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Clues to asthma pathogenesis from microarray expression studies

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Abstract

Asthma is a chronic inflammatory disease characterized by airway hyperresponsiveness (AHR), tissue remodeling, and airflow obstruction. The pathogenesis of asthma is only partly understood, and there is an urgent need for improved therapeutic strategies for this disease. Microarray technology has considerable promise as a tool for discovery of novel asthma therapeutic targets, although the field is still in its infancy. A number of studies have described expression profiles derived from human asthmatic lung tissue, mouse airway tissue, or from key cell types associated with asthma, but to date relatively few studies have exploited these findings to discover new pathways involved in the pathogenesis of asthma. Among the genes to have been identified by array studies and validated by further studies are monokine induced by interferon (IFN)-γ, fatty acid binding proteins (FABP), and complement factor 5 (C5). Here we provide examples of microarray approaches to the discovery of new molecules associated with asthma. We anticipate that these types of analyses will provide considerable insight into asthma pathogenesis and will provide a wealth of new molecules for downstream analyses such as gene deficient mouse studies, or monoclonal antibody production.

Keywords: Asthma; Microarray; Mast cell; Bronchial epithelium; Cytokine

Abbreviations: AHR, airway hyperresponsiveness; FABP, fatty acid binding protein; GM-CSF, granulocyte-macrophage colony stimulating factor; HBE, human bronchial epithelial cells; IFN, interferon; IL, interleukin; MBP, major basic protein; STAT, signal transducer and activator of transcription.

Contents

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1. Introduction

Asthma is a chronic inflammatory disease characterized by airway hyperresponsiveness (AHR), tissue remodeling, and airflow obstruction (Bochner et al., 1994). The incidence of asthma in western countries has increased markedly over the last 20 years, and in countries such as the United States and Australia it now affects ~10% of the population (Woolcock et al., 2001; CDC, 2002). The economic burden of asthma has also increased, with the annual cost in the United States in 1998 estimated at US$12.7 billion (Weiss & Sullivan, 2001). These figures indicate that advances in diagnosis, treatment, or prevention of asthma will lead to major medical and economic benefits to society.

One of the major advances to come out of the genomic revolution has been the development of microarray technology. Using microarrays, it is now possible to profile gene expression for essentially the entire human genome. In this review, we describe some of the recent uses of microarray technology for the study of asthma. Most of these studies are still of a preliminary nature, but initial data strongly suggests that microarrays will lead to major advances in our understanding of asthma etiology and pathogenesis.

1.1. Etiology and pathogenesis of asthma

Asthma is caused by environmental factors in genetically predisposed individuals. Detailed understanding of the etiology and underlying pathogenetic mechanisms in asthma is still lacking, and this is a major impediment to development of improved diagnostic and therapeutic strategies for this disease. Atopy is the strongest predisposing factor for the development of asthma (Lemanske & Busse, 2003), and the majority of asthma is associated with allergic inflammation characterized by the presence of mast cells, basophils, eosinophils, monocytes, and Th2 lymphocytes. The allergic inflammatory response is regulated largely by Th2 cytokines such as interleukin (IL)-4, IL-5, IL-9, and IL-13, all of which impinge on the response in a variety of ways. For example, IL-5 regulates differentiation, activation, and survival of eosinophils, a key effector cell type in allergic inflammation (Foster et al., 2001). IL-4 is a highly pleiotropic cytokine, controlling such events as Th2 cell differentiation, IgE isotype switching, and expression of vascular cell adhesion molecule-1 on endothelial cells. IL-13 shares many functions with IL-4 but in addition is a crucial mediator of airway hyperresponsiveness (Wynn, 2003).

It is becoming increasingly apparent that allergic inflammation does not account for all the features of asthma. For example, a number of genetic studies showed a separation in the inheritance of bronchial hyperresponsiveness and atopy (Holloway et al., 1999; Davies et al., 2003). Studies examining the earliest events in the development of asthma have often demonstrated injury to the lung occurring prior to the development of asthma, or in the absence of eosinophilic inflammation (Cokugras et al., 2001; Payne et al., 2003; Fedorov et al., 2005). Even the role of eosinophils, which have for long been considered central effector cells in asthmatic inflammation, is becoming controversial. In vitro and animal model studies strongly support a role for eosinophils in airway pathology (Foster et al., 2001). However, clinical trials in which the levels of circulating and tissue eosinophils were lowered using anti-IL-5 therapy have questioned a key role for eosinophils in disease (Leckie et al., 2000).

In recent years, there has been increasing focus on the role of airway cells and tissue remodeling in the development of asthma. A number of studies have now shown that airway cells in asthmatic patients differ from those of non-asthmatics. For example, smooth muscle cells from asthmatics appear to have an intrinsically increased rate of proliferation (Johnson et al., 2001) linked to absence of the C/EBP transcription factor (Roth et al., 2004). Epithelial cells from asthmatics show increased susceptibility to rhinovirus infection (Wark et al., 2005) and oxidant-induced apoptosis (Bucchieri et al., 2002), and release more IL-8 and granulocyte-macrophage colony stimulating factor (GM-CSF) when exposed to diesel exhaust particles (Bayram et al., 1998). In response to these and other findings, some researchers have suggested that airway inflammation and remodeling occur as a consequence of increased susceptibility to injury or impaired healing of the airway epithelium (Davies et al., 2003).

