homes than in suburban homes. However, we were surprised to find that the rate of detectable cockroach allergen level was only 12.2% in urban residences, which is low when compared with that in other inner-city populations such as New Orleans (44%), with Baltimore, Boston, New York, and St Louis having detectable cockroach allergen levels of 40%. We speculate that this may reflect the predominance of single-family dwellings in our urban sample compared with high-density residential structures that are present in many large cities. However, like many inner cities, asthma morbidity and mortality is high among the urban population. On the basis of these data, we suggest that although cockroach exposure may partially explain high urban asthma morbidity, it seems unlikely that cockroach allergen exposure is the sole or even the major contributor to the high asthma burden in Detroit children as has been suggested from other studies and cities. Our data also emphasize the potential importance of accounting for regional variation in key environmental allergen exposures when designing optimal intervention strategies directed toward different US urban populations.

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Intact IL-12 signaling is necessary for the generation of human natural killer cells with enhanced effector function after restimulation

To the Editor:
Natural killer (NK) cells are lymphocytes that are important for host defense against viral and bacterial infections, as well as malignant transformation. They have the capacity to kill target cells and produce cytokines on activation without previous sensitization. Although NK cells have been traditionally categorized as members of the innate immune system, recent studies have shown that NK cells have the ability to modify their effector function based on previous cytokine stimulation, activating receptor–mediated stimulation, or both.1-3 The first evidence that NK cells can remember came from studies showing that they mediated recall responses to hapten in mice lacking T and B cells using a contact hypersensitivity model.4 NK cell memory has also been demonstrated in viral infections by using murine models.5 In these models NK cells acquired long-lived memory to influenza A, vesicular stomatitis virus, and HIV antigens.6 Additionally, development of long-lived NK cell memory to mouse cytomegalovirus infection was shown to be dependent on IL-12–mediated signals.7,6 Even without exposure to specific antigens, ex vivo short exposure of mouse and human NK cells to activating cytokines, such as IL-12, IL-15, and IL-18, elicits “memory-like” properties that are defined as enhanced effector functions after restimulation.6,7,8

In this study we tested the role of IL-12–mediated signals in the in vitro generation of human NK cells with enhanced effector function after restimulation. In our opinion the best method to address this issue would involve the use of PBMCs from patients with a deficiency in IL-12 or IL-12 receptor (IL-12R) because we could then exclude that the NK cells used in the experiments had been activated at any time in vivo by IL-12. We have previously described a 19-month-old patient with an IL-12Rβ1 deficiency caused by a complex mutation at exon 14 in the IL12Rβ1 gene (c.1623_1624delinsTT; p.Q541X).9 We showed that IFN-γ production was markedly decreased after stimulation of PBMCs with PHA or PHA plus IL-12 and that there was diminished signal transducer and activator of transcription 4 phosphorylation after IL-12 stimulation.9 Here we specifically tested whether the NK cells from this patient responded to IL-12 stimulation. Flow cytometric analyses showed this patient’s NK cells did not produce IFN-γ when they were stimulated with IL-12 plus IL-15 or IL-12 plus IL-18 (Fig 1). On the other hand, approximately 20% of the NK cells from the age-matched healthy control subject produced IFN-γ when they were stimulated with IL-12 plus IL-18 (Fig 1). When NK cells were stimulated with a combination of the 3 cytokines, almost all NK cells from the healthy control subject produced IFN-γ, whereas only 12% of the NK cells from the patient

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FIG 1. Decreased IFN-γ production by IL-12Rβ1-deficient NK cells in response to IL-12 in combination with IL-15, IL-18, or both. PBMCs were stimulated with different combinations of IL-12 (10 ng/mL), IL-15 (10 ng/mL), and IL-18 (50 ng/mL) for 16 hours or with phorbol 12-myristate 13-acetate (50 ng/mL) and ionomycin (1 μmol/L) for 6 hours. For the last 6 to 8 hours, monensin was added to the culture. Then cell-surface receptor expression and intracellular IFN-γ production were determined by using flow cytometry. Lymphocytes were electronically gated based on forward- and side-scatter parameters, and NK cells were identified by the CD3−CD56+ phenotype.

