

Perturbations of natural killer cell regulatory functions in respiratory allergic diseases

Francesca Scordamaglia, MD,^a Mirna Balsamo, PhD,^d Antonio Scordamaglia, MD,^a Alessandro Moretta, MD,^{b,c} Maria Cristina Mingari, PhD,^{b,e} Giorgio Walter Canonica, MD,^a Lorenzo Moretta, MD,^{b,c,d} and Massimo Vitale, PhD^e *Genoa, Italy*

Background: Allergic diseases are characterized by abnormal responses to allergens favored by an inappropriate regulation of the T_H1-T_H2 polarization. Natural killer (NK) cells give rise to a complex NK/dendritic cell (DC) cross-talk that would help T_H1 responses.

Objective: By analyzing peripheral blood NK cells from 12 patients with either allergic rhinitis or rhinitis and intermittent asthma, we evaluated whether these cells were impaired in their ability to interact with DCs.

Methods: Different circulating NK cell subsets were analyzed by flow cytometry. Mixed NK/DC cultures were performed to assess the reciprocal functional interactions. NK cells were analyzed for their ability to induce DC maturation and cytokine production, and to kill immature DCs. In addition, DCs were assessed for their ability to induce cytokine production by NK cells. **Results:** We first analyzed the CD56⁺⁺CD16^{+/-} cells, a subset of circulating NK cells that is able to respond to DCs by proliferating and producing IFN- γ . Our analysis revealed that this NK cell subpopulation was significantly reduced in most patients. This was reflected by reduced NK cell-mediated IFN- γ production in response to DCs. Also, the capability of promoting DC maturation and/or killing immature DCs, a function sustained by CD56⁺⁺CD16⁺ NK cells, was reduced in most patients.

Conclusions: We suggest that allergic diseases are accompanied by a partial impairment of the NK cell capability of

promoting and maintaining appropriate T_H1 responses. (J Allergy Clin Immunol ■■■■;■■■:■■■-■■■.)

Key words: NK cells, allergy, DC, NK1/NK2, T_H1/T_H2 cytokines, rhinitis, asthma

Allergic respiratory diseases are caused by an abnormal inflammatory response to innocuous antigens characterized by production of specific IgE and consequent activation of residing mast cells as well as of basophils and eosinophils recruited in airways mucosa.^{1,2} It is now well established that the imbalance of T_H1/T_H2 T-cell polarization and the bias toward T_H2 cytokine production plays a pivotal role both in the initiation and in the maintenance of these events.^{2,3} To identify significant therapeutic targets in these frequently disabling diseases, many efforts have been spent in these last years to characterize the regulatory events involved in the balance of T_H1/T_H2 cytokines. On the basis of these studies, several mechanisms involving different cell types have been proposed.³ IL-10-producing regulatory T cells have been shown to control T_H2 responses, and perturbations in their function and/or number have been described in patients with allergy.⁴ A pathogenic role in allergy has also been suggested for the invariant TCR-expressing NK-T cells,^{3,5} although a debate is open on the possibility that high numbers of IL-4⁺IL13⁺ NK-T cells could reach the lungs of patients with asthma.^{6,7} Finally, a key role in the regulation of the T_H2-driven airway inflammation has also been proposed for DCs.⁸ This is also supported by the fact that DCs can be skewed to promote either T_H1 or T_H2 polarization by different stimuli.⁹⁻¹² A role of NK cells in supporting DCs to the induction of appropriate T_H1 responses has recently been highlighted.¹³⁻²⁰ NK cell subsets²¹ characterized by particular functional properties have been proposed to intervene at different stages in the process of DC maturation and DC-mediated T-cell priming.^{22,23} In particular, CD56⁺⁺CD16^{+/-} NK cells respond to maturing DCs by producing IFN- γ and may cooperate with DC in inducing efficient T_H1 response in lymph nodes (LN),²⁴⁻²⁶ whereas CD56⁺⁺CD16⁺ NK cells help DC maturation, production of IL-12, and killing of those DCs that failed to undergo maturation.^{22,23,27,28} Perturbation of these newly described regulatory functions of NK cells may therefore result in an altered regulation of T_H1/T_H2 polarization and contribute to the pathogenesis of allergic disease. This possibility, however, has not yet been analyzed.

In this study, we evaluated peripheral blood NK cells from patients with either allergic rhinitis or rhinitis and intermittent asthma. We provide evidence of altered NK cell-mediated regulatory functions that, in turn, may affect downstream events

From ^athe Clinica Malattie dell'Apparato Respiratorio e Allergologia, Dipartimento di Medicina Interna, ^bthe Dipartimento di Medicina Sperimentale, and ^cthe Centro di Eccellenza per la Ricerca Biomedica, Università di Genova; ^dthe Istituto Giannina Gaslini; and ^ethe Istituto Nazionale per la Ricerca sul Cancro, SC Immunologia Sperimentale D.

Supported by grants awarded by Associazione Italiana per la Ricerca sul Cancro, Istituto Superiore di Sanità, Ministero della Salute (Ricerca Finalizzata Ministeriale 2005), Ministero dell'Università e della Ricerca Scientifica e Tecnologica (Programmi di Ricerca di Interesse Nazionale 2005, project 2005063024_004), Fondo per gli Investimenti della Ricerca di Base-Ministero dell'Università e della Ricerca project-RBNE017B4, European Union FP6, LSHB-CT-2004-503319-AlloStem, and Fondazione Compagnia di San Paolo, Torino, Italy, and the Associazione Ricerca Malattie Immunologiche ed Allergiche (ARMIA) and the Global Allergy and Asthma European Network (GA²LEN).

Disclosure of potential conflict of interest: The authors have declared that they have no conflict of interest.

Received for publication April 3, 2007; revised August 20, 2007; accepted for publication September 27, 2007.

Reprint requests: Giorgio Walter Canonica, MD, Allergy and Respiratory Diseases Clinic, Department of Internal Medicine, University of Genova, Pad Maragliano, Largo R Benzi, 10, 16132 Genova, Italy. E-mail: canonica@unige.it.

