

Peripheral natural killer cells exhibit qualitative and quantitative changes in patients with psoriasis and atopic dermatitis

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Summary

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Conflicts of interest

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Background Psoriasis and atopic dermatitis are the most recurrent skin inflammatory disorders. Despite their distinct aetiology and clinical aspects, these diseases share several immunological features. Besides the largely documented role of T cells, emerging literature supports a potential involvement of innate immune effectors, the natural killer (NK) cells, in both pathologies. In the peripheral blood, NK cells consist of CD3⁺ CD56dim and CD3⁺ CD56bright cell subsets, harbouring a distinct cell surface phenotype, but both endowed with the main NK-cell effector functions: cytotoxicity and cytokine production.

Objectives To determine whether the frequency, the cell surface phenotype and the functional properties of peripheral NK cells were affected in patients with psoriasis or atopic dermatitis.

Methods Peripheral blood mononuclear cells were isolated from 11 patients with psoriasis, nine patients with atopic dermatitis and 16 healthy individuals. By using flow cytometry, we analysed the following parameters of peripheral NK cells: the frequency, the cell surface expression of several NK-cell receptors (NKR) and the activation of the effector functions upon various *in vitro* stimuli.

Results Peripheral NK cells were significantly reduced in both skin diseases. The cell surface expression of various NKR was differently modified in peripheral NK cells of the two cohorts of patients. Finally, NK-cell natural cytotoxicity was affected only in atopic dermatitis, while interferon- γ production was defective in both groups of patients.

Conclusion Psoriasis and atopic dermatitis are associated with quantitative and qualitative changes of peripheral NK cells, mostly shared by both diseases, supporting a common process implicating these innate effectors in skin inflammation.

Psoriasis and atopic dermatitis are two of the most common skin inflammatory disorders.¹ Psoriasis is a genetically determined disease characterized by hyperproliferation and aberrant differentiation of keratinocytes and by inflammatory infiltrates. Psoriasis vulgaris is characterized by erythematous scaly

plaques usually located on the elbows, knees and scalp. Psoriatic arthritis occurs in 5–20% of patients and usually develops several years after the cutaneous manifestations.² Atopic dermatitis is a chronic and relapsing inflammatory disease characterized by pruritic skin lesions usually starting in early

childhood. It depends on gene–gene and gene–environment interactions leading to a disrupted epidermal barrier function, immunodysregulation and, very often, IgE-mediated sensitization to food and environmental allergens.³ Although psoriasis and atopic dermatitis display quite different aetiology and clinical features, the role of the adaptive immune system, especially of T lymphocytes, in the initiation and progression of both diseases was clearly demonstrated.¹ The contribution of effectors of the innate immune system, such as natural killer (NK) cells, is supported by an emerging literature, although data are controversial and thus need further investigation.⁴

Natural killer cells are the third major lymphocyte compartment after T and B cells and exhibit specific phenotypic and functional features. Phenotypically, in humans, NK cells are generally identified as CD3– CD56+ cells. CD3– CD56+ NK cells are highly heterogeneous, as, along their differentiation process, they sequentially acquire cell surface expression of various activating and inhibitory NK-cell receptors (NKR).^{5,6} Moreover, several NKR, including CD56, can also be expressed on rare T-cell subsets.⁷ Nkp46, an activating natural cytotoxicity receptor (NCR), is expressed on NK cells, is conserved in various mammalian species and represents to date the most specific NK-cell marker.⁸ Nkp46 is also found on rare T-cell subsets⁹ and on innate lymphoid cell subsets of the gut and the tonsils.^{10–14} Functionally, NK cells are endowed with two main effector functions: cytotoxicity and cytokine production.⁶ Cytotoxicity relies on the release of perforin and granzyme B contained in NK-cell cytoplasmic granules. Natural cytotoxicity is based on the unique ability of NK cells to recognize and eliminate, without any prior sensitization, targets such as tumours or virally infected cells. NK cells can also kill antibody-coated targets via CD16, the low-affinity receptor for the Fc fragment of immunoglobulins. NK cells also secrete various cytokines and chemokines,¹⁵ which enable NK cells to cross-talk with effectors of both innate and adaptive immunity, thus participating in the regulation of innate and adaptive immune responses.

Natural killer cells are present in the whole body, but they are quite rare in healthy skin.^{10,16} Studies in patients with psoriasis or atopic dermatitis have shown that the frequency of cells expressing NKR, such as CD56, is significantly increased in lesional skin,^{17,18} but rather decreased in the peripheral blood.^{19,20} Injection of circulating NKR+ cells isolated from psoriatic donors into nonlesional skin grafted in immunodeficient mice was sufficient to induce psoriasis-like lesions.²¹ NKR+ cells recruited from the periphery to the lesional skin could contribute to skin disease, although the identity of these NKR+ cells ('bona fide' CD3– CD56+ Nkp46+ NK cells or CD3+ CD56+ NKR+ T cells) is yet to be determined in some studies.

