the AP-1 pathway is downregulated upon exposure to HDM allergen. This could indicate a protective effect by expression of AP-1 as it has been described to bind to NF- κ B-binding site, thereby inhibiting NF- κ B-regulated transcription.

With availability of additional data on the effect of HDM extract on a bronchial epithelial cell-line, a more detailed analysis of this response in airway epithelia can be achieved. In this manuscript, we would like to explore (i) the pros and cons of using a cell-line as a surrogate model for primary epithelium, (ii) the similarities and dissimilarities in the HDM-response of epithelial cells from a nasal or bronchial origin, and (iii) further refine the HDM-response in order to try and identify new targets for medication in allergy and/or asthma.

Material and methods

Data sets

The data sets we used for this comparison were taken from two manuscripts that were previously published; one describing the response of H292 carcinoma cell-line to HDM-allergen (3) and one describing the response of primary cells of allergic and healthy individuals to HDM-allergen (4). For detailed information on cell-culture protocols, experimental conditions, RNA extraction methods, and microarray data validation please refer to these articles. The complete data set from these experiments is accessible through the NCBI Gene Expression Omnibus repository (<http://www.ncbi.nlm.nih.gov/geo/>; accessed 2 January 2008), series accession number GSE9150 and GSE9151.

Experimental procedures

Primary cells were obtained from nasal biopsies of volunteers after getting their informed consent in a locally approved study. The epithelial cell-line NCI-H292 was received as a gift from the Department of Pulmonology, Academic Medical Center, Amsterdam, the Netherlands. Cells were grown to 80% confluence in a six-well plate in their respective culture medium; BEGM (Lonza Clonetics, Breda, the Netherlands) or RPMI-1640 medium (Invitrogen, Breda, the Netherlands) supplemented with 1.25 mM L-glutamine, 100 U/ml penicillin, 100 µg/ml streptomycin, and 10% (v/v) fetal bovine serum (HyClone, Logan, UT, USA). Before stimulation experiment, cells were preincubated with Hanks' balanced salt solution (HBSS) for 24 h. Culture medium was removed and cells were then stimulated with HDM extract diluted in HBSS (2 µg/ml) or with HBSS alone (control condition) for 24 h. Supernatants were removed and stored for further analysis; cells were used for RNA extraction.

Data analysis

Expression values were calculated using the robust multi-array average (RMA) algorithm (6), and statistically analyzed for differ-

ential gene expression using ANOVA [MAANOVA package, version 0.98.8 (7)]. The permutation-based Fs test was used for hypothesis testing (8), and all *P*-values were adjusted for false discovery-rate correction (9). In order to quantify the effect of HDM extract on gene expression, pairwise statistical tests were performed to analyze: (i) the effect of HDM on epithelial cells from healthy individuals separately, (ii) the effect of HDM on epithelial cells from allergic individuals separately, and (iii) the effect of HDM on H292 epithelial cells.

A Gene Set Enrichment Analysis (GSEA) strategy was used to determine if the changes from baseline in the primary epithelial data set was similar to the changes in H292 cells. H292 data was first RMA-normalized and for every probe set in the H292 data a *t*-statistic has been calculated using an empirical Bayes' *t*-test. This *t*-test is an indication of how different a probe set is between H292 HDM and H292 blank. Then the H292 data was ranked by *t*-statistic values. Subsequently, we calculated the 'enrichment statistics' of four defined primary gene sets (upregulated or down-regulated in epithelium from allergic or healthy individuals) in the H292 data set. When the normalized enrichment score (NES) is positive then the primary epithelium gene set is mainly upregulated, if negative it is mainly downregulated in H292. We calculated the statistical significance levels of these enrichments using a gene-permutation strategy.

Results

Comparing H292, primary allergic and primary healthy epithelium on whole array level

The first impression on the similarities (or dissimilarities) was obtained using a correlation analysis on the complete data set from each of the individual microarray chips. Overall, the correlation coefficients between the individual arrays are high ($R > 0.895$), independent of whether the absolute signal levels (Pearson correlation in Fig. 1A) or the relative signal levels (Spearman rank correlation in