FIG 2. Absence of enhanced effector functions after restimulation by IL-12Rβ1–deficient NK cells preactivated with IL-12 plus IL-15 plus IL-18. A, PBMCs were cultured for 16 hours with either low concentrations of IL-15 (1 ng/mL; ie, nonpreactivated) or with IL-12 (10 ng/mL), IL-15 (10 ng/mL), and IL-18 (50 ng/mL) and ionomycin (1 μmol/L; ie, preactivated). Next, cells were washed with PBS and cultured in Iscove modified medium supplemented with 10% human AB serum plus 1 ng/mL IL-15. After 7 days, cells were harvested and incubated with 721.221 cells for 6 hours in the presence of anti-CD107a and anti-CD107b mAbs. The secretion inhibitor monensin was added for the last 5 hours. Then CD107a/b expression (left panel) and TNF-α production (right panel) by NK cells were determined by using flow cytometry. Lymphocytes were electronically gated based on forward- and side-scatter parameters, and NK cells were identified by CD3−CD56+ phenotype. B, PBMCs from the healthy control subject and the second patient were cultured as in Fig 2, A. After 7 days, cells were harvested and incubated with 721.221 cells or with IL-15 plus IL-18 for 16 hours. Monensin was added for the last 5 hours. Then the IFN-γ production by NK cells in response to restimulation with 721.221 cells (left panel) and IL-15 plus IL-18 (right panel) was measured by using flow cytometry.
expressed IFN-γ. Furthermore, on a per-cell basis, the NK cells from the patient produced less IFN-γ than the NK cells from the control subject, as shown by a median fluorescence intensity of 921 for the IFN-γ producing NK cells from the patient versus a median fluorescence intensity of 35,205 for the IFN-γ-producing NK cells from the healthy control subject. We excluded that the patient had a defect in the IFNG gene or its regulation because her NK cells and those from the control subject produced similar amounts of IFN-γ in response to phorbol 12-myristate 13-acetate plus ionomycin (Fig 1). As expected, these results corroborated that NK cells from an IL-12Rβ1–deficient patient do not respond to IL-12.

Next, we evaluated the role of IL-12–mediated signals in the generation of NK cells with enhanced effector functions after restimulation or “memory-like” NK cells, as reported,1,5 after preactivation with cytokines (Fig 2). We stimulated PBMCs with IL-12 plus IL-15 plus IL-18 for 16 hours, followed by washes and a 7-day rest period in vitro, with survival supported by low concentrations of IL-15. After the resting period, cells were harvested and coincubated with 721.221 target cells (EBV-transformed B cells). The expression of CD107 (CD107a and CD107b) as a surrogate for degranulation triggered by 721.221 tumor cells was measured using flow cytometry. Preactivated NK cells from the healthy control subject degranulated more than nonpreactivated NK cells after coincubation with 721.221 cells (15.4% vs 9.4%; Fig 2, A, left panel). However, preactivated and nonpreactivated NK cells from the patient showed similar degranulation after coincubation with 721.221 cells (4.3% vs 4%; Fig 2, A, left panel). These results suggest there is no significant increase in the degranulation capabilities after preactivation with cytokines in NK cells lacking IL-12–mediated signaling.

