

Involvement of human natural killer cells in asthma pathogenesis: Natural killer 2 cells in type 2 cytokine predominance

Haiming Wei, MD, PhD,^a Jian Zhang, MD, PhD,^b Wei Xiao, MD,^c Jinbo Feng,^c
Rui Sun, MD,^{a,b} and Zhigang Tian, MD, PhD^{a,b} Hefei and Jinan, China

Background: T_H2 cells play a pivotal role in the pathogenesis of human asthma. Natural killer (NK) cells are also thought to divide into NK1 and NK2 subsets.

Objective: Whether NK1 or NK2 cells are involved in asthma remains unclear.

Methods: Triple-color flow cytometry for detecting intracellular cytokine and NK cell surface phenotype from asthmatic patients was used. The NK cell cloning and analysis with RT-PCR for cytokine expression and Western blotting for signal transducer and activator of transcription activation were performed.

Results: For the first time, we observed that the ratio of IL-4⁺CD56⁺ NK2 cells in PBMCs of 8 asthmatic patients were higher than in healthy individuals. NK cell clones were then obtained by means of limited dilution, and the average mean of the relative intensity of PCR products for type 2 cytokines significantly increased in the asthmatic patients. Signal transducer and activator of transcription 6, a key transcript factor of type 2 phenotype, was constitutively activated in NK2 clones from asthmatic patients. We cocultured freshly purified NK cells from asthmatic patients with IFN- γ and anti-IL-4 antibody in the presence of IL-15 and found that the content of IL-4⁺ NK2 cells significantly decreased after treatment. Interestingly, NK2-biased status in asthmatic patients was reversed when patients recovered from regular therapy.

Conclusion: The results suggest that the NK2 cell subset is involved in the pathogenesis of asthma. (J Allergy Clin Immunol 2005;115:841-7.)

Key words: Natural killer cells, cytokine, predominance, asthma

Abbreviations used

DC: Dendritic cell
NK: Natural killer
PE: Phycoerythrin
STAT: Signal transducer and activator of transcription
Tc2: Type II cytotoxic T lymphocyte

The prevalence of asthma has dramatically increased worldwide in the past 2 decades.^{1,2} One of the striking advances in the last decade has been the recognition that cytokines play a pivotal role in the development of asthma by regulating the expansion of T_H2 (CD4⁺ T cells) cells.³⁻⁷ In response to allergenic antigens, CD4⁺ T-cell clones from atopic subjects produce IL-4 and IL-5, whereas nonatopic CD4⁺ T-cell clones produce T_H1 cytokines.⁸ Atopic subjects have a higher frequency of IL-4-producing T cells than healthy subjects,⁹ and T-cell clones generated from cord blood lymphocytes of newborns with atopic parents produce higher IL-4 concentrations than neonatal lymphocytes of newborns with nonatopic parents.¹⁰ IL-4 levels are noted to increase in the serum and bronchoalveolar lavage fluid of allergic individuals^{11,12} and dust antigen-stimulated lymphocytes *in vitro*.¹³ Neutralizing IL-4 with anti-IL-4 antibodies or recombinant soluble IL-4 receptor in mice inhibits the development of allergen-specific IgE.¹⁴⁻¹⁸

It is well-recognized that type 2 cytokines are not only produced by CD4⁺ T cells but also by CD8⁺ Tc cells, dendritic cells (DCs), and natural killer (NK) cells, and those cells are called Tc2 (type II cytotoxic T lymphocyte), DC2, or NK2 on the basis of secretion of type 2 cytokines.¹⁹⁻²¹ Until recently, several laboratories described the features of NK1 and NK2 cells *in vitro*²²⁻²⁶; unfortunately, the function of NK1-NK2 cells on immunoregulation, autoimmunity, and allergy *in vivo*, especially in human diseases, remains unknown. In this study we, for the first time, observed that NK2 cells contribute to type 2 cytokine-biased status in patients with asthma.

METHODS

Patients and healthy subjects

The atopic asthmatic patients (n = 8) were recruited from Shandong University, Qilu Hospital, Jinan, China. The patients were in stable condition and free from upper respiratory tract

From ^athe School of Life Sciences, University of Science and Technology of China, Hefei City, and ^bthe School of Pharmacy and ^cQilu Hospital, Shandong University, Jinan City.

Haiming Wei and Jian Zhang contributed equally to this work.

Supported by Outstanding Young Scientist Award and Key Project by Natural Science Foundation of China (30125038, 30230340), Key Basic Science Program by Ministry of Science and Technology of China (2001CB510009, 2003CB515501), and Foundation of Chinese Academy of Science (KSCX2-2-08).

Received for publication December 11, 2003; revised October 28, 2004; accepted for publication November 8, 2004.

Available online January 24, 2005.

Reprint requests: Zhigang Tian, MD, PhD, School of Life Sciences, University of Science and Technology of China, 443 Huangshan Rd, Hefei City, Anhui 230027, China. E-mail: tzg@ustc.edu.cn. Rui Sun, MD, School of Pharmaceutical Sciences, Shandong University, Jinan 250021, China. E-mail: srtzg@yahoo.com.