0091-6749/\$32.00

© 2008 American Academy of Allergy, Asthma & Immunology

doi:10.1016/j.jaci.2007.09.047

Abbreviations used

DC:	Dendritic cell
FACS:	Fluorescence-activated cell sorting
iDC:	Immature dendritic cell
KIR:	Killer immunoglobulin-like receptors
LN:	Lymph node
Mo-DC:	Monocyte-derived dendritic cell
NK:	Natural killer
PBL:	Peripheral blood lymphocyte
SPT:	Skin prick test
TLR:	Toll-like receptor

leading to appropriate T-cell polarization. Thus, our study offers new clues to the role played by NK cells in the pathogenesis of allergic diseases.

METHODS**Patients**

Twelve patients (P1-P12; 9 women and 3 men; mean age, 33.3 years) with a clinical history of allergic rhinitis with or without eye symptoms were studied. Four patients were also affected by intermittent asthma as reported in Global Initiative for Asthma (GINA) guidelines.

Patients presented a skin prick test (SPT) reaction strongly positive (4+) only to *Dermatophagoides pteronyssinus* and/or *Parietaria judaica* pollen. SPTs were performed with a panel of standardized allergens (Stallergenes, Milan, Italy) including mite, grasses, olive, birch, cat and dog dander, *Parietaria*, and *Aspergillus fumigatus*.

At the moment of recruitment, all the patients had no symptoms and were not receiving any anti-inflammatory treatment (including systemic and local steroids, antileukotrienes, antihistamines); moreover, they had never been treated with allergen-specific immunotherapy.

Nine healthy donors (H1-H9) displaying a negative SPT for the same panel of allergens served as controls (7 women and 2 men; mean age, 30.4 years).

Informed consent was obtained from all subjects participating in the study.

mAbs

The following mAbs produced in our laboratory were used in this study: JT3A (IgG_{2a}, anti-CD3), c127 (IgG₁, anti-CD16), AZ20 (IgG₁, anti-NKp30), BAB281 (IgG₁, anti-NKp46), Z231 (IgG₁, anti-NKp44), ECM217 (IgG_{2b}, anti-NKG2D), MA152 (IgG₁, anti-NKp80), Z27 (IgG₁, anti-KIR3DL1), Z199 (IgG_{2b}, anti-NKG2A), A6-136 (IgM, anti-HLA class I), FM184 (IgM, anti-CD1a), and FM95 (IgG₁, anti-CD86). Anti-CCR7 (IgM; BD Pharmingen, San Jose, Calif), anti-CD62L (IgG₁-PC5), anti-CD14 (IgG_{2a}), and anti-CD56 (IgG₁-PC5; Immunotech, Marseille, France) were commercially available. NKVSF1 mAb (IgG₁, anti-KIR2DL1/S1/L2/L3/S2/S4) and 191B8 mAb (IgG_{2a}, anti-NKRP1) were provided by Dr A. Poggi (Genoa, Italy).

Isolation of purified NK cell populations

Both from patients and healthy controls, 20 mL blood was drawn. To obtain peripheral blood lymphocytes (PBLs), PBMCs were isolated on Ficoll-Hypaque gradients and depleted of plastic-adherent cells. Approximately $1/1.5 \times 10^6$ purified NK cells were obtained by negative selection by using the NK cell isolation Kit (Miltenyi Biotec, Bergisch Gladbach, Germany) following the manufacturer's instructions. The NK cell populations were assessed for purity, and only those containing 95% or more of cells homogeneously displaying the CD3⁺CD14⁺CD15⁺CD19⁺ phenotype were selected.

Generation of DCs

Dendritic cells were generated as previously described.²⁷ Briefly, PBMCs were derived from healthy donors, and plastic-adherent cells were cultured in

the presence of IL-4 and GM-CSF (Peprotech, London, United Kingdom) at the final concentration of 20 ng/mL and 50 ng/mL, respectively. After 6 days of culture, cells were characterized by the CD14⁺CD1a⁺CD86⁺ phenotype corresponding to immature DCs (iDCs).

Induction of DC maturation

Immature DCs were plated in 96-well round-bottom microtiter plates at 5×10^4 cells/well either in the absence or in the presence of the NK cells that had been previously cultured overnight in the presence of 50 IU/mL IL-2 (DC/NK ratio: 1/1). DCs were harvested and were assessed for the expression of the maturation marker CD86. As control, optimal DC maturation was induced by the addition of LPS at the final concentration of 1 μ g/mL. When described, DC/NK coculture supernatant was harvested and assessed for IL-12 content by using an ELISA kit from BioSource International (Camarillo, Calif).

NK cell stimulation and cytokine analysis

Dendritic cell-induced NK cell stimulation. Fresh NK cells were cultured in 96-well round-bottom microtiter plates (10^5 cells/well) in the presence of either iDC + 1 μ g/mL LPS (NK/DC ratio: 5/1) or 1 μ g/mL LPS alone as negative control. After 48 hours, supernatants were collected and analyzed for IFN- γ content by specific ELISA Kit (BioSource International). In each experiment, the NK cells derived from a patient were compared with those derived from a control donor for their capability to produce IFN- γ in response to the same DC population. DCs were unique to each experiment. In all cases, DCs were derived from individuals who were unrelated to both patients and controls.

Cytokine-induced NK cell stimulation. Fresh NK cells were cultured for 20 hours in 96-well round-bottom microtiter plates at 2×10^5 cells/well either in the absence or in the presence of IL-2 (100 IU/mL) + IL-12 (2.5 ng/mL). During the last 6 hours, cells were exposed to Golgi-stop (BD Pharmingen) according to the manufacturer's instructions. Subsequently, cells were fixed in 2% paraformaldehyde, permeabilized in 0.5% Saponin, and then stained with anti-IFN- γ -phycoerythrin (4S.B3) or isotype control (BD Pharmingen).

