

NKp46⁺ cells express granulysin in multiple cutaneous adverse drug reactions

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Keywords

acute generalized exanthematous pustulosis; cutaneous adverse drug reaction; drug allergy; drug hypersensitivity; fixed drug eruption; granulysin; granzyme B; maculopapular exanthema; natural killer cells; NKp46 (NCR1/CD335); toxic epidermal necrolysis.

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Abstract

Background: The spectrum of cutaneous adverse drug reactions (cADRs) ranges from benign presentations to severe life-threatening forms such as toxic epidermal necrolysis (TEN). In TEN, granulysin has been shown to be the key cytotoxic molecule. Still, little is known about the expression of granulysin in other cADRs. As an important source of granulysin, natural killer (NK) cells are of major interest in cADRs. Recently, NKp46 has been identified as the most selective NK-cell marker. However, the role of NKp46⁺ cells in cADRs and their contribution to granulysin expression remain to be elucidated.

Methods: Immunohistochemical and immunofluorescence staining of tissue sections from multiple cADRs were quantitatively and qualitatively evaluated. Further, *in vivo* and *in vitro* drug-stimulation tests were performed.

Results: Granulysin is expressed at different levels in multiple cADRs both by NKp46⁺ cells and by CD8⁺ T cells. Even in mild forms of cADRs, granulysin can be induced *in vivo* and *in vitro* in a drug-specific manner. NKp46⁺ cells were found to infiltrate the dermal/epidermal junction particularly in TEN.

Conclusion: The impressive clinical differences of cADRs may not be uniquely explained by the expression of granulysin. Additional factors such as drug-specific activation and recruitment of NKp46⁺ cells to the epidermis may play a role in determining the severity of cADRs. Therefore, unraveling the effects of drugs on NK-cell activation and trafficking may help to better understand the cytotoxic mechanisms behind cADRs.

Adverse drug reactions (ADRs) are one of the major causes of iatrogenic disease (1). Among them, cutaneous ADRs (cADRs) are the most common form (2). However, their pre-

sentation is diverse. It ranges from benign self-limiting reactions such as maculopapular exanthema (MPE) and fixed drug eruption (FDE) to the more severe and systemic acute generalized exanthematous pustulosis (AGEP). At the end of this spectrum, toxic epidermal necrolysis (TEN) represents the severe life-threatening form of cADRs (3).

Although the pathogenesis is not completely understood, cADRs are believed to be immune mediated with lymphocytes playing a key role (4–6). Cytotoxic mechanisms exerted by these immune cells lead to limited or widespread keratinocyte apoptosis, thus determining the severity of the cutaneous reaction (7). Until recently, perforin/granzyme B and Fas/Fas ligand (FasL) released by T and natural killer (NK) cells were considered as the main

Abbreviations

ADR, adverse drug reaction; AGEP, acute generalized exanthematous pustulosis; cADR, cutaneous adverse drug reaction; CM, culture medium; CTL, cytotoxic T cell; FasL, Fas ligand; FDE, fixed drug eruption; GNLY, granulysin; GZMB, granzyme B; IL, interleukin; LTT, lymphocyte transformation test; MPE, maculopapular exanthema; NCR, natural cytotoxicity receptor; NK cells, natural killer cells; PBMC, peripheral blood mononuclear cells; SJS, Stevens-Johnson syndrome; TEN, toxic epidermal necrolysis.

mediators of drug-induced cytotoxicity (6, 8). However, Chung et al. have shown that in Stevens-Johnson syndrome (SJS)/TEN, granulysin is the key cytotoxic molecule. They concluded that in contrast to perforin/granzyme B or Fas/FasL, granulysin is specifically expressed in SJS/TEN, thus providing an explanation for the differing clinical severities (5).