0091-6749/\$30.00

© 2005 American Academy of Allergy, Asthma and Immunology

doi:10.1016/j.jaci.2004.11.026

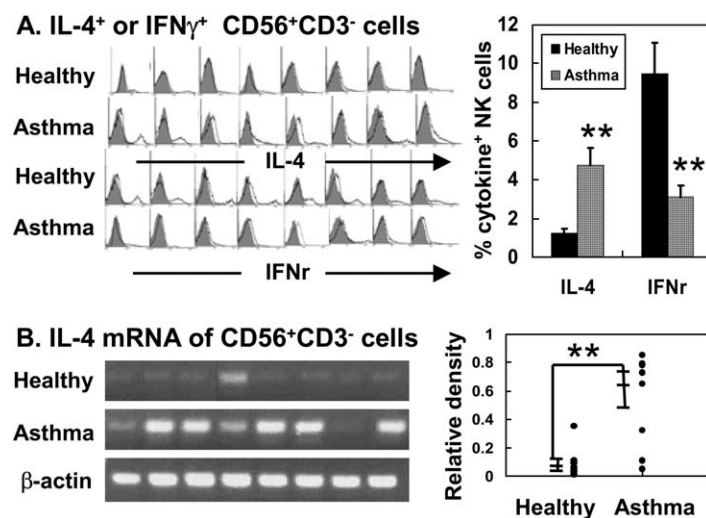


FIG 1. High ratio of IL-4⁺ NK cells in PBMCs of asthmatic patients. **A**, Triple-color staining with CD56, CD3, and IL-4 mAb or IFN- γ mAb. The CD56⁺CD3⁻ cells were gated. **B**, RT-PCR analysis of IL-4 mRNA. CD56⁺ NK cells were purified. All 8 patients and 8 healthy individuals are shown. ***P* < .01. Error bar, SEM.

infections in the 3 months preceding the study. All subjects underwent chest radiography and bronchoscopy. The asthmatic patients fulfilled the criteria for asthma, exhibiting a PC₂₀ of less than 8 mg/mL and reversibility of lung function by at least 15% with a bronchodilator.²⁷ The atopic status was demonstrated by means of positive immediate skin prick test responses to at least one of 10 common inhaled allergens (skin wheal response of >5 mm at 15 minutes). Healthy control subjects (*n* = 10) exhibited no evidence of atopy and had negative skin prick test responses or bronchial hyperresponsiveness (PC₂₀ >18 mg/mL). They had no history of asthma or other allergic diseases (eg, rhinitis) or smoking. Subject characteristics are outlined as follows: mean age of 27 years (range, 15-42 years); 4 female and 4 male subjects; and presence of mild-to-moderate asthma under good control by American Thoracic Society criteria.²⁷ No significant difference was observed between the 2 groups with regard to age or male/female ratio. All patients began to be treated for 10 weeks with oral erythromycin, 200 mg 3 times daily, as previously reported.^{28,29} After 10 weeks' treatment, patients were re-examined for pulmonary function. PC₂₀ values were significantly increased (PC₂₀ >8 mg/mL, 12.47 $\pm$ 3.78 mg/mL). The Ethical Committee of Shandong University School of Medicine carried out the study after approval, and informed consent was obtained from each patient before participation in the study.

Cytokines, antibodies, and primers

Endotoxin-free rhIFN- γ , rhIL-4, and rhIL-15 were purchased from Genzyme (Cambridge, Mass). Anti-human CD3 (IgG1), anti-human CD56 (IgG1), anti-human CD16 (IgG1), anti-human CD14 (IgG1), anti-human CD19 (IgG1), anti-human IL-4, anti-human IFN- γ mouse mAbs, and anti-mouse IgG (H+L) goat PCA, FITC-, CyChrome-, and phycoerythrin (PE)-conjugated streptavidin and isotype control mAbs were all obtained from Becton Dickinson (San Jose, Calif). PCR primers for detecting mRNAs of IL-4, IL-6, IL-10, IFN- γ , TNF- α , TNF- β , and β -actin were designed by us to be 18 to 24 nucleotides long and to have a 100% homology with the particular regions of the genes according to gene sequences. The gene sequences were obtained by using the OLIGO Primer Analysis Software, Version 5.0 (NBA; National Biosciences, Plymouth, Minn). PCR oligomers were produced at the University

of Science and Technology of China Oligonucleotide Synthesis Facility.

NK cell isolation, clonal culture, and shifting toward type 1 cytokines

PBMCs were separated on Ficoll-Hypaque gradients and then passed through a nylon wool column to deplete monocytes and B lymphocytes. The nylon wool nonadherent cells (most of them were NK cells and T lymphocytes) were incubated with anti-CD56 mAb. Human NK cells were then purified from asthma or normal PBMCs to a 95% pure population of CD5⁺CD3⁻, TCR- β ⁻, CD14⁻CD19⁻CD56⁺CD16⁺ lymphocytes by using a modification of the positive immunoselection technique with antibody-coated magnetic beads according to the manipulation manual supplied by the MACS Company (Miltenyi Biotec GmbH, Bergisch Gladbach, Germany). For NK cell cloning, NK cells were cultured in 96-well plastic plates at 37°C in a 5% CO₂ incubator in complete RPMI medium by means of limited dilution in the presence of IL-15 (500 U/mL). Culture medium was exchanged with 50% fresh medium every other day. The single cell clone was expanded continuously for 20 to 25 days, and 1 million NK cells from each colony could be obtained from the long-term culture, as previously reported.³⁰⁻³² For shifting toward the NK1 phenotype, NK cells were incubated with IFN- γ (10 ng/mL) plus anti-IL-4 mAb (10 μ g/mL) in the presence of IL-15 (500 U/mL) for 5 days.^{21,33-35}

