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Intervention of Thymus and Activation-Regulated Chemokine Attenuates the Development of Allergic Airway Inflammation and Hyperresponsiveness in Mice

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Thymus- and activation-regulated chemokine (TARC; CCL17) is a lymphocyte-directed CC chemokine that specifically chemotacts CC chemokine receptor 4-positive (CCR4+) Th2 cells. To establish the pathophysiological roles of TARC in vivo, we investigated here whether an mAb against TARC could inhibit the induction of asthmatic reaction in mice elicited by OVA. TARC was constitutively expressed in the lung and was up-regulated in allergic inflammation. The specific Ab against TARC attenuated OVA-induced airway eosinophilia and diminished the degree of airway hyperresponsiveness with a concomitant decrease in Th2 cytokine levels. Our results for the first time indicate that TARC is a pivotal chemokine for the development of Th2-dominated experimental allergen-induced asthma with eosinophilia and AHR. This study also represents the first success in controlling Th2 cytokine production in vivo by targeting a chemokine. The Journal of Immunology, 2001, 166: 2055–2062.

The specific pathogen-free male C57BL/6 mice (6–8 wk old) were obtained from CLEA Japan (Tokyo, Japan) and bred in a pathogen-free mouse facility of the Department of Molecular Preventive Medicine. All animal experiments complied with the standards set out in the guidelines of University of Tokyo.

Materials and Methods

Animals

OVA was purchased from Sigma (St. Louis, MO). Hamster anti-mouse TARC mAb SH5 was prepared as described previously (23, 24). The specificity of this Ab was evaluated by 1) binding assay using ELISA, 2) calcium mobilization assay, and 3) chemotaxis assay as described below.

Direct ELISA

The specificity of monoclonal anti-mouse TARC SH5 was examined by a direct ELISAs. Recombinant mouse chemokines used for the assay were TARC, liver and activation-regulated chemokine/macrophage inflammatory protein-3α (MIP-3α), macrophage-derived chemokine (MDC), secondary lymphoid chemokine/EChem, EB11-ligand chemokine/MIP-3β, stromal-derived factor-1, RANTES, lymphotactin, MIP-1α, monocyte chemotactic protein-1 (JE), and IL-11 receptor α ligand chemokine/cutaneous T cell-attracting chemokine. They were purchased from PeproTech (Rocky Hill, NJ). In brief, ELISA plates (Costar, Cambridge, MA) were coated with recombinant mouse chemokines at a concentration of 2 μg/ml and

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incubated at 37°C overnight. After washing with PBS containing 0.05% Tween 20 (PBS-T), plates were blocked with PBS containing 1% BSA, 5% sucrose, and 0.03% Na2EDTA. After washing with PBS-T, 0.5 g was added at a concentration of 2 µg/ml and incubated at 37°C for 1 h. After washing with PBS-T, plates were incubated at 37°C for 30 min with biotinylated goat anti-hamster IgG (Vector, Burlingame, CA) at a concentration of 5 µg/ml. After washing with PBS-T, plates were incubated at 37°C for 30 min with HRP-streptavidin (Vector). After washing with PBS-T, bound HRP was developed by tetramethylbenzidine substrate, and OD at 450 nm was measured using a microplate reader.

Calcium mobilization assay
This was conducted using mouse L1.2 pre-B cells stably transfected with mouse CCR4 cDNA as previously described (18). In brief, cells were suspended at 1 × 10^5 cells/ml in HBSS containing 1 mg/ml of BSA and 10 mM HEPES, pH 7.4 (HBSS-BSA), and incubated with 3 nM fura-2/AM fluorescence dye (Molecular Probes, Eugene, OR) at room temperature for 1 h in the dark. After washing twice, cells were resuspended at 5 × 10^5 cells/ml. Cells in 0.1 ml were placed into a fluorescence spectrophotometer (F2000; Hitachi, Tokyo, Japan). Mouse TARC (mTARC; 10 nM) or mouse MDC (mMDC) was added to cells in 0.1 ml in the absence or the presence of 0.5 g at the indicated concentrations, and emission fluorescence at 510 nm was measured upon excitation at 340 and 380 nm with a time resolution of 5 points to obtain the fluorescence intensity ratio (R340/380).