Flow-cytofluorimetric analysis and cytolytic activity

For cytofluorimetric analysis (FACS-Calibur, Becton Dickinson, Franklin Lakes, NJ), cells were stained with the appropriate labeled or unlabeled mAb. In the case of unlabeled mAbs, staining was followed by phycoerythrin-conjugated or fluorescein isothiocyanate-conjugated isotype-specific goat antimouse second reagent (Southern Biotechnology Associated, Birmingham, Ala). In the analysis of cells derived from coculture experiments, DCs were gated on the basis of forward scatter/side scatter parameters and confirmed by the assessment of the expression of CD1a DC marker. Fresh NK cells derived from either healthy donors or patients were cultured overnight in the presence of 50 IU/mL IL-2 and then tested for cytolytic activity in a 4-hour ⁵¹Cr-release assay against allogeneic iDCs.²⁷ Anti-HLA class I mAb (A6-136 IgM) was added in the tests at the final concentration of 5 μ g/mL.

Statistical analysis

The Student *t* test was used to evaluate the different percentages of CD56⁺ NK cells in subjects with allergy and healthy subjects (Fig 1). Because of the variability introduced by the use of different DCs, data shown in Figs 2, 3, and 4 were analyzed by using the Wilcoxon test.

RESULTS**CD56⁺CD16⁺ NK cell population is reduced in size in patients with allergy**

Peripheral blood lymphocytes freshly isolated either from patients with allergy or from healthy donors were assessed by

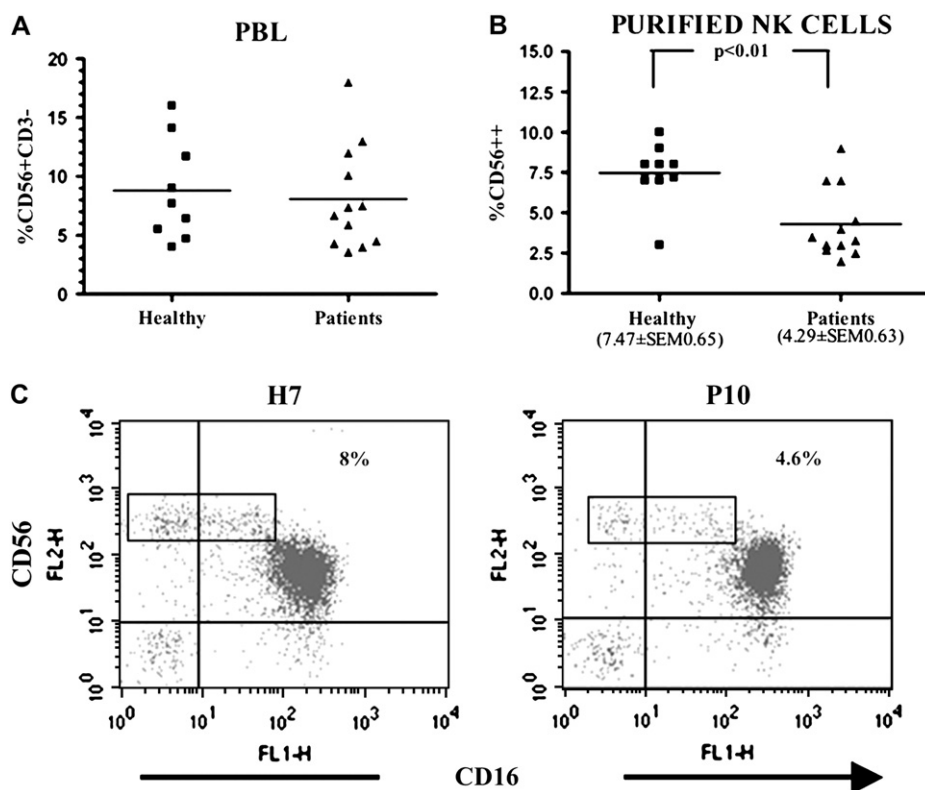


FIG 1. Circulating CD56⁺⁺CD16^{+/-} NK cells are reduced in allergic patients. Percentages of CD56⁺⁺CD3⁻ NK cells in PBL (**A**) and of CD56⁺⁺ cells within the NK cell population (**B**) are shown (healthy individuals, squares; patients, triangles). **C**, CD56 and CD16 expression in purified NK cells from a healthy representative individual (H7) and a representative individual with allergy (P10). The CD56⁺⁺CD16^{+/-} cell population is quantified.

double immunofluorescence staining and cytofluorimetric analysis for the surface expression of CD3 and CD56 antigens. As shown in Fig 1, A, PBLs from patients or healthy controls included similar percentages of CD56⁺⁺CD3⁻ NK cells.

To evaluate in detail more the NK-cell subsets present in PBLs, NK cells were purified from PBMCs by removing cells expressing CD3, CD19, CD14, and CD15 antigens. Cells were further analyzed by fluorescence-activated cell sorting (FACS) for the combined expression of informative surface markers. This analysis revealed that, in the majority of the patients analyzed (9 of 12), the NK-cell subset characterized by the CD56⁺⁺ phenotype was significantly decreased in size [the mean percentage ($\pm$ SEM) of CD56⁺⁺ cells within the whole NK cell population was 4.29 ± 0.63 in patients and 7.47 ± 0.65 in healthy controls ($P < .01$; Fig 1, B)]. The surface phenotype of this subset, known to produce large amounts of IFN- γ ,^{21,26} was identical in patients and normal controls. Thus, CD56⁺⁺ NK cells were CD16^{+/-} (Fig 1, C) KIR⁻NKG2A⁺⁺CD62L⁺⁺CCR7⁺ (not shown).^{21,26} In particular, in both patients and healthy subjects, CD56⁺⁺ NK-cell subsets included similar percentages of CD16⁺ cells (which ranged between 25% and 60%).

Therefore, the current data show that the CD56⁺⁺CD16^{+/-} IFN- γ producing NK cells are reduced in patients with allergy.

Our attention was also focused on the CD56⁻NKRP-1⁺ NK cells because this subset, in contrast with the CD56⁺⁺CD16^{+/-}IFN- γ ⁺ cells, has been shown to produce T_H2-type cytokines (ie, IL-5 and IL-13).²⁹ In this case, however, no differences could be detected between patients and healthy

donors (not shown). Finally, we also evaluated additional informative NK-cell markers including killer immunoglobulin-like receptor (KIR), NKG2A, natural cytotoxicity receptor, NKG2D, NKp80, 2B4, and DNAM1.³⁰ No significant differences could be detected in patients or healthy controls.