RT-PCR

The method used has been previously described.³⁶ Briefly, RNA was extracted from highly freshly purified populations of NK cells or NK cell clones (1 $\times$ 10⁶ of each). RT-PCR was performed by using an RNA PCR kit (Perkin-Elmer, Norwalk, Conn). Twenty-microliter aliquots of the amplified product were fractionated on a 2% agarose gel and visualized by means of ethidium bromide staining. The band intensity of ethidium bromide fluorescence was measured with National Institutes of Health Image Analysis Software, Version 1.61 (National Institutes of Health, Bethesda, Md). The intensities of the bands were determined by using the ratios to β -actin. Some of the cDNA of IL-4, IFN- γ , and β -actin in RT-PCR experiments was

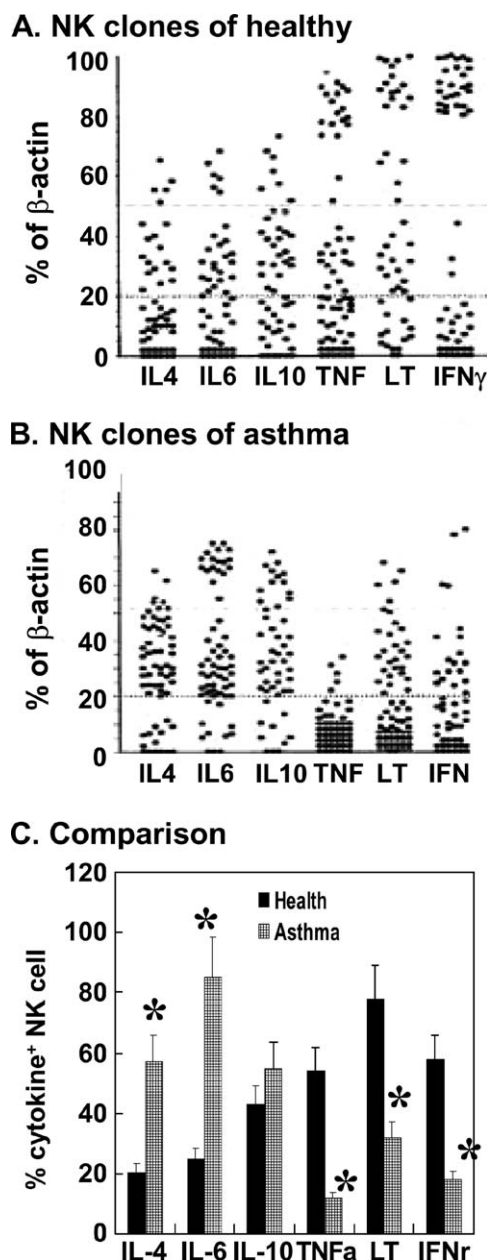


FIG 2. Cytokine paradigm of NK cells clones from healthy individuals and asthmatic patients. Each clone of NK cells was assayed for all 6 cytokine transcripts by means of RT-PCR. The percentage of relative intensity of RT-PCR band to β -actin control was calculated. Each dot is representative of one cytokine mRNA-expressing NK clone. NK cell clones in healthy individuals (A) and in patients (B) were compared in terms of cytokine-producing potential (C). * $P < .05$. Error bar, SEM.

sequenced to verify the actuality of the amplified products. Each experiment had a negative buffer control to exclude the environmental contamination of nonspecific RNA or DNA.

Flow cytometry

To increase the sensitivity of the assay to detect these proteins on the cell surface, NK cells were fixed with 1% (wt/vol) paraformaldehyde for 15 minutes on ice and washed with PBS containing 1%

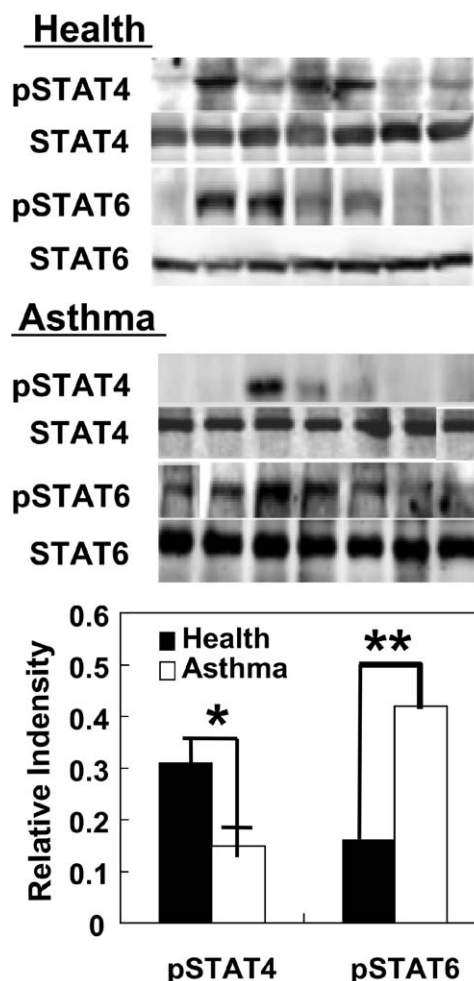


FIG 3. Activation of STAT4 or STAT6 in asthmatic patients. Cytoplasmic protein of purified NK cells was loaded onto a polyacrylamide gel electrophoresis and blotted against primary anti-pSTAT4 or anti-pSTAT6 or anti-unactivated total STAT4 or STAT6 protein antibodies. Protein bands were visualized by means of horseradish peroxidase-conjugated secondary antibody. The relative density of each phosphorylated STAT molecule was calculated for the ratio of total STAT molecules. * $P < .05$. Error bar, SEM.

