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Identification of interleukin-13 related biomarkers using peripheral blood mononuclear cells

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Abstract

Asthma is a chronic disorder characterized by airway inflammation, reversible bronchial obstruction, hyper-responsiveness and remodelling. Data from human *in vitro* studies and experimental *in vivo* models of asthma has implicated interleukin (IL)-13 in the asthma phenotype suggesting that a therapeutic agent against it could be effective in treating asthma. The role of biomarkers is becoming increasingly important in the clinical development of therapeutics. Here we describe the use of the GeneChip® DNA microarray technology platform to explore and identify potential response to therapy biomarkers that are associated with the biology of IL-13. Peripheral blood mononuclear cells (PBMCs) from eight healthy donors were cultured in the presence of IL-13, IL-4, an anti-IL-13 monoclonal antibody (mAb) or an isotype control mAb, and RNA from the treated cells was subjected to microarray analysis. The results revealed a number of genes, such as *CCL17* (TARC), *CCL22* (*MDC*), *CCL23* (*MPIF-1*), *CCL26* (eotaxin 3) and *WNT5A* (human wingless-type MMTV integration site family member 5A), that showed increased expression in the IL-13 and IL-4 treatment groups. Real-time polymerase chain reaction (PCR) subsequently confirmed these results. A follow-up study in PBMCs from five additional healthy donors showed that the neutralization of IL-13 completely blocked IL-13-induced TARC, MDC and eotaxin 3 production at the protein level. These data suggest that *TARC*, *MDC*, eotaxin 3, *CCL23* and *WNT5A* if validated could serve as potential biomarkers for anti-IL-13 therapeutics.

Keywords: *Peripheral blood mononuclear cells, interleukin 13, potential biomarkers*

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Introduction

Asthma is a complex, chronic disorder, with a genetic and an environmental component (Holgate 1999). It is characterized by reversible airway obstruction, airway hyper-responsiveness, airway inflammation and remodelling. Asthma affects an estimated 15 million Americans and the morbidity and mortality associated with it is on the rise in industrialized countries. The pathogenesis of asthma is believed to be driven by the dysregulation of the production of some T helper (Th) 2 cytokines such

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as interleukin (IL)-4, -5, -9 and -13 (Wills-Karp 2004). Data from human *in vitro* studies and experimental *in vivo* animal models of asthma have implicated both IL-4 and IL-13 in the asthma phenotype. IL-13 and IL-4 can mediate their effects via a receptor system that includes IL-4 receptor α (IL-4Rα) and IL-13 receptor α1 (IL-13Rα1) subunits (Hilton et al. 1996). Recent reports using murine models of allergic asthma have shown that the Th2-type cytokine, IL-13, may play a critical role in the pathogenesis of asthma, either by regulating airway inflammation, airway remodelling, mucus hypersecretion or airway hyper-responsiveness (Akbari et al. 2003, Grunig et al. 1998, Venkayya et al. 2002, Walter et al. 2001, Wills-Karp et al. 1998, Yang et al. 2004, 2005), thus making it an attractive target for therapeutic intervention. Also the level of IL-13 has been shown to be upregulated in asthmatics both systemically and in the lungs during an asthma attack (Prieto et al. 2000).

The role of biomarkers is becoming increasingly important in the clinical development of therapeutics. A biomarker can be an indicator of normal biological processes, disease processes, or pharmacological responses to therapeutic intervention (Berkman et al. 2001). Their role ranges from stratifying the patient population in helping to identify responders versus non-responders to determining the efficacy of the therapeutic. Biomarkers can be a valuable tool in making better decisions that will reduce the cost for drug development and enable therapies to reach the right patient population faster.

Here we describe the use of a microarray technology platform to identify potential biomarkers that are associated with the biology of IL-13 using an *in vitro* cell culture system. We have found that several markers, such as *TARC* (thymus and activation regulated chemokine), *MDC* (macrophage-derived chemokine), eotaxin 3, *CCL23* and *WNT5A* (human wingless-type MMTV integration site family member 5A), are regulated by IL-13 treatment. These markers if validated could be used as response to therapy biomarkers in an anti-IL-13 clinical trial.