Natural killer cells from patients with allergy display a reduced capability of producing IFN- γ in response to DC stimulation

After engagement of certain Toll-like receptors (TLRs) by appropriate pathogen-associated molecular patterns (PAMPs), DCs have been shown to promote NK-cell-mediated production of IFN- γ .^{14,26,31} In this DC/NK cell interaction, a prominent role is played by CD56⁺⁺CD16^{+/-} NK cells.²⁶ A reduction of the CD56⁺⁺CD16^{+/-} NK cell component may therefore affect NK cell responses to DC-mediated stimuli.

To verify this possibility, NK cells purified from patients or healthy controls were cultured in the presence of either unrelated allogeneic monocyte-derived DCs (Mo-DCs) + LPS (to induce DC activation via TLR4) or LPS alone (as negative control). Cell supernatants were assayed for the presence of IFN- γ . In this type of experiment, a correct evaluation of NK-cell function requires the use of fresh (not frozen) cells. Therefore, because blood collection from different patients was scheduled at different times, a single patient and a healthy donor (P/H pair) were compared in each experiment. Although a certain degree of variability in the production of IFN- γ exists in different experiments (possibly reflecting

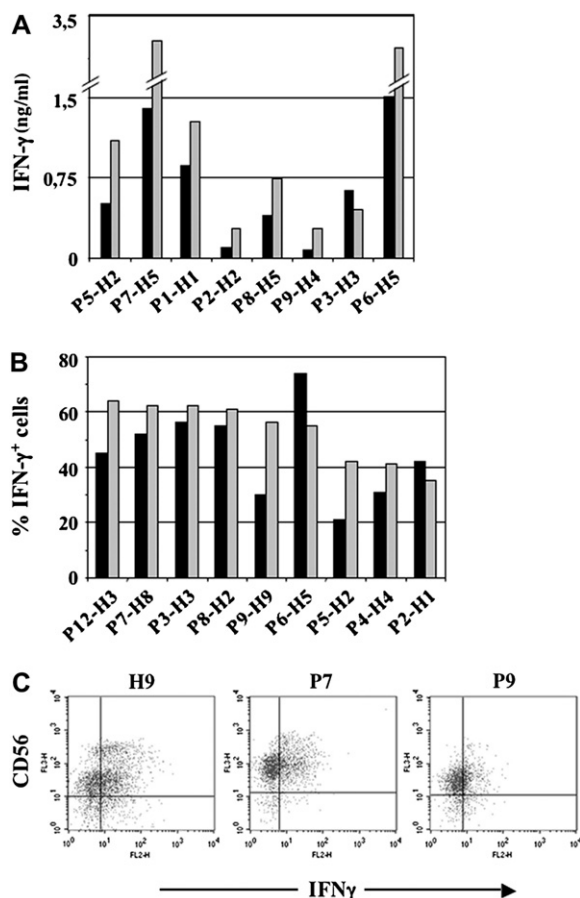


FIG 2. Evaluation of NK-cell-mediated IFN- γ production in patients with allergy or healthy subjects. **A**, IFN- γ content in supernatants from allergic (black) or healthy (gray) NK cells cultured with DCs. **B and C**, NK cells stimulated by cytokines are analyzed by FACS for their IFN- γ content (patients, black; healthy, gray).

differences in the stimulatory capability of the Mo-DCs used), a reduced ability to release IFN- γ in comparison to healthy subjects was detected in 7 of 8 patients ($P < .05$; Fig 2, A; Fig 5).

Natural killer cells from patients and from healthy donors were also compared for their intrinsic ability to produce IFN- γ in response to a T_H1 stimulus. To this end, NK cells were cultured overnight in the presence of IL-2 in combination with IL-12, and then assessed for IFN- γ content by FACS analysis. Although in 7 of 9 patients the percent value of IFN- γ^+ cells was below that of healthy controls, only in some instances a sharp decrease could be appreciated ($P = NS$; Fig 2, B) and involved both the $CD56^{++}$ and the $CD56^{+-}$ NK-cell subsets (Fig 2, C).

Taken together, these data suggest that the defective capability of producing IFN- γ in response to DC-mediated stimuli may be a result of the low percentage of $CD56^{++}$ NK cells within the patients' circulating NK-cell pool (see representative patient 7, Fig 2, A-C). In some cases, however, this defect may also reflect an intrinsic inability of NK cells to produce IFN- γ (see representative patient 9, Fig 2, A-C).

Patients' NK cells induce poor DC maturation

We next assessed whether the capability of promoting effective maturation of DCs could also be compromised in patients' NK cells.

To this end, Mo-DCs derived from unrelated donors were cocultured for 48 hours with NK cells from patients or healthy controls and analyzed for the expression of the maturation marker CD86. In each experiment, NK cells derived from a patient or a healthy subject were comparatively analyzed for their ability to induce maturation of allogeneic Mo-DCs. As shown in Fig 3, Mo-DCs that had been cultured in the presence of patients' NK cells displayed a certain degree of maturation. However, in most cases, the CD86 expression levels (ie, the degree of Mo-DC maturation) induced by patient' NK cells were clearly lower than those induced by healthy NK cells ($P < .05$; Fig 3). This was further confirmed by the fact that CD83 and HLA class II molecules (whose expression levels are generally related to the degree of DC maturation) could be induced at the DC surface less efficiently by patients' than by normal NK cells (not shown).

Previous studies have demonstrated that the process of DC maturation after their encounter with NK cells also results in the induction of IL-12 release (from DCs).^{14,15} Therefore, the failure of patients' NK cells to induce an efficient DC maturation may also result in an impaired capability of promoting IL-12 secretion. This was confirmed by the analysis of IL-12 content in the supernatants from some representative experiments of Mo-DC/NK cell cocultures. As shown in Fig 5 (which summarizes all the values related to the patients), P1, P4, P8, and P9 NK cells induced poor IL-12 release compared with healthy controls whereas in the case of P7 and P11 NK cells, the induction of IL-12 release was comparable or even more efficient than that of healthy NK cells. In all instances, the magnitude of IL-12 release correlated with the Mo-DC maturation level (Figs 3 and 5).