Asthma is a highly heterogeneous disease, and this presents an additional challenge for the design of new therapeutics (Peters, 2003). This heterogeneity is most likely due to a combination of environmental and genetic factors and manifests at many levels including etiology, pathogenesis, prognosis, and response to therapy. To design optimal therapies and diagnostic tools, it will be necessary to fully understand the basis of asthma heterogeneity. Gene profiling technology has the potential to provide important insights in different forms of asthma, and it has already been used successfully to probe heterogeneity in other diseases (Alizadeh et al., 2000).

1.2. Asthma therapies

The most effective asthma therapy at present is treatment with combination inhalers that contain a corticosteroid and a long-acting β2-adrenoceptor agonist. Other drugs efficacious in the treatment of asthma include theophylline, cromolyn, leukotriene antagonists, and anti-IgE monoclonal antibodies. The majority of asthma cases can be well controlled using currently available drugs; however, there is a growing number of asthmatics whose disease is not optimally controlled, particularly those with severe asthma. An additional area for improvement is development of orally active drugs. Unfortunately the pharmaceutical industry has had little success in developing new therapeutic agents for asthma, not least due to the complexity of the disease, a failure to fully understand asthma etiology and pathogenesis, and the lack of suitable animal models. A large number of targets are currently under investigation as potential asthma drug targets (Barnes, 2004); however, few have shown promise so far, and there is a need for the identification of new drug targets.

1.3. Functional genomics

Using microarray technology, it is now relatively straightforward to simultaneously assess transcriptional activity of essentially every gene in an organism's genome. The development of this technology has been an enormous boost to many areas of medical research—drug target discovery, identification of novel transcriptional programs, development of molecular disease classifications, and identification of new prognostic and diagnostic markers. The myriad applications and experimental challenges associated with microarray technology have been widely reviewed elsewhere (Staudt & Brown, 2000; Staudt, 2001; Butte, 2002; Stoughton, 2004) and will not be discussed here. However, it is worth repeating here an important feature of microarray-based experiments and the data they generate. Unlike traditional experimental approaches, a microarray experiment is almost certain to generate novel data. The real challenge is to design a microarray experiment to generate data that can be analyzed in a way that leads to useful and relevant information. This requires careful consideration of numerous factors including sample composition (especially when using tissue samples), methods for data analysis, experimental variation, and number of replicates required.

In this review, we will discuss the literature that has emerged over the last few years in which gene profiling has been employed to address issues relating to the pathogenesis of asthma. The studies have been separated into 2 broad categories: first, those studies involving asthmatic tissue samples, and second, studies involving pure cultures of cell types implicated in the pathogenesis of asthma. The majority of asthma gene profiling studies have been aimed at identifying novel transcriptional programs in cells and tissues that underlie asthmatic pathology. A large number of these studies are of a preliminary nature involving the description of novel gene profiles, and relatively few studies have actually used these profiles to progress our understanding of asthma pathogenesis. We expect that in the next few years there will be many downstream studies, reporting developments arising from novel transcript profiling data. Microarrays also have considerable potential as diagnostic tools, although to date there have been very few efforts to apply this technology to asthma diagnosis.

2. Profiling asthmatic tissue

2.1. Human studies

Our understanding of gene expression in asthma is still at a very basic level, and gene profiling is likely to be profitable for almost any aspect of the pathogenesis of this disease. Some areas of study that we consider to be particularly promising include analysis of the response to therapy; the molecular events underlying asthma heterogeneity; and the response of both airway and inflammatory cells to allergen challenge. The most obvious way to apply gene-profiling technology to the study of asthma is to directly analyze gene expression in human asthmatic tissue samples. As discussed below, this approach can be very difficult and relatively few such studies have been reported. In particular, it is difficult to obtain suitable tissue samples, with biopsies obtained from fiberoptic bronchoscopy being the main source of tissue. Ongoing therapy for asthma or other diseases can also be problematic, by having a confounding effect on gene expression. Nonetheless, a number of reports have emerged in recent years describing gene profiles obtained from asthmatic tissue.

To identify genes expressed in airway epithelium in allergic inflammation, Lilly et al. (2005) used Affymetrix oligonucleotide arrays and endobronchial brushing to analyze gene expression in airway epithelial cells of mild asthmatics before and after segmental allergen challenge. After statistical analysis and post hoc data filtering, 141 up-regulated and 8 down-regulated genes were identified. As well as genes with defined immune function, a large number of genes involved in tissue repair and proliferation were induced. A number of these genes had not previously been associated with asthma, and the data provide the basis for further mechanistic studies. These authors also compared their results to the expression profile previously reported for IL-13-treated airway epithelial cells (Lee et al., 2001) and found very little overlap in the genes that were regulated in both studies. This suggests that many factors in addition to IL-13 are involved in segmental allergen challenge in vivo and emphasizes the need to integrate in vitro and in vivo studies.

Laprise et al. (2004) studied gene expression in bronchial biopsies from healthy controls ($n=4$) and from asthmatics ($n=4$) before and after inhaled corticosteroids (Laprise et al., 2004). Seventy-nine genes had significantly different expression levels between the control and asthmatic groups, and 128 genes showed differential expression between pre- and post-corticosteroid therapy. Corticosteroid therapy reversed ~25% of the genes that were regulated in the asthmatic group, especially genes encoding proteolytic, immune, and extracellular proteins. Although the number of subjects was small, a number of the genes identified had previously been associated with asthma. Together with the study by Lilly et al., this work indicates that bronchial biopsies are suitable tissue source for gene profiling studies and the preliminary data already point to some promising candidates for further study.

A slightly different approach was taken by Guajardo et al. (2005) who studied nasal mucosal cells from healthy children and those with stable and exacerbated asthma, with the assumption that gene expression in nasal mucosal cells reflects gene expression in the respiratory epithelium of the lung. Not surprisingly, a large number of immune genes were up-regulated in the exacerbated asthmatic samples. Cilia-related genes were prominent among the most strongly down-regulated genes in asthmatics, an unexpected finding suggesting that altered ciliary function may contribute to airway obstruction in asthma.