Methods

Peripheral blood mononuclear cell isolation and culture

Peripheral blood mononuclear cells (PBMCs) from healthy donors were isolated using Ficoll (Amersham Biosciences, Piscataway, NJ, USA) and density gradient centrifugation. The PBMCs at 2×10^6 ml^{-1} were cultured in 5 ml RPMI+10% FBS (Invitrogen, Carlsbad, CA, USA) and left untreated or treated with IL-13 (10 ng ml^{-1}) or IL-4 (10 ng ml^{-1}) or an anti-IL-13 monoclonal antibody (mAb) (10 μg ml^{-1}; Centocor Research & Development Inc., Malvern, PA, USA) or an isotype control mAb (10 μg ml^{-1}; Calbiochem, La Jolla, CA, USA) for 24 h. The cells were assayed with one aliquot of cells per donor per treatment group. The IL-13 and IL-4 were purchased from R&D Systems (Minneapolis, MN, USA). Informed consent was obtained from all participating donors.

RNA isolation and microarray

Total cellular RNA was isolated from the cells using the RNeasy mini kit (Qiagen, Inc. Valencia, CA, USA) as per the manufacturer's instructions. The quality and quantity of RNA was assessed using the Agilent 2100 Bioanalyzer (South Plainfield, NJ, USA). Samples that demonstrated high quality (ratio of 28S rRNA to 18S rRNA is greater

than 1.7) were submitted for microarray analysis on the Affymetrix chip. Eighty per cent of the samples passed the QC check.

Microarray processing

RNA amplification, target synthesis and labelling, chip hybridization, washing and staining were performed in accordance with the manufacture's protocol. GeneChip Scanner 3000 was used to scan the chips and fluorescence intensity for each feature of the array was obtained by using GeneChip® Operating Software (Affymetrix, Santa Clara, CA, USA).

Microarray data analysis

In this study, Affymetrix GeneChip® Human Genome U133 Plus 2.0 arrays were used to profile gene expression in human PBMCs from eight donors stimulated with IL-13 or IL-4 at one time point (24 h). Using GeneSpring (Redwood City, CA, USA; version 7.2), chip-to-chip normalization was performed by dividing the averaged intensity of each probe set by the median intensity of a chip. The intensity of each probe set was then normalized to the median intensity of that probe set in the control group. The control groups in this study were the eight untreated samples.

A probe set was regarded as reliably detected if it was called present or marginally present at least once among the 40 samples. Among 54 675 probe sets on a chip, only 36 357 probe sets passed the filtering and were analysed further. Replicate samples were grouped according to their experimental conditions. The average of normalized intensities was used to represent each condition.

Using log 2 transformed normalized intensities, standard ANOVA was conducted in Partek Pro 6.1 (St. Charles, MO, USA) to test treatment effects (untreated, IL13, IL4, anti-IL-13 mAb, and isotype control mAb), and the donor was also considered in the model as a random effect. Post-hoc tests were set up to identify genes showing significant differential expression between each treatment condition and untreated samples. False discovery rate cut-off was set at 0.05. Genes identified by statistical analysis were then filtered by fold change comparison between each treatment condition and untreated samples. The fold change cut-off was set at 1.5.

Reverse transcription and real-time PCR

One microgram of total RNA from each of the treated and untreated groups of PBMCs was used for the reverse transcription (RT) reaction. The RT reaction was performed as per protocol using TaqMan® RT reagents (Applied Biosystems, Foster City, CA, USA) at 37°C for 120 min followed by 25°C for 10 min. Forty nanograms of cDNA per reaction were used in the real-time polymerase chain reaction (PCR) using the ABI Prism® 7900 sequence detection system (ABI Biosystem, Foster City, CA, USA). In the presence of AmpliTaq Gold DNA polymerase (ABI Biosystem), the reaction was incubated for 2 min at 50°C followed by 10 min at 95°C. Then the reaction was run for 40 cycles at 15 s, 95°C and 1 min, 60°C per cycle. Assays-on-Demand™ primers and probes (Applied Biosystems, Foster City, CA, USA) were used in the PCR. The real-time PCR data were analysed using the standard curve method.

PBMC culture and ELISA

PBMCs from five healthy donors were cultured in the presence of IL-13 (10 ng ml^{-1}) or IL-13 (10 ng ml^{-1})+anti-IL-13 mAb (10 μg ml^{-1}) or IL-13 (10 ng ml^{-1})+ isotype control mAb (10 μg ml^{-1}). Culture media supernatants were collected and tested for levels of TARC, MDC and eotaxin 3 protein at 24 h and 48 h time points using human TARC-specific, MDC-specific and human eotaxin 3-specific enzyme-linked immunosorbent assay (ELISA) as per the manufacturer's instructions (R&D Systems, Minneapolis, MN, USA).