In this study, we investigated the frequency, the cell surface phenotype and the functional properties of 'bona fide' CD3– CD56+ Nkp46+ NK cells in the peripheral blood of cohorts of patients with psoriasis or atopic dermatitis, high-

lighting quantitative and qualitative changes of these innate effectors related to the two skin disorders.

Materials and methods

Subjects

Eleven patients with psoriasis and nine patients with atopic dermatitis were enrolled (Table 1). The peripheral blood of the patients with psoriasis was collected according to AORC no. 2008-A00064-51 research project (Assistance Publique-Hôpitaux de Marseille, Marseille, France). The peripheral blood of patients with atopic dermatitis was kindly provided by the biobank 'Allergobiotech', Hospices Civils de Lyon, Lyon, France. Sixteen healthy volunteers were enrolled as controls and included seven men and nine women, mean age 39 years (range 28–56). All subjects were aged > 18 years. Informed consent was obtained from all subjects before entry into the study. All the studies were performed with agreement of the local ethical committees.

Table 1 Characteristics of the patients with psoriasis (PS) and atopic dermatitis (AD), including sex, age, pathology and eventual pharmacological treatment when enrolled in the study

Patient	Sex	Age (years)	Disease	Drug treatment
PS6	F	65	PS vulgaris and arthritis	NT
PS9	F	90	PS vulgaris	NT
PS10	M	48	PS vulgaris and arthritis	NT
PS12	M	38	PS vulgaris and arthritis	Remicade ^a
PS13	M	55	PS vulgaris and arthritis	Methotrexate
PS14	M	61	PS vulgaris	Methotrexate
PS15	F	60	PS vulgaris and arthritis	Remicade ^a
PS16	M	49	PS vulgaris	NT
PS17	M	29	PS vulgaris and arthritis	Acitretin ^b
PS26	M	62	PS vulgaris	NT
PS28	F	46	PS vulgaris	NT
AD5	F	48	AD	Local treatment
AD6	F	45	AD	Local treatment
AD7	M	39	AD	Local treatment
AD8	F	26	AD	Local treatment
AD9	M	37	AD	Local treatment
AD15	M	33	AD	Local treatment
AD16	M	19	AD	Local treatment
AD17	F	44	AD	Local treatment
AD18	F	38	AD	Local treatment

Local treatment, topical treatment of skin lesions with tacrolimus or dermatocorticoids; NT, no pharmacological treatment at the moment of the inclusion in the study. ^aRemicade® (Schering Plough, Levallois-Perret, France). ^bAcitretin® (Stiefel Laboratories, Offenbach am Main, Germany).

Peripheral blood mononuclear cells

Peripheral blood mononuclear cells (PBMCs) were isolated from heparinized venous blood by density gradient centrifugation on Ficoll-Paque (Pharmacia, Uppsala, Sweden), then frozen in a fetal calf serum (FCS) containing 10% dimethyl sulphoxide and kept in liquid nitrogen. The day before the experiment, cells were thawed in prewarmed RPMI supplemented with 10% FCS and 150 IU DNase I (Roche, Basel, Switzerland), then kept overnight at 37 °C in RPMI supplemented with 10% FCS.

Phenotypic analysis

Peripheral blood mononuclear cells (5×10^5 cells) were incubated with 2.5% normal mouse serum, then stained with the following monoclonal antibodies (mAbs): PerCP-Cy5.5-conjugated anti-CD3 (BD Biosciences, Heidelberg, Germany), PE-Cy7-conjugated anti-CD56 (Beckman Coulter, Fullerton, CA, U.S.A.), allophycocyanin (APC)-conjugated anti-NKp46 (BD Biosciences), fluorescein-isothiocyanate (FITC)-conjugated anti-CD94 or anti-CD16 (both from BD Biosciences) and phycoerythrin (PE)-conjugated anti-CD158a,h, anti-CD158b,j and anti-CD158e1,e2 [mix for killer-cell immunoglobulin-like receptor (KIR) staining] or anti-NKp44 or anti-NKp30 (all from Beckman Coulter). FITC-conjugated mIgG1 and IgG2a and PE-conjugated mIgG1 and IgG2a isotype-matched controls were all from Beckman Coulter. After 30 min of incubation at 4 °C, samples were washed once and fixed for 10 min in PBS 1× paraformaldehyde (PFA) 2%. Samples were then washed in PBS 1×, resuspended in PBS 1× bovine serum albumin (BSA) 0.5% and run to the FACS Canto II (BD Biosciences). Data obtained were then analysed by using Diva (BD Biosciences, Germany) or FlowJo software (Tree Star Inc., Ashland, OR, U.S.A.).

