ORIGINAL ARTICLE

Complementary DNA Microarray Analysis of Chemokines and Their Receptors in Allergic Rhinitis

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Abstract

Objective: To analyze the roles of chemokines and their receptors in the pathogenesis of allergic rhinitis by observing the complementary DNA (cDNA) expression of the chemokines and their receptors in the nasal mucosa of patients with and without allergic rhinitis, using gene chips.
Methods: The total RNAs were isolated from the nasal mucosa of 20 allergic rhinitis patients and purified to messenger RNAs, and then reversely transcribed to cDNAs and incorporated with samples of fluorescence-labeled with Cy5-dUPT (rhinitis patient samples) or Cy3-dUTP (control samples of nonallergic nasal mucosa). Thirty-nine cDNAs of chemokines and their receptors were latticed into expression profile chips, which were hybridized with probes and then scanned with the computer to study gene expression according to the different fluorescence intensities.
Results: The cDNAs of the following chemokines were upregulated: *CCL1, CCL2, CCL5, CCL7, CCL8, CCL11, CCL13, CCL14, CCL17, CCL18, CCL19, CCL24,* and *CX3CL1* in most of the allergic rhinitis sample chips. *CCR2, CCR3, CCR4, CCR5, CCR8* and *CX3CR1* were the highly expressed receptor genes. Low expression of *CXCL4* was found in these tissues.
Conclusion: The T helper cell (T_H) immune system is not well regulated in allergic rhinitis. Most of the upregulated genes we identified are of chemokines and their receptors that play important roles in T_H2 response, and some are involved in the induction of allergic reaction, accumulation of inflammatory cells, and degranulation of sensitized cells. These findings can point to new strategies for allergic rhinitis immunotherapy.

Key words: Allergic rhinitis. Chemokine. Differential gene expression. Gene chip. cDNA. T helper type 1 cells.

Resumen

Objetivo: Analizar el papel de las quimiocinas y sus receptores en la patogénesis de la rinitis alérgica, mediante la observación de su expresión del ADN complementario (ADNc), en la mucosa nasal de los pacientes con y sin rinitis alérgica, y utilizando la técnica de micromatriz multigénica (genochip).
Métodos: Se aisló el ARN total de la mucosa nasal de 20 pacientes con rinitis alérgica y se purificó en ARN mensajero, y luego se realizó la transcripción inversa a ADNc y se marcó con fluorescencia con Cy5-dUPT (muestras de pacientes con rinitis) o con Cy3-dUTP (muestras control de mucosa nasal no alérgica). Treinta y nueve ADNc de quimiocinas y sus receptores se colocaron en forma de retícula en genochips de perfil de expresión, los cuales se hibridaron con sondas y se escanearon a continuación por ordenador para estudiar la expresión genética según las distintas intensidades de fluorescencia.
Resultados: El ADNc de las siguientes quimiocinas experimentó un aumento: *CCL1, CCL2, CCL5, CCL7, CCL8, CCL11, CCL13, CCL14, CCL17, CCL18, CCL19, CCL24* y *CX3CL1* en la mayoría de los genochips de muestra de pacientes con rinitis alérgica. *CCR2, CCR3, CCR4, CCR5, CCR8* y X3CR1 fueron los receptores más expresados. Se detectó una baja expresión de *CXCL4* en estos tejidos.
Conclusión: El sistema inmunológico de los linfocitos T cooperadores (T_H) no está bien regulado en la rinitis alérgica. La mayoría de los genes que aumentaron son los correspondientes a las quimiocinas y sus receptores, que juegan un papel importante en la respuesta T_H2, y algunos participan en la inducción de la reacción alérgica, en la acumulación de células inflamatorias y en la desgranulación de las células sensibilizadas. Estos resultados pueden apuntar a nuevas estrategias para la inmunoterapia contra la rinitis alérgica.

Palabras clave: Rinitis alérgica. Quimiocina. Expresión genética diferencial. Micromatriz multigénica. ADNc. Linfocitos T cooperadores del tipo 1.