Results

IL-13 significantly induces expression of multiple genes in PBMCs

DNA microarray technology platform was used to identify genes downstream of IL-13, which could serve as potential response to therapy biomarkers. IL-4 was also included in the treatment groups to attempt to differentiate between IL-13 and IL-4 and identify any markers that could be uniquely regulated by IL-13.

PBMCs from eight different donors were cultured with no stimulant, or IL-13, IL-4, anti-IL-13 mAb or control mAb for 24 h. RNA was isolated from the cells and used for microarray. After analysing the microarray data it became apparent that IL-13 and IL-4 generated a very similar expression profile, i.e. a majority of the genes upregulated by IL-13 were the same as those upregulated by IL-4 at the 1.5-fold cutoff (data not shown). A total of 447 genes were significantly upregulated by IL-13 and 845 genes were significantly upregulated by IL-4 as compared to untreated samples. Ninety-four per cent of the IL-13 upregulated genes were also upregulated by IL-4. This is illustrated by the hierarchical clustering analysis, which clusters the treatment conditions depending upon the similarity of the expression profile (Figure 1). This clustering analysis shows that PBMCs treated with IL-4 or IL-13 are clustered together compared with those that were untreated, which indicates that their expression profile is very similar to each other but quite different from those that are untreated.

After the initial analysis, a subset of genes was selected based on their cellular location (i.e. secreted or cell surface proteins; Table I) and Figure 2 shows a graphical representation of the microarray data for five of these genes. These results suggest that IL-4 and IL-13 induced a similar expression profile in PBMCs for this subset of genes.

The expression profile of CCL17, CCL22, CCL23, CCL26 and WNT5A is confirmed by real-time PCR

TaqMan real-time PCR was used to validate the expression profile of a subset of genes identified from microarray as being modulated by IL-13 or IL-4. As shown in Figure 3, *CCL17*, *CCL22*, *CCL23*, *CCL26* and *WNT5A* were all upregulated by IL-13 or IL-4 while their expression was not modulated by anti-IL-13 mAb or the isotype control mAb. This is quite comparable to the microarray data.

IL-13-induced CCL17, CCL22 and CCL26 proteins are blocked by an anti-IL-13 mAb

In the first set of experiments we were interested in IL-13 and/or IL-4 modulated genes and the effect if any on these genes by anti-IL-13 antibody at baseline. Once we

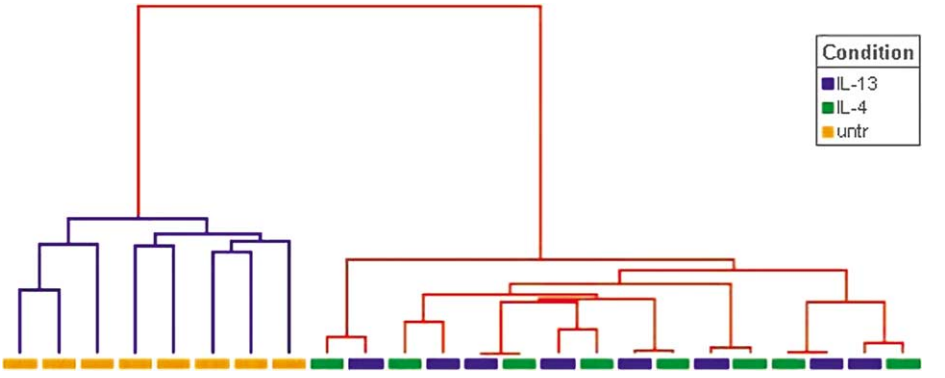

Figure 1. Hierarchical clustering analysis of interleukin (IL)-13 and IL-4 stimulated samples vs. untreated ones. The coloured bars represent treatment conditions: blue, IL-13; green, IL-4; orange, untreated. The IL-13- and IL-4-treated samples cluster together (red dendrograms), as do the untreated samples (blue dendrograms).

identified a set of genes that were modulated by IL-13 and/or IL-4 we performed the second set of experiments to show that these genes are indeed modulated by IL-13 not only at gene level but also at protein level and upon neutralizing the exogenous IL-13 by an anti-IL-13 mAb such an effect is abrogated.