FBS. The cells ($0.2 \times 10^6/0.1$ mL) were then incubated on ice for 30 minutes with unlabeled primary mAbs ($10 \mu\text{g/mL}$). Negative controls were cells incubated without antibodies or incubated with isotype-matched nonreactive Igs. The cells were incubated on ice for 30 minutes in the presence of appropriate biotinylated secondary antibodies (1:500 dilution). The cells were again incubated on ice for 30 minutes in the presence of PE-conjugated streptavidin (1:20 dilution) and then analyzed by means of flow cytometry with FACScalibur, as previously described.³⁷

Intracellular staining for IFN and IL-4 expression was performed by using the PharMingen Intracellular Staining Kit (PharMingen, San Diego, Calif). In brief, cells ($5 \times 10^6/\text{mL}$) were stimulated with PMA (30 ng/mL ; Sigma, St Louis, Mo) and ionomycin ($1 \mu\text{g/mL}$, Sigma). One hour later, monensin ($10 \mu\text{g/mL}$, Sigma) was added to prevent the secretion of the induced cytokines. After 4 hours of culture at 37°C and 5% CO_2 , the cells were harvested and labeled by appropriate antibodies for 30 minutes at 4°C . The surface staining was first performed with PE-conjugated anti-CD56, and then cells

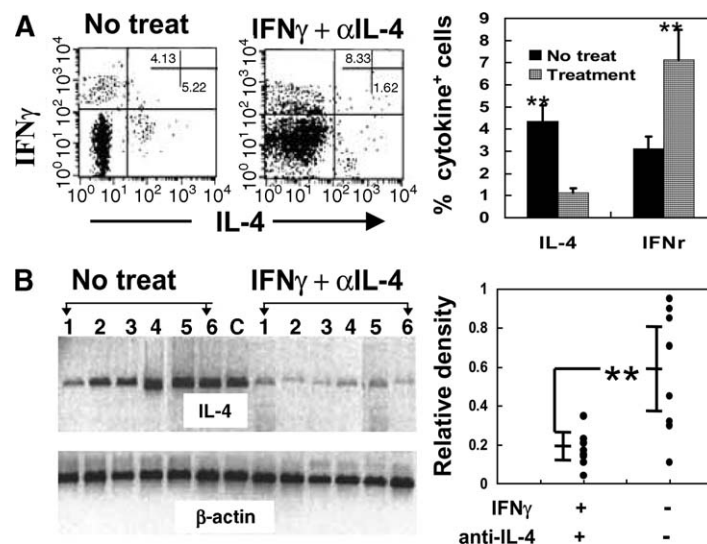


FIG 4. Shifting of NK2 cells of asthmatic patients toward NK1 cells *in vitro*. The purified NK cells were incubated with or without IFN- γ and anti-IL-4 mAb in the presence of IL-15 for 3 days. **(A)**, Triple-color staining. *Left panel* is a representative sample. *Right panel* is the summary of cytometric analysis. **(B)**, RT-PCR of IL-4 mRNA. *Left panel* shows 6 representative samples. *Right panel* is the summary of relative density. ** $P < .01$; Error bar, SEM.

were stained with CyChrome-anti-IFN or FITC-IL-4 mAbs or mouse FITC-IgG1 as a negative control for 30 minutes at 4°C. The CD56⁺ cells were gated after comparison with control antibody, and the intracellular cytokines were then analyzed. To validate the specificity of intracellular cytokine staining, excess unlabeled anti-cytokine antibody or excess cytokine was added to the cell-staining system to competitively inhibit the observed signal in some pre-experiments. Meanwhile, an appropriate fluorescence-labeled control antibody corresponding to each intracellular cytokine was used to diminish the nonspecific staining.

Western blot analysis

Rabbit polyclonal anti-phosphorylated signal transducer and activator of transcription (pSTAT-4) antibody and rabbit polyclonal anti-STAT-4 antibody were purchased from Zymed (San Francisco, Calif). Rabbit polyclonal anti-STAT6 and anti-phospho-STAT6 (pSTAT6) antibodies were obtained from New England Biolabs (Cell Signaling; New England Biolabs, Beverly, Mass). Rabbit IgG was obtained as control antibody from Caltag Laboratories (Burlingame, Calif). An aliquot of 40 μ g of protein from cell lysis was mixed with an equivalent volume of 2 $\times$ protein loading buffer and boiled for 5 minutes before loading onto an SDS-8% polyacrylamide gel. After electrophoresis, proteins were transferred onto nitrocellulose membranes and blotted against primary antibodies. Membranes were washed with 0.05% (vol/vol) Tween 20 in PBS (pH 7.4) and incubated with a 1:4000 dilution of horseradish peroxidase-conjugated secondary antibodies for 45 minutes. Protein bands were visualized by using an enhanced chemiluminescence reaction (Amersham Pharmacia Biotech, Piscataway, NJ), as previously described.³⁶

Statistical analysis

All experiments were repeated at least 4 times, and each sample was performed in triplicate in each experiment. Statistical analyses of the results were performed by using the Wilcoxon signed-rank pair and Mann-Whitney *U* tests. Differences were considered significant when the *P* value was less than .05.

