NKp46-Specific Expression on Skin-Resident CD4⁺ Lymphocytes in Mycosis Fungoides and Sézary Syndrome

TO THE EDITOR

Sézary syndrome (SS), mycosis fungoides (MF), and transformed mycosis fungoides (TMF; Olsen et al., 2007) belong to the heterogeneous group of cutaneous T-cell lymphoma (CTCL). They are characterized as extranodal non-Hodgkin lymphoma from malignant, mature T lymphocytes that home to the skin and persist there (Girardi et al., 2004). SS and MF are the most common CTCL types. Recent genetic and phenotypic studies suggest that MF and SS, previously considered as different stages of the same disease, arise from two distinct T-cell subsets (van Doorn et al., 2009; Campbell et al., 2010). Pathological diagnosis is often hampered by the small proportion of malignant cells and by the morphological similarity to inflammatory skin diseases (ISDs). Thus, a reliable cutaneous biomarker able to distinguish malignant cells from reactive benign T cells in the infiltrate of CTCL is still needed. Most previous studies have focused on blood malignant T lymphocytes in patients with leukemic disease (Vonderheid et al., 2001; Ferenczi et al., 2002; Su et al., 2003; Bensussan et al., 2011). When skin samples were considered, a specific expression of EphA4, Twist, TOX, PDCD1, or CDK158k/KIR3DL2 gene was found in MF or SS patients.

NKp46 has been shown on blood malignant CD4⁺ T lymphocytes in SS but not in MF or in TMF (Bensussan et al., 2011). NKp46 belongs to natural...
Figure 1. Transcriptional expression of NKp46 in epidermotropic T-cell lymphoma skin. (a) CD4, CD8, and CD56 immunostainings in skin biopsies from CTCLs and ISDs. SS/MF/TMF pictures show massive dermal infiltration of CD4+ cells with epidermotropism, sparse CD8+ cells, and no CD 56+ cells. Bar = 300 μm. Histograms represent CD4+, CD8+, and CD56+ cell counts in each CTCL and ISD groups. Bars represent mean ± SD. (b) qRT-PCR of NKp46 in CTCLs, CBCLs, ISDs, and normal skin samples. Reference gene used for normalization was transcription factor IID/TATA-binding protein (TBP). The 2−ΔΔCt method was used for mRNA quantification. mRNA from a Sézary patient expressing NKp46 gene was used as the calibrator. Middle bar represents the mean of the group. All data points represent the average of triplicate tests. NKp46 overexpression in CTCL, but not in ISDs, was also found when NKp46 expression was correlated to CD4 expression. CBCL, cutaneous B-cell lymphoma; CTCL, cutaneous T-cell lymphoma; ISD, inflammatory skin disease; MF, mycosis fungoides; qRT-PCR, quantitative real-time PCR; SS, Sézary syndrome; TMF, transformed mycosis fungoides.
cytotoxicity receptors (NCRs), which induce natural killer (NK)-cell activation upon binding to non-major histocompatibility complex ligands. Expressed on NK cells, regardless of their state of activation, NKp46 is directly involved in target-cell recognition and killing (Sivori et al., 1997).

We analyzed here NKp46 gene expression in skin-resident CD4⁺ T lymphocytes from epidermotropic CTCL, cutaneous diffuse large B-cell lymphoma, leg-type (CBCL), and ISDs. The Institutional Ethics Committee approved this study. Written informed consent was provided according to the Declaration of Helsinki Principles. According to the French Law of August 2004–800 revised in 2011–814, each patient was informed that the part of their sample remaining after the diagnosis had been established could be used for this research and that they could oppose to this. No patients opposed to the research use of their remaining sample.

Included in this study were 15 CTCLs: five MF, at stage IA with only patches and plaques; five TMF, at stage IIA (n = 2) and IIB (n = 3); and five SS: at stage IVa with less than 1 year evolution (male/female = 10/5; median age 63 years, range 35–81). Controls were 10 ISDs (five eczema, four psoriasis, one drug eruption; male/female = 6/4; median age 57 years, range 31–85), five CBCLs (male/female = 3/2; median age 74 years, range 71–83), and five normal skin samples (male/female = 3/2; median age 53 years, range 43–56).

