

Short communication

Natural killer T cells expressing IFN- γ and IL-4 in lesional skin of atopic eczema

Background: The inflammation of atopic eczema (AE) is orchestrated not only by T cells predominantly but also B cells, eosinophils and dendritic cells. Recently, a role of invariant natural killer T (NKT) cells has been reported in bronchial asthma and allergy. Natural killer T cells express a restricted repertoire of T-cell receptor α/β and produce interferon (IFN)- γ and/or interleukin (IL)-4 upon activation.

Aim of the study: To determine the presence of NKT cells in lesional AE skin in comparison with other eczematous disorders and to analyse their cytokine expression.

Methods: Immunofluorescence stainings were carried out using antibodies recognizing NKT cells, CD3+ and CD4+ cells, IFN- γ and IL-4.

Results: Natural killer T cells have been detected in small numbers in the majority of AE specimens as well as in atopy patch test (APT) reactions, allergic contact dermatitis (ACD) and irritant contact dermatitis (ICD). In AE, the proportion of NKT cells among CD3+ cells was approximately 5%. NKT cells expressed both IFN- γ and IL-4 in AE, APT and ACD but predominantly IFN- γ in ICD.

Conclusion: Natural killer T cells are part of the inflammatory infiltrate of AE as well as APT, ACD and ICD, suggesting a pathogenic role of NKT cells in eczematous skin disorders. The pattern of IFN- γ and IL-4 cytokine expression by NKT cells varied depending on the type of eczematous disease.

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The inflammatory infiltrate of atopic eczema (AE) consists mainly of T cells with a predominance of CD4+ compared with CD8+ cells (1). Although a high expression of T helper (Th) 2 cytokines, such as interleukin (IL)-4, IL-5 and IL-13 has been found in the blood and the skin of AE patients, the Th1 cytokine interferon (IFN)- γ is additionally involved and plays a role in the development of spongiosis and chronic lesions (1, 2).

Invariant natural killer T (NKT) cells are a subset of lymphocytes that express a restricted repertoire of T-cell receptor α/β , commonly V α 24 and V β 11 in humans. Natural killer T cells recognize glycolipids rather than peptide antigens which are presented in the context with CD1d (3). Upon activation, NKT cells produce IFN- γ and/or IL-4 and thus may regulate adaptive immune responses including peripheral tolerance and tumour surveillance (3). In 2006, Akbari et al. (4) reported the presence of NKT cells among pulmonary CD4+ cells in bronchial asthma, which is characterized by a Th2 cell and cytokine profile. The purpose of this study was to search for NKT cells in the dermal infiltrate of AE lesions and other eczematous disorders and to determine their cytokine profile.

Methods

Immunofluorescence staining was performed on sections of paraformaldehyde-fixed, paraffin-embedded skin biopsies from acute AE lesions ($n = 21$) from AE lesions treated with topical calcineurin inhibitors ($n = 5$), positive atopy patch test (APT) reactions (application 48 h, reading at 72 h; $n = 3$) as well as allergic contact dermatitis (ACD; positive patch test reaction at 72 h; $n = 3$), irritant contact dermatitis (ICD; positive patch test with sodium lauryl sulphate at 72 h; $n = 3$) and normal skin. To detect NKT cells, a monoclonal anti-human invariant NKT antibody that specifically recognized the complementary-determining region 3 of the V α 24-J α 18 T-cell receptor (6B11; BD Biosciences, San Jose, CA, USA) was used. Double immunofluorescence stainings were carried out with polyclonal antibodies recognizing CD3 (NeoMarkers Inc., Fremont, CA, USA); CD4 (Abcam plc, Cambridge, UK), IL-4 (Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA) and IFN- γ (R&D Systems, Abingdon, UK). Antibody binding was detected with appropriate indocarbocyanine (Cy3)-conjugated (Jackson ImmunoResearch, Suffolk, UK) and Alexa Fluor 488-conjugated (Invitrogen, Paisley, UK) secondary antibodies. Nonspecific binding to Fc γ receptors was blocked with polyclonal human immunoglobulin G. Immunofluorescence staining was evaluated by two independent observers, one blinded, using a confocal laser scanning microscope (LSM 510; Carl Zeiss, Jena, Germany), equipped with argon and helium-neon lasers. Positive cells were counted in 10 consecutive fields of 0.1 mm² at a magnification of 1000 $\times$.