One of the main sources of granulysin is NK cells (9). As lymphocytes of innate immunity, they provide surveillance against virally infected cells and tumor cells and can induce death of autologous cells undergoing various forms of stress, thus recognizing and providing nonmicrobial 'danger' signals to the immune system (10). The study of NK cells has so far been hampered by the lack of specific markers defining this cell subset (11). In humans, NK cells were classically defined as CD3⁺ CD56⁺ lymphocytes. Yet, this and alternative definitions have proven to be difficult as none of the markers used are NK-cell specific but are expressed at various degrees by subsets of T cells (11). Recently, the natural cytotoxicity receptor NKp46 (NCR1, CD335) has been found to be selectively expressed on all peripheral blood NK cells in healthy humans and across mammalian species, thereby leading to the new phenotypical definition of NK cells as CD3⁺ NKp46⁺ lymphocytes (10, 11). In healthy human skin, NKp46⁺ cells have been identified as true NK cells residing exclusively in the dermis (12). Yet, it remains to be elucidated whether these dermal NK cells migrate to the epidermis under inflammatory conditions. Furthermore, their contribution to the immune reaction in cADRs is largely unknown.

Based on the key role of granulysin in TEN, we sought to investigate its expression and cellular source in other forms of cADRs, namely FDE, MPE and AGEP. By taking advantage of the refined definition of NK cells as NKp46⁺ cells, we further aimed at determining their contribution to the infiltrate and to granulysin expression in cADRs. Finally, we investigated the *in vivo* and *in vitro* expression of granulysin in drug-stimulation tests of drug-allergic patients.

Materials and methods

All antibodies used in this study are listed in Table S1.

Samples from patients

The study was approved by the Medical Ethics Committee of the Canton of Berne, Switzerland. Written informed consent was obtained from all patients, and the Declaration of Helsinki protocols were followed. The study included patients with TEN ($n = 11$, mean 60 ± 18 years), FDE ($n = 13$, mean age 61 ± 22 years), MPE ($n = 12$, mean age 51 ± 18 years) and AGEP ($n = 11$, mean age 45 ± 20). Diagnosis of MPE and FDE was made on the basis of typical history, clinical manifestation, histopathology, time course and exclusion of other causes such as viral or bacterial infections and autoimmune diseases (13, 14). Toxic epidermal necrolysis and AGEP were diagnosed based on criteria proposed by Bastuji-Garin et al. and Sidoroff et al., respectively (15, 16). Punch biopsies were obtained from lesional skin at a

site of pronounced inflammation. Normal skin was obtained from eight healthy women (mean age 35 ± 16 years).

Immunohistochemical staining

Immunostaining was performed using the streptavidin-biotin complex/alkaline phosphatase method, as previously described with modifications (17): 2- to 3-m-thick paraffin-embedded sections were dewaxed, rehydrated and pretreated as shown in Table S1. They were then incubated with the primary antibody or an isotype-matched control antibody for 1 h at room temperature, followed by a biotinylated secondary antibody and the streptavidin-biotin complex/alkaline phosphatase method (K0376; DakoCytomation, Glostrup, Denmark). All sections were developed in new fuchsin-naphthol (KO624; DakoCytomation) and counterstained with hematoxylin. Quantitative analysis was performed by using the digital image analysis system NIS-Elements Software BR 2.30 (Nikon, Tokyo, Japan), as previously described (17).

The distribution pattern of granulysin⁺ and NKp46⁺ cells was analyzed qualitatively in an investigator-blinded fashion in two independent evaluations: If positively stained cells were found predominantly in the dermis without contact to the dermal/epidermal junction, the infiltration was judged as 'dermal.' Samples, where positively stained cells were predominantly found at the dermal/epidermal junction or within the epidermis, were classified as 'junctional/epidermal.'

Immunofluorescence double staining

Tissue sections were serially incubated overnight with a mouse anti-human granulysin antibody followed by incubation with a FITC-labeled goat anti-mouse IgG2a antibody. Sections were then incubated for 1 h with either anti-NKp46, anti-CD8, or anti-CD4, followed by isotype-specific Alexa Fluor 594-conjugated goat anti-mouse or rabbit anti-goat antibodies (Table S1). Finally, cells were stained with diaminodiphenylindol nucleic acid stain (D1306; Invitrogen, Carlsbad, CA). The specificity of the reaction was confirmed by using irrelevant isotype-matched antibodies.