Functional analysis

Peripheral blood mononuclear cells (5×10^5 cells) were incubated for 4 h at 37 °C in the presence of Golgi Stop (BD Biosciences); FITC-conjugated anti-CD107a and anti-CD107b antibodies (5 µL of each; BD Biosciences); and medium; human erythroleukaemia K562 cell line (2×10^5 cells); mouse mastocytoma P815 (2×10^5 cells) preincubated with anti-P815 mAbs (50 µg mL⁻¹; Biovalley, Illkirch, France); phorbol 12-myristate 13-acetate (PMA) (2 ng mL⁻¹) and ionomycin (500 ng mL⁻¹). At the end of incubation, cells were harvested and stained with PerCP-Cy5.5-conjugated anti-CD3 (BD Biosciences) and APC-conjugated anti-CD56 (Beckman Coulter) in PBS 1× BSA 0.5% for 30 min. Cells were then washed, fixed for 10 min in PBS 1× PFA 2%, then washed in Perm/Wash Buffer 1× (BD Biosciences). Cells were then incubated in Perm/Wash Buffer 1× with PE-conjugated anti-interferon (IFN)-γ mAb (BD Biosciences), then washed and resuspended in PBS 1× BSA 0.5%. Samples were run to the FACS Canto II (BD Biosciences). Data obtained were then analysed by using Diva or FlowJo software.

Statistical analysis

The unpaired Student t-test was used to compare data obtained from the different cohorts of subjects (*P = < 0.05, **P = < 0.005, ***P = < 0.0001).

Results

CD3[−] CD56⁺, CD3⁺ CD56⁺ and CD3⁺ CD56[−] cell distribution in the peripheral blood of patients with psoriasis and atopic dermatitis

Peripheral CD3[−] CD56⁺ NK cells enclose CD3[−] CD56^{bright} and CD3[−] CD56^{dim} cell subsets. CD3[−] CD56^{bright} NK cells are rare in the blood, but numerous in secondary lymphoid organs, while CD3[−] CD56^{dim} NK cells are predominant in the blood⁵ (Fig. 1a). As expected, CD56 was also found on a fraction of T cells⁷ (Fig. 1a). We analysed the distribution of CD3[−] CD56^{bright} and CD3[−] CD56^{dim} NK-cell subsets, with respect to CD3⁺ CD56⁺ T cells and CD3⁺ CD56[−] conventional T cells, in the peripheral blood of healthy individuals and of patients with psoriasis and atopic dermatitis. The percentages of both CD3[−] CD56^{bright} and CD3[−] CD56^{dim} NK-cell subsets were significantly reduced in patients with psoriasis, while only the CD3[−] CD56^{dim} NK-cell subset was affected in patients with atopic dermatitis (Fig. 1b,c). Regarding T cells, CD3⁺ CD56⁺ T cells were not modified in either of these two skin pathologies, while conventional T-cell percentages were reduced in patients with atopic dermatitis (Fig. 1d,e). In contrast to T cells, CD3[−] CD56^{bright} and CD3[−] CD56^{dim} NK-cell subsets homogeneously expressed the NKp46 marker, thus confirming their 'bona fide' NK-cell identity (Fig. 2).

In conclusion, the reduction of peripheral NKR⁺ cells in patients with psoriasis and atopic dermatitis mainly concerns CD3[−] CD56⁺ NKp46⁺ NK cells. Moreover, the two peripheral NK-cell subsets are differentially affected in the two cohorts of patients.

Phenotypic analysis of peripheral natural killer cell subsets in patients with psoriasis and atopic dermatitis

CD3[−] CD56^{bright} and CD3[−] CD56^{dim} NK-cell subsets exhibit distinct subset-specific cell surface phenotypes.⁵ Besides NKp46, human NK cells express other NCR, such as NKp30 and NKp44. Like NKp46, NKp30 is homogeneously expressed by both NK-cell subsets, while NKp44 is detectable exclusively on a fraction of CD3[−] CD56^{bright} cells (Fig. 3a). During the last stages of NK-cell maturation, NK cells acquire the expression of other NKR, such as the CD94/NKG2 heterodimers, KIR molecules and CD16. CD94 is present on almost all CD3[−] CD56^{bright} NK cells, but only on about half of CD3[−] CD56^{dim} NK cells (Fig. 3a). In contrast, CD16 and KIR are mostly present on CD3[−] CD56^{dim} NK cells, but found only on rare fractions of CD3[−] CD56^{bright} NK cells (Fig. 3a). We investigated whether these subset-specific phenotypes were affected in peripheral NK-cell subsets of patients with