RESULTS

Increased IL-4⁺ NK2 cells in asthmatic patients

As shown in Fig 1, the ratio of IL-4⁺CD56⁺ NK2 cells in the blood of 8 asthmatic patients was higher than that in healthy individuals, as determined by means of double staining, whereas the ratio of IFN- γ ⁺CD56⁺ NK1 cells in these patients was lower (Fig 1, A). IL-4 mRNA transcripts of freshly purified NK cells from 8 patients demonstrated significantly higher image density (0.63 ± 0.16) than those of healthy individuals (0.09 ± 0.06 ; Fig 1, B), which was further confirmed by means of double intracellular staining of purified NK cells with anti-IL-4 and anti-IFN- γ antibodies (see Fig E1 in the Journal's Online Repository at www.mosby.com/jaci), demonstrating that IL-4⁺ NK2 cell levels were increased and IFN- γ ⁺ NK1 cell levels were decreased in patients with asthma.

We then successfully constructed libraries of NK cell clones from 5 patients and 5 healthy individuals. Each library from one patient or one healthy individual contained more than 100 clones. The relative density to normalized β -actin in RT-PCR of cytokines in each clone (total of 100 clones) was calculated, and the paradigm of cytokine expression in all cell clones is shown in Fig 2. NK cell clones from healthy individuals produced more type 1 cytokines (Fig 2, A), but those from asthmatic patients produced more type 2 cytokines (Fig 2, B). When asthmatic patients and healthy subjects were compared in terms of the frequency of cytokine-producing NK clones, all cytokine levels except IL-10 were significantly changed, and asthmatic patients contained a higher ratio of NK2 cells in their peripheral blood (data not shown here).

We also examined activation of STAT4 or STAT6, common signal pathways of type 1 or type 2 cytokines, in purified NK cells from asthmatic patients and healthy subjects. In accordance with the results in Figs 1 and 2, the phosphorylation of STAT4 or STAT6 was downregulated or upregulated in asthmatic patients (Fig 3), respectively, indicating STAT6 of NK2 cells from patients with asthma were constitutively activated.

Reversing of IL-4⁺ NK2 cells into IFN- γ ⁺ NK1 cells *in vitro* and in recovered asthmatic patients after clinical therapy

Shifting from T_H2 toward T_H1 was thought to be an important feature of the T_H1-T_H2 paradigm and clinically important in disease relapse. We cocultured purified NK cells from 8 asthmatic patients with IFN- γ and anti-IL-4 antibody in the presence of IL-15 and found that the percentages of IL-4⁺CD56⁺ NK2 cells were significantly decreased from 4.17% $\pm$ 1.03% to 1.02% $\pm$ 0.16%, and the percentages of IFN- γ ⁺CD56⁺ cells were increased from 3.75% $\pm$ 0.47% to 6.87% $\pm$ 1.24%, respectively, after treatment (Fig 4, A). These results were further confirmed by means of RT-PCR, showing that the relative density of IL-4 mRNA transcript significantly decreased (Fig 4, B).

Clinically, T_H2-biased status might be reversed when the patients experienced remission or recovered after regular therapy. Accordingly, we observed that NK2-biased status in asthmatic patients was reversed when patients recovered from regular therapy without using immune inhibitors, such as corticosteroids. The purified NK cells from asthmatic patients before or after therapy were stored frozen, and we then compared the ability to transcript IL-4 or IFN- γ mRNA in RT-PCR experiments. As shown in Fig 5, the IL-4 mRNA- or IFN- γ mRNA-producing potentials of NK cells were significantly decreased or increased, respectively, after recovery from active disease status, further confirming that NK2 status is parallel to disease progression of asthma.

DISCUSSION

Importance of increased IL-4⁺ NK2 cells in asthmatic patients

Not only were Tc1-Tc2 and DC1-DC2 involved in differentiation of T_H1-T_H2 cells, but they were also directly related to the pathogenesis of a variety of autoimmune diseases.³⁸⁻⁴⁰ NK cells were considered to divide into NK1 and NK2 cells on the basis of secretion of type 1 cytokines or type 2 cytokines, respectively,²²⁻²⁶ although phenotypic and functional cauterizations of these 2 NK cell groups are still unknown. In this article we, for the first time, observed that the percentage of NK2 cells of asthmatic patients was much higher than that of healthy individuals, whereas percentages of NK1 cells in these patients were much lower (Fig 1). We observed that type 2 cytokine-expressing NK clones were biased in PBMCs from asthmatic patients when compared with the NK cell clone library from healthy subjects (Fig 2), suggesting that