Introduction

Chemokines, which together with their receptors are involved in allergic reaction, are secreted basic proteins of 8 to10 kd. According to a review of the field, their roles include regulating leukocyte trafficking in vivo through a family of G protein-coupled receptors and they have important roles in regulating allergic rhinitis [1]. Dendritic cells in the nose pick up an antigen and carry it to regional lymph nodes, where naive T and B cells are activated and then leave the lymph nodes and travel back to the nasal mucosa. There, they accumulate, activating T helper type 2 (T_H2) cells, eosinophils, and mast cells. Chemokines are the molecules that control the movement of these cells in vivo. Even dendritic cells and lymphocyte trafficking are regulated by chemokines.

Dendritic cell-derived I-309 (CCL1), DC-CK1 (CCL18), macrophage-derived chemokine (MDC, CCL22), RANTES (regulated on activation, normal T-cell expressed and secreted, CCL5), eotaxin-1 (CCL11), eotaxin-2 (CCL24), eotaxin-3 (CCL26) are chemokines that can be found in nasal tissue for eosinophil and T_H2 cell trafficking and recruiting [2]. CCL11, CCL22, TARC (thymus and activation-regulated chemokine, CCL17), and CCL1 can activate T_H2 cells, which are regulated by the transcription factor STAT6 and influenced by interleukin (IL) 4 and IL-13 secreted from T_H2 cells [3]. Chemokine receptors such as CCR3, CCR4, and CCR8 have also been found to be upregulated at sites of T_H2 inflammation [4]. Thus, there is direct influence on T_H2 cell development and the T_H1/T_H2 cell balance is affected.

Microarray technology has provided a powerful tool for the study of complicated biological process in cells and tissues. For example, complementary DNA (cDNA) microarray analysis has been used to determine the differential expression of genes in tumors and infectious diseases [5,6]. For this study, a chip containing 39 genes of chemokines and their receptors was designed to further investigate the latent mechanisms of chemokines and their receptors in the pathogenesis of allergic rhinitis. Microarray hybridization was performed according to the standard Affymetrix protocol [7].

Materials and Methods

Tissue Specimens

Human inferior turbinate nasal mucosa was obtained from 20 patients with perennial allergic rhinitis (12 women, 8 men; mean age, 35 years; range, 22–50 years) and 3 nonallergic patients (2 women, 1 man; mean age, 40 years; range, 21–45 years). All underwent surgery for nasal obstruction. All patients in the group with allergic rhinitis had a history of sneezing, secretion and nasal obstruction during natural exposure to allergen, a positive skin prick test to mixed grass pollen and/or tree pollen and/or weeds and a positive screening for specific immunoglobulin (Ig) E. Subjects in the nonallergic group with no history of allergy had negative skin-prick tests to a panel of common aeroallergens (mixed grass pollen, tree pollen, weeds, house dust mite, cat hair, dog hair and molds) and a negative screening for specific IgE. The specimens were obtained after the patients refrained from the use of topical or systemic corticosteroid therapy for 2 weeks.

Construction of cDNA Microarrays

The cDNA clones of 39 chemokines and receptors with the same insert orientation in the pGEM-T vector plasmids were purchased from United Gene Holding, Ltd (Shanghai, China), and the cDNA inserts were amplified with standard polymerase chain reaction (PCR) procedure using universal M13 primers (M13F: CGCCAGGGTTTTCCCAG TCACG A and M13R: AGCGGATAACAATTTCACACAGG) [8], then purified according to the protocol of Schena et al [9] with the length of PCR production about 1000 to 3000 base pairs. The purity of the PCR product was examined through agarose electrophoresis and then it was dissolved in 3% sodium chloride-sodium citrate buffer (SSC). On sialylated slides (TeleChem Inc, Sunnyvale, California, USA) the target genes were spotted with the 7500 Spotting Robotics (Cartesian Inc, Newton, Massachusetts, USA). After spotting, the slides were hydrated (2 hours), dried (0.5 hours, room temperature), ultraviolet cross-linked (65 mJ/cm), and then treated with 0.2% sodium dodecyl sulfate (SDS), water (H_2O), and 0.2% sodium tetrahydroborate (10 minutes). The slides were dried again and left ready for use.