Chemoattract assay
L1.2 pre-B cells stably transfected with mouse CCR4 were washed twice with phenol-red-free RPMI 1640 medium containing 1 mg/ml BSA, and 0.1 ml of cell suspension containing 2.5 × 10^5 cells was applied to each of the upper wells of a Transwell plate (pore size 30 µm, Costar). Mouse TARC or mouse MDC at 10 nM was preincubated with or without the indicated concentrations of 0.5 g for 30 min and added to the lower wells in a volume of 0.6 ml. After 4 h at 37°C migrated cells were determined by measuring dsDNA with PicoGreen dsDNA quantitation reagent (Molecular Probes). Values were expressed as the percentage of input cells that migrated to the lower wells. All assays were performed in triplicate.

Induction of murine asthma model
Pulmonary eosinophilia in response to OVA was generated in mice as described previously (25, 26). In brief, the model of murine lung eosinophilia used here consists of an initial phase of sensitization and a second phase of induction of the allergic response. Mice were first sensitized with i.p. injection of OVA (0.1 mg/mouse) in 0.2 M PBS (Sigma) on days 1 and 8. The mice were challenged by inhalation of aerosolized 1% OVA for 20 min on days 15–21 to induce the response. At different times after the last allergen challenge, animals were killed under anesthesia with barbiturate. P.I.P. (i.p. and aerosolized) was administered to mice on a similar schedule as in negative controls.

Effect of anti-TARC Ab
In the series of blocking experiments, mice were injected with neutralizing mAb against mTARC 5H5 (50 µg/mouse i.p.) 30 min before OVA administration on days 8–21, and then analyzed 6 h after allergen challenge on day 21. OVA-treated control mice were injected with the same amount of compounds (heterologous injection; Dako, Santa Barbara, CA) at the same time points as during the treatments. The dose and time schedules of Ab treatment were basically decided according to previous reports with similar experimental strategy (27).

Bronchoalveolar lavage (BAL)
BAL was performed as previously described (11). Briefly, at various time points after the aerosol exposure, the lungs were lavaged via a tracheal cannula with 0.7 ml of PBS three times. The recovered BAL fluid was immediately centrifuged (1000 rpm, 2 min, 4°C), and cells in BAL fluid were washed twice and resuspended in 1 ml of PBS. The number of cells was determined by hemocytometer. Samples were applied to glass slides by cytocentrifugation (5 × 10^4 cells/slide), air-dried for 10 min, and then subjected to Wright-Giemsa stain (Fisher Diagnostics, Pittsburgh, PA). The percentages of eosinophils, lymphocytes, neutrophils, and macrophages were determined by counting at least 500 cells/slide using standard morphologic criteria.

Histology
Lung specimens were fixed in 10% neutral buffered formalin and paraffin embedded. De-paraffinized sections (3 µm thick) were stained with hematoxylin and eosin and analyzed under a light microscope.

Immunohistochemistry
Lung specimens were embedded in Technovit OCT compound (Miles, Elkhart, IN), frozen in liquid nitrogen, and cut with a cryostat into 7-µm-thick sections. After fixation of endogenous peroxidase activity (28), the sections were incubated with TARC mAb (5H5 hamster anti-mouse TARC mAb), anti-mouse CD4 mAb (RM4-5; PharMingen, San Diego, CA), Hamster anti-mouse TARC mAb (5H5)-treated sections were incubated sequentially with HRP-conjugated anti-hamster IgG (Southern Biotechnology Associates, Birmingham, AL). Rat anti-mouse CD4 mAb (RM4-5; PharMingen)-treated sections were incubated with alkaline phosphatase-labeled anti-rat IgG and anti-hamster IgG (Jackson ImmunoResearch Laboratories, West Grove, PA), respectively. After visualization with 3,3′-diaminobenzidine (Wako Chemicals, Dallas, TX) or alkaline phosphatase substrate kit I (Vector), slides were counterstained with Mayer’s hematoxylin. Control hamster (Rockland, Gilbertsville, PA) and rat (Sigma) IgG did not stain the same samples in any experiments (10).