Microarrays have considerable potential as tools for molecular classification and the development of improved strategies for diagnosis, although to date few studies have focused on asthma. After screening peripheral blood from atopic asthmatics, non-atopic asthmatics, and healthy controls,

Brutsche et al. (2002) developed a composite atopy gene expression (CAGE) score that was superior to total IgE in differentiating atopic from non-atopic subjects. This study used low-density arrays and relatively few subjects, but as a proof-of-principle study, it clearly indicates the potential of this approach in the development of diagnostic strategies.

2.2. Animal models

A number of gene profiling studies have been undertaken using animal models of asthma. With the exception of 1 study using a monkey model of asthma (Zou et al., 2002), all studies have used the mouse model of acute allergic airway inflammation. Although the usual caveats concerning animal models apply, these studies have the advantage of obtaining sufficient quantities of well-defined tissue samples, free of uncontrolled genetic and environmental heterogeneity.

A particularly elegant use of gene profiling was reported by Karp et al. (2000) to identify the genetic basis of allergen-induced airway hyperresponsiveness (AHR) susceptibility in mouse strains of differing susceptibility. These investigators had previously used high- and low-responder mouse strains to identify 2 distinct quantitative trait loci (QTL) on chromosome 2 that regulate AHR. Using gene profiling, a panel of genes differentially expressed between allergen-treated lungs of high- and low-responder strains was identified. Only one of these genes, complement factor 5 (C5) was located within the QTL intervals. SNP-based genotyping identified a mutation in C5 in high-responder mice that resulted in deficiency of C5 mRNA and protein, and subsequent heightened susceptibility to allergen-induced AHR. These findings may be directly relevant to human disease, because a recent study has reported on a haplotype within the C5 gene associated with protection against childhood asthma (Hasegawa et. al., 2004).

Marc Rothenberg's lab has conducted an extensive series of studies based on gene profiling lung tissue from mouse models of allergic airway inflammation. Two mouse models were used: (1) a model based on systemic sensitization with ovalbumin (OVA), with subsequent aerosol challenge with OVA, and (2) repeated intranasal challenge with *Aspergillus fumigatus* allergen. Although these 2 models vary considerably in terms of induction (systemic vs. mucosal), their ultimate pathologic profile is similar, involving tissue eosinophilia, mucus hypersecretion, and AHR. Using these 2 models, an asthma gene expression signature was developed. The expression of 4.7% of all genes on the chip was regulated following antigen exposure. A large number of the genes induced at the early time point (3 hr) were associated with pathogen recognition and initiation of the immune response such as CD14, CD83, IL-1β, and CXCL1. By 18 hr following allergen exposure, expression of genes associated with adaptive immunity and tissue remodeling became more prominent.

Seventeen of the 28 chemokines represented on the chip were up-regulated, including several not previously associated with allergic airway inflammation. Surprisingly, expression of a substantial number of interferon (IFN)-regulated, Th1-associated chemokines was enhanced, including monokine induced by IFNγ (Mig) and IFNγ-inducible protein of 10 kDa (IP-10) (Fulkerson et al., 2004a, 2004b). Further experiments indicated that Mig induced a dose-dependent inhibition of chemokine-induced eosinophil chemotaxis, and neutralization of Mig enhanced airway eosinophilia in the model of allergic airway inflammation (Fulkerson et al., 2004a). Thus, the results provide evidence for a regulatory pathway by which both Th1- and Th2-associated chemokines regulate eosinophil responses. This study provides a good example of the utility of gene profiling for discovery of novel disease-associated genes, in which array-based discoveries lead to generation of novel hypotheses that can be tested in biological systems.

A notable feature of the data from mouse model gene profiles was the strong up-regulation of genes involved in arginine metabolism, including arginase I, arginase II, and the cationic amino acid transporter 2 (Zimmermann et al., 2003). The transcriptional changes correlated with increased lung arginase activity. Arginase expression was also enhanced in human asthmatic lung. Previous studies had already implicated nitric oxide synthase (NOS)-mediated arginine metabolism in the pathogenesis of asthma (Meurs et al., 2003), and the findings by Zimmermann now suggest a pivotal and complex role for arginine metabolism in asthma, involving interplay between NOS and arginase (Zimmermann et al., 2003; King et al., 2004a).

These investigators have subsequently mined the array data set to establish further novel insights into asthma pathogenesis. In addition to arginase, a number of novel genes potentially involved in asthma pathogenesis were described and their lung expression characterized in more detail. This included ADAM8 (King et al., 2004b), small proline rich protein 2 (Zimmermann et al., 2005), and trefoil factor 2 (Nikolaidis et al., 2003). Most of this work is descriptive in nature, and its true value will become apparent when the function of these novel asthma genes is tested in appropriate models.

An interesting feature of the gene profiling studies by Rothenberg's lab was uncovered when they analyzed the effect of signal transducer and activator of transcription (STAT)-6 on gene expression in the asthma model. Although STAT6-mediated responses are central to the development and manifestations of allergic airway inflammation (Kuperman et al., 1998, 2002), a substantial number of genes were regulated in a STAT6-independent manner. In addition, an additional program of gene expression was induced in STAT6 KO mice undergoing allergic airway inflammation, a finding that will need to be considered in any STAT6-based therapeutic approaches.