Tissue Treatment

Total RNA extraction was done as follows: the allergic rhinitis nasal mucosa tissue and the normal tissue stored in liquid nitrogen were ground completely into tiny granules in a 100 mm ceramic mortar (RNase free) and homogenized in Trizol solution (Gibco Inc, Gaithersburg, Maryland, USA); after centrifugation, the supernatant was extracted with phenol and chloroform (1:1) twice and then with phenol and chloroform (5:1) once, discarding the organic phase each time; the aqueous phase was then precipitated by an equal volume of isopropanol at low temperature; centrifuged and the pellet dissolved with distilled, deionized, nuclease-free water.

mRNA Purification

The messenger RNAs (mRNAs) were purified with the Oligotex mRNA Midi Kit (Qiagen Inc, Hilden, Germany, USA). The mRNAs were used according to the concentration of total RNA and purified through Oligo dT cellulose columns.

Probe Preparation

The fluorescent cDNA probes were prepared through reverse transcription and then purified, referring to the protocol of Schena et al [9]. The probes from normal tissue were labeled with fluorescent Cy3-dUTP, and those from allergic rhinitis patients were labeled with fluorescent Cy5-

dUTP. The probes were mixed and precipitated by ethanol, and resolved in a 20 mL hybridization solution (5 × SSC + 0.4% SDS + 50% formamide + 5 × Denhardt solution).

Hybridization and Washing

The chips were prehybridized with hybridization solution plus 3 mL denatured salmon sperm DNA at 42ºC for 6 hours. After denaturing at 95ºC for 5 minutes, the probe mixtures were added on the prehybridized chips and then covered with glass. The chips were incubated at 42ºC for 15 to 17 hours. The slides were washed in solutions of 2 × SSC + 0.2% ×SDS; 0.1 × SSC + 0.2% SDS; and 0.1 × SDS at 60ºC; each washing lasted 10 minutes and the chips were then dried at room temperature.

Detection and Analysis

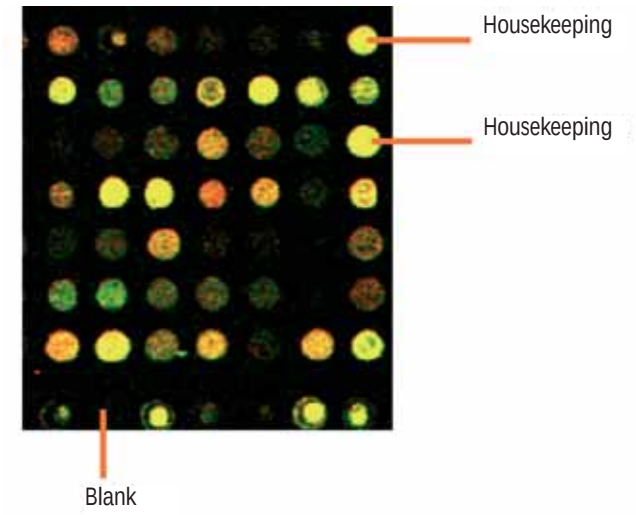
The chips were scanned by a ScanArray 3000 laser scanner (General Scanning Inc, Billerica, Massachusetts, USA) at 2 wavelengths. The acquired images were analyzed by ImaGene 3.0 software (BioDiscovery Inc, El Segundo, California, USA). The intensity of each spot at the 2 wavelengths represented the quantity of Cy3-dUTP and Cy5-dUTP fluorescence. All raw signal microarray intensities were normalized by locally weighted linear regression for within-slide normalization and by mean or median normalization for cross-slide normalization [10]. Every chip was spotted with negative controls (rice U2 RNA, hepatitis C virus coat protein, and the spotting solution as the blanks) and housekeeping genes, including β-actin, glyceraldehyde-3-phosphate dehydrogenase, cyclophilin A, and ribosomal protein L13a to normalize all signal intensities. Experiments were done in duplicate, and the Pearson correlation coefficients *(r)* and 95% confidence intervals were calculated according to Gao et al [10]. These corrected, normalized signals were used to calculate the ratios of Cy3 to Cy5 fluorescence by computer. The following criteria were used to identify the differentially expressed genes [9]. Upregulation was indicated by a) a coefficient of variation (CV) <1, b) the ratio of Cy3 to Cy5 fluorescence ≥2, and c) >16 different chips (80% of the total number). Downregulated genes were indicated by a) a CV <1, b) a ratio of Cy3 to Cy5 fluorescence ≤0.5, and c) >16 different chips.