Fluorescent immunohistochemistry
To better identify the cell types that were stained for TARC protein, we performed studies with fluorescent micrographs. After inhibition of endogenous peroxidase activity, the sections were incubated with hamster anti-mouse CD11c mAb (N418, Serotec, Oxford, U.K.), Hamster anti-mouse CD11c mAb (N418, Serotec)-treated sections were incubated with alkaline phosphatase-labeled anti-hamster IgG (Jackson ImmunoResearch Laboratories). After visualization with alkaline phosphatase substrate kit I (Vector), the sections were washed with water and PBS. After inhibition of endogenous fluorescence, the sections were incubated with hamster anti-mouse TARC mAb (5H5). Hamster anti-mouse TARC mAb (5H5)-treated sections were incubated sequentially with FITC-conjugated anti-hamster IgG (Southern Biotechnology Associates). To enhance the fluorescent staining, the sections were incubated with FITC-conjugated anti-FITC IgG (Southern Biotechnology Associates). CD11c mAb (red) and TARC mAb (green) fluorescence could be examined simultaneously under epifluorescence microscopy at a wavelength exciting FITC (490 nm). Control hamster IgG (Rockland) did not stain the same samples in any experiment. In these studies TARC expression was stained in green, whereas CD11c-positive cells were stained in red.

In addition, negative cells were visualized by a transillumination with a green filter (29).

Chemokine and cytokine gene expression analysis in the lung
Total RNA was isolated from lung specimens using RNazol B (Biotecx, Houston, TX), according to the manufacturer’s instructions, reverse transcribed into cDNA, and amplified. The levels of cytokine and chemokine expression were determined with the novel method of real-time quantitative PCR using the ABI 7700 sequence detector system (PE Applied Biosystems, Foster City, CA) (18). The sense primer for TARC was 5′-CAGGAAGTGGTGAGCTGGTATA-3′, and the antisense primer was 5′-GGTGTTGCTCGTGATTATA-3′. The sense primer for GAPDH was 5′-ATGATGACTCCACTCTCCGAGGACTCCACTCCAGC-3′, and the antisense primer was 5′-GGTGTTGCTCGTGATTATA-3′. The sense primer for eotaxin was 5′-AGAGCTCCACCAGCCGTTCTTATFF-3′, and the antisense primer was 5′-GGTGTTGCTCGTGATTATA-3′. The sense primer for RANTES was 5′-CATATGCGTCTGAGCACAATGG-3′, and the antisense primer was 5′-GGTGTTGCTCGTGATTATA-3′. The sense primer for Eotaxin was 5′-AGAGCTCCACCAGCCGTTCTTATFF-3′, and the antisense primer was 5′-GGTGTTGCTCGTGATTATA-3′.

The 7700 sequence detector system (PE Applied Biosystems, Foster City, CA) (18). The sense primer for TARC was 5′-CAGGAAGTGGTGAGCTGGTATA-3′, and the antisense primer was 5′-GGTGTTGCTCGTGATTATA-3′. The sense primer for GAPDH was 5′-ATGATGACTCCACTCTCCGAGGACTCCACTCCAGC-3′, and the antisense primer was 5′-GGTGTTGCTCGTGATTATA-3′. The sense primer for eotaxin was 5′-AGAGCTCCACCAGCCGTTCTTATFF-3′, and the antisense primer was 5′-GGTGTTGCTCGTGATTATA-3′. The sense primer for RANTES was 5′-CATATGCGTCTGAGCACAATGG-3′, and the antisense primer was 5′-GGTGTTGCTCGTGATTATA-3′. The sense primer for Eotaxin was 5′-AGAGCTCCACCAGCCGTTCTTATFF-3′, and the antisense primer was 5′-GGTGTTGCTCGTGATTATA-3′.

The 7700 sequence detector system (PE Applied Biosystems, Foster City, CA) was used to discriminate between the different expression levels of the different genes. The expression levels of the different genes were calculated by the 2^-Delta Delta CT method, and the data were presented as the fold change in expression relative to the control group.

The 7700 sequence detector system (PE Applied Biosystems, Foster City, CA) was used to discriminate between the different expression levels of the different genes. The expression levels of the different genes were calculated by the 2^-Delta Delta CT method, and the data were presented as the fold change in expression relative to the control group.
Measurement of cytokine production

The release of cytokines in anti-TARC Ab-treated sensitized mice or control Ab-treated sensitized littermates was determined by ELISA. BAL fluids were collected 48 h after Ag challenge on day 21. BAL fluids concentrated by freeze-drying were assayed using commercially available ELISA kits for IL-4, IL-13, and IFN-γ (Endogen, Boston, MA). Absorbance values were converted to the concentration of each cytokine in the BAL fluid (picograms per milliliter) by interpolation to the respective standard curve. The detection limits of the assay for IFN-γ, IL-4, and IL-13 were 8, 9, and 9 pg/ml, respectively.