In order to validate the modulation of some of the genes by IL-13 at the protein level, PBMCs were isolated from an additional five healthy donors. The cells were cultured for 24 h or 48 h with just media or stimulated with IL-13 or IL-13+anti-IL-13 mAb or IL-13+isotype control mAb. ELISA was used to detect proteins in cell culture supernatants. This confirmed the upregulation of *CCL17*, *CCL22* and *CCL26* by IL-13. As shown in Figure 4, IL-13 (10 ng ml^{-1} at 24 h and 48 h time points) stimulates the protein expression of *CCL17*, *CCL22* and *CCL26*. Also neutralization of IL-13 by an anti-IL-13 mAb results in completely blocking the IL-13-induced *CCL17*, *CCL22* and *CCL26*. This result confirms that the production of these chemokines by PBMCs could be regulated by IL-13.

Table I. List of some of the genes upregulated by interleukin (IL)-13 and IL-4.

Potential biomarkers associated with IL-13 biology and IL-4 biology	GenBank Accession Number
CCL17 or *TARC* (thymus and activation regulated chemokine)	NM_002987
CCL26 or Eotaxin 3	AF096296
CCL23 or *MPIF-1* (myeloid progenitor inhibitory factor 1)	U58913
CCL22 or *MDC* (macrophage-derived chemokine)	NM_002990
CD1 A,B,C	M28825, NM_001764, NM_001765
WNT5A (human wingless-type MMTV integration site family member 5A)	NM_003392
IgE CD23A	NM_002002
hIL-17 Rβ	NM_018725

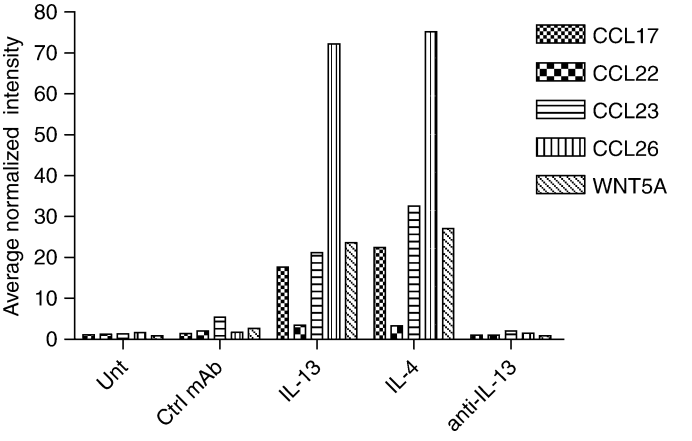

Figure 2. Microarray data showing the average normalized intensity levels of *CCL17*, *CCL22*, *CCL23*, *CCL26* and *WNT5A* upon treatment of peripheral blood mononuclear cells (PBMCs) from eight donors with interleukin (IL)-13, IL-4, anti-IL-13 monoclonal antibody (mAb) or isotype control (ctrl) mAb for 24 h. Each of these genes was identified using a false discovery rate of 0.05. Unt, untreated.

Discussion

It is becoming increasingly evident that there is a great need for biomarkers to become an integral part of drug discovery and development. Biomarkers can be especially valuable in helping to make early decisions on lead compounds and thereby drive down the cost of drug development. The microarray technology platform provides a unique approach in profiling the entire genome and thereby provides insight into potential markers that can help stratify a patient population or predict response to therapy.

The asthmatic patient population is highly heterogeneous both in terms of symptoms and response to therapy. Biomarkers can play a key role in identifying responders versus non-responders and thereby lead to the development of more effective therapies. A number of studies have been described where the microarray platform has been used to identify asthma signature genes using *in vivo* models (Zimmermann et al. 2003, Zou et al. 2002) or *ex vivo* models (Brutsche et al. 2002, Hansel et al. 2005, Syed et al. 1999). These can be useful in identifying disease-specific biomarkers. On the other hand, numerous studies have focused on primary lung cells such as bronchial epithelial cells, bronchial smooth muscle cells and lung fibroblasts and the effect of a particular cytokine such as IL-13 (Lee et al. 2001, Yuyama et al. 2002, Syed et al. 2005) or IL-4 (Yuyama et al. 2002) on these cell types. These studies can identify target-specific biomarkers.