Results

Microarray experiments were performed with cDNA probes corresponding to allergic rhinitis nasal mucosa labeled with Cy5 (red) fluorescence and cDNA from control nasal mucosa labeled with Cy3 (green) fluorescence. Therefore, the red spots indicated the upregulated genes, the green spots the downregulated genes, and the yellow spots the genes that were similarly expressed between the allergic rhinitis and control samples (figure).

The following genes were differently expressed in the microarray analysis of nasal mucosa tissue from allergic rhinitis patients: *CCL1, CCL2, CCL3, CCL4, CCL5, CCL7, CCL8, CCL11, CCL13, CCL16, CCL17, CCL18, CCL19, CCL20, CCL22, CCL23, CCL24, CCL25, CX3CL1, CXCL1, CXCL2, CXCL3, CXCL4, CXCL5, CXCL6, CXCL10, CXCL11, CX3CL1, XCL1,* and *XCL2*. The *CCL1, CCL2, CCL5, CCL7, CCL8, CCL11, CCL13, CCL14, CCL17, CCL18, CCL19, CCL24,* and *CX3CL1* genes were upregulated on most samples (number of high-expression chips >80%); *CXCL4* was found to be downregulated (low expression, Table1).

Genes for chemokine receptors were differentially expressed as follows: *CCR2, CCR3, CCR4, CCR5, CCR6, CCR7, CCR8, XCR1,* and *CX3CR1*. The *CCR2* receptor gene was upregulated on most of the samples (numbers of high-expression chips >80%, with up to 5-fold higher expression in the experiments), and the *CCR3, CCR4, CCR5, CCR8,* and *CX3CR1* receptor genes were also upregulated on most allergic rhinitis samples (Table 2). No further differences in gene expression were observed.



Gene chip scanning map of expressed genes of chemokines and receptors in allergic rhinitis. The chip profile contains 30 genes of chemokines and 9 genes of chemokines receptors plus the negative controls (rice U2 RNA gene, HCV coat protein, and spotting solution as blanks) and 12 housekeeping genes, including β-actin, glyceraldehyde-3-phosphate dehydrogenase, cyclophilin A, and ribosomal protein L13a to normalize the 2 overall intensities by a coefficient. The intensity of each spot at the 2 wavelengths represents the amount of Cy3-dUTP and Cy5-dUTP, respectively. The red spots indicate the upregulated genes, the green spots the downregulated genes, and the yellow spots the genes that are similarly expressed between tissues from allergic rhinitis patients

Discussion

Chemokines are a group of cytokines associated with allergic inflammation responsible for activation of leukocytes such as T and B lymphocytes, monocytes, neutrophils, eosinophils, and basophils, specifically by inducing the

