Differential Expression of Th2 Chemokine Receptors on T Cells from Atopic and Nonatopic Asthmatics in Response to Der p 1-Pulsed Dendritic Cells

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Background: *In vitro polarized human Th2 cells preferentially express the chemokine receptors CCR3 and CCR4 and migrate to their ligands: eotaxin, monocyte-derived chemokine (MDC) or CCL22, thymus- and activation-regulated chemokine (TARC) or CCL17. However, little is known about the regulation of these chemokine receptor axes by Der p 1-pulsed dendritic cells in house dust mite (HDM)-sensitive and non-atopic asthmatics.*
Objective: *The aim was to investigate the modulatory effects of Der p 1-pulsed DCs on the expression of CCR3 and CCR4 on $CD4^+$ T cells of HDM-sensitive and non-atopic asthmatics.*
Material and Method: *Using real-time RT-PCR and flow cytometry analysis, the expression of CCR3 and CCR4 were assessed in autologous $CD4^+$ T cells after co-incubation with Der p 1-pulsed DCs from these two asthmatic groups. We also determined the mRNA expression of CCR4 ligands TARC/CCL17 and MDC/CCL22 in monocyte-derived DCs after Der p 1 pulsation.*
Results: *We performed flow cytometry analysis of $CD4^+$ T cells from HDM-sensitive and non-atopic asthmatics, taken 24 and 48 h after co-incubation with Der p 1-pulsed DCs. We demonstrated that after co-incubation, there was a significant increase in $CCR3^+$ and $CCR4^+$ $CD4^+$ T cells from HDM-sensitive asthmatics, which began to occur at 24 h and 48 h respectively, and corresponded to their expression at mRNA levels. In contrast, only CCR4 mRNA but not protein expression was increased in non-atopic $CD4^+$ T cells. After Der p 1 pulsation, mRNA expression of CCR4-specific ligands (CCL17 and CCL22) was also markedly upregulated in HDM-sensitive DCs whereas only CCL17 gene expression was increased in non-atopic DCs.*
Conclusion: *These data support the role of DCs in differential regulation of CCR3 and CCR4 on $CD4^+$ T cells from HDM-sensitive and non-atopic asthmatics after Der p 1 exposure.*

Keywords*: Der p 1, Dendritic cells, Th2 chemokine receptors, Asthma*

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Dendritic cells (DCs) have a unique capacity to stimulate na□ve T cells *in vivo*[1]. Airway DCs capture inhaled antigen (Ag), undergo maturation and migrate to the draining mediastinal lymph nodes to initiate the Ag-specific T cell response[2-5]. Ag-loaded mature DCs stably express MHC-associated antigenic peptide and attract and stimulate naive T cells to induce a primary immune response[6]. This interaction between DCs and T cells occurs through newly expressed costimulatory molecules such as CD80, CD86, and CD40, interacting with their ligand expressed on T

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cells[1,7]. By controlling the strength and duration of TCR triggering, the pattern of costimulatory molecule expression, and the production of polarizing cytokines, DCs determine the outcome of the primary T cells response of polarized Th1 and Th2[8].

T lymphocyte are involved in initiating and maintaining airway inflammation in asthma[9]. Activated $CD4^+$ T cells producing IL-4, IL-5, and IL-13 (Th2 cells) have been identified in bronchoalveolar lavage and bronchial biopsies of both atopic and non-atopic asthmatic patients[10,11]. Trafficking of activated T cell into inflammatory sites is a tightly regulated and controlled process directed by multiple molecules, in particular chemokines and chemokine receptors[12,13]. A flurry of recent in vitro studies has demonstrated that Th1 and Th2 cells express distinct sets of chemokine receptors that might regulate the recruitment and localization of these cells to inflammatory sites[14,15]. CXCR3 and CCR5 have been associated with the Th1 cells[16,17], whereas CCR3 and CCR4 have been associated with Th2 cells[18-20].