Flow cytometry

All flow cytometry staining and intracellular staining were performed on either peripheral blood mononuclear cells (PBMCs) (drug-stimulation assay) or single-cell suspension (cells isolated from skin) using the BD Cytotix/Cytoperm kit according to the manufacturer's protocol (BD Biosciences). Stained cells were analyzed on a FACSCanto (BD Bioscience, Basel, Switzerland), and data were analyzed with FlowJo software (Tree Star).

Isolation and phenotypic characterization of lymphocytes from positive patch test

A punch skin biopsy was obtained from a bullous patch test reaction from an amoxicillin-allergic MPE patient. The biopsy was minced in a culture dish with culture medium.

Emigrated lymphocytes were collected, filtered (45- μ m filter), and directly stained for fluorescence activated cell sorting (FACS) analysis.

Drug stimulation of PBMCs from drug-allergic donors

Culture medium (CM) was prepared as previously described (6). PBMCs were thawed and cultured at 5×10^5 cells/well in triplicates in a 96-well U-bottom culture plate at 37°C in a 5% CO₂ incubator in the presence/absence of culprit/tolerated drugs at the indicated final concentrations (Table S2). Culprit/tolerated drugs were determined by lymphocyte transformation test (LTT), as previously described (6, 18). After 68 h, Brefeldin A (Sigma Chemicals Co, Buchs, Switzerland) was added (10 μ g/ml) for the final 4 h to enhance the detectability of granulysin by intracellular FACS staining.

Statistical analysis

Statistical analysis was performed by means of nonparametrical tests: equality of population medians among different cADRs

was tested by Kruskal–Wallis test, followed by pair-wise comparison by Mann–Whitney *U*-test with Bonferroni–Holm correction. Spearman rank correlation was used for analysis of correlations followed by testing of linear regression (*F*-test). Paired samples were analyzed by Wilcoxon signed rank test.

Results

Granulysin is expressed in multiple cADRs at the dermal/epidermal interface

In all cADRs studied, granulysin was expressed at significantly higher levels than in normal skin, where granulysin⁺ cells were almost absent (Fig. 1). The highest numbers of granulysin⁺ cells were found in TEN and FDE, with statistically highly significant differences from healthy skin. Analysis of tissue distribution revealed that granulysin-expressing cells are predominantly found at the dermal/epidermal interface with a substantial proportion infiltrating the epidermis, regardless of the cADR (Fig. 1C). In TEN, granulysin was frequently expressed in areas surrounding epidermal necrosis