Table 1. Differently Expressed Chemokines in Allergic Rhinitis

Chemokine	Gene Bank	Gene Product	No. (%) of High-Expression Chips	No. (%) of Similar-Expression Chips	No. (%) of Low-Expression Chips	Regulation
CCL1	*NM_002981*	I-309/SCYA1	17 (85%)	3 (15%)	0 (0%)	↑
CCL2	*NM_002982*	MCP-1, MCAF/SCYA2	16 (80%)	3 (15%)	1 (5%)	↑
CCL3	*NM_002983*	MIP-1a/SCYA3	2 (10%)	15 (75%)	3 (15%)	–
CCL4	*NM_002984*	MIP-1b	5 (25%)	13 (65%)	2 (10%)	–
CCL5	*NM_002985*	RANTES/SCYA5	17 (85%)	3 (15%)	0 (0%)	↑
CCL7	*NM_006273*	MCP-3/SCYA7	18 (90%)	2 (10%)	0 (0%)	↑
CCL8	*NM_005623*	MCP-2/SCYA8	16 (80%)	4 (20%)	0 (0%)	↑
CCL11	*NM_002986*	Eotaxin/SCYA11	16 (80%)	2 (10%)	2 (10%)	↑
CCL13	*NM_005408*	MCP-4, CKβ10/SCYA13	19 (95%)	1 (5%)	0 (0%)	↑
CCL14	*NM_032962*	CC1, HCC-1, NCC-2, CCCK-1/-3, CKβ1, MCIF/ SCYA14	16 (80%)	3 (15%)	1 (5%)	↑
CCL16	*NM_004590*	NCC-4, LEC, HCC-4, LMC, Mtn-1, LCC-1, CKbeta12	2 (10%)	16 (80%)	2 (10%)	–
CCL17	*NM_002987*	TARC/ SCYA17	17 (85%)	2 (10%)	1 (5%)	↑
CCL18	*NM_002988*	DC-CK1, PARC, MIP-4, AMAC-1, CKβ7/SCYA18	17 (85%)	1 (5%)	2 (10%)	↑
CCL19	*NM_006274*	Exodus-3, ELC, MIP-3β, CKβ11/SCYA19	17 (85%)	3 (15%)	0 (0%)	↑
CCL20	*NM_004591*	MIP-3a/SCYA20	3 (15%)	15 (75%)	2 (10%)	–
CCL22	*NM_002990*	MDC	12 (60%)	7 (35%)	1 (5%)	–
CCL23	*NM_005064*	MIP-3, MPIF-1, CKβ8-1	5 (25%)	15(75%)	0 (0%)	–
CCL24	*NM_002991*	MPIF-2, CKβ6, eotaxin-2/ SCYA24	16 (80%)	2 (10%)	2 (10%)	↑
CCL25	*NM_005624*	TECK	2 (10%)	13 (65%)	5 (25%)	–
CX3CL1	*NM_002996*	Fractalkine/SCYD1	16 (80%)	4 (20%)	0 (0%)	↑
CXCL1	*NM_001511*	GROa/MGSA	9 (45%)	5 (25%)	6 (30%)	–
CXCL2	*NM_002089*	MIP-2a/GROb	8 (40%)	10 (50%)	2 (10%)	–
CXCL3	*NM_002090*	GRO3	13 (65%)	5 (25%)	2 (10%)	–
CXCL4	*NM_002619*	Platelet factor-4/ SCYB4	1 (5%)	3 (15%)	16 (80%)	↓
CXCL5	*NM_002994*	ENA-78	2 (10%)	13 (65%)	5 (25%)	–
CXCL6	*NM_002993*	GCP-2	3 (15%)	8 (40%)	9 (45%)	–
CXCL10	*NM_001565*	IP-10	5 (25%)	13 (65%)	2 (10%)	–
CXCL11	*NM_005409*	I-TAP	1 (5%)	14 (70%)	5 (25%)	–
XCL1	*NM_002995*	Lymphotactin, SCM-1b, ATAC	7 (35%)	8 (40%)	5 (25%)	–
XCL2	*NM_003175*	SCM-1b	5 (25%)	6 (30%)	9 (45%)	–