We also measured TNF-α production by NK cells after coincubation with 721.221 cells (Fig 2, A, right panel). Similar to the degranulation results, preactivated NK cells from the healthy control subject produced more TNF-α than nonpreactivated NK cells (0.51% vs 0.1%; Fig 2, A, right panel), whereas there were no differences in TNF-α production when preactivated and nonpreactivated NK cells from the patient were compared (0.02% vs 0.04%; Fig 2, A, right panel). A patient with a different homozygous null IL12RB1 mutation (c.1495C>T; p. Q499X) and impaired signal transducer and activator of transcription 4 phosphorylation on IL-12 stimulation (see Fig E1 in this article’s Online Repository at www.jacionline.org) was also tested to confirm that the observed results were related to the impaired IL-12 signaling rather than with a particular mutation. Similar to the results obtained with cells from the first patient, preactivated NK cells from the healthy control subject produced more IFN-γ than nonpreactivated NK cells after restimulation with 721.221 cells (20.4% vs 14.7%) and IL-15 plus IL-18 (54.1% vs 29.7%; Fig 2, B). However, preactivated and nonpreactivated NK cells from the second patient showed similar IFN-γ production after restimulation with 721.221 cells (1.29% vs 3.06%) and IL-15 plus IL-18 (3.60% vs 1.94%; Fig 2, B). These results indicate that IL-12RB1–deficient NK cells do not have the ability to acquire enhanced effector functions after restimulation. In addition to the defect in restimulation, the IL-12RB1–deficient NK cells also have a defect in primary activation. The deficiency in IFN-γ production after target and IL-15 plus IL-18 stimulation and in the absence of preactivation suggests that human NK cells require in vivo priming with IL-12 to acquire full functional reactivity and is consistent with a study by Guia et al.10 Our observations are partly in contradiction to those published by Romee et al.,8 who showed that after preactivation of human NK cells with IL-12 plus IL-15, restimulation with K562 target cells led to an increase in IFN-γ production without an increase in the cell-surface expression of CD107a on NK cells. Although we do not fully understand the reasons for this discrepancy, the use of different target cells and staining with both anti-CD107a and anti-CD107b mAbs in our experiments might explain the differences. In fact, our results are in accordance with those obtained with the mouse cytomegalovirus infection model, which demonstrated that degranulation by mouse memory NK cells was enhanced after stimulation with anti-NK1.1 mAb and that the generation of memory NK cells was dependent on IL-12–mediated signals.6

Here, using human IL-12Rβ1–deficient NK cells, we have demonstrated the essential role of IL-12–mediated signals in the generation of human NK cells with enhanced effector function after restimulation. These findings provide the rationale for including brief stimulations or preactivation with IL-12 in combination with other cytokines, such as IL-15 and IL-18, to generate NK cells with enhanced effector functions (ie, “memory-like”) that might be of great relevance in the field of NK cell–based immunotherapy and might also unveil a new aspect of pathophysiology to be considered in IL-12Rβ1–deficient patients.

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Seasonal increases in peripheral innate lymphoid type 2 cells are inhibited by subcutaneous grass pollen immunotherapy

To the Editor:

We report for the first time the effects of grass pollen subcutaneous immunotherapy (SCIT) on the novel subset of innate lymphoid type 2 cells (ILC2s) in patients with seasonal allergic rhinitis (SAR). Allergic rhinitis is an IgE-mediated chronic inflammation of the nasal mucosa, thought largely to be confined to the grass pollen season (July-August 2013) and outside (December 2013) the grass pollen season. Clinical symptoms were evaluated using self-reported visual analog scales. One-hundred microliters of peripheral blood was immunostained with fluorochrome-labeled mAbs for surface markers: allophycocyanine-lineage cocktail (CD3, CD14, CD16, CD19, CD20, CD56; clones: UCHT-1, HCD14, 3G8, HB19, 2H7, HCD56; BioLegend, London, United Kingdom), peridinin chlorophyll protein-Cy5.5 antihuman CD127 (clone: eBioDR5R5; eBioscience, Hatfield, United Kingdom), phycoerythrin-anti ST2 (clone: 97203; R&D), V450 antihuman CRTH2 (clone: BW16; BD Biosciences, Oxford, United Kingdom), and phycoerythrin-Cy7 antihuman CD117 (clone: 104D2; eBioscience). Erythrocytes were subsequently lysed, fixed, and permeabilized to allow intracellular staining with fluorescein isothiocyanate antihuman IL-13 (clone: PVW13-1; eBioscience). Lineage-negative lymphocytes that expressed prostaglandin D2 receptor (CRTH2) and IL-7 receptor (CD127) were considered ILC2s (Fig 1, A).