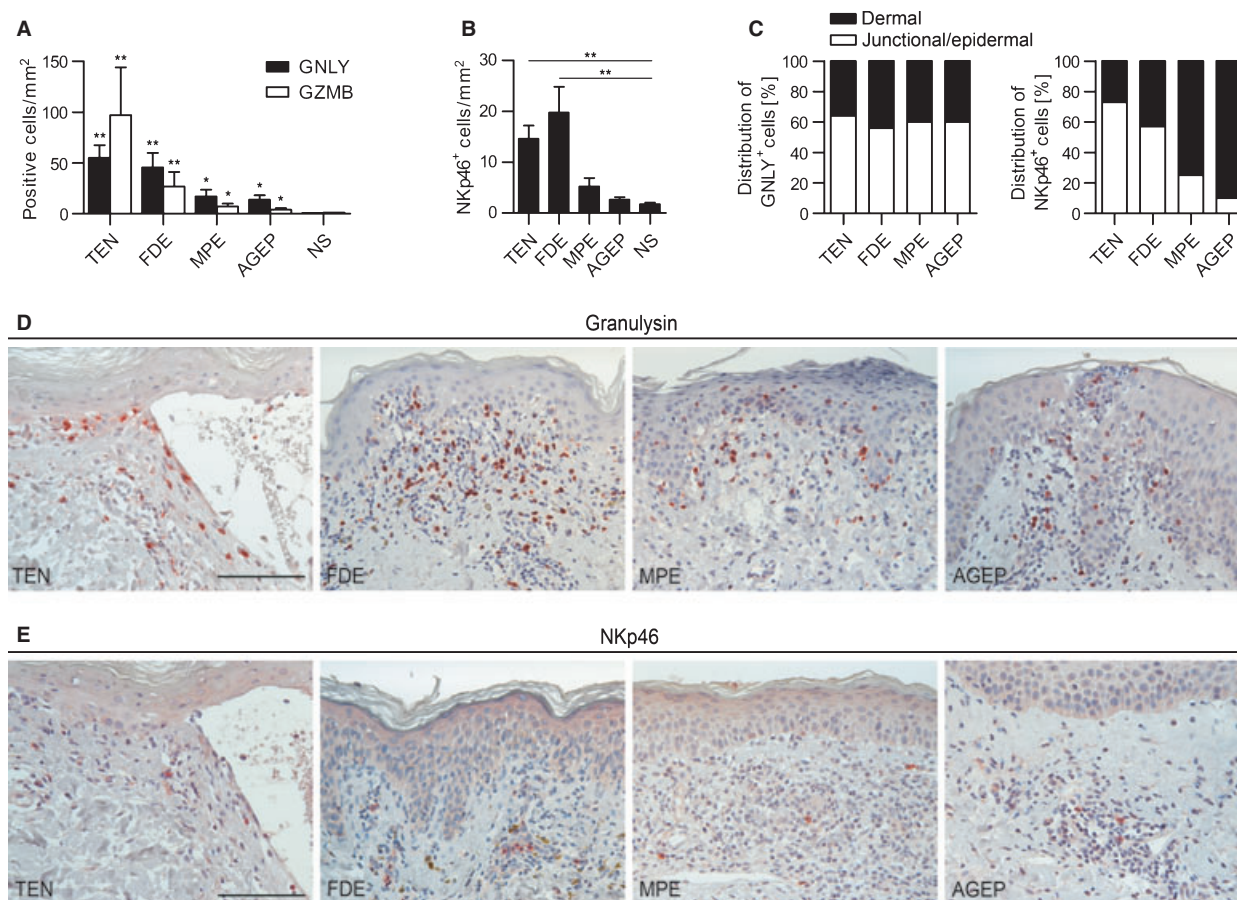


Figure 1 Tissue expression of granulysin, granzyme B, and NKp46 in cutaneous adverse drug reactions (cADRs). (A) Quantitative analysis of granulysin and granzyme B expression (Mean + SEM). (B) Quantitative analysis of NKp46 expression (Mean + SEM). (C) Distribution of granulysin⁺ or NKp46⁺ cells within dermal or epidermal/

junctional compartment. (D and E) Representative examples of tissue samples of cADRs stained with granulysin (D) and NKp46 (E). Magnification: 200x, bars = 100 μ m (A and B). **P* < 0.05, ***P* < 0.01. Asterisks in (A) represent *P*-values obtained from pair-wise comparison with normal skin (NS).

and within blisters (Fig. 1D). In the remaining cADRs, granulysin⁺ cells were regularly found in close proximity to keratinocytes showing vacuolar degeneration. Interestingly, granzyme B was expressed at comparable levels to those of granulysin in all cADRs investigated.

NKp46 is expressed at different cutaneous layers in cADRs

NKp46⁺ cells were found significantly more frequent in both TEN and FDE when compared to normal skin, where a mild infiltration was observed (Fig. 1B). In MPE and AGEF, NKp46⁺ cells were not significantly increased. In contrast to granulysin⁺ cells, NKp46⁺ cells showed differing distribution patterns depending on the type of cADR (Fig. 1C): In TEN, the majority of NKp46⁺ cells were found at the dermal/epidermal interface and within blisters. To a lesser extent, this was also observed in FDE whereas in MPE and AGEF, NKp46⁺ cells were confined to the dermal infiltrate.

NKp46⁺ cells differentially contribute to skin-infiltrating lymphocytes in cADRs

We assessed the relative contribution of NKp46⁺ cells to the lymphocyte infiltrate in cADRs (Fig. 2A). Interestingly, NKp46⁺ cells make up approximately 13% of the lymphocytes in TEN, but considerably smaller proportions in the remaining cADRs where they represent <4% of the lymphocyte infiltrate.