Analysis of the patients' NK cell capability of killing immature Mo-DC (iDC)

Natural killer cells, in the presence of appropriate activating stimuli (ie, certain TLR ligands and/or cytokines such as IL-2 or IL-12), become capable of killing iDCs (while sparing mature Mo-DCs).^{19,23,27,31,32} It has been suggested that this phenomenon may represent a mechanism by which NK cells can eliminate those DCs that, during an immune response, do not undergo maturation and, consequently, do not induce proper T_H1 responses.^{19,23} In this context, the evaluation of the patients' NK cell capability of killing iDCs appears to be a reliable approach to verify the possible role of NK cells in the pathogenesis of allergic diseases.

In this set of experiments, NK cells isolated from patients or healthy controls after overnight culture in the presence of IL-2 were comparatively analyzed for their ability to kill allogeneic iDCs in a ^{51}Cr release cytolytic assay. Also in this case, NK cells derived from a patient and a healthy subject were analyzed in each experiment. To avoid interferences in the NK-cell-mediated DC killing because of the possible occurrence of killer immunoglobulin-like receptors-HLA class I inhibitory interactions between NK cells (from different donors) and DCs, the cytolytic assays were also performed in the presence of a HLA class I-specific mAb.²⁷ Indeed, after mAb-mediated blocking of KIR/HLA class I interactions, NK cells can kill iDCs independent of their KIR haplotype. Therefore, under these experimental conditions, actual cytolytic activity against iDCs can be properly evaluated.

As shown in Fig 4, in most cases, patients' NK cells displayed a reduced ability to kill iDCs compared with healthy

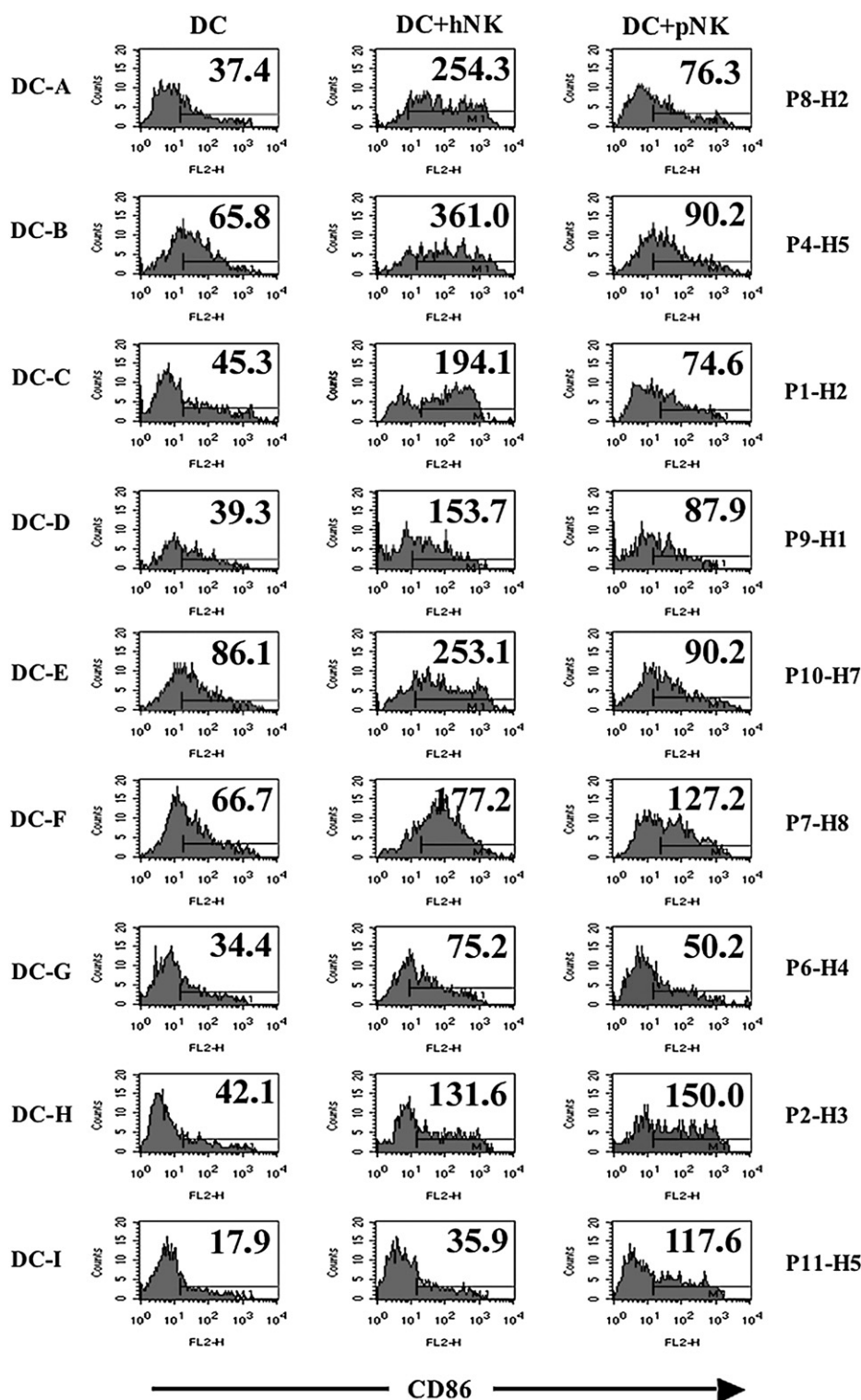


FIG 3. NK cells of patients with allergy induce poor DC maturation. Cytofluorimetric analysis of CD86 expression on DCs that have been cultured either in the absence (DC) or in the presence of healthy (DC+hNK) or patient (DC+pNK) NK cells. On the left and the right sides are indicated the DC and the P/H pairs analyzed in each experiment. Numbers indicate the CD86 mean fluorescence intensity.

NK cells ($P < .04$), although the levels of DC killing varied in different experiments. Remarkably, in some cases, a major difference existed between patient and healthy NK cell cytotoxicity. In each P/H pair analyzed, similar differences between

patient and healthy NK cells were detectable at both 10:1 (Fig 4) and 5:1 (not shown) effector/target ratios. Similar results were obtained in an additional cytolytic assay against a different DC target (not shown).