The proportion of LinCD127+CRTH2+ cells (ILC2s) in peripheral blood was comparable to that reported in a previous study (1%-20% of lineage negative lymphocytes) and they expressed the IL-33 receptor (ST2) (Fig 1, A and F). ILC2s were 58% higher in the SAR group during the pollen season than during out of season (P = .007). In contrast, there was no change in ILC2s over the same time period for SCIT and NA groups (Fig 1, C; see Tables E3 and E4 in this article’s Online Repository at www.jacionline.org). During the pollen season, the proportions of ILC2s were elevated in the SAR group (median [interquartile range], 14.8 [2.99]) compared with the SCIT group (9.34 [3.45]; P = .03) and NA (7.34 [4.86]; P < .001). Because CD117 (c-kit) has been reported as a surface marker for identifying a subtype of ILC2s, we enumerated the proportion of CD117+ ILC2s in our population. Similarly, CD117+ ILC2s were more prominent in the SAR group than in SCIT and NA groups (Fig 1, B and D and Tables E3 and E4). We report that peripheral blood ILC2s during the pollen season are elevated in patients with SAR than in NA patients (2-fold; P < .001). A possible explanation for these findings is that we immunostained ILC2s from whole blood rather than isolated peripheral blood mononuclear cells. Also, our observed elevation of ILC2s was detectable only during the pollen season. These findings highlight the effect of natural grass pollen allergen exposure in increasing ILC2 numbers in peripheral blood as has recently been observed following experimental nasal challenge with cat allergen in cat-allergic subjects. Although most subjects with SAR were not monosensitized, their symptoms were largely confined to the grass pollen season (P < .001) (Fig 1, G; see Table E2 in this article’s Online Repository at www.jacionline.org), with no perennial symptoms requiring more than occasional antihistamine use. SCIT-treated subjects reported markedly less seasonal symptoms than did untreated subjects with SAR (Fig 1, G, and Table E2). Moreover, in allergic individuals, there was a correlation between visual analog scales and the proportion of ILC2s during the pollen season (r = 0.52, P = .02) (Fig 1, H).

IL-13 has been recognized as the major cytokine produced by ILC2s and plays a major role in allergic inflammation. We therefore identified the proportion of ex-vivo IL-13+ ILC2s in peripheral blood. The seasonal increase in IL-13+ ILC2s was inhibited after SCIT treatment. IL-13+ ILC2s were almost 2-fold higher in the SAR group (3.06 [0.78]) than in both SCIT...
FIG E1. Genetic and phenotypic characterization of IL-12Rβ1 deficiency in the second patient. A, Coding exons and adjacent splicing sites within the IL12RB1 gene from the second patient were PCR amplified and sequenced with the Sanger technique. Results showed a mutation at exon 13, adding to a premature stop codon. The test result was IL12RB1 NM_005535 c.1495C>T, p.Q499X homozygous. B, IL-12Rβ1 cell-surface expression on PBMCs from 2 healthy donors and the second patient. Cells were stimulated with anti-CD3 and anti-CD28 (1 μg/mL) antibodies plus IL-2 (10 ng/mL) for 5 days. After that, cells were treated with IL-12 (100 ng/mL) for 30 minutes. Lymphocytes were electronically gated based on forward- and side-scatter parameters. Histograms in gray show the binding of isotype control antibody, and histograms in black show the binding of anti–IL-12Rβ1 antibody. PE, Phycoerythrin. C, PBMCs were cultured as in Fig E1, B. After that, cells were treated with IL-12 (100 ng/mL) for 30 minutes, and signal transducer and activator of transcription 4 phosphorylation (p-STAT4) was detected by means of flow cytometric analysis. Lymphocytes were electronically gated based on forward- and side-scatter parameters. Binding of anti-p-STAT4 antibody to unstimulated (black) and stimulated (red) cells is shown.