Pooling the data of all cADRs included in the study, we found significant correlations between numbers of granulysin⁺ cells and both NKp46⁺ and CD8⁺ cells but not with CD4⁺ cells (Fig. 2B).

NKp46⁺ cells and CD8⁺ T cells are a major source of granulysin in cADRs

To analyze the cellular source of granulysin in cADRs, we performed double immunofluorescence on tissue sections of all cADRs included in this study. Results from samples of a MPE patient are shown in Fig. 3A–C. We readily found colocalization of granulysin with NKp46 and CD8 but only rarely with CD4 in all cADRs analyzed (Fig. 3 and data not shown).

Within blisters of TEN patients, we detected granulysin⁺ cells co-expressing NKp46, thus demonstrating that granulysin-producing NKp46⁺ cells distinctly infiltrate the epidermis in TEN (Fig. 3D).

Bullous skin patch reaction to amoxicillin is infiltrated by NKp46⁺ cells and CD8⁺ T cells expressing granulysin

To confirm our data obtained by immunohistochemistry, we analyzed skin-infiltrating lymphocytes from a positive bullous skin patch test from an amoxicillin-allergic MPE patient by flow cytometry (Fig. 4). The infiltrating lymphocytes mainly consisted of CD4⁺ T cells, with CD8⁺ T cells and NK cells making up 14% and 6.8% of the lymphocytes, respectively. Granulysin expression, however, was highest in activated NKp46⁺ cells, as evidenced by its high co-expression with the leukocyte activation marker CD69. Granulysin was further expressed by around 10% of CD8⁺ T cells and by few CD4⁺ T cells.

Drug-specific upregulation of granulysin in PBMCs from drug-allergic patients

We further sought to investigate whether granulysin can be induced by drugs *in vitro* in PBMCs of drug-allergic MPE patients (Fig. 5). Culprit and tolerated drugs of the individual patients were determined by LTT (Table S2). Most prominently NK cells, but also CD8⁺ T cells, significantly upregulated granulysin after 72 h of drug stimulation. A small subpopulation of CD4⁺ T cell also showed upregulation of granulysin. This response was drug specific because only the culprit drug but not tolerated drugs induced granulysin and PBMCs from healthy but drug-exposed individuals failed to upregulate granulysin in response to the drugs used in the assay (data not shown).

Discussion

In this study, we show that granulysin is expressed at different levels in multiple cADRs and that skin-infiltrating NKp46⁺ cells and CD8⁺ T cells are a prominent source thereof. We observed that NKp46⁺ cells make up a larger proportion of the lymphocyte infiltrate in TEN than in

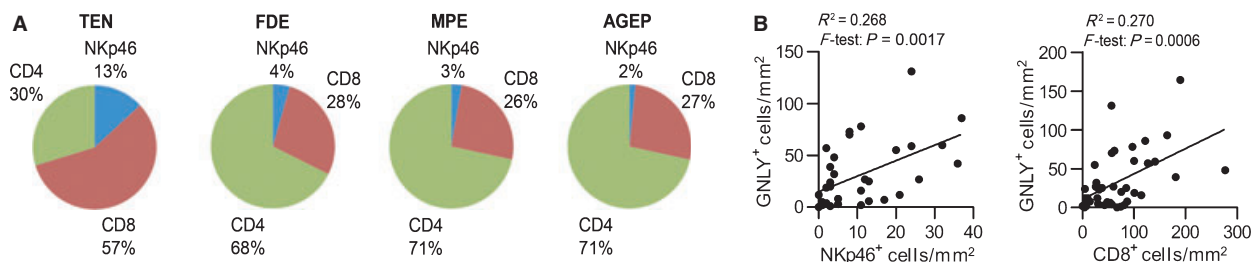


Figure 2 Composition of lymphocyte infiltrate in cutaneous adverse drug reactions (cADRs). (A) Relative contribution of NKp46⁺, CD8⁺, and CD4⁺ cells to the infiltrate in cADRs (cADRs) as calculated from quantitative analysis of immunohistochemically

stained tissue sections. (B) Correlation of NKp46 and CD8 expression with granulysin expression in pooled samples of all cADRs included in the study. Statistics: Spearman test followed by F-test of linear regression.