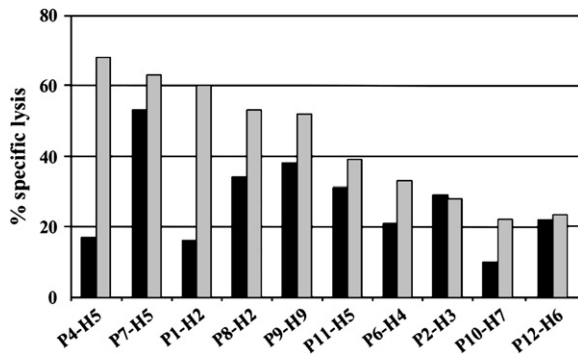


FIG 4. Analysis of the ability of patients' NK cells to kill iDCs. Patients (black) and healthy (gray) NK cells are compared in a cytolytic assay for their ability to kill allogeneic iDCs. NK cells from each P/H pair were assessed against a different iDC target. E/T ratio, 10/1.

DISCUSSION

In this study, we provide evidence that the regulatory functions of human NK cells may be impaired in allergic respiratory diseases. Importantly, NK cells appear to be affected in their ability to properly interact with DCs, a phenomenon that is believed to favor the polarization toward T_H1 -type adaptive responses.¹³⁻²⁰ As summarized in Fig 5, in most patients, circulating NK cells were characterized by low proportions of $CD56^{++}CD16^{+/-}$ IFN- γ -producing cells^{21,26} and by a consequent reduced ability to produce IFN- γ after interaction with DCs. In addition, patients' NK cells were less efficient in promoting DC maturation and/or in killing immature DCs.

In respiratory allergies, a bias toward T_H2 -type responses is detectable in circulating T lymphocytes.^{4,33,34} The precise mechanism by which this systemic immunologic disorder results in local allergic inflammation is not fully understood; however, it is conceivable that T_H2 -polarized T cells present in local lymphoid tissues may play a major role. The $CD56^{++}CD16^{+/-}$ NK cells express an array of surface receptors (including CCR7 and CD62L) that allow their homing into LNs.^{21,24,25} There, after interaction with mature DCs, $CD56^{++}CD16^{+/-}$ NK cells can produce large amounts of IFN- γ and favor the T_H1 polarization toward a T_H1 phenotype. The fact that $CD56^{++}CD16^{+/-}$ NK cells are reduced in the peripheral blood of patients with allergy may result in an insufficient recruitment in LNs and in an imbalanced regulation of T_H1 - T_H2 polarization.

Different from $CD56^{++}CD16^{+/-}$ NK cells, $CD56^{+}CD16^{+}$ NK cells can traffic to peripheral tissue, where they can support maturation of DCs that are capable of promoting optimal T-cell priming and T_H1 polarization.^{19,22,23} NK cells have been shown to promote the expression of costimulatory molecules (such as CD86) and the production of IL-12 in DCs.^{14,15} IL-12 is crucial in the induction of T_H1 polarization. In addition, NK cells are capable of killing immature DCs. Because of this functional capability, NK cells may contribute to eliminate those DCs that failed to undergo complete maturation and would thus induce suboptimal T-cell activation or even T_H2 polarization.^{19,22,23} Our analysis provides evidence that, in most patients, these NK cell functions are impaired (Fig 5), thus suggesting that NK cells reaching inflamed peripheral tissues may fail to keep in check the process of DC maturation. In this context, it is important to note that DCs of the airways in the absence of polarizing stimuli were reported to promote T_H2 -type responses.^{8,35} The impairment of

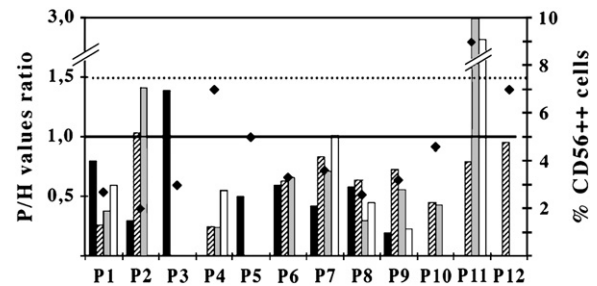


FIG 5. Synoptic data of the NK cell phenotype and functions of allergic patients analyzed. Diamonds, Percent of $CD56^{++}$ NK cells. Bars, Patient/healthy donor (P/H) values ratio related to all of the NK cell functions analyzed (data from Fig 2, B, are not reported because they are not statistically significant). Black, IFN- γ induced on NK-DC coculture. Striped, DC killing. Gray, CD86 mean fluorescence intensity (DC maturation). White, IL-12 produced by DCs on NK-cell stimulation. The solid line refers to data in the healthy controls (P/H ratio = 1). The dotted line indicates the mean percentage of $CD56^{++}$ cells in healthy controls.

NK-cell function may therefore be crucial to the pathophysiology of the disease.

Taken together, our present observations suggest that different NK-cell subsets and different NK-cell functions may be affected in allergy. Although we do not know why and how these perturbations of the NK-cell homeostasis would be induced, it is conceivable that the particular cytokine milieu characteristic of patients with allergy may play a role. For example, it has been shown that the development of $CD56^{++}CD16^{+/-}$ IFN- γ -producing NK cells within circulating lymphocytes is inhibited by IL-13, whereas it is promoted by IL-12.^{36,37} In addition, IL-4 has been shown to inhibit the NK-cell capability of inducing DC maturation and killing iDCs.³⁸ In this context, a recent report showing an abnormal frequency of IL-4-producing NK2 cells in PB from patients with asthma³⁹ may suggest that the dysregulation of the NK cytokine profile may by itself contribute to the NK-cell impairment.