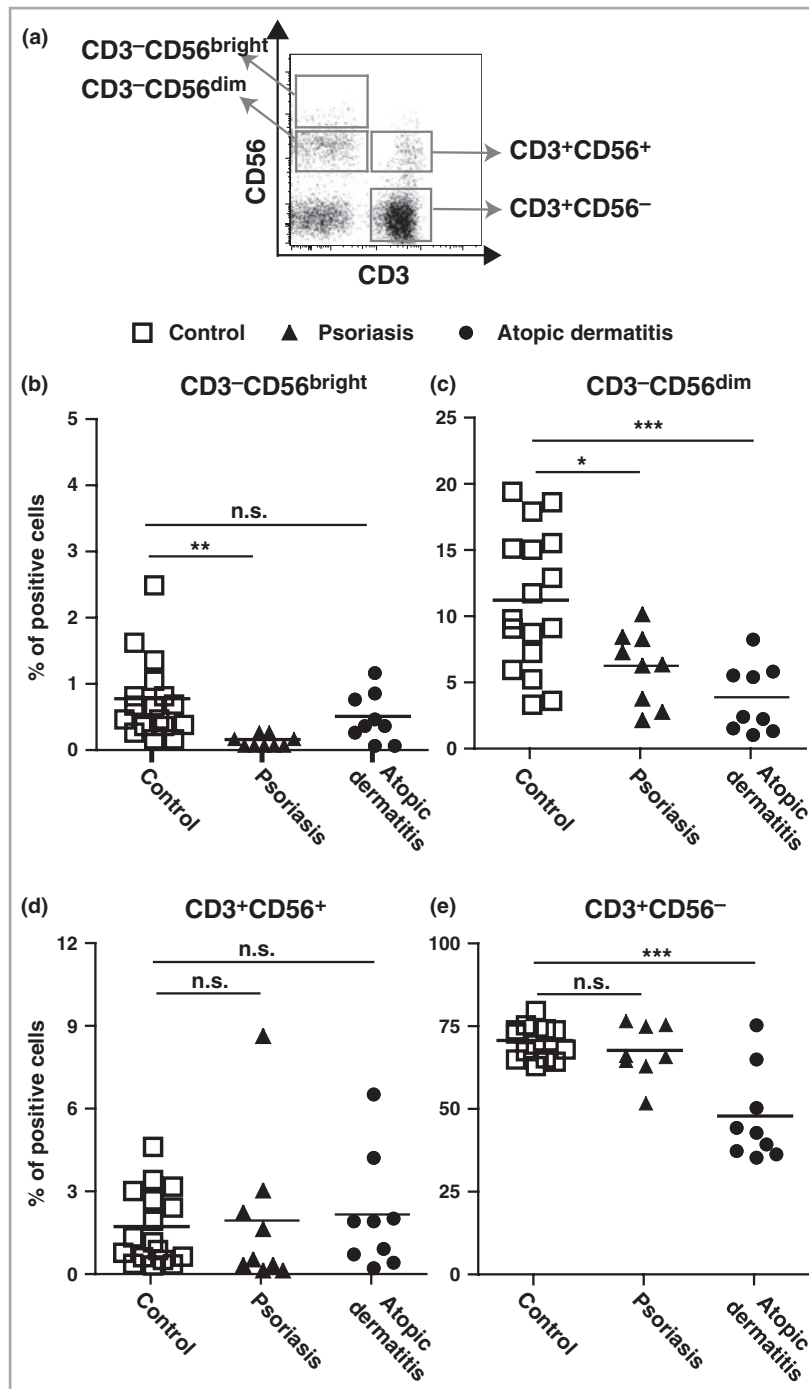


Fig 1. CD3⁻ CD56⁺, CD3⁺ CD56⁺ and CD3⁺ CD56⁻ cell distribution in the peripheral blood of patients suffering from psoriasis and atopic dermatitis. (a) Flow cytometry analysis of CD56 and CD3 expression on peripheral blood mononuclear cells (PBMCs) isolated from a healthy control, performed by gating on lymphocytes. The various CD3/CD56 subsets are indicated by grey boxes. One representative experiment of at least 10. (b–e) Percentages of CD3⁻ CD56^{bright} (b), CD3⁻ CD56^{dim} (c) NK cells, and of CD3⁺ CD56⁺ (d) and CD3⁺ CD56⁻ (e) T cells within lymphocytes of PBMCs of control healthy individuals (empty squares), patients with psoriasis (filled triangles) and patients with atopic dermatitis (filled circles). Controls, $n = 16$; patients with psoriasis, $n = 9$; patients with atopic dermatitis, $n = 9$. * $P < 0.05$; ** $P < 0.005$; *** $P < 0.0001$; n.s., not significant.