Results

Number of NKT cells

Normal skin did not contain any NKT cells. In contrast, single NKT cells were detected among dermal infiltrating cells in AE, APT, ACD and ICD, respectively (Table 1). They were predominantly found in areas with dense perivascular infiltrates. We did not detect any NKT cells in the epidermis. In lesional AE skin, NKT cells were identified in 19 of 21 biopsy specimens. Overall, the number of NKT cells was relatively low with a mean of 3 (range 0–8) cells per mm² (Fig. 1). The proportion of NKT among CD3+ cells in the dermis was in average 5%, and ranged between 0 and 9%. Double immunofluorescence staining ($n = 9$) revealed that all NKT cells expressed CD4. In skin biopsies taken after topical calcineurin inhibitor treatment, a reduction of NKT cells was observed in all patients. In three patients, NKT cells were no longer detectable, in the other two patients, NKT cells were still detected in 0.5% and 5% of infiltrating CD3+ cells, respectively. The mean number of NKT cells in positive APT reactions was 4 (range 1–7) cells/mm² with a mean proportion of 6% (range 5–12%) of CD3+ cells. Accordingly, the NKT cell numbers were almost identical in AE and APT. The absolute numbers of NKT cells were 6 cells/mm² in ACD and 9 cells/mm² in ICD, and thus slightly higher compared with AE. Among infiltrating CD3+ cells, the relative numbers of NKT cells were 6% (range 5–8%) in ACD and 9% (range 7–12%) in ICD.

Cytokine expression by NKT cells

In all investigated specimen positive for NKT cells from lesional AE, APT, ACD and ICD, both IFN- γ and IL-4 expressing NKT cells were identified (Table 1). The proportion IFN- γ to IL-4 expressing NKT cells was approximately 3 : 2 in AE, APT and ACD and 5 : 1 in ICD. In AE, NKT cells represented approximately 8% of all IL-4 + CD4+ cells. In positive APT reactions, the relative proportion of NKT cells among IL-4 + CD4+ cells was 14%. All of the IL-4 + CD4+ cells were NKT cells in ACD and ICD. This high percentage was largely due to the low numbers of IL-4 + CD4+ T cells and not the consequence of a particular high infiltration of NKT cells in ACD and ICD skin. The proportion of IFN- γ + NKT cells among IFN- γ + CD4+ cells was

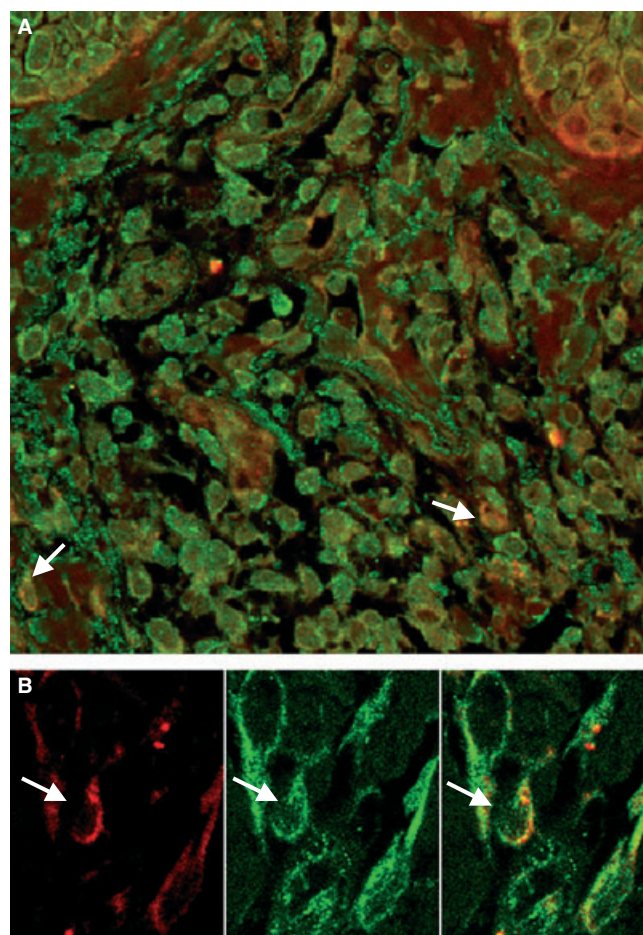


Figure 1. Natural killer T (NKT) cells among infiltrating T lymphocytes in lesional skin of atopic eczema. Skin tissue specimens were stained with anti-human invariant NKT antibody (red) and anti-CD3 antibody (green) to detect NKT cells (orange). (A) 400 $\times$, (B) 1000 $\times$.

similar, varying between 17% in APT and ACD, 18% in AE and 20% in ICD.