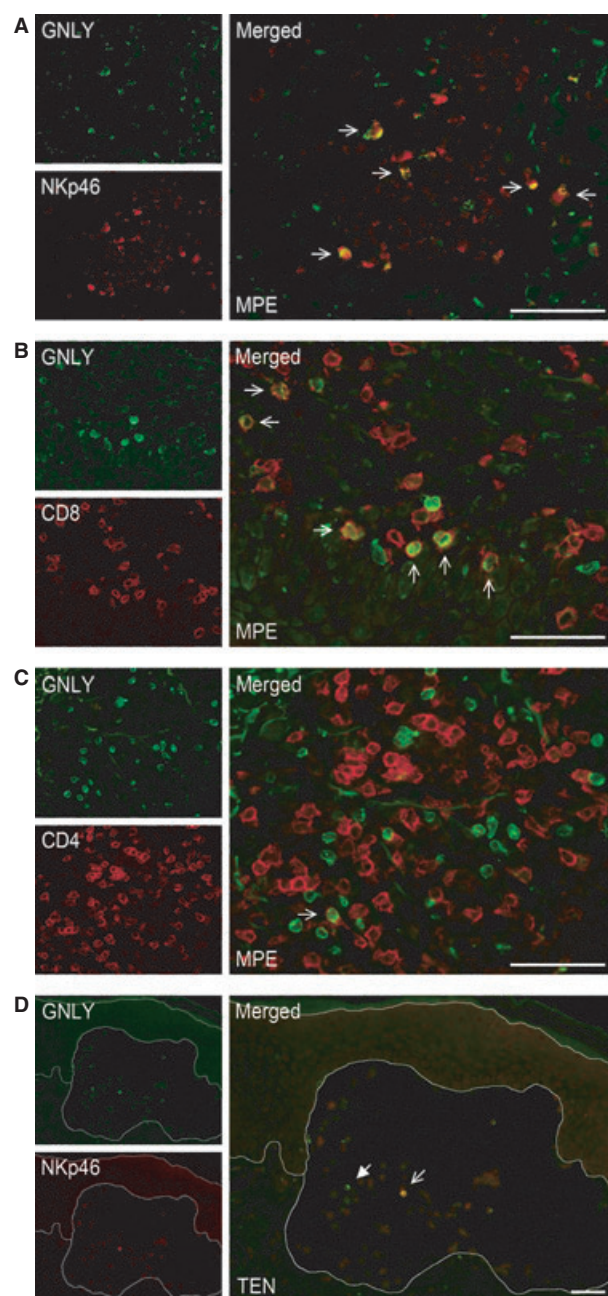


Figure 3 Cellular source of granulysin in cADR. (A–C) Expression of granulysin (green) and NKp46, CD8, and CD4 (all in red) in maculopapular exanthema. Arrows indicate double-positive cells. Magnification 400 $\times$, bars = 200 μ m. (D) Expression of granulysin (green) and NKp46 (red) within a blister of a toxic epidermal necrolysis sample; closed arrow = granulysin single-positive cell, open arrow = granulysin⁺ NKp46⁺ cell. White lines show borders of epidermis and blister. Original magnification 100 $\times$, bar = 200 μ m.

less cytotoxic cADRs and, intriguingly, that NKp46⁺ cells specifically accumulate at the dermal/epidermal junction in TEN but not in other cADRs. Activated granulysin-expressing NKp46⁺ cells were also found in a bullous skin patch

test elicited in a drug-allergic patient, thus showing that this pathogenetic element of cADRs can be induced in an *in vivo* test. Finally, we demonstrate that granulysin can be induced in a drug-specific manner in lymphocytes of drug-allergic donors *in vitro*.