It has recently been shown that the chemokine receptor/ligand CCR3/CCR3 ligand eotaxin pathway and CCR4/CCR4 ligand monocyte-derived chemokine (MDC/CCL22) pathway play a critical role in the homing of antigen-specific Th2 cells to the lung after allergen challenge[21,22]. These two pathways act in a coordinated cooperative manner, with the CCR3/eotaxin pathway being critical in the acute response after the initial challenge[21]. The CCR4/MDC pathway is proposed to be primarily responsible for the long-term recruitment of antigen-specific Th2 cells *in vivo* after the repeated exposure to allergen. These pathways have been investigated in airway epithelial cells, however, little is known about the role of dendritic cells in modulating CCR3 and CCR4 expression when they prime T cells to a specific antigen, such as the house dust mite protein Der p1. In addition, very limited information is available on the expression of these specific chemokine receptors in atopic and non-atopic asthmatic patients during DC priming.

Herein, we demonstrated that Der p1-pulsed DCs significantly induced both CCR3 and CCR4 expression on autologous HDM allergic $CD4^+$ T cells after co-incubation in a Der p 1 concentration-dependent manner. Obviously, CCR3 expression was induced earlier than CCR4 expression. In contrast, only CCR4 mRNA but not protein expression was observed in $CD4^+$ T cells from non-atopic asthmatic patients, possibly resulting from delayed induction of CCR4 transcription which occurred at 48 hours. Therefore, a longer time course study is required to determine the resulting CCR4 protein expression. We also found the induction of Th1 chemokine receptor CCR5 and CXCR3 expression by Der p 1-pulsed DCs in both HDM-sensitive and non-atopic asthmatic patients. Finally, Der p 1 pulsation induced both CCL17 and CCL22 transcription in DCs from HDM-sensitive asthmatics and only CCL17 gene expression in those of non-atopic asthmatics.

Material and Method

Subjects

Nine non-atopic patients with asthma and 10 atopic asthmatic subjects who had been non-smokers were included in the study. Asthmatic subjects had a baseline of > 60% predicted FEV_1 and demonstrated reversibility of FEV_1 12% or more after 2.5 mg albuterol nebulization, and the provocative concentration of methacholine causing a 20% reduction in FEV_1 (PC_{20}) $\leq$ 4 mg/ml. Allergic status was defined by the presence of a positive skin prick test to at least one of four common aeroallergens (grass pollen, cat dander, *Dermatophagoides pteronyssinus*, *Aspergillus fumigatus*) (Greer Lab, Lenoir, NC). Non-atopic asthmatic subjects were defined by negative skin prick test to common allergen (*e.g.*, grass pollen, cat dander, *Dermatophagoides pteronyssinus*, *Aspergillus fumigatus*) and by undetectable specific IgE antibodies in the sera. All patients provided written informed consent for participation following an approval of the study from the Ethics Committee of Siriraj Hospital.

Generation of monocyte-derived DCs

PBMCs were isolated from heparinized blood by Isoprep (Robbins Scientific, Sunnyvale, CA) density gradient centrifugation and monocytes were enriched by adherence. Briefly, PBMCs were incubated for 60 min in RPMI 1640 medium (Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum (FBS; Biochrom AG, Berlin, Germany) at 37°C. After the non-adherent cells were harvested, the remaining monocytes were incubated in RPMI 1640 supplemented with 10% FBS, 500 U/mL interleukin-4 (IL-4, Genzyme, Cambridge, MA), and 400 U/mL granulocyte-macrophage colony-stimulating factor (GM-CSF, Xiamen Amoytop Biotech, Fujian, China). Cells were fed with fresh medium every other day. After 7 days of culture the resulting immature DCs (iDC) were pulsed with either 10 or 100 ng/mL Der p 1 (Indoor Biotech, Charlottesville, VA), or PBS. At the same time DCs were additionally stimulated with 1000 U/mL tumor necrosis factor-α (TNF-α,

a gift from Dr. Daniela Maennel, University of Regensburgh, Germany) to induce their maturation.

Preparation of CD4$^+$ T-cells

$CD4^+$ T-cells were prepared from nonadherent PBMCs using magnetic sorting (Miltenyi Biotech, Gladbach, Germany). The purity of $CD4^+$ T cells after purification as determined by flow cytometry was over 97%.