psoriasis and atopic dermatitis. The distribution of NKp30 and NKp44 markers was not significantly affected in either of the two NK-cell subsets of the two cohorts of patients (data not shown). CD94⁺ cell percentages were significantly reduced in CD3⁻ CD56^{bright} NK cells of patients with psoriasis and in CD3⁻ CD56^{dim} NK cells of patients with atopic dermatitis (Fig. 3b, upper panel). KIR⁺ cell percentages were significantly increased in CD3⁻ CD56^{bright} NK cells of patients with psoriasis, but decreased in CD3⁻ CD56^{dim} NK cells of patients with atopic dermatitis (Fig. 3b, middle panel).

Finally, CD16⁺ cell percentages were found reduced in CD3⁻ CD56^{bright} NK cells of patients with atopic dermatitis and in CD3⁻ CD56^{dim} NK cells of patients with psoriasis (Fig. 3b, lower panel).

NK-cell subset-specific cell surface phenotypes are thus differently modified in peripheral NK cells of patients with psoriasis and atopic dermatitis, with major consequences for CD94 and KIR in CD3⁻ CD56^{dim} NK cells of patients with atopic dermatitis and for CD16 in CD3⁻ CD56^{dim} NK cells of patients with psoriasis.

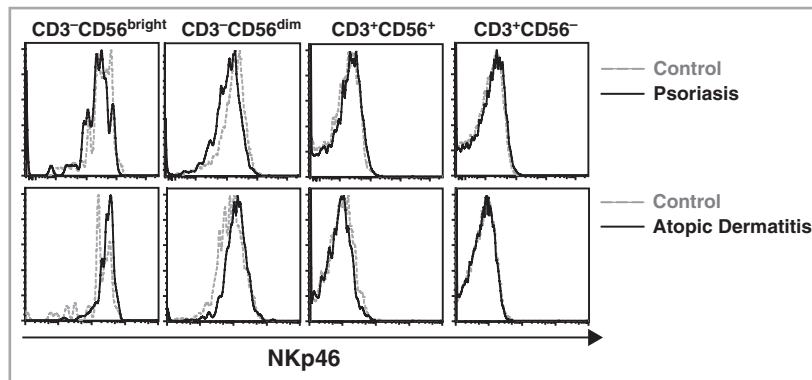


Fig 2. Natural killer (NK)p46 expression on CD3⁻ CD56⁺, CD3⁺ CD56⁺ and CD3⁺ CD56⁻ cells in the peripheral blood of patients with psoriasis and atopic dermatitis. Flow cytometry analysis of NKp46 expression on indicated cell subsets of peripheral blood mononuclear cells (PBMCs) isolated from healthy control or indicated patients. Cell surface expression on one representative control out of three (long dashed grey line) vs. one representative patient out of five (black filled line, patients with psoriasis, upper panels; patients with atopic dermatitis, lower panels) is depicted.

Functional analysis of peripheral natural killer cells in patients with psoriasis and atopic dermatitis

We determined whether peripheral NK cells in patients with psoriasis and atopic dermatitis were fully functional in terms of cytotoxicity and of production of the IFN- γ cytokine. CD107 cell surface expression is a read-out of NK-cell degranulation, an early key process of NK-cell cytotoxicity.²² By combining cell surface anti-CD107 and intracellular anti-IFN- γ stainings, we compared in the same flow cytometry-based assay the cytotoxicity and the IFN- γ production of peripheral NK cells of controls and of patients with psoriasis and atopic dermatitis. At steady state, CD107⁺ and IFN- γ ⁺ NK-cell percentages were comparable between controls and patients (Fig. 4a, medium). Natural cytotoxicity was evaluated upon coculture with the human NK cell-sensitive tumour cell line K562. In this case, CD107⁺ NK-cell percentages were significantly reduced in patients with atopic dermatitis, compared with controls and patients with psoriasis (Fig. 4a, K562). Antibody-dependent cellular cytotoxicity (ADCC) was measured upon coculture with the mouse NK cell-resistant tumour cell line P815 previously coated with anti-P815 antibodies (ADCC). CD107⁺ NK-cell percentages in ADCC were comparable between controls and patients (Fig. 4a, ADCC). IFN- γ production induced upon natural cytotoxicity or ADCC conditions was not significantly affected in patients with respect to controls (Fig. 4b, K562 and ADCC). Finally, we stimulated PBMC with a combination of PMA and ionomycin (P/I), a chemical stimulation that strongly activates both NK-cell effector functions bypassing the triggering of NK-cell surface receptors. In this case, the IFN- γ production, but not cytotoxicity, was severely affected in NK cells of both cohorts of patients, compared with controls (Fig. 4a,b).