Here we describe the use of microarray technology to identify genes associated with the biology of IL-13, which if validated can provide information about the potential response to an anti-IL-13 mAb therapy. Through an *in vitro* study we have identified a panel of potential biomarkers, associated with IL-13 biology. Such a panel of biomarkers can be used as a tool to predict response to an anti-IL-13 therapeutic antibody if a similar expression pattern is observed *in vivo* post-treatment in a disease population. These potential biomarkers were, however, also upregulated by IL-4, which is not unexpected since IL-4 and IL-13 can signal via the same IL-13 receptor complex. However, the concentration of IL-4 used in this study was probably more

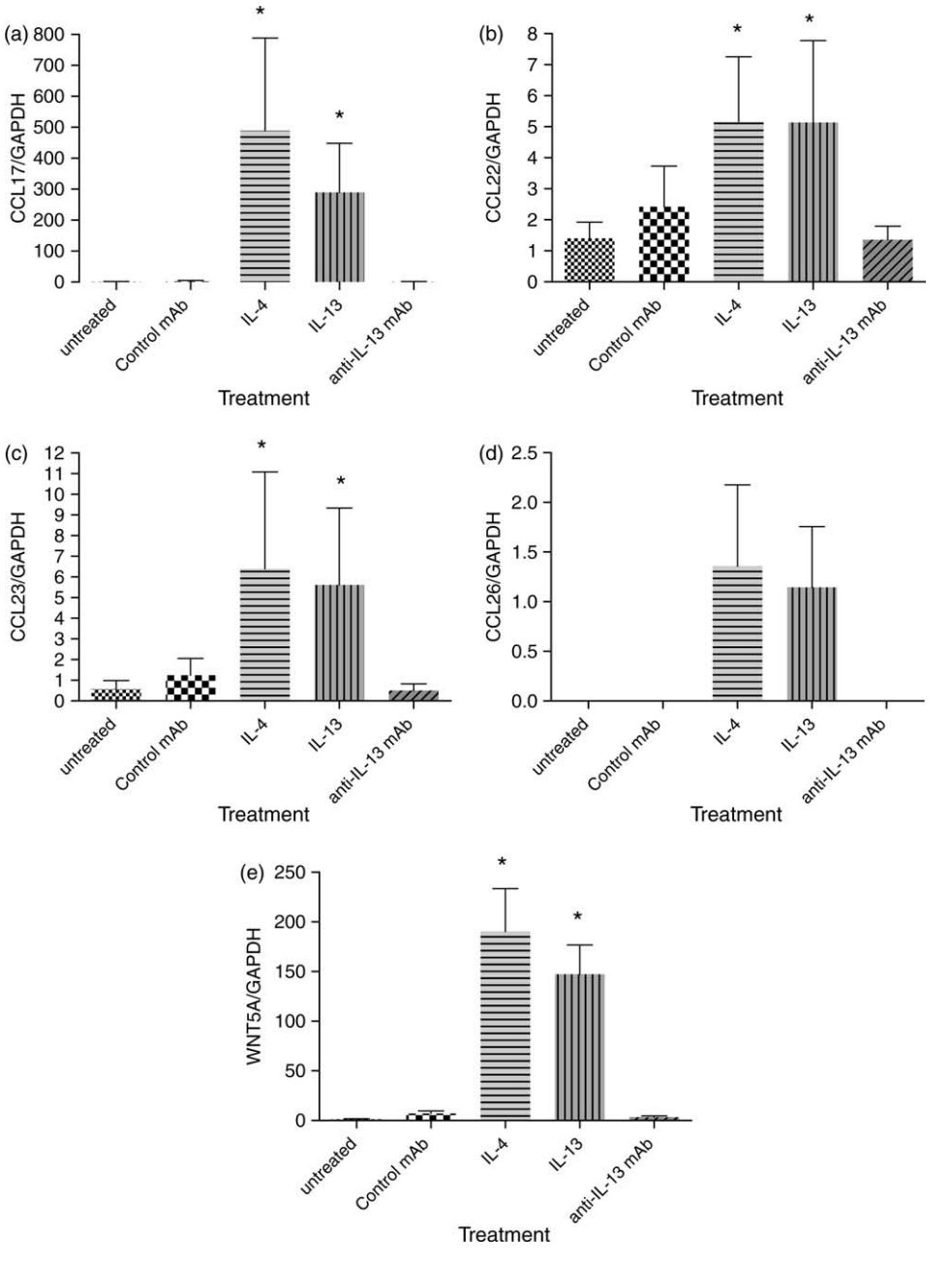

Figure 3. Real-time polymerase chain reaction (PCR) (Taqman®) analysis showing the level of (A) *CCL17*, (B) *CCL22*, (C) *CCL23*, (D) *CCL26* and (E) *WNT5A* upon treatment of peripheral blood mononuclear cells from eight donors with interleukin (IL)-13, IL-4, anti-IL-13 monoclonal antibody (mAb) or isotype control mAb for 24 h. The quantity of each gene is normalized to GAPDH. Values shown are mean $\pm$SD, $n = 8$; $^{\star}p < 0.01$ compared with untreated. The level of *CCL26* in the untreated, control mAb and anti-IL-13 mAb treatment groups was undetectable.