Co-culture of DCs and autologous CD4$^+$ T-cells

Der p 1-pulsed or unpulsed DCs were seeded in 96-well plate at 1 x 10^4 cells per well. Autologous $CD4^+$ T lymphocytes were added to make a ratio of DCs to lymphocytes equal to 1:10, and incubated for 24 or 48 h.

Fluorescence-activated cell sorting (FACS) analysis

Flow cytometric analysis was performed as previously described[23]. Briefly, dendritic cells were washed and incubated with the corresponding primary monoclonal antibodies for 60 min at 4°C, washed twice, and resuspended in PBS containing 1% bovine serum albumin Flow cytometric analysis on 20,000 cells was performed using a FACSort (Becton Dickinson, San Jose, CA). The employed primary monoclonal antibodies were antibodies raised against human CCR3, CCR4, CXCR3 and CCR5 (1:100; Chemicon International Inc, Temecula, CA).

RNA isolation and Real-time quantitative RT-PCR analysis

Total RNA was prepared using an RNeasy Mini kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. Total RNA was reverse transcribed into cDNA using Improm-II Reverse Transcription system (Promega, Madison, WI). Real-time RT-PCR was performed using FastStart Universal SYBR Green I Master with (Roche Diagnostics, USA) an ABI PRISM 7900 thermal cycler (Applied Biosystems, Warrington, UK) according to the manufacturer's instructions. Primers were purchased from First BASE Laboratory (Singapore): CCR3 sense: 5'-TCT AAC CTT TGC AGC CAC ATT T-3', antisense: 5'-TTC CCG GCA AAG GAA TAA CT-3', CCR4 sense: 5'-CTC CCT TTT TGG GGC TAC TA-3', antisense: 5'-GTC GTG GAG TTG AGA GAG TA-3', CXCR3 sense: 5'-CTA TGA CTA TGG AGA AAA CGA GAG TGA-3', antisense: 5'-AAG GCC CGG TCG AAG TTC-3', CCR5 sense: 5'-CTG ACA TCT ACC TGC TCA AC-3', antisense: 5'-CTG CAG GTG TAA TGA AGA CC-3', TARC (CCL17) sense: 5'-GCA AAG CCT TGA GAG GTC TTG A-3', antisense: 5'-CGG TGG AGG TCC CAG GTA GT-3', MDC (CCL22) sense: 5'-GTC CTG TTC CCA TCA GCG AT-3', antisense: 5'-CAG GCT GGA GAC AGA GAT GGA-3', GAPDH sense: 5'-GAA ATC CCA TCA CCA TCT TCC-3', antisense: 5'-AAA TGA GCC CCA GCC TTC TC-3'. 7300 System SDS software version 1.4.0.25 was used to analyze relative quantification of the target cDNA according to the ACt method.

Statistical analysis

Mean $\pm$ standard deviation of at least 3 independent experiments is shown. The statistical significance of differences in ΔCt values and percentage of CCR^+ or $CXCR3^+$ $CD4^+$ T-cells between before and after co-incubation with Der p 1-pulsed DCs was determined by Mann-Whitney U test. Any difference with a value of $p < 0.05$ was considered significant.

Results

Der p 1-pulsed dendritic cells induced an increased expression of both CCR3 and CCR4 on HDM allergic CD4$^+$ T cells

Recent evidence has shown that effector Th cells are polarized with respect to their chemokine receptor expression as well as their cytokine production, and that Th2 cells preferentially express CCR3 and CCR4[14,15,18]. In addition, Der p 1-pulsed monocyte-derived DCs could induce Th2 polarization in allergic patients[23,24]. These findings were confirmed in the *in vitro* Der p 1-pulsed DC-co-incubated T cell model used for this study. Autologous $CD4^+$ T cells showed significantly increased expression of CCR3 and CCR4 mRNA after co-incubation with Der p 1-pulsed DCs from HDM-sensitive asthmatics (Table 1).