Measurement of specific airway responsiveness (sRaw)

We measured the sRaw (centimeters of H2O per liter per second) in unanesthetized mice with the double-chamber plethysmograph (27) on day 23. The noninvasive technique is based on measurement of the time delay between thoracic and mouth volume changes, and we calculated the airway resistance (30, 31). Airway responsiveness to i.v. methacholine challenge was defined by the sRaw. In brief, mice were positioned in the double chamber. Preliminary experiments demonstrated a significant dose-response relationship between the methacholine dose and the sRaw, where 50 mg/kg of methacholine seemed an optimal dose (data not shown). After establishment of a stable state, methacholine was injected i.v. (50 mg/kg), and sRaw was measured for 5 min.

Measurement of the number of CD4-positive cells

CD4-positive cells were quantified in the area 100 μm beneath the epithelial basement membrane in several nonoverlapping high power fields until all the available area was covered. The final result, expressed as the number of positive cells per square millimeter, was calculated as the average of all cellular counts.

Statistical analysis

Results are expressed as the mean ± SE. Statistical significance analyses were performed unless otherwise indicated by two-way ANOVA, and multiple comparisons were made by Fisher’s test. p < 0.05 was accepted as statistically significant.

Results

Specificity of anti-mouse TARC mAb

The specificity of anti-mouse TARC mAb 5H5 was first examined by a direct ELISA. 5H5 bound only to recombinant mouse TARC protein but not to other tested mouse CC chemokines, including MDC, liver and activation-regulated chemokine/MIP-3α, secondary lymphoid chemokine/6Ckine, EB11-ligand chemokine/MIP-3β, stromal-derived factor-1, RANTES, lymphotactin, MIP-1α, MCP-1 (JE), and IL-11 receptor α locus chemokine/cutaneous T cell-attracting chemokine. Furthermore, we checked the cross-reactivity of 5H5 with mMDC by measuring the calcium mobilization and chemotaxis in mouse L1.2 pre-B cells that were stably transfected with mouse CCR4 cDNA. 5H5 completely inhibited mouse TARC-induced calcium mobilization (Fig. 1A) and chemotaxis (Fig. 1B). In contrast, such inhibition was not seen in mouse MDC-induced calcium mobilization or chemotaxis. Thus, 5H5 was concluded to be a highly specific neutralizing mAb to mouse TARC and was used for in vivo administration and the immunohistochemical studies described below.

Increase in lung TARC mRNA levels in a murine model of asthma

To evaluate the changes in TARC mRNA expression during the development of a murine model of asthma, total lung RNA was extracted 3, 6, 24, and 48 h after the last Ag inhalation (on day 21), and the levels of TARC mRNA were examined by a real-time quantitative PCR. Lung TARC mRNA expression was detectable in the untreated lung, which was significantly increased at 3–6 h after the last OVA challenge, and the levels were subsequently enhanced up to 24 h (Fig. 2).

Detection of TARC protein by immunohistochemistry

To confirm the production of TARC protein and to identify the producing cells in the lung, immunohistochemical staining was performed. Bronchial epithelial cells specifically expressed TARC in the lung of untreated animals (Fig. 3B). We studied TARC expression by immunohistochemical analysis 3, 6, 24, and 48 h after the last Ag inhalation. The results showed that the expression peaked at 6 h (data not shown). In the OVA-treated group, there was strong staining for TARC, mainly in bronchial epithelium, peribronchial lesions, and infiltrating cells (Fig. 3C). To better identify the cell types that were stained for TARC protein expression, we performed studies using fluorescent microfluorographs. We chose CD11c, since TARC is known to be preferentially produced by DC, and CD11c (leukocyte integrin CR4 α subunit) has been used as a marker for most dendritic cells (32). TARC expression was stained in green, and CD11c-positive cells were
stained in red. Peripheral bronchial epithelial cells and endothelial cells (Fig. 3E) were stained in green, namely those expressing TARC protein. In contrast, CD11c-positive cells adjacent to this kind of structural cell were rarely stained in yellow (double positive).