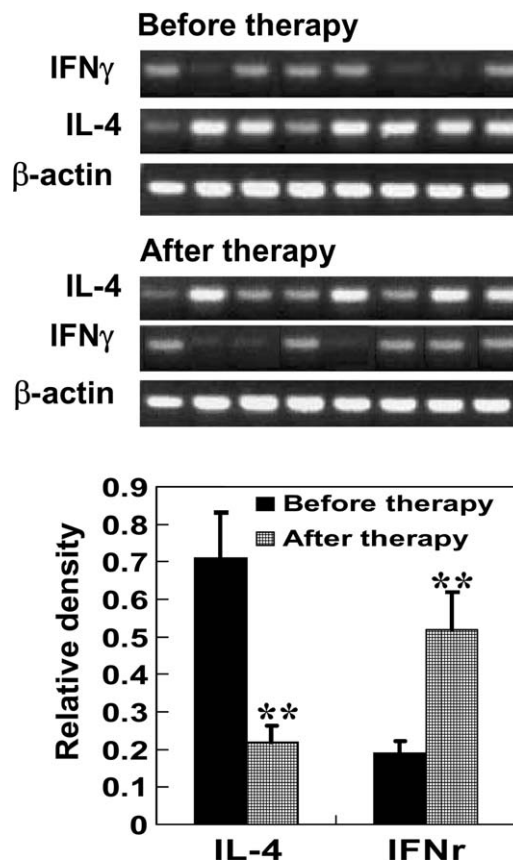


FIG 5. Shifting of NK2 cells of asthmatic patients toward NK1 cells after recovery from clinical therapy. After 10 weeks of erythromycin-containing treatment, PC₂₀, an index of bronchial sensitivity, was increased significantly. The purified NK cells were obtained before or after therapy and stored frozen at -85°C . The RT-PCR experiment was performed when all samples were obtained. The relative density to the ratio of β -actin was calculated. ** $P < .01$. Error bar, SE.

asthmatic patients contained a relatively higher ratio of NK2 cells in addition to T_H2 cells in peripheral blood. Many studies have revealed that type 1 or 2 cytokine-mediated signals are primarily transduced by the Jak-Stat signaling cascade.⁴¹ STAT4 regulates transcription of type 1 cytokines, and in contrast, STAT6 regulates that of type 2 cytokines, with the underlying mechanisms well explained by the pathogenesis of asthma.⁴² We found that STAT6 was constitutively activated in patients with asthma (Fig 3), indicating that NK2-biased status in asthma was correlated with STA6 overactivation, which is the same as what happened in T_H2-biased asthma. We speculate that a high level of serum type 2 cytokines in asthmatic patients is possibly in part the result of biased NK2 cells, although we could not determine whether NK2 cells appear as a beginning inducer of asthma or as a secondary result induced by T_H2 in asthma. The research with animal asthma models will be helpful in addressing this argument by seeing whether the appearance of NK2 cells is parallel to asthma pathogenesis and whether

depletion of NK cells influences serum IL-4 concentrations during asthma progress.

Although the total amount of NK cells in peripheral blood is much lower than that of T lymphocytes, NK cells are characterized by rapid responsiveness, extensive activation, and strong potentials of cytokine production. Therefore the cytokine-producing NK cells should play a pivotal role in the inflammatory process, and NK2-biased mechanisms underlying the pathogenesis of asthma should be paid more attention. Because the asthmatic subjects were atopic and the healthy subjects were non-atopic, it is possible that differences in NK1 and NK2 cell populations might be only related to atopic status rather than being specific for asthma. Therefore similar results might be observed if subjects with allergic rhinitis were studied. It remains completely unclear what the function of such NK2 cells might be. Although it was found that NK2 cells exert weaker cytotoxicity than NK1 cells,²²⁻²⁶ the roles in T- or B-cell help or in IgE switching-antibody production are still unclear.

Importance of reversing IL-4⁺ NK2 cells into IFN- γ ⁺ NK1 cells of asthmatic patients

The T_H1-T_H2 paradigm is characterized by shifting, and shifting from T_H2 toward T_H1 was thought to be especially of clinical importance. We found that the percentage of IL-4⁺CD56⁺ NK2 cells was significantly decreased after treatment (Fig 4), indicating that NK2-biased status in patients with asthma might possibly be reversed after treatment. Clinically, T_H2-biased status might be reversed when the patients experienced remission or recovered after regular therapy.⁴³⁻⁴⁵ Several *in vitro* studies show that macrolides affect several inflammatory processes, such as migration of neutrophils, the oxidative burst in phagocytes, and the production of proinflammatory cytokines, although the precise mechanisms are not clear.⁴⁶ Controlled clinical trials involving both children and adults with chronic asthma treated with macrolides have been performed.^{28,29,47} In this study we treated patients with chronic asthma with erythromycin and found that NK2-biased status in asthmatic patients was reversed when patients recovered from therapy with erythromycin (Fig 5). The percentage of IFN- γ ⁺CD56⁺ NK1 cells in the peripheral blood of asthmatic patients was remarkably increased, but that of IL-4⁺CD56⁺ NK2 cells was significantly decreased (Fig 5). Although how the NK1 cells increased after macrolide therapy remains unclear and the method to correlate changes in NK1/NK2 immune deviation with a therapeutic response is still elusive, the observation that NK2 cell content was parallel to the symptom severity is still novel and attractive and needs to be further investigated.