To localize the expression of CCR3 and CCR4 with $CD4^+$ T cells, we used flow cytometry to determine that both CCR3 and CCR4 were indeed present in $CD4^+$ T cells from HDM-sensitive asthmatics after co-incubation with Der p 1-pulsed DCs. Immunocytochemistry staining for CCR3 and CCR4 showed that $CD4^+$ T cells were CCR3 (7.3%) and CCR4 (29%) positive, and double positive cells were very few prior to incubation with Der p 1-pulsed DCs. Consistent with the pattern of CCR3 and CCR4 mRNA expression, the proportion of T cells that expressed CCR3 was significantly increased after 24 h of co-incubation with DCs pulsed with two different concentrations of Der p 1 while increased numbers of T cells that expressed CCR4 was detectable after 48 h of the co-culture (Table 2). However, there was a decrease in the number of $CCR3^+$

$CD4^+$ T cells at 48 h, compared with that at 24 h of incubation whereas the converse was true for CCR4.

To determine the pattern of CXCR3 and CCR5 mRNA expression in autologous $CD4^+$ T cells after co-incubation with Der p 1-pulsed DCs, we performed real-time RT-PCR in RNA extracted from HDM-sensitive $CD4^+$ T cells. We did not find any significant CXCR3 and CCR5 gene expression after the co-incubation (Table 1). Flow cytometric analysis consistently showed that there was no significant change in the number of CXCR3- or CCR5-expressing $CD4^+$ T cells after the interaction with DCs throughout the period of co-incubation (Table 2).

Der p 1-pulsed dendritic cells induced an increased expression of CCR4 on $CD4^+$ T cells from non-atopic asthmatics

To determine whether Th2 chemokine receptor expression in response to co-incubation with Der p 1-pulsed DCs occurred with $CD4^+$ T cells from non-atopic asthmatic patients, we incubated autologous non-atopic T cells with Der p 1-pulsed DCs, and measured CCR3 and CCR4 mRNA and protein expression. The co-cultured $CD4^+$ T-cells markedly upregulated CCR4 mRNA starting after the co-incubation for 48 h (Table 1), and this upregulation was slower than that seen in HDM-sensitive asthmatics. However, there was no significant change in the proportion of $CD4^+$ T cells that expressed CCR4 and CCR3 after the co-culture (Table 1).

Similar to HDM-sensitive asthmatic patients, the expression of Th1 chemokine receptors at both mRNA (Table 1) and protein (Table 2) levels in non-atopic T cells was not modulated by Der p 1-pulsed DC.

Der p 1-pulsed DC from HDM-sensitive asthmatics expressed CCR4 chemokine (CCL17 and CCL22)

Increased expression of mRNA encoding TARC/CCL17 and MDC/CCL22 was observed in Der p 1-pulsed DCs from HDM-sensitive asthmatics as compared with control DCs without Der p 1 pulsation (Table 3). In contrast, monocyte-derived DCs from non-atopic asthmatics showed increased expression of only TARC/CCL17 but not MDC/CCL22 mRNA after Der p 1 pulsation ($p = 0.03$) although this induction was later (48 h) than that seen in the HDM-sensitive asthmatic group (Table 3).

Discussion

We present an *in vitro* study investigating the expression of CCR3 and CCR4 receptors in $CD4^+$ T cells of HDM-sensitive and non-atopic asthmatic patients after exposure to Der p 1-pulsed DCs. As measured by both mRNA and protein expression in $CD4^+$ T cells from HDM-sensitive asthmatics, expression of CCR3 and CCR4 was significantly induced by Der p 1-pulsed DCs. In addition, the mRNA expression of CCR4 ligands TARC/CCL17 and MDC/CCL22 was markedly upregulated in DCs after Der p 1 pulsation. Increased expression of mRNA encoding CCR4 and TARC/CCL17 was observed in non-atopic asthmatic patients after co-incubation with Der p 1-pulsed DCs and this was reflected in significantly higher percentages of $CD4^+$ T cells expressing CCR4. The upregulation of CCR4 mRNA expression occurred earlier in $CD4^+$ T cells from HDM-sensitive asthmatics (24 h) than those from non-atopic asthmatics (48 h). Expression of CXCR3 and CCR5 receptors did not differ in T cells of HDM-sensitive and non-atopic asthmatic patients however measured.