In conclusion, the natural cytotoxicity of circulating NK cells is selectively affected in atopic dermatitis, while NK cells of both groups of patients exhibit profound defects in IFN- γ production when induced by P/I stimulation.

Discussion

In this study, we reported quantitative and qualitative changes of the two main circulating NK-cell subsets in patients with psoriasis and atopic dermatitis. Interestingly, peripheral NK-cell frequency and IFN- γ production were reduced in both cohorts of patients, while NKR expression and natural cytotoxicity were differently affected in the two skin disorders studied. Our results thus emphasize that there are important alterations in circulating NK cells in both psoriasis and atopic dermatitis, despite their different clinical features.

The decrease of peripheral NK cells reported here is consistent with previous reports,^{19,20} but extend these earlier findings by clearly demonstrating that the circulating NKR⁺ cells affected are mainly 'bona fide' CD3⁻ CD56⁺ NKp46⁺ NK cells belonging to both CD3⁻ CD56^{bright} and CD3⁻ CD56^{dim} NK-cell subsets. This reduction could be due to chemokine-dependent NK-cell recruitment from the periphery to the inflamed skin. Circulating NK cells express chemokine receptors, such as CCR2, CCR5, CXCR3 and CX3CR1.²³ Consistent with the reduction of peripheral CD94⁺ CD3⁻ CD56^{bright} NK cells reported here, an accumulation of CXCR3⁺ CD94/NKG2A⁺ CD3⁻ CD56^{bright} NK cells, probably due to CXCL10 production by psoriatic keratinocytes, was detected in psoriatic skin lesions.¹⁷ A potential CCL2-dependent recruitment of peripheral NK cells to lesional atopic dermatitis skin was also reported.¹⁸ It would be crucial to investigate in atopic dermatitis the cell surface expression of CD94, KIR and CD16 NKR on skin-infiltrating NK cells.

Alternatively, the reduction of NK-cell frequency in patients with psoriasis and atopic dermatitis could be due to an impaired cell survival, consequent to chronic inflammatory conditions or to pharmacological treatments. NK-cell changes reported here were independent of drug treatment of patients when enrolled in the study, thus excluding the latter hypothesis. In patients with new-onset psoriasis, NK-cell frequencies have been found normal, although NK cells exhibited an