REFERENCES

1. Beasley R, Crane J, Lai CKW, Pearce N. Prevalence and etiology of asthma. *J Allergy Clin Immunol* 2000;105(suppl):S466-72.
2. Wills-Karp M. Immunologic basis of antigen-induced airway hyper-responsiveness. *Annu Rev Immunol* 1999;17:255-81.
3. Umetsu DT, McIntire JJ, Akbari O, Macaubas C, DeKruyff RH. Asthma: an epidemic of dysregulated immunity. *Nat Immunol* 2002;3:715-20.
4. Neurath MF, Finotto S, Glimcher LH. The role of Th1/Th2 polarization in mucosal immunity. *Nat Med* 2002;8:567-73.
5. Steinke JW, Borish L. Th2 cytokines and asthma. Interleukin-4: its role in the pathogenesis of asthma, and targeting it for asthma treatment with interleukin-4 receptor antagonists. *Respir Res* 2001;2:66-70.
6. Hamelmann E, Gelfand EW. IL-5-induced airway eosinophilia—the key to asthma? *Immunol Rev* 2001;179:182-91.
7. Wills-Karp M. IL-12/IL-13 axis in allergic asthma. *J Allergy Clin Immunol* 2001;107:9-18.
8. Parronchi P, Carli M, De Manetti R, Simonelli C, Piccinni MP, Macchia D, et al. Aberrant interleukin (IL)-4 and IL-5 production in vitro by CD4⁺ helper T cells from atopic subjects. *Eur J Immunol* 1992;22:1615-20.
9. Chan SC, Brown MA, Willcox TM, Li SH, Stevens SR, Tara D, et al. Abnormal IL-4 gene expression by atopic dermatitis T lymphocytes is reflected in altered nuclear protein interactions with IL-4 transcriptional regulatory element. *J Invest Dermatol* 1996;106:1131-6.
10. Piccinni MP, Beloni L, Giannarini L, Livi C, Scarselli G, Romagnani S, et al. Abnormal production of T helper 2 cytokines interleukin-4 and interleukin-5 by T cells from newborns with atopic parents. *Eur J Immunol* 1996;26:2293-8.
11. Daher S, Santos LM, Sole D, De Lima MG, Nasipz CK, Musatti CC. Interleukin-4 and soluble CD23 serum levels in asthmatic atopic children. *J Invest Allergol Clin Immunol* 1995;5:251-4.
12. Walker C, Bauer W, Braun RK, Menz G, Braun P, Schwarz F, et al. Activated T cells and cytokines in bronchoalveolar lavages from patients with various lung diseases associated with eosinophilia. *Am J Respir Crit Care Med* 1994;150:1038-48.
13. Leonard C, Tormey V, Burke C, Poulter LW. Allergen-induced cytokine production in atopic disease and its relationship to disease severity. *Am J Respir Cell Mol Biol* 1997;17:368-75.
14. Coyle AJ, Le Gros G, Bertrand C, Tsuyuki S, Heusser CH, Kopf H, et al. Interleukin-4 is required for the induction of lung Th2 mucosal immunity. *Am J Respir Cell Mol Biol* 1995;13:54-9.
15. Corry DB, Folkesson HG, Warnock ML, Erle DJ, Matthay MA, Wiener-Kronish JP, et al. Interleukin 4, but not interleukin 5 or eosinophils, is required in a murine model of acute airway hyperreactivity. *J Exp Med* 1996;183:109-17.
16. Renz H, Bradley K, Enssle K, Loader JE, Larsen GL, Gelfand EW. Prevention of the development of immediate hypersensitivity and airway hyperresponsiveness following *in vivo* treatment with soluble IL-4 receptor. *Int Arch Allergy Immunol* 1996;109:167-76.
17. Henderson W, Chi E, Maliszewski C. Soluble interleukin-4 receptor inhibits airway inflammation following allergen challenge in a mouse model of asthma. *J Immunol* 2000;164:1086-95.
18. Dabbagh K, Takeyama K, Lee HM, Ueki IE, Lausier JI, Nadel JA. IL-4 induces mucin gene expression and goblet cell metaplasia *in vitro* and *in vivo*. *J Immunol* 1999;162:6233-7.
19. Vukmanovic-Stejić M, Vyas B, Gorak-Stolinska P, Noble A, Kemeny DM. Human Tc1 and Tc2/Tc0 CD8 T-cell clones display distinct cell surface and functional phenotypes. *Blood* 2000;95:231-40.
20. Liu YJ, Kadowaki N, Rissoan MC, Soumelis V. T cell activation and polarization by DC1 and DC2. *Curr Top Microbiol Immunol* 2000;251:149-59.
21. Deniz G, Akdis M, Aktas E, Blaser K, Akdis CA. Human NK1 and NK2 subsets determined by purification of IFN- γ -secreting and IFN- γ -non-secreting NK cells. *Eur J Immunol* 2002;32:879-84.
22. Loza MJ, Zamai L, Azzoni L, Rosati E, Perussia B. Expression of type 1 (interferon) and type 2 (interleukin-13, interleukin-5) cytokines at distinct stages of natural killer cell differentiation from progenitor cells. *Blood* 2002;99:1273-81.
23. Cooper MA, Fehniger TA, Caligiuri MA. The biology of human natural killer-cell subsets. *Trends Immunol* 2001;22:633-40.
24. Rosmaraki EE, Douagi I, Roth C, Colucci F, Cumano A, Di Santo JP. Identification of committed NK cell progenitors in adult murine bone marrow. *Eur J Immunol* 2001;31:1900-9.
25. Cooper MA, Fehniger TA, Turner SC, Chen KS, Ghaehri BA, Ghayur T, et al. Human natural killer cells: a unique innate immunoregulatory role for the CD56 (bright) subset. *Blood* 2001;97:3146-51.