All biopsies were performed before any treatment, because steroids or methotrexate impairs NK-cell phenotype including NKp46 expression, or they suppress NK-cell proliferation (Chiossone et al., 2007; Dauguet et al., 2010). Diagnoses were reviewed

![Figure 2](image-url)

**Figure 2.** Transcriptional expression of NKp46 in cutaneous CD4⁺ laser-microdissected cells in epidermotropic T cell lymphomas. (a) PCR products of laser-microdissected cells on agarose gel electrophoresis. CD4 and NKp46 mRNA are detected in SS, MF, and TMF, with a bright distinctive band at 128 and 86 bp, respectively, but CD8 mRNA and CD56 mRNA are not detected. In ISDs and CBCLs, only CD4 mRNA is detected. (b) qRT-PCR of NKp46 in CD4⁺ laser-microdissected cells in CTCLs, ISDs, and CBCLs. The median level of NKp46 mRNA in CD4⁺ laser-microdissected cells is higher in the nine pooled CTCLs when compared with five CBCLs (P < 0.05) or five ISDs (P < 0.05). The reference gene used for normalization was the transcription factor IID/TATA-binding protein (TBP). The 2⁻⁰⁻ΔC'T method was used for mRNA quantification. mRNA from a Sézary patient expressing the NKp46 gene was used as the calibrator. Bars represents mean ± SD. CBCL, cutaneous B-cell lymphoma; CTCL, cutaneous T-cell lymphoma; ISD, inflammatory skin disease; MF, mycosis fungoides; PBMC, peripheral blood mononuclear cell; qRT-PCR, quantitative real-time PCR; SNK, NK lymphoma cell lines; SS, Sézary syndrome; TMF, transformed mycosis fungoides.
Nkp46 Expression on Skin-Resident CD4+ Cells in CTCLs

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according to the WHO-EORTC classification (Olsen et al., 2007).

Phenotypic analyses, performed on formalin-fixed paraffin-embedded sections, showed that CD3+ lymphocytes were prominent in dermal infiltrates of all CTCLs. CD4+ was the major T lymphocyte subtype with median cell count/field of 95 (range 29–132) in SS, 94 (range 49–98) in MF, and 310 (range 101–352) in TMF. The CD4/CD8 ratios were over 4:1 in all CTCLs (median 53, range 7–372). CD56 cells were rare, with median cell count/field of 0.1 (range 0–0.2) in SS, 0.2 (range 0–0.2) in MF, and 0.1 in TMF (range 0–0.1; Figure 1a). When compared with ISDs, there was no significant difference, because in ISDs the CD4+ median cell count/field was 102 (range 70–130), CD4/CD8 ratio was 1.5, and CD56 median cell count/field was 3 (range 1–5).

Nkp46 gene expression level was assessed in whole tissue sections using real-time (RT)-PCR. No significant difference was found between SS, MF, and TMF. However, we found a significant overexpression of Nkp46 in the 15 CTCLs, with a median expression level of 3.1 (range 0.56–8.17) compared with ISDs (P<0.05), CBCLs (P<0.05), or normal skin (P<0.05) (Figure 1b). On agarose gel electrophoresis, a distinct bright band, only found in the CTCL group, confirmed the RT-PCR data. By using RT-PCR, we found that T-plastin transcripts were detected at similar levels in CTCL and ISD groups.

To ascertain whether Nkp46 mRNA expression level was linked to CD4+ T lymphocytes, we laser-microdissected immunostained CD4+ cells from frozen skin sections (Supplementary Figure S1a online). We checked the laser-microdissected cells using endpoint reverse transcription PCR (CD3, CD4 positive, CD8, CD56 negative) and agarose gel electrophoresis (Figure 2a). On performing quantitative RT-PCR (qRT-PCR), the median Nkp46 gene expression level was found to be 7.3 (range 3.7–86.18) in the 15 CTCLs, without significant difference across MF–TMF–SS. In addition, Nkp46 gene expression levels in CD4+ laser-microdissected cells were significantly higher when CTCLs were compared with CBCLs (P<0.05) and with ISDs (P<0.05)(Figure 2b). T-plastin mRNA was not expressed in CD4+ T lymphocytes from CTCL patients, although it was reported in leukemic T cells (Su et al., 2003).

Laser microdissection combined with qRT-PCR enabled us to demonstrate that Nkp46 was overexpressed in CTCLs only, and that Nkp46 expression was specifically found in laser-microdissected CD4+ T lymphocytes from SS, MF, and TMF. An aberrant Nkp46 expression had been reported on circulating Sézary cells but not in circulating cells of MF or TMF (Bensussan et al., 2011). If, early in the evolution of MF and TMF, malignant cell distribution is restricted to the skin, Nkp46 would only be detected in skin-resident CD4+ T lymphocytes and not in circulating blood cells. Our results imply that Nkp46 specificity is not limited to SS but extends to other epidermotropic CTCLs, even at an early stage.