Table 2. Differently Expressed Chemokine Receptors in Allergic Rhinitis

Chemokine Receptor	Gene Bank	Ligand	No. (%) of High-Expression Chips	No. of Similar-Expression Chips	No. (%) of Low-Expression Chips	Regulation
CCR2	*NM_000647*	CCL2	17 (85%)	2 (10%)	1 (5%)	↑
CCR3	*NM_001837*	CCL11, CCL24	18 (90%)	2 (10%)	0 (0%)	↑
CCR4	*NM_005508*	CCL17, CCL22	16 (80%)	3 (15%)	1 (5%)	↑
CCR5	*NM_000579*	CCL4	17 (85%)	2 (10%)	1 (5%)	↑
CCR6	*NM_031409*	CCL20	7 (35%)	12 (60%)	1 (5%)	–
CCR7	*NM_001838*	CCL19, CCL21	2 (10%)	3 (15%)	15 (75%)	–
CCR8	*NM_005201*	CCL1	16 (80%)	3 (15%)	1 (5%)	↑
CX3CR1	*NM_001337*	CX3CL1	17 (85%)	3 (15%)	0 (0%)	↑
XCR1	*NM_005283*	XCL1, XCL2	8 (40%)	6 (30%)	6 (30%)	–

exocytosis of secondary granules. It is now generally accepted that some chemokines and their receptors play essential roles in the pathogenesis of allergic rhinitis. The role is supported by the detection of eotaxin-1, RANTES, MCP-3, and MCP-4 expression in the nasal mucosa and nasal secretions after nasal allergen challenge of allergic rhinitis patients, with recruitment of basophils, eosinophils, and mast cells in the target tissues with allergic inflammation [11,12]. As the major non-IgE histamine releasing factors, chemokines may play important roles in the late phase when histamine is released during allergic responses. In order to further clarify the mechanisms of chemokines and their receptors in allergic rhinitis, we selected certain chemokine cDNAs and their receptors for gene chip analysis to observe gene expression in the nasal mucosa of allergic rhinitis patients.

We found that cDNA for many chemokines and their receptors were differentially expressed in nasal mucosa from allergic rhinitis patients compared with normal mucosa, and some genes were highly expressed in most of the chips (>80%). Among them were such chemokines as CCL1, CCL5, CCL7, CCL8, CCL11, CCL13, CCL14, CCL17, CCL18, CCL19, and CCL24, which were associated with activation and development of T_H2 cells. T_H lymphocytes developed into either T_H1 or T_H2 cells. They can be classified on the basis of their cytokine production profile in the immune system: T_H1 cells yield interferon-γ and transforming growth factor-β in the action of induction of macrophages and killer T cells for cellular immunity or delayed hypersensitivity, mostly in bacterial and viral infections [13]. T_H2 cells produce IL-3, IL-4, IL-10 and other cytokines that are mainly involved in an IgE-mediated delayed type-1 hypersensitivity reaction characteristic of facilitating the recruitment of eosinophils [14]. The highly expressed chemokine genes in the experiment were the ones related to T_H2 responses such as eotaxin-1 (*CCL11*), eotaxin-2 *(CCL24)*, MCP-3 *(CCL7)*, and MCP-4 *(CCL13)*, which affect the T_H1/T_H2 balance by enhancing T_H2 responses.

Genes for macrophage-derived chemokines MCP-1 (*CCL2*), MCP-2 (*CCL8*), MCP-3 (*CCL7*), MCP-4 (*CCL13*) were found to be highly expressed in most of the samples (>80%), and the MCP-4 was highly expressed in almost all samples (95%). CCL2 may act as a recruiter of regulatory and effector $CD4^+$ and $CD8^+$ T leukocytes, stimulating histamine or leukotriene release from mast cells or basophils and inducing fibrosis due to TGF-β and procollagen. All this leads to an enhancement of T_H2 polarization [15]. Actions of *CCL8* and *CCL7* that are relevant to allergic responses are activating and stimulating basophils to release inflammatory mediators and regulating IgE responses [16]. *CCL13* which, even reached 5-fold higher expression than in control samples, can induce emigration of monocytes and lymphocytes, as well as recruitment and activation of airway eosinophils through increasing calcium ions in the cytoplasm of those cells; *CCL13* has also been shown to activate eosinophils, leading to degranulation and it can induce basophils to release histamine and provoke inflammatory disorders [16]. The mRNAs of *CCL13* have been found expressed in pulmonary alveolar lavage fluid in animal models of nasal polyps and asthma [17]. The high expression of *CCL13* in our study confirmed the hypothesis that MCP-4 plays a key role in the pathogenesis of allergic rhinitis.