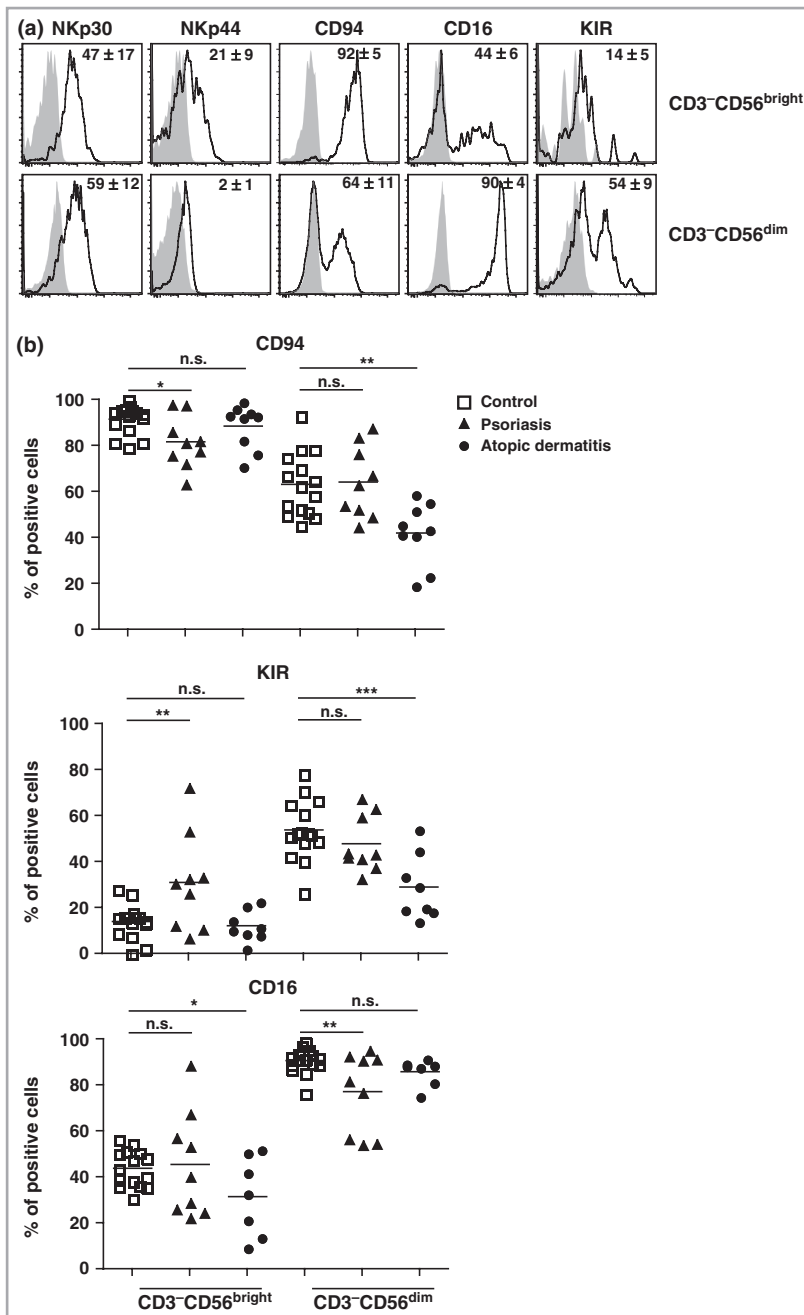


Fig 3. Phenotypic analysis of natural killer (NK)-cell subsets in the peripheral blood of patients with psoriasis and atopic dermatitis. (a) Flow cytometry analysis of the expression of indicated NK-cell markers in CD3- CD56^{bright} and CD3- CD56^{dim} NK-cell subsets of peripheral blood mononuclear cells (PBMCs) isolated from a healthy control. Specific staining is indicated by bold black line, while isotype-matched control staining is depicted by a filled grey line. Results are representative of at least 16 independent experiments performed by using different healthy donors. Percentages (mean ± SEM) of positive cells are indicated. (b) Percentages of positive cells for indicated NK-cell markers in CD3- CD56^{bright} and CD3- CD56^{dim} NK-cell subsets of PBMCs of indicated cohorts of controls or of patients. Controls, n = 16; patients with psoriasis, n = 9; patients with atopic dermatitis, n = 9. *P < 0.05; **P < 0.005; ***P < 0.0001; n.s., not significant.

upregulation of CD95/Fas and a downregulation of CD94 expression.²⁴ The decrease of peripheral NK cells would be a late event appearing during the chronic phase of disease progression. Fas upregulation on NK cells of patients with psoriasis²⁴ could render them more prone to apoptosis. In patients with atopic dermatitis, NK cells display a preferential apoptosis via a monocyte-dependent mechanism upon P/I stimulation.²⁰ Whether other stimuli can induce *in vitro* and *in vivo* a preferential apoptosis of NK cells in patients with psoriasis and atopic dermatitis remains to be investigated.

The expression of NKR, such as CD94, KIR and CD16, was altered in NK cells of patients with atopic dermatitis and

psoriasis. For CD94, our findings are consistent with those from a previous report in psoriasis²⁴ and extend them to patients with atopic dermatitis. Variations in NKR+ cell percentages within peripheral NK-cell subsets could be due to the migration of specific NK-cell fractions to inflamed sites or rather to the downregulation of NKR cell surface expression upon inflammation. To discriminate between these two hypotheses, a monitoring of NKR expression on both circulating and skin-infiltrating NK cells along disease progression would be highly informative.

Natural killer-cell effector functions were differently affected in patients with psoriasis and atopic dermatitis. Cytotoxicity