The present data confirm previous studies[21,22] suggesting that the expression of CCR3 and CCR4 in isolated peripheral blood $CD4^+$ T cells from

Table 1. Real-time RT-PCR analysis of the mRNA expression of Th1 and Th2 chemokine receptors in T cells after co-incubation with Der p 1-pulsed DC

		Co-incubation (hrs)	Ct Mean (SD) Control DC	Ct Mean (SD) DC+Dp 100 ng /mL	p-value
HDM-sensitive asthmatics	$CCR3^+$	24 (n = 9)	0.8(0.3)	1.7(0.4)	0.0012
		48 (n = 9)	0.8(0.3)	1.8(0.4)	0.0002
	$CCR4^+$	24 (n = 9)	2.0(0.7)	3.6(1.3)	<0.0001
		48 (n = 9)	1.8(0.3)	3.8(0.9)	<0.0001
	$CXCR3^+$	24 (n = 6)	3.1(0.5)	3.2(0.5)	0.102
		48 (n = 6)	1.9(0.3)	2.4(0.6)	0.309
	$CCR5^+$	24 (n = 9)	2.1(0.6)	2.1(0.6)	0.54
		48 (n = 9)	1.7(0.4)	1.9(0.5)	0.38
Non-atopic asthmatics	$CCR3^+$	24 (n = 8)	1.2(0.1)	1.3(0.2)	0.328
		48 (n = 8)	1.5(0.2)	1.6(0.2)	0.87
	$CCR4^+$	24 (n = 8)	1.4(0.3)	1.5(0.4)	0.536
		48 (n = 9)	2.2(0.3)	3.6(1.2)	0.0008
	$CXCR3^+$	24 (n = 4)	1.5(0.4)	1.6(0.5)	0.88
		48 (n = 4)	2.8(0.9)	3.1(1.0)	0.48
	$CCR5^+$	24 (n = 8)	1.7(0.3)	1.7(0.3)	1.0
		48 (n = 9)	3.5(0.8)	4.0(1.2)	0.25

Table 2. Flow cytometric analysis of the number of T cells expressing Th1 and Th2 chemokine receptors after co-incubation with Der p 1-pulsed DCs