Discussion

This study demonstrated that NKT cells migrate into the skin during eczematous inflammation in AE, APT, ACD and ICD, and make up a small proportion of infiltrating T cells. The presence of NKT cells in lesional skin of AE

Table 1. Numbers of NKT cells, CD3+ cells as well as IFN- γ and IL-4 expressing NKT and CD4+ cells in atopic eczema (AE), atopy patch test (APT), allergic contact dermatitis (ACD) and irritant contact dermatitis (ICD) (mean $\pm$ SE cells/mm²)

	NKT cells	CD3+ cells	IFN- γ + CD4+ cells	IFN- γ + NKT cells	IL-4 + CD4+ cells	IL-4 + NKT cells
AE	3 $\pm$ 1	77 $\pm$ 11	16 $\pm$ 3.1	3 $\pm$ 0.8	25 $\pm$ 3.5	1.6 $\pm$ 0.4
APT	4 $\pm$ 1.7	53 $\pm$ 6	15 $\pm$ 3.5	2.5 $\pm$ 0.7	14 $\pm$ 7.3	1.5 $\pm$ 0.4
ACD	6 $\pm$ 1	96 $\pm$ 3	18 $\pm$ 2.4	3 $\pm$ 1	1 $\pm$ 0.6	2 $\pm$ 0.7
ICD	9 $\pm$ 0.6	99 $\pm$ 13	25 $\pm$ 3.5	5 $\pm$ 0.9	1 $\pm$ 0.9	1 $\pm$ 0

NKT, natural killer T cells; IFN, interferon; IL, interleukin.

represents a new finding. Previously published work suggested that the number of NKT cells in the peripheral blood of AE patients was decreased compared with healthy individuals due to a specific recruitment of NKT cells into AE sites (5). However, according to our observations and the results earlier reported in ACD (6), it is more likely that NKT cells are recruited together with T cells into the skin. Moreover, in agreement with our data, the presence of a significant number of NKT cells at elicitation sites of ACD (6) as well as in an experimental model of contact hypersensitivity (7) has been reported. Interestingly, in nonallergic eczema like ICD, we also detected NKT cells at an even higher proportion of infiltrating T cells compared with AE or APT.

Natural killer T cells were shown to play a role in asthma and allergy. In animal asthma models, NKT cell-deficient mice failed to develop allergen-induced airway hyper-responsiveness, airway eosinophilia as well as immunoglobulin E (IgE) and Th2 cytokine production after allergen challenge (8, 9). This failure could be reversed by the transfer of NKT cells (8, 9). Thus, the observation that NKT cells are present among pulmonary CD4⁺CD3⁺ T cells in asthma patients seemed logical, although the reported frequency of 60% was rather high (4). Indeed, these data were subsequently corrected by Vijayanand et al. (10) and a number of other studies demonstrating the presence of NKT cells in much lower numbers, fewer than 2% of T cells, in airway biopsies, bronchialveolar lavage fluid and sputum of asthma patients.

Natural killer T cells are able to produce both Th1 and Th2 cytokines. Resting NKT cells constitutively express IL-4 and IFN- γ mRNA without relevant cytokine production (11). Different mechanisms of NKT cell activa-

tion resulting in IFN- γ and/or IL-4 cytokine production have been described (12). Natural killer T cells are activated by either microbial antigens or cytokines derived from dendritic cells such as IL-12 or both (12). These principal mechanisms might also play a role in eczematous diseases including AE. However, type of stimulation as well as surrounding milieu of NKT cells seem to determine whether NKT cells bias Th1 or Th2 responses. Ligand-activated NKT cells were shown to exert a potent inhibitory effect on Th2 cell differentiation and subsequent IgE production by producing IFN- γ , whereas the IL-4 production had only a minor effect on Th2 cell differentiation (13). In contrast, upon CD226 engagement, NKT cells from asthma patients induced a Th2 bias of CD3⁺ T cells (14). In AE skin and APT reactions, we observed a mixed cytokine pattern of NKT cells expressing both IFN- γ and IL-4. Furthermore, our results confirmed previous observations regarding the expression of IL-4 and IFN- γ by NKT cells during the elicitation phase of ACD (6). In contrast, NKT cells expressed almost exclusively IFN- γ in ICD.

In this study, we demonstrated the presence of cytokine-expressing NKT cells in AE and other eczematous diseases, although their numbers and cytokine pattern differed between the different pathologic conditions. The role of NKT cells and their cytokines in the pathogenesis of eczematous disorders remains unknown.

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