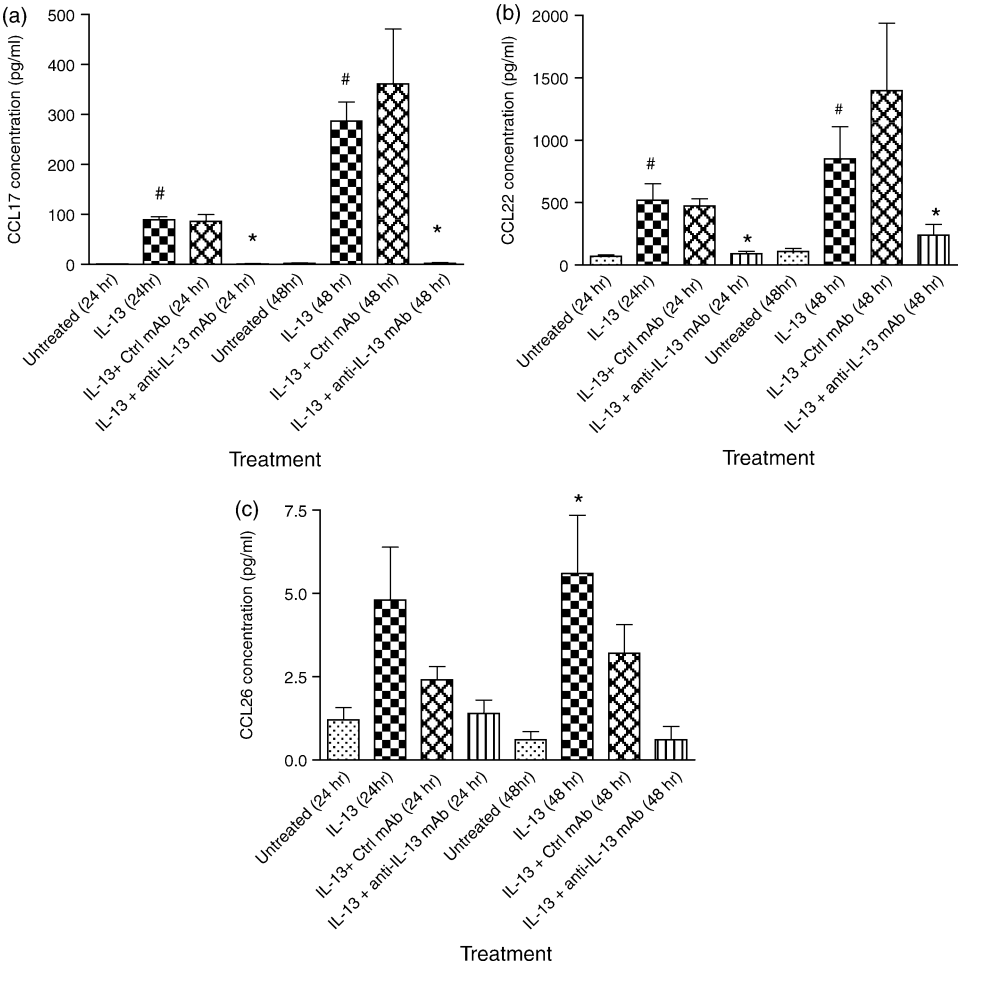

Figure 4. Effect of neutralizing interleukin (IL)-13. Peripheral blood mononuclear cells were incubated with IL-13, IL-13+anti-IL-13 monoclonal antibody (mAb), or IL-13+isotype control mAb for 24 h and 48 h time points. The levels of (A) *CCL17*, (B) *CCL22* and (C) *CCL26* were assessed in the cell culture supernatants using enzyme-linked immunosorbent assay (ELISA). Each bar represents mean±SD, $n=5$; $^{\#}p<0.05$ compared with untreated or $^{\star}p<0.05$ compared with IL-13+control Ab treated.

than tenfold higher than that considered as the pathological concentration of IL-4. Therefore this might have resulted in many more genes been regulated by IL-4 than might be expected. In spite of the overlap between IL-4- and IL-13-regulated genes, the panel of genes described in this study can still be used as IL-13-related biomarkers in a setting where IL-13 is believed to be a major player in the disease pathogenesis. All the genes identified by microarray were validated by real-time PCR and, of those, three were taken forward for confirmation at protein level. Both IL-13-induced TARC and MDC proteins were totally blocked by neutralizing IL-13 and these data are statistically significant. In the case for eotaxin 3, a very low level of eotaxin 3 protein was detected upon IL-13 stimulation, possibly due to the relatively smaller population of eotaxin 3-producing cells in PBMCs. All these potential biomarker candidates can also be produced by other structural cells in response to IL-13 and/or IL-4

stimulation. Therefore, the level of these markers could be higher in patient serum, sputum or bronchoalveolar lavage (BAL) samples.