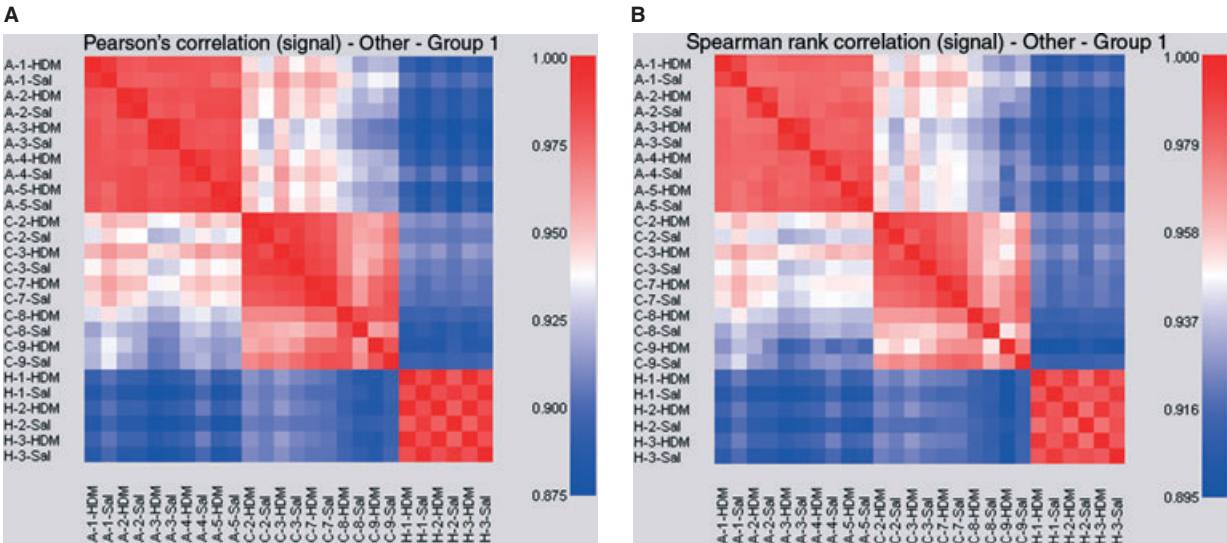


Figure 1. Correlation analysis of the expression profiles on the different individual genes based on (A) the absolute signal levels and (B) the relative rank of the signal levels. The individual arrays are labeled to indicate their origin (A = primary Allergic, C = primary healthy, and H = H292), the replicate number (1–5 for allergic, 1–3 for H292, and 2–3 + 7–9 for healthy), and their exposure to house dust mite (HDM) extract [Sal = vehicle only (saline), HDM = HDM extract].

Fig. 1B) of the arrays are used for analysis. Detailed study of Fig. 1 does show however that all arrays within a certain class (primary healthy, primary allergic, and H292) resemble each other more closely than they resemble arrays of the two other classes. Furthermore, primary epithelium of allergic or healthy individuals is more similar to each other than either of them is to the H292 cell-line.

Comparing allergic and healthy epithelium to H292 based on the effect of HDM extract

Although correlation analysis gives some clues about relationships on a global level, it is not well suited to investigate the individual responses of genes to HDM extract. Using a factorial design with multiple testing corrections, we identified 8995 probe-sets that are significantly different between vehicle and HDM extract conditions, in at least one of the three classes (primary healthy, primary allergic, and H292).

The Venn-diagram (Fig. 2) shows for this collection of probe-sets the similarity in their responses among the different groups. Several conclusions can be drawn. First, there is only a limited overlap in the response to HDM with just one of 8995 probe-sets showing a similar response in all three groups and 140 probe-sets showing a similar response shared between two of the three groups. Secondly, the response to HDM is far more extensive in H292 (8279 probe-sets) than in primary healthy (555 probe-sets) or allergic (301 probe-sets) epithelium. Thirdly, the response in H292 is more similar to the response in primary healthy epithelium than to the response in allergic epithelium. Also, the Venn-diagram shows, as we have reported before (4), that there is hardly any overlap of similar behavior (nine probe-sets) between the effects of HDM on primary epithelium from healthy and allergic individuals.

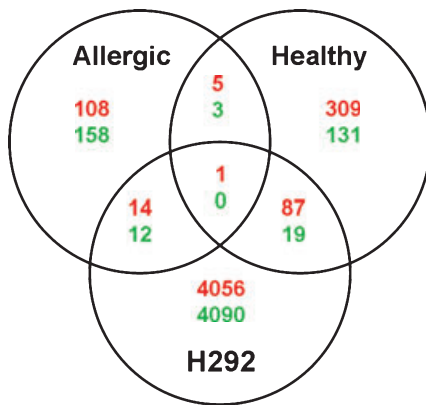


Figure 2. Venn-diagram showing statistically significant genes (black), statistically upregulated genes (red), and statistically downregulated genes (green). If genes show similar characteristics (upregulated or downregulated in both groups) they will appear in the overlapping boxes.