The highly expressed chemokine of *CCL5* is thought to be associated with allergic inflammation, as it is involved in the recruitment and activation of eosinophils in reactions after antigen challenge [18]. *CCL5* has been found to be highly expressed in the epithelial and endothelial cells of the lower nasal mucosa in allergic rhinitis, with amounts of mRNA and proteins in proportion to antigen stimulation [19]. *CCL5* can spur nasal mucosa to induce eosinophil cells and basophil cells to release histamine [20]. As eosinophils emerge in the antigen-induced early-phase, *CCL5* contributes to eosinophil-mediated inflammatory responses in the earlier period of allergic reaction.

Eotaxin was another highly expressed chemokine in our results, consistent with other reports [21]; increased production of eotaxin is known to induce activation and migration of eosinophil cells. Eotaxin has 3 isoforms: eotaxin-1 (*CCL11*), eotaxin-2 (*CCL24*), and eotaxin-3 (*CCL26*). However, the expressed level of *CCL26* is so low in vivo [22] that it was not chosen for our chip array. CCL11 (one of the most important chemokines involved in tissue inflammation and that plays a key role in asthma and rhinitis) and CCL24 were both upregulated in our study. Eotaxin can be derived from all kinds of inflammatory cells, especially macrophages and eosinophils after antigen challenge. We think the underlying mechanism is that eotaxin accelerates basophilic cell degranulation, regulates T_H2 cell by modulation of eosinophil accumulation, at last leads to an imbalance of the T_H1/T_H2 ratio, and then further induces allergic reaction through the IgE − mast-cell − F εRI cascade [23].

The highly expressed chemokine gene *CCL17* is the only one of this type that acts on the T cell. The amount of CCL17 has been found to be higher in epithelial cells obtained from patients with allergic rhinitis than in those from nonallergic patients [24]. The special ligand of CCL17 is CCR4. Some researchers have shown that *CCL17* is highly expressed in epithelial cells of the inferior turbinate mucosa in allergic rhinitis and is regulated by IL-4, IL-13, and interferon cytokines [25]. *CCL17* has been known to facilitate the recruitment, activation, and development of T_H2 cells and its major role is the development of naïve T cell and enhancement of allergic airway hypersensitivity [25]. The highly expressed CCL1 is another chemokine that controls the trafficking of T_H2 cells to sites of allergic inflammation [26].

Meanwhile, cytokines and chemokines produce marked effects via the receptors of chemokines. But cytokines and chemokines and chemokine receptors do not have a one-to-one relationship. Several cytokines and chemokines can be combined on the same receptor, which therefore uses different ligands in the inflammatory reaction. Different chemokine receptors are expressed in T_H1 or T_H2 cells and can be seen as the markers of them, and even for eosinophils, basophils, and mast cells. Among them, the most important eosinophil chemokine receptor is CCR3, and CCR3 ligands such as eotaxin-1, eotaxin-2, eotaxin-3, and RANTES have been shown to activate eosinophils to release granules. Ligands of CCR4 are CCL8, CCL7, CCL13, CCL5, and CCL17. The ligands of the CCR5 are MIP-1, MIP-2, and CCL5. CCL1 interacts with CCR8. All these receptors have been known to belong to T_H2-cell dominance. CCR2, whose ligands are CCL2, CCL8, CCL7, and CCL13, is thought to activate polarization of T_H1, and CCR1, whose ligands are CCL5, MIP-1,MIP-2, and MIP-3, activates 2 types of T cells in a similar manner. Allergic rhinitis is a systemic inflammatory reaction triggered by T_H2 cell-mediated immune responses and genes of chemokine receptors *CCR3, CCR4, CCR5,* and *CCR8* were upregulated in our experiment, but the CCR1 had no differential expression between the allergic rhinitis samples and the controls. CCR3, the receptor of eotaxin, has been reported to be expressed at high levels on eosinophils but not expressed in monocytes or neutrophilic granulocytes [27]. CCR3 plays its role in activated eosinophil granule release through its ligands CCL11, CCL24, and CCL13 and urges eosinophils to move to a local inflammation site [28]. CCR4 and CCR8 are expressed on key leukocytes associated with allergic inflammation, such as T_H2 cells, eosinophils, mast cells, and basophils. CCL17 is a ligand of CCR4, and CCL1 a ligand of CCR8. All this establishes a subset of chemokines or chemokine receptors that are potentially important in modulating immune responses by amplifying T_H2 cell responses [29].