Anti-TARC Ab attenuated pulmonary eosinophilia in murine asthma models

To evaluate the specific contribution of TARC to the development of lung inflammation in this OVA model, blocking experiments of this chemokine were performed using specific neutralizing Ab. First, we examined the cell profiles of BAL fluids in groups of anti-TARC Ab-treated and control Ab-treated mice. There were marked increases in total cell number, mostly eosinophils, but also macrophages and lymphocytes, in BAL fluids obtained from OVA-treated mice as described previously (11) (Fig. 4). Control Ab did not affect any of the changes induced by OVA treatment in this model of asthma (Fig. 4). Treatment with anti-TARC Ab strikingly decreased the total cell number and the number of eosinophils as well as lymphocytes recovered in the lavage fluid compared with those in the group treated with control Ab (Fig. 4). In contrast, the number of macrophages was not changed by treatment with anti-TARC Abs. These results established that TARC played a pivotal role in the induction of lymphocyte and eosinophil infiltration in the airways.

Histological changes by anti-TARC Ab treatment

In accordance with the changes found in BAL fluid preparations, neutralization of TARC reduced the number of infiltrating cells into the lung in response to OVA, most of which appeared to be mononuclear lymphocytes and eosinophils by hematoxylin-eosin and Wright-Giemsa stainings (Fig. 5, A–D).

Anti-TARC Ab attenuated Ag-induced AHR in a murine model of asthma

Eosinophilic inflammation is clearly a hallmark of allergic asthma, and considerable evidence suggests an association between pulmonary eosinophil infiltration and AHR in human asthma (33). To determine the role of TARC in the development of allergen-induced AHR, measurements of airway reactivity to i.v. methacholine were performed on day 23. Animals sensitized and challenged by OVA with the treatment of control Ab showed significantly higher sRaw in response to methacholine compared with saline control animals given control Ab (Fig. 6). The baseline sRaw tended to be higher than that in unsensitized animals, but the difference was not significant. OVA-sensitized and challenged mice treated with anti-TARC Ab showed significantly lower sRaw in response to methacholine compared with those treated with control Ab (Fig. 6). With the anti-TARC Ab treatment, the increase in sRaw was inhibited by 64% ($p < 0.005$), and the sRaw was not
To elucidate whether anti-TARC mAb inhibited T lymphocyte infiltration and saline injection and saline inhalation at each time point after methacholine injection. The difference in baseline airway resistance is due to Ag exposure without methacholine. These results indicate that the development of Ag-induced AHR was significantly decreased with anti-TARC Ab.

Anti-TARC Ab suppressed the accumulation CD4\(^+\) cells in the lung

To elucidate whether anti-TARC mAb inhibited T lymphocyte infiltration in the lung, we also evaluated the number of CD4\(^+\) T cells in the airways by anti-TARC Ab treatment. Anti-TARC mAb treatment markedly decreased the degree of infiltration of CD4\(^+\) T cells (number of CD4-positive cells per square millimeter, 58.5 ± 6.19 (±SEM) and 26.5 ± 3.00 (±SEM) in control Ab group and anti-TARC Ab group, respectively; p < 0.001, by Student’s t test; Fig. 5, E and F).

Anti-TARC Ab selectively suppressed the local concentrations of Th2-type cytokines in the lung

Th2 cytokines such as IL-4 and IL-13 are required for pulmonary eosinophilia and induction of AHR (9, 33). To determine whether the blockage of TARC shows any effect on the local production of these cytokines in this model of asthma, we measured the levels of IL-4 and IL-13 as well as that of a Th1-type cytokine IFN-\(\gamma\). BAL fluids were obtained 48 h after last OVA inhalation, and IL-4 and IL-13 levels were significantly increased in the OVA treatment group (Fig. 7). Blockage of TARC significantly decreased OVA-induced production of these two cytokines. The levels of IFN-\(\gamma\) in BAL fluids were not statistically different between the groups given control Ab treatment and those given TARC Ab treatment at 48 h after the last OVA inhalation (Fig. 7).

Effect of anti-TARC Ab on chemokine expression in the lung

To evaluate the changes in chemokine expression during the development of a murine model of asthma, the levels of eotaxin, RANTES, and MDC mRNA were examined by a real-time quantitative PCR. Lung eotaxin mRNA expression was detectable in the untreated lung, which was significantly increased at 6–24 h after the last OVA challenge, and the levels were suppressed by anti-TARC Ab (Fig. 8A). Lung RANTES mRNA expression was increased, but not significantly, after OVA challenge, and the levels tended to be reduced, but were not significantly changed, by anti-TARC Ab (Fig. 8B). MDC mRNA expression was significantly increased after OVA challenge, but anti-TARC Ab treatment did not significantly affect MDC mRNA expression (Fig. 8C).