The data from the expression profiles of the individual genes shows that the response induced by HDM in the bronchial cell-line H292 is more similar to primary healthy epithelium (overlap of 106 probe-sets) than to the response in allergic epithelium (overlap of 26 probe-sets). To further explore this observation we used a GSEA-based approach (10). Four gene sets were created; statistically downregulated in allergic (173 genes), statistically upregulated in allergic (128 genes), statistically downregulated in healthy (153 genes), statistically upregulated in healthy (402 genes), and compared these gene sets to the expression profiles in H292. Table 1 summarizes the GSEA-analysis and shows that primary epithelial from healthy individuals behave as expected. Upregulated genes of the primary cells are enriched in the upregulated (NES = +2.961, $P = 0.000$) gene set of H292, whereas the downregulated genes are enriched in the downregulated (NES = +2.007, $P = 0.000$) gene set of H292. The situation for the gene sets in allergic epithelium is strikingly different. Now both upregulated (NES = +1.296, $P = 0.068$) and downregulated genes (NES = +1.900, $P = 0.000$) are enriched in the upregulated gene set of H292. Moreover, significance level of the comparison between upregulated allergic vs upregulated H292 ($P = 0.068$) is far below the significance level of upregulated healthy vs upregulated H292 ($P = 0.000$), confirming that the H292 response to HDM extract is more similar to the response in healthy primary epithelium than to the response in allergic epithelium.

Identification of a core-response to HDM extract in airway epithelium

In our earlier studies, we have used genes from primary epithelium that change their expression in response to HDM extract to form a regulatory network. When we map these genes in the H292 data set (see Supplementary Table 1A–D), we find that some of these genes are affected in a similar fashion, suggestive of a core-response to HDM extract in airway epithelium. Four of these genes have shared response between H292 and allergic epithelium; two are upregulated (SerpB3 and PSAT1) and two are downregulated (complement factor C3 and

Table 1. Gene set enrichment analysis

Gene Set	Gene pool size	NES	Nominal P -value
Allergic downregulated	173	+1.900	0.000
Allergic upregulated	128	+1.296	0.068
Healthy downregulated	153	–2.007	0.000
Healthy upregulated	402	+2.961	0.000

Robust multi-array-normalized data of H292 was compared to defined probe-sets from primary epithelium according to methods described in the Material and methods section. Positive value for NES indicates that this gene set of primary epithelium is predominantly upregulated in H292, whereas negative values indicate that the data set is predominantly downregulated in H292. Nominal significance levels are indicated.

NES, normalized enrichment score.

IVNS1ABP). The comparison between H292 and primary epithelium from healthy individuals reveals a larger overlap with 24 genes that are upregulated in both groups (CCL20, IL-8, CXCL2, CXCL1, IL-1B, AREG, TNFAIP3, HBEGF, PTGS2, BMP2, LDLR, PLAUR, PLA2, NFKB2, NFKB1, JUN, ATF3, EGR1, NPC1, TICAM1, EPHA2, CTGF, DUSP1, and SPRY1) and one gene that is downregulated in both groups (TLR-3).

In order to see how the genes that have shown to overlap between the healthy controls, allergic and H292 are interacting we used a network analysis. In our network we look at direct interaction, and filter out the interactions that are not of a regulatory type. We are then left with a network that contains 24 genes (of the 29 unique genes that show overlap; see Fig. 3).

Comparing H292, primary allergic, and primary healthy epithelium on the mediator level

The response of different cell types to distinct stimuli is frequently evaluated by analyzing expression profiles of secreted mediators. Not only can this be a convenient approach, but has also the rationale that through these secreted mediators a cell may affect downstream processes. This holds especially true for immunological processes. Not only can the secreted chemokines recruit immuno-competent cells to local tissues, but cytokines or growth factors can also affect the functions of these cells. In order to evaluate the response of HDM extract on epithelial cells at this mediator level, we examined this class of molecules in more detail.

In total, we find that: of the 147 probe-sets that are ascribed to cytokines and chemokines, 32 probe-sets change their expression significantly in H292, 10 probe-sets are significantly altered in primary healthy epithelium, and only one probe-set is altered in allergic epithelium. This single probe-set in allergic epithelium is specific for IL-8. Whereas in allergic epithelium IL-8 is downregulated, it is upregulated both in H292 and primary epithelium from healthy individuals. Of the 10 probe-sets that display an altered expression due to HDM extract exposure in healthy epithelium, we find that eight are also altered in H292. More than just investigating the statistical significance of the HDM-affected genes, it would be more relevant to consider the fold-change of these probe-sets between primary epithelium and H292. Figure 4B shows again that only for the comparison between the mediators of H292 and primary healthy epithelium there is a clear positive correlation, even though the fold-changes in H292 are clearly bigger than those in the healthy primary epithelium.