The relevance of these genes to disease is very important for them to be effective biomarkers. To that end, a number of the genes discussed above have been linked to diseases where IL-13 is believed to play a role. Increased level of TARC has been reported in serum and induced sputum of asthmatics (Sekiya et al. 2002), plasma of children during asthma exacerbation (Leung et al. 2003) and serum of patients with atopic dermatitis (Hijnen et al. 2004). This provides evidence that TARC might be involved in the pathogenesis of such disorders. Eotaxin 3 gene expression has been shown to be upregulated in bronchial biopsies of asthmatics after allergen challenge implying that eotaxin 3 may be important in late-phase eosinophil recruitment to the airways of asthmatics (Berkman et al. 2001). Also, *CCL22* was shown to be upregulated in BAL after a segmental allergen challenge of asthmatics (Pilette et al. 2004). Of the panel of potential biomarkers described above, *WNT5A* and *MPIF-1* (*CCL23*) have not been previously reported to be regulated by IL-13. These are potentially novel IL-13- or IL-4-related biomarkers, which could also be useful in a disease setting where IL-13 or IL-4 plays a major role.

The data suggest that *TARC*, *MDC*, eotaxin 3 (at mRNA and protein level) and *WNT5A* and *MPIF*-1 (at mRNA level) if validated could serve as potential biomarkers for anti-IL-13 therapeutics. It is important to note that the ultimate validity of a potential biomarker or panel of biomarkers can only be tested in the clinic. However, the purpose of this study and studies like this is to identify potential candidates, which can be put forth for validation in a clinical setting.

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References

Akbari O, Stock P, Meyer E, Kronenberg M, Sidobre S, Nakayama T, Taniguchi M, Grusby MJ, DeKruyff RH, Umetsu DT. 2003. Essential role of NKT cells producing IL-4 and IL-13 in the development of allergen-induced airway hyperreactivity. Nature Medicine 9:582–588.

Berkman N, Ohnona S, Chung FK, Breuer R. 2001. Eotaxin-3 but not eotaxin gene expression is upregulated in asthmatics 24 hours after allergen challenge. American Journal of Respiratory Cell and Molecular Biology 24:682–687.

Brutsche MH, Joos L, Carlen Brutsche IEA, Bissinger R, Tamm M, Custovic A, Woodcock A. 2002. Array-based diagnostic gene-expression score for atopy and asthma. Journal of Allergy and Clinical Immunology 109:271–273.

Grunig G, Warnock M, Wakil AE, Venkayya R, Brombacher F, Rennick DM, Sheppard D, Mohrs M, Donaldson DD, Locksley RM, et al. 1998. Requirement for IL-13 independently of IL-4 in experimental asthma. Science 28:22261–2263.

Hansel NN, Hilmer SC, Georas SN, Cope LM, Guo J, Irizarry RA, Diette GB. 2005. Oligonucleotide-microarray analysis of peripheral-blood lymphocytes in severe asthma. Journal of Laboratory and Clinical Medicine 145:263–274.

Hijnen D, de Bruin-Weller M, Oosting B, Lebre C, de Jong E, Bruijnzeel-Koomen C, Knol E. 2004. Serum thymus and activation-regulated chemokine (TARC) and cutaneous T cell-attracting chemokine (CTACK) levels in allergic diseases: TARC and CTACK are disease-specific markers for atopic dermatitis. Journal of Allergy and Clinical Immunology 113:334–340.

Hilton DJ, Zhang J-G, Metcalf D, Alexander WS, Nicola NA, Willson TA. 1996. Cloning and characterization of a binding subunit of the interleukin 13 receptor that is also a component of the interleukin 4 receptor. Proceedings of the National Academy of Sciences 93:497–501.

Holgate S. 1999. The epidemic of allergy and asthma. Nature 402:B2–B4.