Discussion

In the present study we have demonstrated that 1) the expression of TARC was constitutively seen in the lung and was up-regulated in a murine model of allergic asthma; 2) the specific Ab against TARC attenuated OVA-induced airway eosinophilia; 3) the Ab diminished the degree of AHR; 4) the Ab reduced infiltration of CD4\(^+\) cells in the airways; and 5) this Ab also decreased Th2 cytokine levels and eosinophil-chemotactic chemokine expression in the lung. These findings suggested that TARC is a pivotal chemokine for the development of allergen-induced tissue eosinophilia and AHR, which are the most important features of bronchial asthma. To the best of our knowledge, this is the first report clearly indicating the role of TARC in the development of asthma.

A number of clinical studies showed that there was an intense infiltration of inflammatory cells, including T cells, especially CD4\(^+\) cells, as well as eosinophils. There was a significant correlation between the number of CD4\(^+\) cells in BAL fluids and the degree of AHR in asthmatic patients (34). Increasing evidence suggests that T lymphocytes, in particular CD4\(^+\) T cells of the Th2 type, play an essential role in the development of the eosinophilic inflammatory response commonly found in asthma (35, 36). Elevated IL-4, IL-5, and IL-13 levels in bronchial biopsies (36, 37), BAL cells, and blood (37) of allergic asthmatic patients have been
chemoattractant for T cells, especially of the Th2 type, was identified to be a specific ligand for CCR4 (18) and to be a selective chemoattractant for T lymphocytes (17). TARC was subsequently identified as a molecule that could traffic effector T lymphocytes into inflamed areas of the lung.

Inflammation. It is likely that certain chemokines play roles in asthma. However, it remains unclear how the recruitment of T cells, especially CD4+ T cells, into the lung is elicited during allergic inflammation. It might be predominant in generating and perpetuating the late phase of the response (11). IL-4, IL-5, and IL-13 (4–7) have been strongly implicated in eosinophil accumulation and the following AHR (39, 40) in a number of experimental models of asthma. Mice sensitized with OVA showed maximal lung monocyte/macrophage accumulation at early stages of inflammatory response, followed by an increase in eosinophil and T lymphocyte numbers at later stages of the response (11). IL-4, IL-5, and IL-13 (4–7) have been strongly implicated in generating and perpetuating the late phase asthmatic response, including recruitment of activated eosinophils into airways, AHR, and airflow limitation (8–10). Recent reports strongly suggested that Th2 cells play an essential role in the development of asthmatic airway inflammation. However, it remains unclear how the recruitment of T cells, especially CD4+ cells, into the lung is elicited during allergic inflammation. It is likely that certain chemokines play roles in trafficking effector T lymphocytes into inflamed areas of the lung.

TARC is the first CC chemokine to be shown to selectively chemoattract T lymphocytes (17). TARC was subsequently identified to be a specific ligand for CCR4 (18) and to be a selective chemoattractant for T cells, especially of the Th2 type, CD4+ human T lymphocytes (20–22). TARC has been reported to be expressed in dendritic cells and possibly in macrophages. In the present experiment we performed fluorescent microfluorographic studies. TARC was stained in green, and CD11c-positive cells were stained in red as a marker for dendritic cells (32). The results suggested that bronchial epithelial cells and endothelial cells were potential sources of this chemokine (Fig. 3E).

Since the number of inflammatory cells in the BAL might be different from that in the tissue itself, we also studied the degree of CD4-positive cell infiltration in the tissues. Our present findings of blocking experiments with anti-TARC Ab clearly indicated that inhibition of TARC decreased BAL lymphocytes and airway infiltration of CD4-positive T cells, possibly Th2 cells, which produce Th2 cytokines such as IL-4 and IL-13. Decreased local production of these cytokines seemed to attenuate eosinophil accumulation and the following AHR (39, 40) in a number of ways. Since Th2 cells themselves are capable of producing chemokines, a decrease in Th2 cells-derived chemokines such as RANTES might be involved. Besides, recent studies emphasized the importance of airway epithelial-derived chemokines, including eotaxin, in the pathogenesis of asthma (41). Airway epithelial cells produce these chemokines in response to Th2 type cytokines, including IL-4 and IL-13 from T cells (42, 43). Finally, locally recruited eosinophils also produce chemoattractants for themselves, such as RANTES, eotaxin, and lipids such as leukotriene C4 and platelet-activating factor (41).