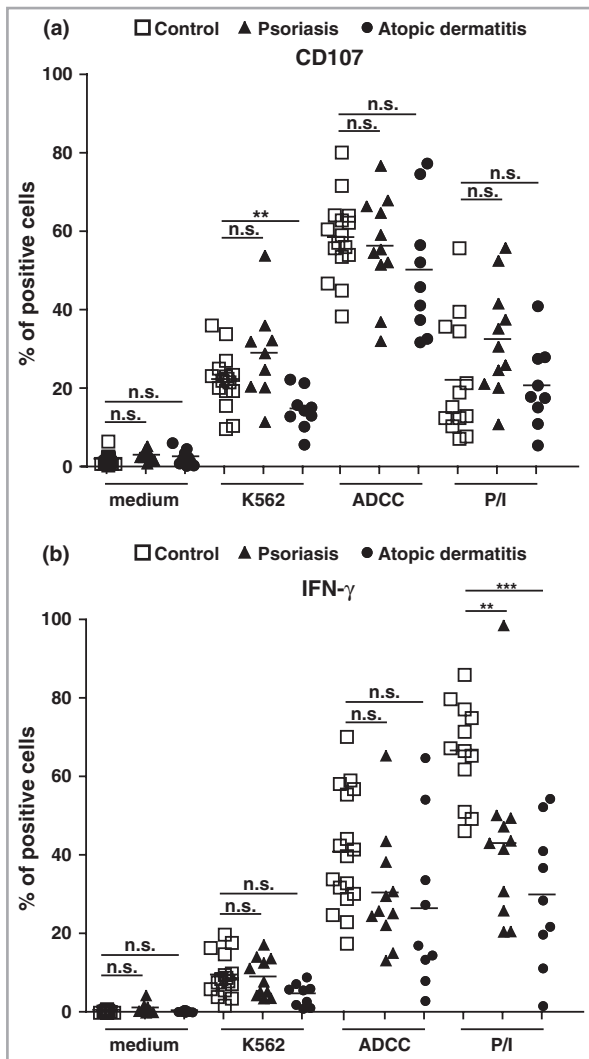


Fig 4. Functional analysis of natural killer (NK) cells in the peripheral blood of patients with psoriasis and atopic dermatitis. (a, b) Percentages of CD107+ (CD107a+ and CD107b+) cells (a) or of interferon (IFN)- γ + (b) cells in CD3-CD56+ NK cells of peripheral blood mononuclear cells (PBMCs) isolated from indicated cohorts of controls or of patients. Gating was performed on CD3-CD56+ NK cells within lymphocytes. PBMC were stimulated with medium; K562 tumour cell line (K562); P815 tumour cell line and antibodies anti-P815 (ADCC); phorbol 12-myristate 13-acetate (2 ng mL⁻¹) and ionomycin (500 ng mL⁻¹) (P/I). The effector/target (E/T) ratio for incubation with tumour cells was 500 000 PBMC/200 000 tumour cells, E/T = 2.5/1. Controls, n = 11–16; patients with psoriasis n = 9–11; patients with atopic dermatitis, n = 9. *P < 0.05; **P < 0.005; ***P < 0.0001; n.s., not significant.

was unaffected in patients with psoriasis, while NK cells of patients with atopic dermatitis showed a defect in natural cytotoxicity. In contrast, NK cells of both cohorts of patients were defective in IFN- γ production on P/I stimulation. In patients with atopic dermatitis, this defect has been related to an enhanced NK-cell apoptosis,²⁰ but this aspect has not yet been investigated in psoriasis. Unlike circulating NK cells, NK

cells isolated from skin psoriatic lesions produce *ex vivo* high amounts of IFN- γ and tumour necrosis factor- α proinflammatory cytokines.¹⁷ An open question is whether NK cells exert a disease-promoting or disease-regulatory role in skin diseases, as previously reported for other autoimmune and inflammatory diseases.⁶ Through IFN- γ secretion NK cells can promote type-1 polarization of T cells,²⁵ which in turn massively infiltrate psoriatic skin lesions²⁶ and contribute to the chronic phase of atopic dermatitis.⁴ Limiting NK-cell numbers and function could regulate Th1 polarization and dampen inflammation. However, in atopic dermatitis, defective NK cell-dependent Th1 polarization has been postulated to promote Th2 polarization during initial acute phase and to increase susceptibility to viral infections.²⁰ NK cells can modulate immune responses through different mechanisms, such as the cross-talk with dendritic cells²⁷ or the secretion of anti-inflammatory cytokines, such as interleukin-10.^{28,29} Moreover, Fas/CD95high NK cells are present in the blood of patients with multiple sclerosis during remission phase and contribute to the suppression of IFN- γ production by autoimmune CD4+ T lymphocytes.³⁰ Whether in psoriasis circulating Fas/CD95high NK cells²⁴ can exert a regulatory role remains to be determined.

In conclusion, our findings demonstrate that two distinct skin diseases, such as psoriasis and atopic dermatitis, although clinically quite different, associate with similar quantitative and qualitative changes of circulating NK cells. We believe that the experimental settings reported here will be crucial for monitoring NK-cell frequency, phenotype and effector functions in both blood and skin in future longitudinal studies and will contribute to an improvement in the knowledge regarding the role of NK cells in various skin inflammatory diseases.

What's already known about this topic?

- The frequency of cells expressing natural killer (NK) cell receptors (NKR) is decreased in the peripheral blood of patients with psoriasis or atopic dermatitis.
- The expression of the NKR CD94 is downregulated in patients with psoriasis.
- Cytokine production is affected in peripheral NK cells of patients with atopic dermatitis.

What does this study add?

- In both psoriasis and atopic dermatitis peripheral NKR+ cells are decreased and they are mainly *bona fide* NK cells.
- Phenotypic analysis shows that the expression of various NKR on the two main peripheral NK-cell subsets is differently modified in the two skin diseases.
- Natural cytotoxicity was found to be affected only in patients with atopic dermatitis, while interferon- γ production was defective in the NK cells of both groups of patients.

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