Discussion

In this article, we have presented a detailed analysis of the similarities and differences of the effect of HDM

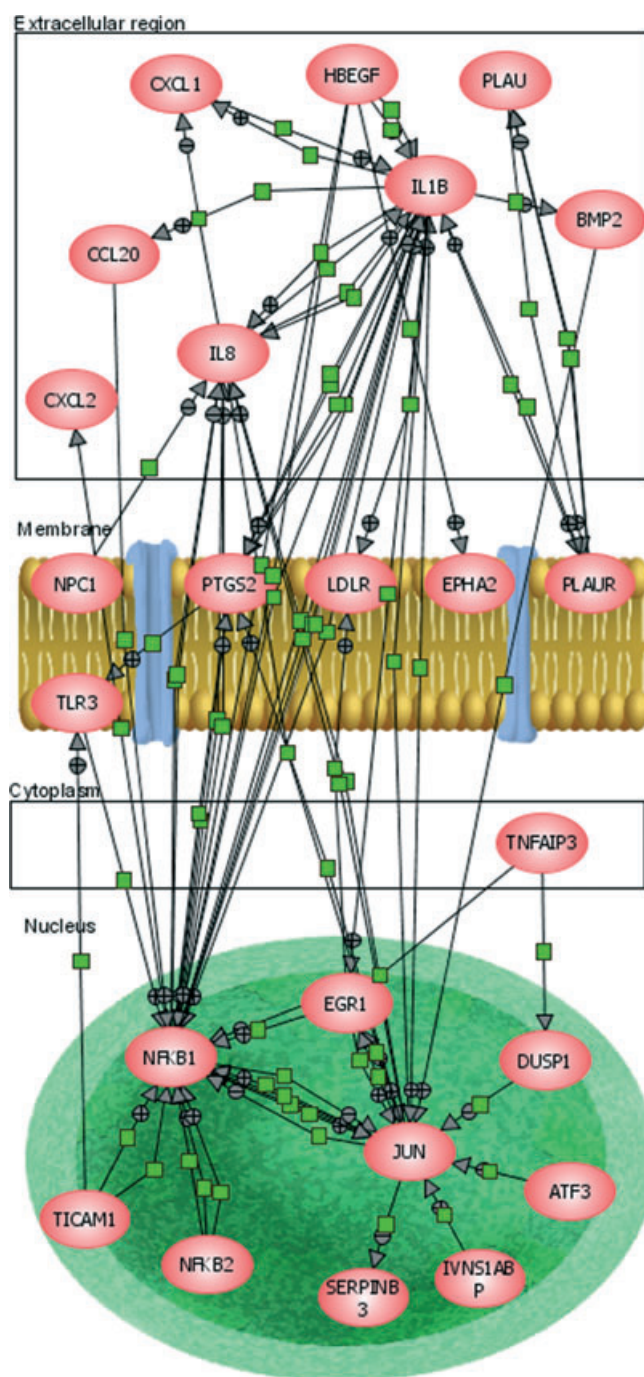


Figure 3. Network analysis of genes overlapping (statistical and direction) between allergic, healthy, and H292.

extract on the expression profiles in airway epithelial cells. Given the diverse background of the epithelia tested (cell-line vs primary cells, allergic vs healthy, and bronchial origin vs nasal origin) some relevant conclusions can be drawn. These conclusions will have implications for our understanding of the advantages of using a cell-line as a model and may even help to understand the contribution of airway epithelia to the allergic response.

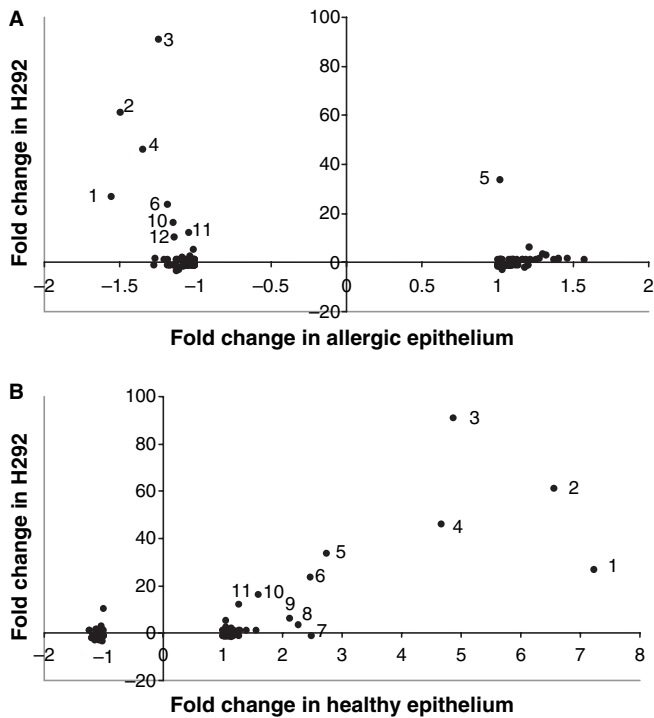


Figure 4. Comparison of the change in expression for soluble mediators between H292 and (A) primary allergic epithelium and (B) primary healthy epithelium. The labels for the indicated probe-sets are: IL-8 (numbers 1 and 2), CCL20 (number 3), CXCL2 (number 4), IL-1A (number 5), CXCL1 (number 6), CXCL10 (number 7), IL-1B (numbers 8 and 9), CXCL3 (number 10), IL-6 (number 11), and IL-11 (number 12).