		Co-incu bation (hrs)	% of $CD4^+$T-cells: Mean (SD)			p-value 1 vs. 2 1 vs. 3
			Control DC1	DC+Dp 10ng/mL2	DC+Dp 100 ng/mL3	
HDM-sensitiveasthmatics	$CCR3^+$	24 (n = 8)	7.3 (2.9)	15.8 (5.8)	19.3 (3.9)	0.0019 0.0002
		48 (n = 10)	4.4 (3.5)	9.9 (5.4)	12.8 (4.0)	0.025 0.001
	$CCR4^+$	24 (n = 8)	29.0 (20.5)	33.2 (22.4)	33.3 (19.9)	0.64 0.57
		48 (n = 10)	22.0 (12.2)	44.6 (12.9)	47.5 (15.4)	0.0029 <0.0001
	$CCR3^+CCR4^+$	24 (n = 8)	0.7 (0.8)	1.3 (0.4)	1.5 (0.56)	0.17 0.10
		48 (n = 10)	1.1 (1.8)	2.3 (2.4)	2.2 (2.3)	0.05 0.07
	$CXCR3^+$	24 (n = 6)	4.6 (3.7)	6.0 (3.8)	8.2 (6.4)	0.69 0.58
		48 (n = 6)	6.5 (3.8)	11.0 (8.4)	10.5 (9.1)	0.7 0.93
	$CCR5^+$	24 (n = 6)	2.9 (4.6)	3.6 (7.1)	1.1 (1.2)	1.0 0.81
		48 (n = 6)	4.5 (5.9)	5.2 (7.2)	3.7 (6.7)	1.0 0.48
	$CXCR3^+CCR5^+$	24 (n = 6)	2.7 (5.0)	3.6 (6.2)	2.5 (1.8)	0.39 0.13
		48 (n = 6)	3.0 (4.0)	4.9 (8.7)	4.1 (7.5)	0.93 0.93
Non-atopicasthmatics	$CCR3^+$	24 (n = 7)	0.4 (0.4)	0.43 (0.4)	0.5 (0.4)	1.0 0.94
		48 (n = 7)	4.2 (6.4)	2.2 (3.2)	2.3 (3.3)	1.0 0.69
	$CCR4^+$	24 (n = 7)	36.6 (32.5)	50.0 (34.2)	46.2 (33.1)	0.38 0.38
		48 (n = 7)	27.2 (20.4)	49.3 (25.3)	47.9 (29.1)	0.128 0.097
	$CCR3^+CCR4^+$	24 (n = 7)	1.3 (1.4)	1.7 (1.9)	2.4 (2.1)	0.84 0.79
		48 (n = 7)	2.4 (2.1)	2.5 (2.1)	1.9 (2.2)	0.84 0.3
	$CXCR3^+$	24 (n = 6)	17.0 (23.5)	19.8 (31.2)	19.7 (26.5)	0.93 0.58
		48 (n = 6)	16.5 (14.5)	19.2 (25.6)	20.6 (25.7)	0.699 0.93
	$CCR5^+$	24 (n = 6)	2.9 (3.6)	4.3 (6.4)	5.7 (9.9)	1.0 0.93
		48 (n = 6)	2.9 (3.7)	4.0 (5.2)	3.9 (6.3)	0.93 0.81
	$CXCR3^+CCR5^+$	24 (n = 6)	5.9 (6.9)	5.3 (6.6)	7.6 (11.8)	0.74 0.81
		48 (n = 6)	5.6 (7.1)	6.3 (7.1)	9.0 (11.8)	1.0 0.93

Table 3. Real-time RT-PCR analysis of the mRNA expression of Th2 chemokine in DCs after Der p1 pulsation

		Duration of incubation (hrs)	ACt Mean (SD)		p-value
			Control DC	DC+Dp 100 ng /mL	
HDM-sensitive asthmatics	CCL17	24 (n=9)	1.7(0.3)	3.5(0.3)	<0.0001
	CCL22	24(n=9)	1.1(0.4)	2.0(0.8)	0.0056
Non-atopicasthmatics	CCL17	48(n=6)	2.4(0.3)	3.5(0.6)	0.03
	CCL22	48(n=5)	2.2(0.8)	3.3(1.6)	0.42

allergic individuals could be induced by antigen exposure. Induction of CCR3 and CCR4 expression by Der p 1-pulsed DCs in HDM-sensitive asthmatics possibly reflects in vivo recruitment of Th2 cells after HDM-allergen exposure. Previous studies proposed that both CCR3 and CCR4 pathway are involved in early stage of Th2 recruitment in vivo but predominance of the CCR4 pathway may be necessary for the long-term recruitment of Th2 cells[21]. A progressive increase in CCR4 and, to a lesser extent CCR3, in parallel with strong upregulation of the CCR4 ligands TARC/CCL17 and MDC/CCL22 in the present study might be circumstantial evidence supporting the predominant use of the CCR4 pathway for Th2 recruitment *in vivo*. The increased number of $CCR4^+$ or $CCR3^+$ T cells from HDM-sensitive asthmatics may be due to increased proliferation of Th2 cells because our previous study in another group of HDM-sensitive asthmatics suggested that Der p 1-pulsed DCs could simultaneously induce T cell proliferation and Th2 polarization[23].