The chips showed that in addition to upregulated T_H2 chemokine receptor genes, there were some T_H1-associated chemokines receptors such as the *CCR2* and *CCR5* that were also highly expressed. It is known that CCR2 is the only chemokine receptor expressed on basophils and is capable of powerful chemotaxis for basophil with consistent mediator release, even with histamine release, leading to allergic symptoms [28]. In the experiment, CCR2 in most of samples was highly expressed, as much as 2- to 5-fold higher than the controls, indicating that *CCR2* was involved in allergic inflammation for its action on basophils. CCR5 is also thought of as an inflammation-related chemokine receptor, as it takes part in cell-mediated immunity induced by T_H1 cells. After the T_H1 cells are activated, the increased *CCR5* expression facilitates the T_H1 cells to move to the area of inflammation, and accelerates the clearance of harmful antigens from the body.

Upregulated genes of fractalkine (*CX3CL1*) and its receptor CX3CR1 were shown in the experiment. CX3CL1 is a unique chemokine expressed on activated endothelial cells. It can fulfill the dual functions of an adhesion molecule and a chemoattractant and functions as a vascular gateway by attracting CX3CR1-expressing natural killer cells, cytotoxic T cells, and macrophages with immediate cytolytic function [31]. It binds to the CX3CR1, which is a pertussis toxin-sensitive G-protein-coupled receptor and capable of inducing locomotion and mobilization of intracellular calcium; it also activates the heterotrimeric G proteins [32], which mediate both leukocyte migration and adhesion. CX3CL1 and its receptor may favor contact within follicles between activated T helper lymphocytes and activated B lymphocytes, thus contributing to the maturation of the B lymphocyte response [33]. The CX3CL1/CX3CR1 system has been found to play important roles in various clinical conditions, such as cardiovascular disease, graft rejection, human immunovirus infection and inflammatory diseases. The CX3CL1/CX3CR1 system is considered as an amplification circuit of polarized T_H1 responses, and its upregulation may contribute to the recruitment of effector T_H lymphocytes, but the mechanism is still unclear.

The only obviously lowly expressed chemokine gene was platelet factor-4 *(CXCL4)*, which is an asymmetrically associated

homotetrameric (70 residues/subunit) chemokine [34]. Many cells in vivo (eosinophils, basophils, neutrophils, monocyte-macrophage cells, mast cells, platelets, and endothelial cells) may stimulate CXCL4 to emerge under certain conditions. Previous studies have shown that the platelet activation in the local site was a feature of the late inflammatory response to antigen challenge and that CXCL4 might play an important role in allergic inflammation [35]. However, recent studies have shown that patients undergoing continuous natural exposure to sensitizing allergens had no altered platelet activity in vivo, as reflected by plasma levels of the chemokines [36,37], so indicating that clinical manifestations of allergy might come with different platelet activity, possibly inhibition of CXCL4 in allergic rhinitis.

Using gene chips, we conducted a large-scale simultaneous gene expression analysis and obtained more detailed and accurate data through large numbers of samples in allergic rhinitis. The results showed local T_H1/T_H2 imbalance and upregulation of the chemokines attracted to the T_H2 cells. Some chemokines and receptors apparently play key roles in allergic rhinitis through direct influence on T_H2 cell maturation and drift. These data suggest an attractive strategy for drug or other therapy affecting the shifting T_H1/T_H2 balance.

Acknowledgments

We thank Shanghai United Gene Holding, Ltd for their cDNA microarray service. This work was supported by the National Science Foundation of China (No.3027140).

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Manuscript received December 15, 2006; accepted for publication March 19, 2007.

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