In the past few years, the concept that NK cells may play a regulatory role in the adaptive responses has become increasingly evident. The existence, within circulating lymphocytes, of NK cells skewed toward either type 1 (NK1) or type 2 (NK2) cytokine (ie, IFN- γ or IL-4, IL-5, IL-13, respectively)-secreting phenotype has been reported by several groups.⁴⁰⁻⁴² The imbalance between these 2 NK cell types has been proposed to play a role in the pathogenesis of allergic diseases.^{43,44} Although these 2 functionally distinct NK-cell types cannot be unequivocally identified by a defined surface phenotype, NK2 cells have been suggested to be part of the small NKRP-1⁺ $CD56^{-}$ NK cell subset,²⁹ whereas NK1 cells seem to constitute a more heterogeneous NK-cell population including both $CD56^{++}CD16^{+/-}$ and part of $CD56^{+}CD16^{+}$ NK cells.⁴² After the recent advances on the definition of the NK/DC interactions, however, the concept of the NK-cell regulatory function cannot be restricted to the type of cytokines secreted, but would need to be extended to the functional outcomes resulting from the NK/DC interactions. By the analysis of these functions, we suggest that allergic diseases are characterized by an impairment of peripheral blood NK cells to interact with DCs and promote and maintain appropriate T_H1 responses. Our findings are not in contrast with the possibility that alterations in NK1/NK2 balance may occur in allergy. Indeed, the NK-cell population known to interact efficiently with DCs may be, at least in part,

overlapping with the NK1-cell subset, which includes both CD56⁺⁺CD16^{+/-} and CD56⁺CD16⁺ cells. In addition, similar to the NK1 functional capabilities, the ability to induce substantial DC maturation and to eliminate iDCs is enhanced by IL-12 and inhibited by IL-4.³⁸ Therefore, the impairment of the NK-cell function described in this study is in accordance with previous studies indicating the role of NK1 cells in the control of T_H2-driven inflammation.^{44,45}

In conclusion, our results confirm previous data suggesting a role for the NK1/NK2 dysregulation in allergy. More importantly, they provide new insights in elucidating the downstream mechanisms by which this imbalance may favor the development of allergic diseases.

We thank Dr A. M. Riccio for performing statistical analyses.

Clinical implications: It would be interesting to evaluate whether effective therapeutic strategies, including specific immunotherapy, are able to restore the NK cell-promoted T_H1 responses in patients with allergy.