Recent evidence indicates that Der p 1-pulsed DCs could effectively induce Th2 polarization in vitro and therefore activate IL-4 production[23]. IL-4 in combination with TNF-a upregulates TARC/CCL17 production by an airway epithelial cell line. Therefore, it is tempting to speculate that a strong mRNA expression of CCR4 ligands TARC/CCL17 and MDC/CCL22 in dendritic cells after Der p 1 pulsation in the present study may result from IL-4 effects similar to the observation seen in airway epithelial cells after allergen challenge with human asthmatic patients. IL-4-induced CCR4 ligands may chemoattract $CCR4^+$ T cells, which in turn are induced to express more IL-4, establishing a mechanism for amplifying Th2 response[25]. These data support the proinflammatory role of DCs via the CCR4/ligands axis in the pathogenesis of airway inflammation in asthma. Previous studies of antigen-induced airway inflammation have indeed provided evidence for a crucial role of the CCR4/MDC (CCL22) and CCR4/TARC (CCL17) axes regulated by airway epithelium in the recruitment of Th2 cells into the lung[21,26]. Repeated allergen challenge resulted in an increased frequency of $CCR4^+$ expressing Th2 cells in the inflamed airways, and anti-MDC as well as anti-TARC antibodies suppressed cell influx and airway hyperresponsiveness[26,27]. Therefore, the previous and present studies may imply that both dendritic cells and airway epithelial cells work in cooperative manner in directing Th2 recruitment seen in asthma by regulating CCR4/ligand axis after allergen exposure.

Very limited data is available on the pattern of Th chemokine and chemokine receptor expression on T cells from non-atopic asthmatics in response to HDM allergen exposure. In this study, we demonstrated that Der p 1-pulsed DCs had the increase of TARC/CCL17 mRNA expression, and $CD4^+$ T cells following co-incubation with Der p 1-pulsed DCs expressed more CCR4 but not CCR3. However, unlike HDM-sensitive T cells, we could only detect the upregulation of CCR4 transcripts in non-atopic T cells, typically preceding CCR4 protein expression, but in fact there was no significant change in its protein levels. This may be due to the fact that it took a longer period of time for the induction of CCR4 gene expression by Der p 1-pulsed DCs in non-atopic T cells (48 h) than in HDM-allergic T cells (24 h). Therefore, it may require a longer time course study to demonstrate significant differences in CCR4 protein expression in non-atopic T cells. All data suggested that the CCR4/TARC (CCL17) might be predominantly used for Th2 recruitment in non-atopic asthmatics, although, we could not exclude the contribution of the CCR3/CCR3 ligand axis regulated by airway epithelial

cells to Th2 recruitment in non-atopic asthmatics because this information is as yet unavailable.

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การแสดงออกของตัวรับ Th2 chemokine บน T cell ของผู้ป่วยโรคหืดชนิดภูมิแพ้ และชนิดไม่แพ้ในการตอบสนองต่อ dendritic cell ที่ทำปฏิกิริยากับ Der p 1