Gene profiling has also been used in an attempt to unravel the differential effects of IL-4 and IL-13 in asthma. Although IL-4 and IL-13 both act through the same receptor on non-hematopoietic cells, IL-13 appears to have a more potent effect than IL-4 on AHR, pulmonary fibrosis, and goblet cell hyperplasia (Wills-Karp & Chiaramonte, 2003). Gene profiling was used to test the hypothesis that the enhanced activity of IL-13 was due to induction of unique genes by this cytokine. A limited number of genes were identified that were induced by

IL-13, but not by IL-4. By analyzing expression of these genes, it became apparent that IL-4 possesses some counter-regulatory properties not shared by IL-13 that can suppress STAT6-mediated responses (Finkelman et al., 2005).

2.3. Considerations relating to tissue profiling studies

There are a number of issues that need to be considered in designing gene profiling studies of asthmatic lung and biopsy samples.

2.3.1. Source of tissue

For obvious reasons, large samples of asthmatic human lung tissue are not readily available for study. The best approach is to profile bronchial biopsy samples (Laprise et al., 2004; Lilly et al., 2005), although this has a number of limitations including the small size of biopsies, the difficulty in ensuring collection of fully representative samples, and the limited regions of the lung that are accessible by fiberoptic biopsy protocols.

2.3.2. Ongoing therapy

Ongoing therapy can potentially have a major effect on gene expression profiles. Most moderate and severe asthmatics are taking corticosteroids, which are known to have major transcriptional effects. It is difficult to fully control for this issue. The extent of the problem is illustrated by a microarray study that specifically examined the effect of corticosteroid treatment on gene expression in asthmatics (Laprise et al., 2004). This study identified a greater number of genes regulated by corticosteroids than genes regulated by asthma itself.

2.3.3. Tissue heterogeneity

For any tissue sample, multiple cell types contribute to the gene expression profile. This cellular heterogeneity adds considerable complexity to data interpretation and has the potential to obscure pathologically important transcripts in specific cell types. This problem is particularly prominent for inflammatory diseases, in which a large number of leukocytes infiltrate the tissue site, making comparisons with uninflamed control tissue difficult. This has the potential to overwhelm the data analysis with thousands of differentially regulated genes. The mouse model studies described above (Section 2.2) were able to avoid this problem by focusing on time points early after allergen challenge prior to the major leukocyte influx (King et al., 2004b). In asthma, different proportions of specific airway cells such as epithelial cells, smooth muscle cells, and fibroblasts in healthy and asthmatic subjects contribute to sampling heterogeneity. Finally, asthmatic changes such as oedema, inflammation, and mucous gland hypertrophy can interfere with the depth of sampling and biopsy constituents (Laprise et al., 2004).

2.3.4. Addressing the challenges of profiling tissue samples

The issues above present significant obstacles to meaningful functional genomic analysis of asthmatic tissue samples. The problem of tissue heterogeneity requires that more extensive validation of the array results be undertaken than in cellular studies. In particular, immunohistochemistry or in situ hybridization is necessary to understand the pathologic basis for differential gene expression across samples.

Laser capture microdissection (LCM) has considerable potential for overcoming the difficulties associated with tissue heterogeneity. This technology allows isolation of specific cell types or even single cells (Luo et al., 1999; Kamme & Erlander, 2003) and is a particularly powerful strategy in combination with gene profiling (Kamme & Erlander, 2003; Peterson et al., 2004; Player et al., 2004). To date there is only 1 report detailing the application of this approach to asthma, in which LCM was used to define the phenotype of smooth muscle cells in asthma (Woodruff et al., 2004). The gene profiling data indicated no difference in gene expression in SMC from healthy and asthmatic subjects, which supported the authors' conclusion that the major defect in asthmatic SMC is increased proliferation.

An additional approach to tackle tissue heterogeneity is to integrate gene expression profiles derived from both tissues and cells. For example, Peterson et al. (2004) used a panel of expression profiles from purified leukocyte subsets to assist in characterizing the expression pattern of glomeruli from lupus nephritis. By doing so, they were able to obtain additional information about the nature of leukocyte infiltration in the glomeruli, and how it contributed to specific patterns of gene expression. This approach has not yet been utilized for asthma studies.

3. Profiling purified and cultured cells

An alternative approach for identifying new genes that contribute to the pathogenesis of asthma is to establish gene expression profiles for pure populations of cell types central to disease pathogenesis. This is especially valuable when in vitro models can be established that mimic aspects of the pathogenesis of asthma, for example, mast cells activated by cross-linking FcεR1 or comparison of Th1 and Th2 cells.

3.1. Mast cells

Mast cells are key effector cells during the immune response to pathogens, but their effector functions are also responsible for many of the symptoms associated with allergic diseases such as asthma. Mast cell products, such as histamine and tryptase, which are stored within preformed cytoplasmic granules, are rapidly released following IgE-dependent activation and can be readily detected in bronchial alveolar lavage samples from asthmatic patients (Wenzel et al., 1988; Broide et al., 1990, 1991). Despite their important role in disease, mast cells are relatively understudied because they are difficult to obtain in high numbers. Microarray technology has made a major contribution to mast cell research in recent years, by identifying novel transcriptional programs underlying mast cell function.