Treatment with anti-TARC Ab dramatically decreased the number of eosinophils in BAL samples and histology. Studies of mRNA levels of eotaxin in the lung clearly showed a decrease after anti-TARC treatment, whereas the levels of RANTES did not significantly change, suggesting that decreased eotaxin expression might be predominantly involved in this setting.

There is accumulating evidence that shows a critical role for a variety of chemokines in the sequential local migration of inflammatory cells. Gonzalo and colleagues (44) indicated that the coordinated action of CC chemokines in the lung orchestrates allergic inflammation and airway hyper-responsiveness in a murine model of asthma. RANTES expression was up-regulated during the early phase of airway inflammation, suggesting a role in the development of asthma. However, another report (45) failed to show the significance of this CC chemokine in asthma. In our model of asthma, quantitative evaluation of RANTES mRNA expression did not show any significant change (Fig. 8B); therefore, its importance remains unknown.

Recent investigations have revealed that CCR3 and CCR4 are expressed on Th2 cells, whereas CCR5 is preferentially expressed on Th1 cells (15, 21, 22, 46, 47). The ligands for CCR4 include a CC chemokine MDC in addition to TARC. MDC not only shares common features with TARC: it has 32% homology with TARC in amino acid sequence and is a potent chemokine for T cells (48). MDC has been reported to be expressed by dendritic cells, which also produce TARC. Gonzalo et al. (49) described the role of MDC in a murine asthma model similar to ours. In their hands, blocking of MDC by the polyclonal Ab resulted in prevention of AHR associated with significant reduction of inflammatory eosinophils in the lung interstitium, but not in BAL (49). Since the mAb against...
TARC used in our experiments was highly specific for TARC and did not cross-react with MDC (Fig. 1, A and B), our data strongly suggest that TARC is also an essential chemokine for T cells in the development of allergic inflammation in addition to MDC, although further study is necessary to better elucidate the mutual roles of TARC and MDC in T cell migration.

It would be important to study CCR4 expression on CD4+ infiltrating T lymphocytes in the airways. We attempted to study CCR4 expression on BAL cells by FACS, but the commercially available Ab (Santa Cruz Biotechnology, Santa Cruz, CA) detects the C-terminals of intracellular domains of CCR4, and therefore, it was unsuccessful. As for the results obtained by cryostat sections, there was an intense staining for airway epithelial cells and endothelial cells, and accurate evaluation of CCR4-positive T cells were not possible (data not shown).

During the preparation of this manuscript, an important paper appeared related to this study. Chvatchko et al. reported that CCR4 deletion had no effect on a Th2-dependent model of allergic airway inflammation in mice (50). However, we must keep in mind that the findings obtained from their knockout mice cannot always be applied to the actual pathophysiology of the disease, because they are mature animals that have lacked the targeted gene since birth. In our studies we directly assessed the importance of TARC in the development of a murine model of asthma using anti-TARC-specific neutralizing mAb and found that 1) the expression of TARC was constitutively seen in the lung and was up-regulated in murine models of allergic asthma; 2) the specific Ab against TARC attenuated OVA-induced airway eosinophilia, the degree of AHR, and the infiltration of CD4 positive T cells, eosinophils, and mast cells in bronchial biopsies obtained from atopic and nonatopic (intrinsic) asthma patients with mild asthma. Am. J. Respir. Crit. Care Med. 154:1804-1809.

In conclusion, our results demonstrate that a CC chemokine, TARC, is essentially involved in the development of AHR and eosinophilia through the recruitment of Th2-type CD4-positive T lymphocytes in a murine model of bronchial asthma. Therefore, TARC could be a novel target for intervention therapy of asthma.

References

FIGURE 8. Increase in eotaxin expression in murine models of asthma. Real-time quantitative PCR analysis of eotaxin and RANTES mRNA expression in the lung. We isolated total RNA from the lung at the following points: untreated and 3, 6, 24, and 48 h after OVA exposure. Total RNA was reverse transcribed with reverse transcriptase and amplified by a real-time quantitative PCR according to the manufacturer’s instructions. The quantity of cytokine mRNA was expressed relative to the calibrator. This result represents four independent experiments. * Values significantly different from those of PBS groups (p < 0.05). Significance was determined by one-way ANOVA, followed by Fisher’s least significant difference test for multiple comparisons.