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T-helper lymphocyte ชนิด type 2 (Th2 cell) เป็นเซลที่สำคัญในก่อให้เกิดหลอดลมอักเสบในผู้ป่วยโรคหืด เหตุผลหนึ่งเพราะ Th2 cell มีตัวรับ (Th2 chemokine receptor) ได้แก่ CCR3 และ CCR4 ซึ่งเมื่อ CCR4 ถูกกระตุ้นด้วย chemokine เช่น CCL22 และ CCL17 ที่สร้างจาก dendritic cell (DC) ในหลอดลมของผู้ป่วยโรคหืด จะทำให้ Th2 cell เคลื่อนที่เข้าไปในหลอดลมมากขึ้น อย่างไรก็ตาม เป็นมีการศึกษาเกี่ยวกับการควบคุมการทำงานของ chemokine receptor ชนิดนี้ในเชิงเปรียบเทียบระหว่างผู้ป่วยโรคหืดชนิดแพ้ตัวไรฝุ่นและไม่แพ้สารก่อภูมิแพ้ (non-atopic asthma) น้อยมาก ดังนั้นเพื่อศึกษาผลการควบคุมของ Der p 1-pulsed dendritic cell ต่อการแสดงออกของ CCR3 และ CCR4 บนผิว $CD4^+$ T cell ของผู้ป่วยโรคหืดชนิดแพ้ตัวไรฝุ่นและชนิดไม่แพ้สารก่อภูมิแพ้ ผู้วิจัยวิเคราะห์การแสดงออกของ CCR3 และ CCR4 ใน T cell หลังจากการสัมผัสกับ Der p 1-pulse DC ในเชิงปริมาณของยีนส์ (mRNA) และโปรตีนด้วยวิธี real-time RT-PCR และ flow cytometry ตามลำดับ รวมทั้งการแสดงออก CCL17 และ CCL22 ใน dendritic cell ผลการศึกษาพบว่าหลังจาก T cell ของผู้ป่วยโรคหืดชนิดแพ้ไรฝุ่นสัมผัสกับ Der p 1-pulsed DC เป็นเวลานาน 24 ชั่วโมง จึงเริ่มมีการเพิ่มขึ้นของปริมาณของ CCR4 mRNA และ CCR3 mRNA รวมทั้ง CCR3 protein และอีก 24 ชั่วโมงต่อมา จึงเริ่มมีการแสดงออกของ CCR4 protein เพิ่มขึ้น ขณะที่การสัมผัสกับ Der p 1-pulsed DC ทำให้ T cell ของผู้ป่วยโรคหืดชนิดไม่แพ้สารก่อภูมิแพ้ มีการแสดงออกเพิ่มขึ้นเฉพาะ CCR4 mRNA เท่านั้น ซึ่งเกิดขึ้นหลังสัมผัสเป็นเวลานาน 48 ชั่วโมง โดยที่ไม่พบการเปลี่ยนแปลงระดับของ CCR4 protein ทั้งนี้อาจเป็นเพราะ การสร้าง CCR4 mRNA ใน non-atopic T cell เกิดขึ้นช้ากว่า HDM-sensitive T cell ทำให้การสร้างโปรตีนเกิดขึ้นช้า ตามไปด้วย อาจใช้เวลานานกว่า 48 ชั่วโมง ซึ่งในการศึกษานี้กำหนดเวลาการสัมผัสสูงสุดเพียง 48 ชั่วโมง อาจไม่เพียงพอสำหรับที่ จะตรวจพบการเปลี่ยนแปลงของ CCR4 protein นอกจากนี้ยังพบว่า Der p 1 กระตุ้นการสร้าง CCL17 และ CCL22 mRNA ของ DC ที่มาจากผู้ป่วยโรคหืดชนิดแพ้ไรฝุ่นเพิ่มขึ้น แต่กระตุ้นการสร้าง CCL17 mRNA ของ DC ที่มาจากผู้ป่วยโรคหืดชนิดไม่แพ้สารก่อภูมิแพ้เพิ่มขึ้นเท่านั้น ซึ่งเกิดขึ้นช้ากว่าการสร้าง CCL17 mRNA ของ DC ที่มาจากผู้ป่วยโรคหืดชนิดแพ้ไรฝุ่น จากข้อมูลทั้งหมดชี้ให้เห็นว่าเมื่อ DC สัมผัสกับโปรตีนของไรฝุ่นจะกระตุ้นการทำงานของ Th2 chemokine receptor system ในผู้ป่วยโรคหืดที่เดิมแพ้ไรฝุ่นเพิ่มขึ้น ซึ่งการตอบสนองนี้ในผู้ป่วยโรคหืดที่แพ้ไรฝุ่นเกิดขึ้นเร็วและสมบูรณ์กว่าในผู้ป่วยโรคหืดชนิดไม่แพ้สารก่อภูมิแพ้ และอาจเป็นกลไกหนึ่งที่ทำ ให้การอักเสบของหลอดลมเพิ่มขึ้นอย่างรวดเร็วหลัง จากสัมผัสกับไรฝุ่นในผู้ป่วยโรคหืดที่แพ้ไรฝุ่นและในทางตรงกันข้ามการอักเสบนี้ในผู้ป่วยโรคหืดที่ไม่แพ้สารก่อภูมิแพ้อาจเกิดขึ้นยากกว่าและช้ากว่า