Cross-linking of high affinity IgE receptor (FcεR1) on mast cells by multivalent antigens is the main mode of mast cell activation during an asthmatic response. Early microarray studies conducted on in vitro cultured mast cells have therefore focused on profiling gene expression following IgE-dependent activation (Nakajima et al., 2001, 2002; Sayama et al., 2002). Sayama et al. (2002) observed up-regulated expression of 18 cytokines and 13 chemokines, which highlights the importance of this cell type in mediating inflammation. In particular, the authors noted the expression and up-regulation of IL-11, which had not been previously described in mast cells (Sayama et al., 2002). IL-11 has been implicated in airway inflammation and remodeling and was originally thought to be produced by eosinophils as IL-11 expression colocalized with major basic protein (MBP) expression in the subepithelial layer of asthmatic airways (Minshall et al., 2000). Although MBP was originally considered a highly eosinophil-specific product, microarray data have now revealed that mast cells also express high levels of MBP (Nakajima et al., 2001) necessitating a reinterpretation of the IL-11 immunostaining data.

During an asthmatic response, a mast cell residing in the lung will be exposed not only to allergens but also to cytokines such as IL-4, IL-5, IL-9, and IL-13. Comparing the effects of Th2 cytokines on mast cell activation via IgE receptor cross-linking is useful for understanding the complex interactions between mast cells and factors present in their immediate cellular environment during an asthmatic response. Lora et al. (2003) showed that priming with IL-4, IL-5, and IL-9 modified gene expression following FcεR1 cross-linking (Lora et al., 2003). For example, IL-4 favored the induction of genes involved in cell cycle arrest, consistent with previous reports that IL-4 stimulated mast cell apoptosis in vitro (Yeatman et al., 2000). These findings are useful for understanding mast cell homeostasis, as disease states are commonly associated with increased mast cell presence.

There is also growing evidence to suggest that mast cells are involved in fibrosis, a common outcome of long-term inflammation. To identify genes involved in this pathway, 2 independent studies used microarrays to identify growth factor expression in mast cells (Okumura et al., 2005; Wang et al., 2005). Both studies found that activated mast cells express and secrete the growth factor amphiregulin. Amphiregulin is a member of the epidermal growth factor family (EGF) and has been shown to (1) bind EGF receptor (Johnson et al., 1993); (2) promote fibroblast, epidermal keratinocyte, and tumor cell growth; and (3) induce a phenotype similar to inflammatory psoriasis when overexpressed in mice (Cook et al., 1997). Recombinant amphiregulin stimulated fibroblast proliferation, which correlated with increased expression of c-*fos* (Okumura et al., 2005), and also induced expression of mucin genes, MUC2 and MUC5AC, in a human pulmonary mucoepidermoid carcinoma cell line (Wang et al., 2005). These results suggest that mast cell-derived amphiregulin can directly influence fibrotic events and goblet cell hyperplasia, which are common causes of morbidity related to long-term asthma.

Cell surface receptors associated with promoting T-cell, B-cell, and dendritic cell interactions, such as CD40L, CD82, SLAM, CD83, 4-1BB ligand, and OX40 ligand, have also been identified on mast cell microarrays (Sayama et al., 2002; Okumura et al., 2003; Kashiwakura et al., 2004). Interestingly, T-cell costimulation by OX40L has been shown to promote the differentiation of naïve T-cells into Th2 cells (Ohshima et al., 1998). Further microarray analysis of mast cell subtypes showed that OX40L is preferentially expressed on cultured tonsillar mast cells but is also expressed at low levels on activated lung and peripheral blood-derived mast cells (Kashiwakura et al., 2004). The authors also observed that the enhanced T-cell proliferation observed during co-culture with mast cells could be blocked by an anti-OX40L antibody. Tonsillar mast cells are also more commonly found in close association with T-cells, suggesting that there is cross-talk occurring between the 2 cell types.

3.2. Airway epithelial cells

The airway epithelium has a multi-faceted role in asthma, acting not just as a physical barrier, but also as an active participant in key processes such as tissue remodeling and the inflammatory response. The central role of the airway epithelium in asthma has led a number of investigators to focus on this cell type in array studies designed to identify novel transcriptional programs underlying disease.

There is considerable evidence that bronchial epithelial cell responses in asthma are directly regulated by IL-4 and IL-13 (Richter et al., 2001; Kuperman et al., 2002; Cohn et al., 2004). To characterize such responses, Yuyama et al. (2002) profiled human bronchial epithelial cells (HBE) from Clonetics following stimulation with IL-4 or IL-13. The gene expression profiles induced by IL-4 and IL-13 were highly similar, consistent with the shared receptor/signaling systems between IL-4/IL-13. Twelve genes were identified that were consistently regulated by both IL-4 and IL-13 in multiple cultures. Among these genes were the cysteine and serine protease inhibitors squamous cell carcinoma antigen-1 (SCCA1) and SCCA2, which the authors subsequently found to be increased in the serum of asthmatic subjects, with a possible role in modulating activity of house dust mite allergens (Yuyama et al., 2002; Sakata et al., 2004).

We have also developed gene expression profiles for HBE stimulated with IL-4 and IL-13, with overall results very similar to those reported by Yuyama et al. Our studies utilized higher density arrays and consequently a larger panel of differentially regulated genes was obtained. One of the genes most highly up-regulated by IL-4 and IL-13 was the adipocyte fatty acid binding protein (FABP) aP2. This surprising in vitro result modeled in vivo allergic inflammation because aP2 expression was increased in airway epithelium of mice undergoing allergic airway inflammation. Furthermore, using KO mice we demonstrated a key role for aP2, and the related FABP mal1, in regulating allergic airway inflammation (Shum et al., submitted). This finding underscores the value of the discovery-driven microarray approach, because it would be very hard to predict Th2 cytokine-regulated expression of aP2 in HBE based on the current literature.