The whole array analysis has shown a high level (80.1%) of correlation ($R > 0.895$) between all individual arrays, independent of their origin. Given that this analysis is the summation of approximately 55 000 probe-sets and that all cells tested are of epithelial origin, this might not come as a surprise. However, this does not imply that despite the ability of cell-lines to respond to protease activity, H292 would be the perfect model for primary nasal epithelium (11). When we investigate the response of each epithelium to HDM extract on the macro-level a different picture emerges. Now only a very limited number of genes behave in a similar fashion in all three epithelia. This is not surprising. Our previous analysis of the response to HDM extract in primary epithelium obtained from both healthy and allergic individuals had already revealed a limited overlap between these two cell types. Inclusion of a third factor (H292) in this analysis will only serve to reduce the number of similarities. When we investigate the response of H292 to HDM, we observe that both the fold-changes are more pronounced and that the number of genes affected is higher than in primary epithelium. The latter observation might well be related to the limited variation that can be expected in a triplicate H292 experiment. The experimen-

tal variation in a cell-line is likely to be significantly less than the variation in primary epithelia, where intra-individual differences will result in a reduced statistical power to detect differences. In this respect a cell-line is a clear advantage, as also small differences can be detected, differences that for the process under investigation, can be as important as bigger differences. Whether the more pronounced fold-changes induced by HDM in H292 are also related to the cell-line nature of H292 is unclear, but its bronchial nature should be considered. For instance, the response to lipopolysaccharide (LPS) is also greater in bronchial epithelia than in nasal epithelia (data not shown). As nasal epithelia are more likely to come in contact with environmental triggers, a mechanism seems to be in place that partly protects nasal epithelia from eliciting a too strong response. Despite the observation that the response in H292 is more similar to the response in healthy primary epithelium, these considerations must lead to the conclusion that on the macro-level the response in H292 cannot be used as a detailed model for the response of primary nasal epithelium to HDM extract.

Even though H292 cannot be used as a true model for the reason explained above, our detailed analysis of the similarities between the response in H292 and primary epithelium provided clues toward a better understanding of the HDM-response *per se* and how this response could contribute to allergy. This would be as a consequence of a further delineation of the responses we had first described in the analysis of primary healthy *vs* primary allergic epithelium. Just 29 genes of the regulatory network we had identified in primary epithelium respond in H292 in an identical fashion. Nearly all (24 of 29) genes show only one of the two distinct expression patterns that we had first identified in the transcription factors of the primary epithelium network. One class is characterized by high expression in non-HDM-exposed allergic samples that does not further increase after HDM-exposure, whereas these same transcription factors have low expression in non-HDM-exposed healthy samples and are upregulated by HDM. Genes with this expression pattern are: IL-8, IL-1B, CXCL3, TNFAIP3, HBEGF, PTGS2, BMP2, PLAUR, PLAU, NFKB2, NFKB1, NPC1, TICAM1, EPHA2, CTGF, and SPRY1. The second class is similar to the first class, but now the expression levels in allergic epithelium are relatively low (and remain unaffected by HDM-exposure), while they still go up in healthy epithelium. Genes with this expression pattern are: CCL20, CXCL2, AREG, LDLR2, JUN, ATF3, EGR1, and DUSP1. The four genes (Serp1B3, PSAT1, complement factor C3, and IVNS1ABP) that have a shared expression between H292 and primary allergic epithelium cannot be clustered in a similar fashion.

Previously, we indicated an aberrant interaction between the NF- κ B and AP-1 transcription families that could be responsible for the activated state in primary epithelium from allergic individuals. Now we show, not only that four of these transcription factors (NFKB2,