26. Chakir H, Camilucci AA, Filion LG, Webb JR. Differentiation of murine NK cells into distinct subsets based on variable expression of the IL-12R beta 2 subunit. *J Immunol* 2000;165:4985-93.
27. American Thoracic Society Board of Directors. Standards for the diagnosis and care of patients with chronic obstructive pulmonary disease (COPD). *Am Rev Respir Dis* 1987;136:225-44.
28. Miyatake H, Taki F, Taniguchi H, Suzuki R, Takagi K, Satake Y. Erythromycin reduces the severity of bronchial hyperresponsiveness in asthma. *Chest* 1991;99:670-3.
29. Amayasu H, Yoshida S, Ebana S, Yamamoto Y, Nishikawa T, Shoji T, et al. Clarithromycin suppresses bronchial hyperresponsiveness associated with eosinophilic inflammation in patients with asthma. *Ann Allergy Asthma Immunol* 2000;84:594-8.
30. Briard D, Brouty-Boye D, Azzarone B, Jasmin C. Fibroblasts from human spleen regulate NK cell differentiation from blood CD34+ progenitors via cell surface IL-15. *J Immunol* 2002;168:4326-32.
31. Mrozek E, Anderson P, Caligiuri MA. Role of interleukin-15 in the development of human CD56+ natural killer cells from CD34+ hematopoietic progenitor cells. *Blood* 1996;87:2632-40.
32. Carson WE, Fehniger TA, Haldar S, Eckhart K, Lindemann MJ, Lai CF, et al. A potential role for interleukin-15 in the regulation of human natural killer cell survival. *J Clin Invest* 1997;99:937-43.
33. Agarwal SK, Marshall GD Jr. Dexamethasone promotes type 2 cytokine production primarily through inhibition of type 1 cytokines. *J Interferon Cytokine Res* 2001;21:147-55.
34. Asselin S, Conjeaud H, Minty A, Fradelizi D, Breban M. Stable polarization of peripheral blood T cells towards type 1 or type 2 phenotype after polyclonal activation. *Eur J Immunol* 1998;28:532-9.
35. Akdis CA, Blesken T, Akdis M, Wuthrich B, Blaser K. Role of interleukin 10 in specific immunotherapy. *J Clin Invest* 1998;102:98-106.
36. Tian Z, Shen X, Feng H and Gao B. IL-1 β attenuates IFN α / β -induced antiviral activity and STAT1 activation in the liver: involvement of proteasome-dependent pathway. *J Immunol* 2000;165:3959-65.
37. Tian ZG, Woody MA, Sun R, Welniak LA, Raziuddin A, Funakoshi S, et al. Recombinant human growth hormone promotes hematopoietic reconstitution after syngeneic bone marrow transplantation in mice. *Stem Cells* 1998;16:193-9.
38. Kemeny DM, Vyas B, Vukmanovic-Stejic M, Thomas MJ, Noble A, Loh LC, et al. CD8+ T cell subsets and chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 1999;160(suppl):S33-7.
39. Todate A, Chida K, Suda T, Imokawa S, Sato J, Ide K, et al. Increased numbers of dendritic cells in the bronchiolar tissues of diffuse panbronchiolitis. *Am J Respir Crit Care Med* 2000;162:148-53.
40. Bertorelli G, Bocchino V, Zhou X, Zanini A, Bernini MV, Damia R, et al. Dendritic cell number is related to IL-4 expression in the airways of atopic asthmatic subjects. *Allergy* 2000;55:449-54.
41. Darnell JE Jr. STATs and gene regulation. *Science* 1997;277:1630-5.
42. Pernis AB, Rothman PB. JAK-STAT signaling in asthma. *J Clin Invest* 2002;109:1279-83.
43. Brand U, Bellinghausen I, Enk AH, Jonuleit H, Becker D, Knop J, et al. Allergen-specific immune deviation from a TH2 to a TH1 response induced by dendritic cells and collagen type I. *J Allergy Clin Immunol* 1999;104:1052-9.
44. Lim CS, Zheng S, Kim YS, Ahn C, Han JS, Kim S, et al. Th1/Th2 predominance and proinflammatory cytokines determine the clinicopathological severity of IgA nephropathy. *Nephrol Dial Transplant* 2001;16:269-75.
45. Altfeld M, Addo MM, Kreuzer KA, Rockstroh JK, Dumoulin FL, Schliefer K, et al. TH1 to TH2 shift of cytokines in peripheral blood of HIV-infected patients is detectable by reverse transcriptase polymerase chain reaction but not by enzyme-linked immunosorbent assay under nonstimulated conditions. *J Acquir Immune Defic Syndr* 2000;23:287-94.
46. Zalewska-Kasubaska J, Gorska D. Anti-inflammatory capabilities of macrolides. *Pharmacol Res* 2001;44:451-4.
47. Jaffe A, Bush A. Anti-inflammatory effects of macrolides in lung disease. *Pediatr Pulmonol* 2001;31:464-73.