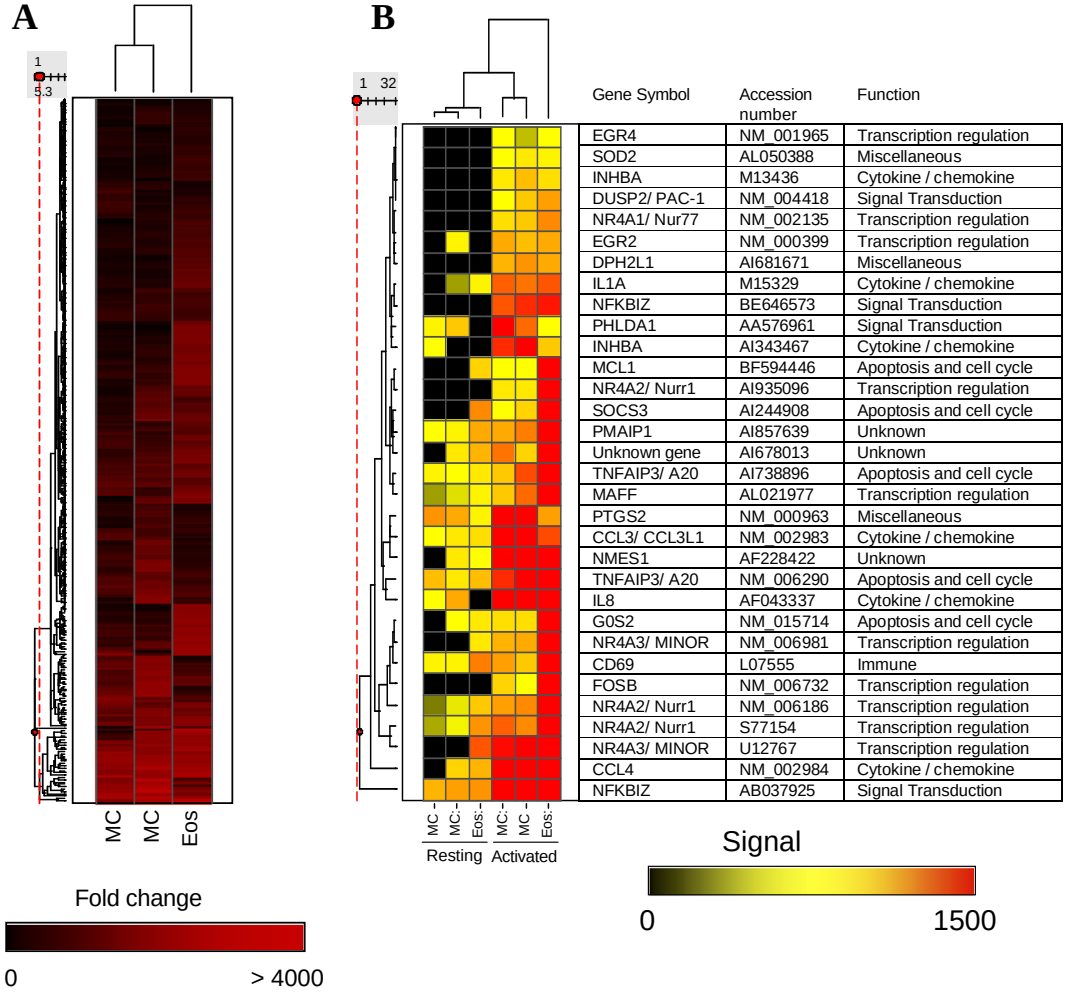
An entirely different pattern of gene expression in IL-13 stimulated bronchial epithelial cells was described by Lee et al. (2001), most likely related to a considerably shorter incubation time with IL-13 (6 vs. 18 hr). This illustrates an important caveat in these types of studies, namely, that transcription is a dynamic process and interpretation of these "snapshots" of gene expression needs to be made with care.

3.3. Airway smooth muscle cells

Similar studies have been performed in IL-13 stimulated smooth muscle cells (Lee et al., 2001; Jarai et al., 2004; Syed et al., 2005). Each of these studies reported panels of differentially regulated genes in response to IL-13. A notable feature of the data was the limited overlap between expression profiles of IL-13-treated bronchial smooth muscle cells, lung fibroblasts, and bronchial epithelial cells (Lee et al., 2001). Thus, in asthma the effects of IL-13 on the lung may be mediated through distinct mechanisms in several different cell types.

3.4. Th1/Th2 cells

The factors affecting Th1/Th2 differentiation and effector function are key determinants of immunity. Excessive Th2 responses to allergens are thought to have a major role in asthma pathogenesis, especially during the early stages of disease (Busse & Lemanske, 2001). A number of studies have applied gene profiling to identify novel genes associated with Th1/Th2 cells (Rogge et al., 2000; Chtanova et al., 2001; Hamalainen et al., 2001; Lu et al., 2004) and Tc1/Tc2 cells (Chtanova et al., 2001). These studies have been valuable in providing new targets for research into this key area of the immune response. In addition to identifying novel targets, Lu et al. profiled gene expression during the course of Th1/Th2 differentiation, and by doing so they were able to obtain a broad overview of the differentiation process. Th differentiation could be divided into 2 phases, an initial activation phase characterized by switching on of replication-related genes, and a differentiation phase characterized by expression of func-