NFKB1, JUN, and ATF3) can be found to respond in H292, but also that other genes with this type of response are conserved between H292 and primary epithelium. Some cytokines, chemokines, or growth factors of the core-response have been well described as classical players in allergy or inflammation; IL-1 β , IL-8, CXCL2, CXCL3, CCL20, CTGF, HBEGF, and AREG (12–19). Also, some relative new players in the allergy field are related to the regulation of critical signaling effects; C3, SERPINB3, DUSP1, SPRY1, EPHA2, PLA2, and PLAUR (20–27). Moreover, three genes; TLR3, TICAM1, and IVNS1ABP (28–30) can be directly linked to the defense against viral infections. This is highly relevant given the above-mentioned dual relationship between allergy and viral infection. So early viral infections in life have been linked to the protection against the later development of allergic disease and conversely concurrent viral infections have been linked to the exacerbation of allergic and asthmatic symptoms (31). TICAM1 (Toll-like receptor adaptor molecule 1, also known as TRIF) is an essential part of the TLR3-mediated signaling cascade that is activated through viral PAMPs, leading to an anti-viral response and the production of interferons (32). IVNS1ABP (influenza virus NS1A-binding protein) is the potential target of the viral protein NS1A through which influenza virus interferes with the RNA metabolism of the infected cell (30). More debatable could be the link between allergy and obesity through LDLR or NPC1, given that both these proteins can be linked to lipid metabolism (33, 34). Also, here more genes, which can be linked to nutrition like the SLC-class of amino acid transporters, are part of the larger regulatory network in primary epithelium.

A number of studies have focused on individual genes or proteins in relation to allergy or inflammation. Although, this approach is valid, it does not take into account the complex nature of the immunological response. In some instances (IL-1 β or IL-8) it is not clear whether these genes are typical for the allergic response in epithelium. More likely, these genes correspond to a strong common response triggered by environmental factors. Therefore, one should be careful to draw general conclusions based on outcome of a relatively few parameters. Our analysis of the

HDM-response in epithelia shows that this is also true in a narrowly defined experimental system. Most importantly, the HDM-responses in three different epithelia reveal the involvement of a limited number of genes. Moreover, the activated state of the allergic epithelium itself influences the ability of allergic epithelium to respond to HDM extract and can even result in a reduction in the expression level of genes that are highly expressed at baseline. These genes are involved in the regulation in signaling events inside the cell (through receptors, phosphatases, and adaptor molecules), the regulation of downstream events (through cytokines, chemokines, and growth factors), and regulation of these factors (through transcription factors). Not only do these data show the active and well-defined involvement of airway epithelia in the allergic response, but also identify potent new targets for treatment.

Supplementary material

The following supplementary material is available for this article online:

Table S1. Genes that are in the Venn diagram and show statistical change in two groups with a similar direction of change. 1A) genes up-regulated both in allergic and H292. 1B) genes that are down-regulated both in allergic and H292. 1C) genes that are up-regulated in healthy and H292. 1D) genes that are down-regulated in healthy and H292.

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Supplemental table 1 genes that are in the Venn diagram and show statistical change in two groups with a similar direction of change. 1A) genes up-regulated both in allergic and H292. 1B) genes that are down-regulated both in allergic and H292. 1C) genes that are up-regulated in healthy and H292. 1D) genes that are down-regulated in healthy and H292.

In bold are indicated the genes that are also in the network analysis (4) of allergic and healthy controls.

Supplemental table 1A

ID	Symbols	Names	FC all	fc h292
209720_s_at	SERPINB3	serpin peptidase inhibitor, clade B (ovalbumin), member 3	1.67	2.47
223062_s_at	PSAT1	phosphoserine aminotransferase 1	2.12	2.10
209719_x_at	SERPINB3	serpin peptidase inhibitor, clade B (ovalbumin), member 3	1.62	1.88
224209_s_at	GDA	guanine deaminase	1.95	2.72
209908_s_at	TGFB2	transforming growth factor, beta 2	1.47	2.51
242722_at	LMO7	LIM domain 7	1.29	2.50
		solute carrier family 30 (zinc transporter), member 1		
228181_at	SLC30A1		1.29	1.58
207059_at	PAX9	paired box gene 9	1.58	1.52
236067_at	MBNL2	muscleblind-like 2 (Drosophila)	1.30	1.46
242204_at	WFDC5	WAP four-disulfide core domain 5	2.65	1.30
1569206_at	TCP11L2	t-complex 11 (mouse) like 2	1.38	1.16
232810_at	AIG1	androgen-induced 1	1.29	1.16
225105_at	LOC387882	NA	1.51	1.09

Supplemental table 1B

ID	Symbols	Names	FC all	fc h292
217767_at	C3	complement component 3	-1.62	-1.12
201362_at	IVNS1ABP	influenza virus NS1A binding protein	-1.50	-1.40
226796_at	LOC116236	NA	-1.30	-1.09
221534_at	Bles03	NA	-1.15	-1.20
225240_s_at	MSI2	musashi homolog 2 (Drosophila)	-1.33	-1.35
205573_s_at	SNX7	sorting nexin 7	-1.44	-1.46
244317_at	KIAA1324L	KIAA1324-like	-1.23	-1.50
226910_at	COMMMD2	COMM domain containing 2	-1.43	-1.53
217853_at	TNS3	tensin 3	-1.51	-1.78
		polypeptide N-acetylgalactosaminyltransferase 11		
219013_at	GALNT11		-1.33	-2.00
212336_at	EPB41L1	erythrocyte membrane protein band 4.1-like 1	-1.70	-2.11
205623_at	ALDH3A1	aldehyde dehydrogenase 3 family, member A1	-1.50	-3.98