REFERENCES

1. Canonica GW. Introduction to nasal and pulmonary allergy cascade. *Allergy* 2002; 57(suppl 75):8-12.
2. Herrick CA, Bottomly K. To respond or not to respond: T cells in allergic asthma. *Nat Rev Immunol* 2003;3:405-12.
3. Umetsu DT, Dekruyff RH. Immune dysregulation in asthma. *Curr Opin Immunol* 2006;18:727-32.
4. Akdis M, Verhagen J, Taylor A, Karamloo F, Karagiannidis C, Cramer R, et al. Immune responses in healthy and allergic individuals are characterized by a fine balance between allergen-specific T regulatory 1 and T helper 2 cells. *J Exp Med* 2004;199:1567-75.
5. Akbari O, Stock P, Meyer E, Kronenberg M, Sidobre S, Nakayama T, et al. Essential role of NKT cells producing IL-4 and IL-13 in the development of allergen-induced airway hyperreactivity. *Nat Med* 2003;9:582-8.
6. Akbari O, Faul JL, Hoyte EG, Berry GJ, Wahlstrom J, Kronenberg M, et al. CD4⁺ invariant T-cell-receptor⁺ natural killer T cells in bronchial asthma. *N Engl J Med* 2006;354:1117-29.
7. Vijayanand P, Seumois G, Pickard C, Powell RM, Angco G, Sammut D, et al. Invariant natural killer T cells in asthma and chronic obstructive pulmonary disease. *N Engl J Med* 2007;356:1410-22.
8. Lambrecht BN, Hammad H. Taking our breath away: dendritic cells in the pathogenesis of asthma. *Nat Rev Immunol* 2003;3:994-1003.
9. Banchereau J, Briere F, Caux C, Davoust J, Lebecque S, Liu YJ, et al. Immunobiology of dendritic cells. *Annu Rev Immunol* 2000;18:767-811.
10. Gagliardi MC, Sallusto F, Marinaro M, Langenkamp A, Lanzavecchia A, De Magistris MT. Cholera toxin induces maturation of human dendritic cells and licenses them for Th2 priming. *Eur J Immunol* 2000;30:2394-403.
11. Romani L. Immunity to fungal infections. *Nat Rev Immunol* 2004;4:1-23.
12. Napolitani G, Rinaldi A, Berton F, Sallusto F, Lanzavecchia A. Selected Toll-like receptor agonist combinations synergistically trigger a T helper type 1-polarizing program in dendritic cells. *Nat Immunol* 2005;6:769-76.
13. Zivvogel L. Dendritic and natural killer cells cooperate in the control/switch of innate immunity. *J Exp Med* 2002;195:9-14.
14. Gerosa F, Baldani-Guerra B, Nisii C, Marchesini V, Carra G, Trinchieri G. Reciprocal activating interaction between NK cells and dendritic cells. *J Exp Med* 2002; 195:327-33.
15. Piccioli D, Sbrana S, Melandri E, Valiante N-M. Contact-dependent stimulation and inhibition of dendritic cells by natural killer cells. *J Exp Med* 2002;195: 335-41.
16. Mocikat R, Braumuller H, Gummy A, Egeter O, Ziegler H, Reusch U, et al. Natural killer cells activated by MHC class I (low) targets prime dendritic cells to induce protective CD8 T cell responses. *Immunity* 2003;19:561-9.
17. Borg C, Jalil A, Laderach D, Maruyama K, Wakasugi H, Charrier S, et al. NK cell activation by dendritic cells (DCs) requires the formation of a synapse leading to IL-12 polarization in DCs. *Blood* 2004;104:3267-75.
18. Kalinski P, Giermasz A, Nakamura Y, Basse P, Storkus WJ, Kirkwood JM, et al. Helper role of NK cells during the induction of anticancer responses by dendritic cells. *Mol Immunol* 2005;42:535-9.
19. Moretta A, Marcenaro E, Sivori S, Della Chiesa M, Vitale M, Moretta L. Early liaisons between cells of the innate immune system in inflamed peripheral tissues. *Trends Immunol* 2005;26:668-75.
20. Cooper MA, Fehniger TA, Fuchs A, Colonna M, Caligiuri MA. NK cell and DC interactions. *Trends Immunol* 2004;25:47-52.
21. Cooper MA, Fehniger TA, Caligiuri MA. The biology of human natural killer-cell subsets. *Trends Immunol* 2001;22:633-40.
22. Moretta A. Natural killer cells and dendritic cells: rendezvous in abused tissues. *Nat Rev Immunol* 2002;2:957-65.
23. Moretta L, Ferlazzo G, Bottino C, Vitale M, Pende D, Mingari MC, et al. Effector and regulatory events during natural killer-dendritic cell interactions. *Immunol Rev* 2006;214:219-28.
24. Fehniger TA, Cooper MA, Nuovo GJ, Cella M, Bacchetti F, Colonna M, et al. CD56^{bright} natural killer cells are present in human lymph nodes and are activated by T cell-derived IL-2: a potential new link between adaptive and innate immunity. *Blood* 2003;101:3052-7.
25. Ferlazzo G, Thomas D, Lin SL, Goodman K, Morandi B, Muller WA, et al. The abundant NK cells in human secondary lymphoid tissues require activation to express killer cell Ig-like receptors and become cytolytic. *J Immunol* 2004;172: 1455-62.
26. Vitale M, Della Chiesa M, Carlomagno S, Romagnani C, Thiel A, Moretta L, et al. The small subset of CD56^{bright} CD16⁺ natural killer cells is selectively responsible for both cell proliferation and interferon-gamma production upon interaction with dendritic cells. *Eur J Immunol* 2004;34:1715-22.
27. Della Chiesa M, Vitale M, Carlomagno S, Ferlazzo G, Moretta L, Moretta A. The natural killer cell-mediated killing of autologous dendritic cells is confined to a cell subset expressing CD94/NKG2A, but lacking inhibitory killer Ig-like receptors. *Eur J Immunol* 2003;33:1657-66. Erratum in: *Eur J Immunol* 2003;33: 2947.
28. Vitale M, Della Chiesa M, Carlomagno S, Pende D, Aricò M, Moretta L, et al. NK-dependent DC maturation is mediated by TNF- α and IFN- γ released upon engagement of the NKP30 triggering receptor. *Blood* 2005;106:566-71.
29. Loza MJ, Peters SP, Zangrilli JG, Perussia B. Distinction between IL-13⁺ and IFN- γ - α natural killer cells and regulation of their pool size by IL-4. *Eur J Immunol* 2002;32:413-23.
30. Moretta L, Bottino C, Pende D, Vitale M, Mingari MC, Moretta A. Different checkpoints in human NK-cell activation. *Trends Immunol* 2004;25:670-6.
31. Ferlazzo G, Tsang ML, Moretta L, Melioli G, Steinman RM, Münz C. Human dendritic cells activate resting natural killer (NK) cells and are recognized via the NKP30 receptor by activated NK cells. *J Exp Med* 2002;195:343-51.
32. Sivori S, Falco M, Della Chiesa M, Carlomagno S, Vitale M, Moretta L, et al. CpG and double-stranded RNA trigger human NK cells by Toll-like receptors: induction of cytokine release and cytotoxicity against tumors and dendritic cells. *Proc Natl Acad Sci U S A* 2004;101:10116-21.
33. Parronchi P, Macchia D, Piccinni MP, Biswas P, Simonelli C, Maggi E, et al. Allergen- and bacterial antigen-specific T-cell clones established from atopic donors show a different profile of cytokine production. *Proc Natl Acad Sci U S A* 1991;88: 4538-42.
34. Jenmalm MC, Van Snick J, Cormont F, Salzman B. Allergen-induced Th1 and Th2 cytokine secretion in relation to specific allergen sensitization and atopic symptoms in children. *Clin Exp Allergy* 2001;31:1528-35.
35. Stumbles PA, Thomas JA, Pimm CL, Lee PT, Venaille TJ, Proksch S, et al. Resting respiratory tract dendritic cells preferentially stimulate T helper cell type 2 (Th2) responses and require obligatory cytokine signals for induction of Th1 immunity. *J Exp Med* 1998;188:2019-31.
36. Loza MJ, Perussia B. The IL-12 signature: NK cell terminal CD56⁺ high stage and effector functions. *J Immunol* 2004;172:88-96.
37. Perussia B, Chen Y, Loza MJ. Peripheral NK cell phenotypes: multiple changing of faces of an adapting, developing cell. *Mol Immunol* 2005;42:385-95.
38. Marcenaro E, Della Chiesa M, Bellora F, Parolini S, Millo R, Moretta L, et al. IL-12 or IL-4 prime human NK cells to mediate functionally divergent interactions with dendritic cells or tumors. *J Immunol* 2005;173:3992-8.
39. Wei H, Zhang J, Xiao W, Feng J, Sun R, Tian Z. Involvement of human natural killer cells in asthma pathogenesis: natural killer 2 cells in type 2 cytokine predominance. *J Allergy Clin Immunol* 2005;115:841-7.
40. Warren HS, Kinnear BF, Phillips JH, Lanier LL. Production of IL-5 by human NK cells and regulation of IL-5 secretion by IL-4, IL-10, and IL-12. *J Immunol* 1995; 154:5144-52.
41. Peritt D, Robertson S, Gri G, Showe L, Aste-Amezaga M, Trinchieri G. Differentiation of human NK cells into NK1 and NK2 subsets. *J Immunol* 1998;161: 5821-4.

42. Deniz G, Akdis M, Aktas E, Blaser K, Akdis CA. Human NK1 and NK2 subsets determined by purification of IFN-gamma-secreting and IFN-gamma-nonsecreting NK cells. *Eur J Immunol* 2002;32:879-84.
43. Walker C, Checkel J, Cammisuli S, Leibson PJ, Gleich GJ. IL-5 production by NK cells contributes to eosinophil infiltration in a mouse model of allergic inflammation. *J Immunol* 1998;161:1962-9.
44. Aktas E, Akdis M, Bilgic S, Disch R, Falk CS, Blaser K, et al. Different natural killer (NK) receptor expression and immunoglobulin E (IgE) regulation by NK1 and NK2 cells. *Clin Exp Immunol* 2005;140:301-9.
45. Broide D, Schwarze J, Tighe H, Gifford T, Nguyen MD, Malek S, et al. Immunostimulatory DNA sequences inhibit IL-5, eosinophilic inflammation, and airway hyperresponsiveness in mice. *J Immunol* 1998;161:7054-62.