Gene Symbol	Accession number	Function
EGR4	NM_001965	Transcription regulation
SOD2	AL050388	Miscellaneous
INHBA	M13436	Cytokine / chemokine
DUSP2/ PAC-1	NM_004418	Signal Transduction
NR4A1/ Nur77	NM_002135	Transcription regulation
EGR2	NM_000399	Transcription regulation
DPH2L1	AI681671	Miscellaneous
IL1A	M15329	Cytokine / chemokine
NFKBIZ	BE646573	Signal Transduction
PHLDA1	AA576961	Signal Transduction
INHBA	AI343467	Cytokine / chemokine
MCL1	BF594446	Apoptosis and cell cycle
NR4A2/ Nurr1	AI935096	Transcription regulation
SOCS3	AI244908	Apoptosis and cell cycle
PMAIP1	AI857639	Unknown
Unknown gene	AI678013	Unknown
TNFAIP3/ A20	AI738896	Apoptosis and cell cycle
MAFF	AL021977	Transcription regulation
PTGS2	NM_000963	Miscellaneous
CCL3/ CCL3L1	NM_002983	Cytokine / chemokine
NMES1	AF228422	Unknown
TNFAIP3/ A20	NM_006290	Apoptosis and cell cycle
IL8	AF043337	Cytokine / chemokine
G0S2	NM_015714	Apoptosis and cell cycle
NR4A3/ MINOR	NM_006981	Transcription regulation
CD69	L07555	Immune
FOSB	NM_006732	Transcription regulation
NR4A2/ Nurr1	NM_006186	Transcription regulation
NR4A2/ Nurr1	S77154	Transcription regulation
NR4A3/ MINOR	U12767	Transcription regulation
CCL4	NM_002984	Cytokine / chemokine
NFKBIZ	AB037925	Signal Transduction

Fig. 1. Genes up-regulated in both activated mast cells and eosinophils. Humn cord blood-derived mast cells were activated by FcεRI cross-linking for 2 hr. Peripheral blood eosinophils were activated with PMA for 2 hr. The mast cell microarray experiments were performed in duplicated and hierarchical clustering of genes was performed using Spotfire Decision site. (**A**) Hierarchical clustering of the 301 genes that were identified as being up-regulated upon activation in both mast cells and eosinophils. The color-coded scale indicates fold increase, from black, which represents no change, to bright red representing strongest up-regulation. (**B**) Transcripts up-regulated included 32 that were increased by at least 3-fold. The expression is displayed, from black representing no expression, to yellow indicating moderate expression, to red representing highest expression. MC, mast cell; Eos, eosinophil.

tional gene groups and transcription factors important in Th1/2 biology (Lu et al., 2004). This type of analysis can facilitate hypothesis generation. For example, based on expression kinetics during Th1 and Th2 differentiation the authors speculated that the transcription factor Txk is important during the early phase of Th1 differentiation.

3.5. Eosinophils

Despite ongoing controversy (Adamko et al., 2003), the balance of evidence indicates a major effector role for eosinophils in asthma. Eosinophils readily undergo apoptosis in the absence of survival factors such as IL-5 and GM-CSF, and this is a promising area for therapeutic intervention. To identify pathways involved in eosinophil survival, Temple et al. (2001) developed gene expression profiles from IL-5 treated eosinophils. This identified 80 genes, only a minority of which were likely to be directly involved in apoptosis. To narrow this list down further, the results were compared with expression profiles from an IL-5-dependent human erythroleukemic cell line following IL-5 withdrawal. Four genes were coordinately regulated in both cellular systems, Pim-1, DSP-5, CD24, and SLP-76, each of which has already been implicated in apoptosis in other cellular systems. This illustrates a theme to be developed further in Section 3.6, that incorporating multiple distinct cellular models can add considerable power to an array experiment and its interpretation. The study by Temple et al. was subsequently extended by an independent group who showed that GM-CSF induces a very similar expression profile to IL-5, and that this experimental system also mimics some of the changes that an eosinophil undergoes following migration into the airways (Bates et al., 2004). A similar study looked at responses of eosinophils to stem cell factor (SCF). In this system, about 13% of the genes on an "inflammation-specific" array were regulated by SCF, indicating that this growth factor has a much greater effect on eosinophil function than was previously appreciated (Oliveira et al., 2002).

3.6. Integrating array data from multiple cell types

The cellular gene profiling experiments detailed above have been relatively simple in design and scope, mostly comparing untreated cells with cells treated to reflect some aspect of asthma pathology. By increasing the number of experimental variables, such as cell or tissue type, treatment groups, or time points, the power (and complexity) of gene profiling can be markedly enhanced. For example, increasing the number of time points gives much greater insight into a dynamic process, as illustrated above by studies into Th1/Th2 differentiation (Lu et al., 2004).

To identify novel inflammatory disease genes, we developed gene profiles from 24 distinct leukocyte subsets, including major subsets involved in asthma such as eosinophils, mast cells, basophils, Th2 cells, and dendritic cells (Liu et al., manuscript in preparation). A similar database comprising gene expression profiles from numerous distinct leukocyte subsets was recently reported (Abbas et al., 2005). Integrating multiple array data sets has allowed us to identify genes likely to be involved in allergic inflammation based on their expression patterns. An example of the type of analysis we have performed is shown in Fig. 1, in