Supplemental table 1C

ID	Symbols	Names	fc con	fc h292
205476_at	CCL20	chemokine (C-C motif) ligand 20 (MIP3alpha)	4.88	90.92
202859_x_at	IL8	interleukin 8	6.56	61.16
209774_x_at	CXCL2	chemokine (C-X-C motif) ligand 2 (GRObeta)	4.68	46.03
204470_at	CXCL1	chemokine (C-X-C motif) ligand 1 (GROalpha)	2.48	23.32
205239_at	AREG	amphiregulin (schwannoma-derived growth factor)	1.64	20.84
202643_s_at	TNFAIP3	tumor necrosis factor, alpha-induced protein 3	3.36	12.28

203821_at	HBEGF	heparin-binding EGF-like growth factor	2.98	9.85
1557285_at	AREG	amphiregulin (schwannoma-derived growth factor)	1.85	7.75
38037_at	HBEGF	heparin-binding EGF-like growth factor	2.61	6.77
1554997_a_at	PTGS2	prostaglandin-endoperoxide synthase 2	4.09	6.66
39402_at	IL1B	interleukin 1, beta	2.12	6.09
205067_at	IL1B	interleukin 1, beta	2.27	3.25
202068_s_at	LDLR	low density lipoprotein receptor	1.68	2.82
210845_s_at	PLAUR	plasminogen activator, urokinase receptor	8.34	2.81
207535_s_at	NFKB2	NFKB2 (p100)	2.53	2.72
202679_at	NPC1	Niemann-Pick disease, type C1	1.30	2.48
205289_at	BMP2	bone morphogenetic protein 2	2.81	2.46
205290_s_at	BMP2	bone morphogenetic protein 2	2.57	2.37
239876_at	NFKB1	NFKB1 (p105)	2.27	2.04
211668_s_at	PLAU	plasminogen activator, urokinase	2.16	1.98
213191_at	TICAM1	toll-like receptor adaptor molecule 1	1.95	1.97
203499_at	EPHA2	EPH receptor A2	1.61	1.88
217584_at	NPC1	Niemann-Pick disease, type C1	1.62	1.80
201464_x_at	JUN	v-jun sarcoma virus 17 oncogene homolog (avian)	1.52	1.73
209101_at	CTGF	connective tissue growth factor	2.81	1.51
201044_x_at	DUSP1	dual specificity phosphatase 1	1.71	1.45
205479_s_at	PLAU	plasminogen activator, urokinase sprouty homolog 1, antagonist of FGF signaling	1.91	1.42
212558_at	SPRY1	(Drosophila)	1.55	1.32
1554980_a_at	ATF3	activating transcription factor 3	3.80	1.28
211506_s_at	IL8	interleukin 8	7.23	26.66
227404_s_at	EGR1	early growth response 1	3.23	2.23
201694_s_at	EGR1	early growth response 1	2.71	1.57
221841_s_at	KLF4	Kruppel-like factor 4 (gut)	1.52	11.12
204622_x_at	NR4A2	nuclear receptor subfamily 4, group A, member 2 v-maf musculoaponeurotic fibrosarcoma oncogene	1.81	4.29
36711_at	MAFF	homolog F (avian)	2.02	4.08
217997_at	PHLDA1	pleckstrin homology-like domain, family A, member 1	1.44	3.97
209457_at	DUSP5	dual specificity phosphatase 5	2.68	3.32
204621_s_at	NR4A2	nuclear receptor subfamily 4, group A, member 2	1.94	3.07
239331_at	NA	NA	2.05	2.99
229242_at	NA	NA	1.43	2.86
233488_at	RNASE7	ribonuclease, RNase A family, 7	1.59	2.66
219496_at	C2orf26	chromosome 2 open reading frame 26	1.59	2.52
221765_at	UGCG	UDP-glucose ceramide glucosyltransferase	1.41	2.40
213506_at	F2RL1	coagulation factor II (thrombin) receptor-like 1	2.31	2.29
204011_at	SPRY2	sprouty homolog 2 (Drosophila)	1.59	2.29
240861_at	NA	NA	1.63	2.28
231697_s_at	TMEM49	transmembrane protein 49	1.59	2.18
203313_s_at	TGIF	TGFB-induced factor (TALE family homeobox)	1.92	1.95
225756_at	CSNK1E	casein kinase 1, epsilon protein phosphatase 1, regulatory (inhibitor) subunit	1.19	1.94
224692_at	PPP1R15B	15B	1.36	1.91
230503_at	NA	NA	2.29	1.91
225582_at	KIAA1754	KIAA1754	1.49	1.80
242294_at	NA	NA	1.33	1.79
210260_s_at	TNFAIP8	tumor necrosis factor, alpha-induced protein 8	1.81	1.70