Lee JH, Kaminski N, Dolganov G, Grunig G, Koth L, Solomon C, Erle DJ, Sheppard D. 2001. Interleukin-13 induces dramatically different transcriptional programs in three human airway cell types. American Journal of Respiratory Cell and Molecular Biology 25:474–485.

Leung TF, Wong CK, Lam CWK, Li AM, Ip WK, Wong GWK, Fok TF. 2003. Plasma TARC concentration may be a useful marker for asthmatic exacerbation in children. European Respiratory Journal 21:616–620.

Pilette C, Francis JN, Till SJ, Durham SR. 2004. CCR4 ligands are up-regulated in the airways of atopic asthmatics after segmental allergen challenge. European Respiratory Journal 23:876–884.

Prieto J, Lensmar C, Roquet A, van der Ploeg I, Gigliotti D, Eklund A, Grunewald J. 2000. Increased interleukin-13 mRNA expression in bronchoalveolar lavage cells of atopic patients with mild asthma after repeated low-dose allergen provocations. Respiratory Medicine 94:806–814.

Sekiya T, Yamada H, Yamaguchi M, Yamamoto K, Ishii A, Yoshie O, Sano Y, Morita A, Matsushima K, Hirai K. 2002. Increased levels of a TH2-type CC chemokine thymus and activation-regulated chemokine (TARC) in serum and induced sputum of asthmatics. Allergy 57:173–177.

Syed F, Blakemore SJ, Wallace DM, Trower MK, Johnson M, Markham AF, Morrison JFJ. 1999. CCR7 (EBI1) receptor down-regulation in asthma: differential gene expression in human CD4+ T lymphocytes. QJM 92:463–471.

Syed F, Panettieri R, Tliba O, Huang C, Li K, Bracht M, Amegadzie B, Griswold D, Li L, Amrani Y. 2005. The effect of IL-13 and IL-13R130Q, a naturally occurring IL-13 polymorphism, on the gene expression of human airway smooth muscle cells. Respiratory Research 6:9.

Venkayya R, Lam M, Willkom M, Grunig G, Corry DB, Erle DJ. 2002. The Th2 lymphocyte products IL-4 and IL-13 rapidly induce airway hyperresponsiveness through direct effects on resident airway cells. American Journal of Respiratory Cell and Molecular Biology 26:202–208.

Walter DM, McIntire JJ, Berry G, McKenzie ANJ, Donaldson DD, DeKruyff RH, Umetsu DT. 2001. Critical role for IL-13 in the development of allergen-induced airway hyperreactivity. Journal of Immunology 167:4668–4675.

Wills-Karp M. 2004. Interleukin-13 in asthma pathogenesis. Immunological Reviews 202:175–190.

Wills-Karp M, Luyimbazi J, Xu X, Schofield B, Neben TY, Karp CL, Donaldson DD. 1998. Interleukin-13: central mediator of allergic asthma. Science 282:2258–2261.

Yang G, Li L, Volk A, Emmell E, Petley T, Giles-Komar J, Rafferty P, Lakshminarayanan M, Griswold DE, Bugelski PJ, et al. 2005. Therapeutic dosing with anti-interleukin-13 monoclonal antibody inhibits asthma progression in mice. Journal of Pharmacology and Experimental Therapeutics 313:8–15.

Yang G, Volk A, Petley T, Emmell E, Giles-Komar J, Shang X, Li J, Das AM, Shealy D, Griswold DE, et al. 2004. Anti-IL-13 monoclonal antibody inhibits airway hyperresponsiveness, inflammation and airway remodeling. Cytokine 28:224–232.

Yuyama N, Davies DE, Akaiwa M, Matsui K, Hamasaki Y, Suminami Y, Yoshida NL, Maeda M, Pandit A, Lordan JL. 2002. Analysis of novel disease-related genes in bronchial asthma. Cytokine 19:287–296.

Zimmermann N, King NE, Laporte J, Yang M, Mishra A, Pope SM, Muntel EE, Witte DP, Pegg AA, Foster PS, et al. 2003. Dissection of experimental asthma with DNA microarray analysis identifies arginase in asthma pathogenesis. Journal of Clinical Investigation 111:1863–1874.

Zou J, Young S, Zhu F, Gheyas F, Skeans S, Wan Y, Wang L, Ding W, Billah M, McClanahan T, et al. 2002. Microarray profile of differentially expressed genes in a monkey model of allergic asthma. Genome Biology 3:research0020.1– research0020.13.