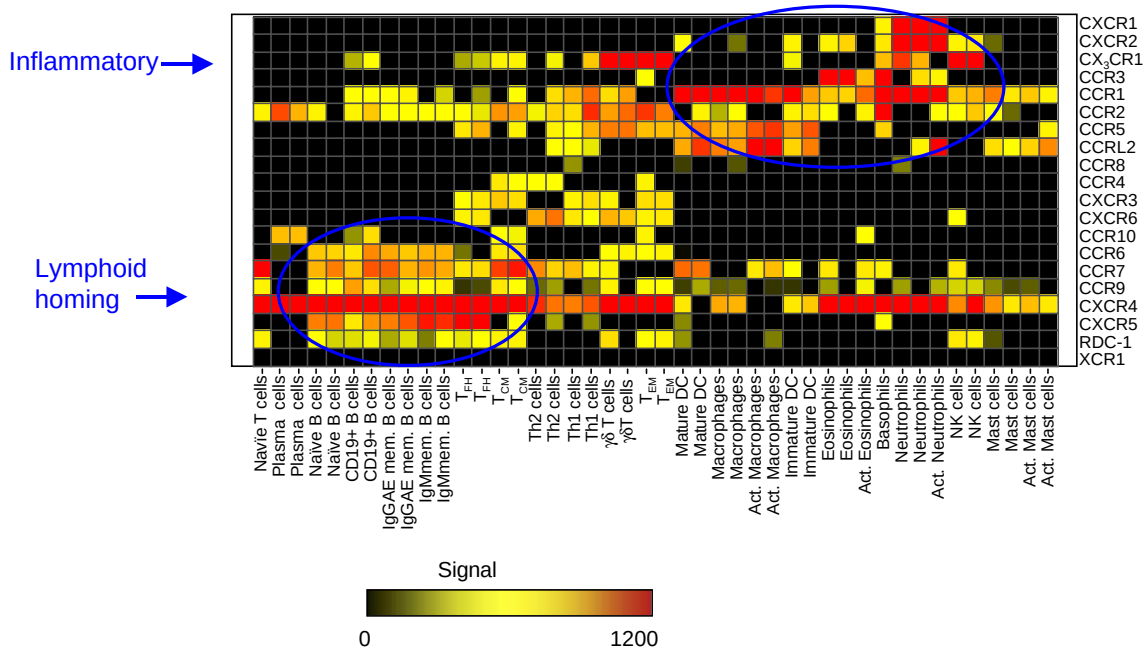


Fig. 2. The expression of all the chemokine receptors represented in Affymetrix U133A and B GeneChips on all the leukocyte subsets we profiled are displayed including CCR1-10, CXCR1-6, CX_3CR1, XCR1, as well as putative chemokine receptors RDC-1 and CCRL2. Receptors and leukocyte subsets were clustered together base on the patterns of expression. Circles represent expression patterns associated with "lymphoid" chemoattractant receptors (typified by CCR7), which marks in particular leukocyte types associated with lymph node homing, and "inflammation"-associated receptors, typically expressed on inflammatory leukocytes such as neutrophils. CD19+ B-cells, peripheral blood B-cells; Mem, memory; T_{FH}, follicular helper T cells; T_{CM}, central memory T cells; T_{EM}, effector memory T cells; Act., activated.

which genes up-regulated in both mast cells and eosinophils following activation (mast cells with FcεR1 cross-linking, and eosinophils with PMA) were identified. Because mast cells and eosinophils are the major effector leukocytes in allergic inflammation, this set of genes is likely to contain a high proportion of genes involved in allergic effector function. This type of analysis is made even more powerful by "subtracting" gene profiles from other non-allergic cell types such as macrophages and neutrophils (Liu et al., manuscript submitted). An additional strategy that we and others have adopted is to identify genes whose expression is restricted to a single leukocyte subset (Liu et al., manuscript submitted) (Nakajima et al., 2001, 2004; Abbas et al., 2005). In our experience, these strategies identify numerous genes already implicated in asthma, but also highlight novel candidates for further study.

Array experiments typically assess the expression of thousands of genes in a limited number of cell types or tissues. However, the reverse of this approach—the study of select genes or molecular families across large numbers of data sets provides a very effective mechanism to understand the role of genes of interest in biology. We have a strong interest in certain molecular families, such as the chemokines and chemokine receptors, and the TNF superfamily. Fig. 2 shows a heat map representation of all the chemoattractant receptors in human leukocytes. This type of analysis allowed us to make associations between the different receptors. For instance, 1 poorly characterized receptor, RDC-1, showed an expression pattern resembling that of the so-called "lymphoid" or "constitutive" homing receptors such as CCR7 or CXCR4. "Inflammatory" receptors such as CXCR1 and CCR3 are expressed on cell types associated with inflammation, such as neutrophils and eosinophils. With the public availability of large data sets for various cell types or biological paradigms, we believe that interrogation of individual genes or gene families will become increasingly common.

Large array data sets can be mined for specific types of targets. For example, Nakajima et al. screened a gene expression library comprising 10 different cell types to identify cell surface receptors and ion channels (i.e., "druggable" targets) expressed on specific granulocyte subsets (Nakajima et al., 2004).

The analysis of gene profiles obtained from tissue samples can be enhanced by combining the data with profiles obtained from specific cell types. This strategy has not been employed in asthma-related studies, but its value has been clearly demonstrated in other systems. For example, combining gene profiling data from lupus nephritis glomeruli and individual leukocyte subsets greatly facilitated interpretation of the glomerular clustering data (Peterson et al., 2004). Along similar lines, gene profiles of normal leukocyte subsets have been invaluable in characterizing gene expression profiles of lymphoid malignancies by providing molecular insights into the cell of origin and underlying pathogenetic mechanisms (Shaffer et al., 2002).

4. Conclusion

Gene profiling using microarray technology has almost unlimited potential for discovery-based research and molecular diagnosis. The power of this approach is also one of its great challenges because the size of the data sets requires careful and complex analysis to extract biologically meaningful data. In most cases, an array experiment is not an end in itself, but a starting point for hypothesis generation and further experimentation. This is the current status of the application of microarrays to asthma research. Gene profiles have been generated, but with few exceptions, they have not yet been used to make significant advances in our understanding of asthma. We anticipate numerous advances in asthma research in the coming years based on initial microarray studies.

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