225557_at	AXUD1	AXIN1 up-regulated 1	1.99	1.68
235165_at	PARD6B	par-6 partitioning defective 6 homolog beta (C. elegans)	2.11	1.65
209493_at	PDZD2	PDZ domain containing 2	1.58	1.61
221840_at	PTPRE	protein tyrosine phosphatase, receptor type, E	1.49	1.60
32069_at	N4BP1	NA	1.16	1.57
1566901_at	TGIF	TGFB-induced factor (TALE family homeobox)	2.69	1.55
240991_at	LOC392271	NA	2.05	1.53
1558783_at	WTAP	Wilms tumor 1 associated protein	2.26	1.53
209535_s_at	NA	NA	1.40	1.52
1554783_s_at	ARHGEF2	rho/rac guanine nucleotide exchange factor (GEF) 2	1.23	1.48
1555411_a_at	CCNL1	cyclin L1	1.88	1.47
224606_at	KLF6	Kruppel-like factor 6	2.14	1.44
208033_s_at	ATBF1	AT-binding transcription factor 1	1.22	1.42
207439_s_at	SLC35A2	solute carrier family 35 (UDP-galactose transporter), member A2	1.31	1.41
227099_s_at	LOC387763	NA	1.66	1.39
225991_at	TMEM41A	transmembrane protein 41A	1.47	1.39
203463_s_at	EPN2	epsin 2	1.14	1.38
219334_s_at	OBFC2A	oligonucleotide/oligosaccharide-binding fold containing 2A	1.33	1.38
1556220_at	NA	NA	1.50	1.37
211962_s_at	ZFP36L1	zinc finger protein 36, C3H type-like 1	2.32	1.36
1557810_at	CCT5	chaperonin containing TCP1, subunit 5 (epsilon)	1.48	1.32
214629_x_at	RTN4	reticulon 4	1.13	1.29
203068_at	KLHL21	kelch-like 21 (Drosophila)	1.53	1.28
225648_at	STK35	serine/threonine kinase 35	1.20	1.27
229396_at	OVOL1	ovo-like 1(Drosophila)	3.69	1.26
235037_at	TMEM41A	transmembrane protein 41A	1.39	1.25
203002_at	AMOTL2	angiomin like 2	1.92	1.25
215528_at	NA	NA	1.76	1.23
227489_at	CCDC45	coiled-coil domain containing 45	1.36	1.20
203739_at	ZNF217	zinc finger protein 217	1.61	1.19
201328_at	ETS2	v-ets erythroblastosis virus E26 oncogene homolog 2 (avian)	1.44	1.14
1557394_at	DLGAP4	discs, large (Drosophila) homolog-associated protein 4	1.35	1.12
224624_at	LRRC8A	leucine rich repeat containing 8 family, member A	1.34	1.11

Supplemental table 1D

ID	Symbols	Names	fc con	fc h292
206271_at	TLR3	toll-like receptor 3	-1.51	-1.75
226243_at	LOC391356	NA	-1.27	-1.14
223213_s_at	ZHX1	zinc fingers and homeoboxes 1	-1.27	-1.14
204784_s_at	MLF1	myeloid leukemia factor 1	-1.37	-1.20
226151_x_at	CRYZL1	crystallin, zeta (quinone reductase)-like 1	-1.16	-1.23
218048_at	COMMD3	COMM domain containing 3	-1.19	-1.23
229353_s_at	NUCKS1	nuclear casein kinase and cyclin-dependent kinase substrate 1	-1.31	-1.29
227925_at	FLJ39051	NA	-1.49	-1.29
223442_at	NICN1	nicolin 1	-1.18	-1.34
203714_s_at	TBCE	tubulin-specific chaperone e	-1.21	-1.39

202735_at	EBP	emopamil binding protein (sterol isomerase)	-1.25	-1.40
219962_at	ACE2	angiotensin I converting enzyme (peptidyl-	-1.60	-1.52
215016_x_at	DST	dipeptidase A) 2	-1.41	-1.81
242093_at	NA	dystonin	-1.35	-2.02
		NA		
202436_s_at	CYP1B1	cytochrome P450, family 1, subfamily B, polypeptide	-1.89	-2.14
		1		
211361_s_at	SERPINB13	serpin peptidase inhibitor, clade B (ovalbumin),	-1.23	-2.27
		member 13		
221521_s_at	Pfs2	NA	-1.39	-3.28
209917_s_at	TP53AP1	TP53 activated protein 1	-1.25	-3.60
204734_at	KRT15	keratin 15	-1.18	-3.79