This aberrant Nkp46 expression recently reported in TMF skin, together with other NK-specific receptors (Ortonne et al., 2012), could be linked, in the three types of CTCLs studied here, to lymphocyte reprogramming toward a NK phenotype (Meresse et al., 2006), an hypothesis supported by expression of cytotoxic proteins as granzyme B and T cell–restricted intracellular antigen in CTCL cells (Vermeer et al., 1999). However, no alteration of the KIRs and Nkp46 gene locus on 19q13.4 was found using genomic microarrays or array-based comparative genomic hybridization. The transcriptional signature of this reprogramming could result from induction of receptors and adaptors normally restricted to the NK lineage (Meresse et al., 2006). Another cause for this aberrant expression of NK markers in CTCL could be deregulation of the transcription factor-E2A implicated in T- and NK-cell lineage development (Steininger et al., 2011).

Laser microdissection combined with qPCR enabled us to identify Nkp46 overexpression in skin-resident CD4+ T lymphocytes from 15 CTCLs. In the same patients, RT-PCR on whole tissue sections enabled us to characterize a significant Nkp46 overexpression in CTCLs and to distinguish them from ISDs, CBCLs, or normal skin, with a sensitivity and a specificity of 89% and 100%, respectively, and with a positive predictive value of 100%. Therefore, as long as a specific antibody is not available, RT-PCR on whole tissue sections could be helpful for day-to-day diagnostics, particularly in the early stages of epidermotropic CTCL. Further study on large patient sample series will be necessary to assess the value of Nkp46 as a biomarker of epidermotropic CTCL.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/jid

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Generalized Pustular Psoriasis Triggered by Amoxicillin in Monozygotic Twins with Compound Heterozygous IL36RN Mutations: Comment on the Article by Navarini et al.

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TO THE EDITOR

We read with great interest the recent report regarding IL36RN mutations in acute generalized exanthematous pustulosis (AGEP) by Navarini et al. (2013). They analyzed IL36RN mutations in 96 cases with AGEP and found only one homozygous mutation (p.Leu27Pro) and three heterozygous mutations. p.Leu27Pro is a founder mutation causing generalized pustular psoriasis (GPP) homozygously in African populations, and the AGEP patient described by Navarini et al. (2013) with the homozygous mutation p.Leu27Pro is an African woman (Marrakchi et al., 2011; Navarini et al., 2013). She had a previous history of drug-induced type-unknown skin reaction triggered by uncertain antibiotics. She showed pustules covering 85% of her body surface, including the mouth, and a 39.4°C fever triggered by amoxicillin.

A positive patch test to amoxicillin was noted. Treatment of the patient was not described. No recurrence was observed during the 18-month follow-up.

In a recent report, we analyzed 11 patients with GPP not accompanied by psoriasis vulgaris (PV) (GPP-alone; Sugiura et al., 2013). Among the 11 GPP-alone patients, there was a monozygotic twin with the compound heterozygous mutations p.Arg10X and p.Arg10ArgfsX1 in IL36RN whose pustules and high fever had primarily been triggered by amoxicillin.

The patients were 6-year-old Japanese male identical twins. At the age of 2 years, they had erythema with pustules on the whole body and fever over 38°C after amoxicillin intake. In one of the twins, blood examination revealed a white blood cell count of 24,300/μl and a C-reactive protein concentration of 1.17 mg dl⁻¹. Bacterial culture for the pustules was negative. A skin biopsy from a pustular eruption on the trunk revealed a spongiform pustule of Kogoj in the epidermis (Figure 1c). The results of these examinations were similar to those of the other twin. Patch tests were conducted on one of the twins, and it was found to be positive for amoxicillin.

Skin eruptions including pustules of the twins were improved with a dosage of 6 mg kg⁻¹ oral cyclosporine. After the first episode, each twin has episodic systemic pustules unrelated to amoxicillin intake (Figure 1a and b). Neither twin has ever showed typical scaly erythema corresponding to PV. The two patients, monozygotic twins, had the compound heterozygous mutations p.Arg10X and p.Arg10ArgfsX1 in IL36RN (Sugiura et al., 2013). Thus, they were diagnosed as having GPP-alone with IL36RN mutations that had once been triggered by amoxicillin.

Amoxicillin- or penicillin-related drug-induced GPP has been reported for a long time, although the pathomechanisms of how penicillin induces GPP have never been addressed (Ryan and Baker,

Abbreviations: AGEP, acute generalized exanthematous pustulosis; DITRA, deficiency of IL-36 receptor antagonist; GPP, generalized pustular psoriasis; IL36RN, IL-36 receptor antagonist; PV, psoriasis vulgaris.

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