Granulysin has been shown to be the key molecule responsible for disseminated keratinocyte death in SJS-TEN (5). In accordance with these findings, granulysin expression was most pronounced in TEN in our study. However, our data suggest that granulysin is also involved in cytotoxic mechanisms in other types of cADRs: Immunohistochemical analysis revealed that granulysin⁺ cells are found in spatial proximity to areas of epidermal necrosis and keratinocyte degeneration across all cADRs investigated. Expression of granulysin did not differ significantly from that of granzyme B, another cytotoxic molecule expressed by cytotoxic T cells (CTLs) and NK cells. No difference was furthermore detected between TEN and other cADRs when analyzing the tissue distribution of granulysin⁺ cells. Moreover, we were able to illustrate that granulysin can be upregulated in a drug-specific manner in patients who showed only mild ADRs clinically: On the one hand, *in vivo* drug stimulation of an MPE patient with a skin patch test led to profound upregulation of granulysin in NK cells. On the other hand, *in vitro* drug stimulation of PBMCs from MPE patients produced similar results. Therefore, the impressive clinical difference between TEN and other cADRs might not be solely explained by a qualitative difference in granulysin expression. It appears that granulysin represents one of multiple cytotoxic pathways used by CTLs and NK cells in all cADRs and that the distinct clinical manifestations result from quantitative differences in their expression rather than differential release of specific cytotoxic mediators. In fact, cytotoxic pathways like granule-mediated exocytosis of perforin and granzyme B as well as the Fas-FasL interaction have all been implicated in the pathogenesis of cADRs including TEN by various studies (4, 8, 19–21). Interestingly, we also found comparable infiltration with granulysin⁺ cells in FDE and TEN, which further argues against a specific expression of granulysin in TEN. In summary, these data point toward additional factors that either limit or extend keratinocyte death in the course of cADRs.

One of these limiting factors has been identified in FDE as CD4⁺ regulatory T cells (Tregs) infiltrating the epidermis where they control the harmful immune reaction mediated by CTLs, thus preventing progression to TEN (22, 23). Corresponding to these observations and previous reports (4), we detected considerably higher numbers of CD4⁺ T cells in FDE than in TEN. Similar mechanisms controlling the cytotoxic effects of granulysin might also be in place in MPE and AGEF, where we show that granulysin-expressing cells are clearly outnumbered by CD4⁺ T cells.

Interesting evidence pointing toward factors extending keratinocyte injury in cADRs was found in our study by analyzing the tissue expression of NKp46⁺ cells. We made three important observations: First, increased absolute numbers of NKp46⁺ cells are found in TEN and FDE but only in TEN, they make up a significant proportion of the lymphocyte infiltrate. Second, NKp46⁺ cells are recruited

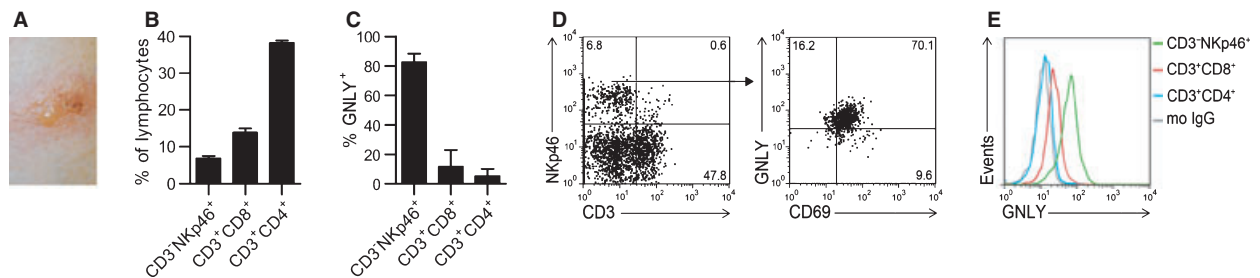


Figure 4 Expression of granulysin in lymphocytes infiltrating bulous patch test from drug-allergic maculopapular exanthema patient. (A) Clinical picture of positive patch test. (B) Contribution of NKp46⁺ cells to the lymphocyte infiltrate. Graph shows percentage of the indicated cell type within live lymphocytes isolated from the

patch test. (C) Granulysin expression in skin-infiltrating lymphocytes. (D) Granulysin is prominently expressed in activated NKp46⁺, CD69⁺ cells. (E) Histogram showing differential expression of granulysin in skin-infiltrating lymphocytes. (B and E) Results derive from FACS analysis of cells isolated from skin.

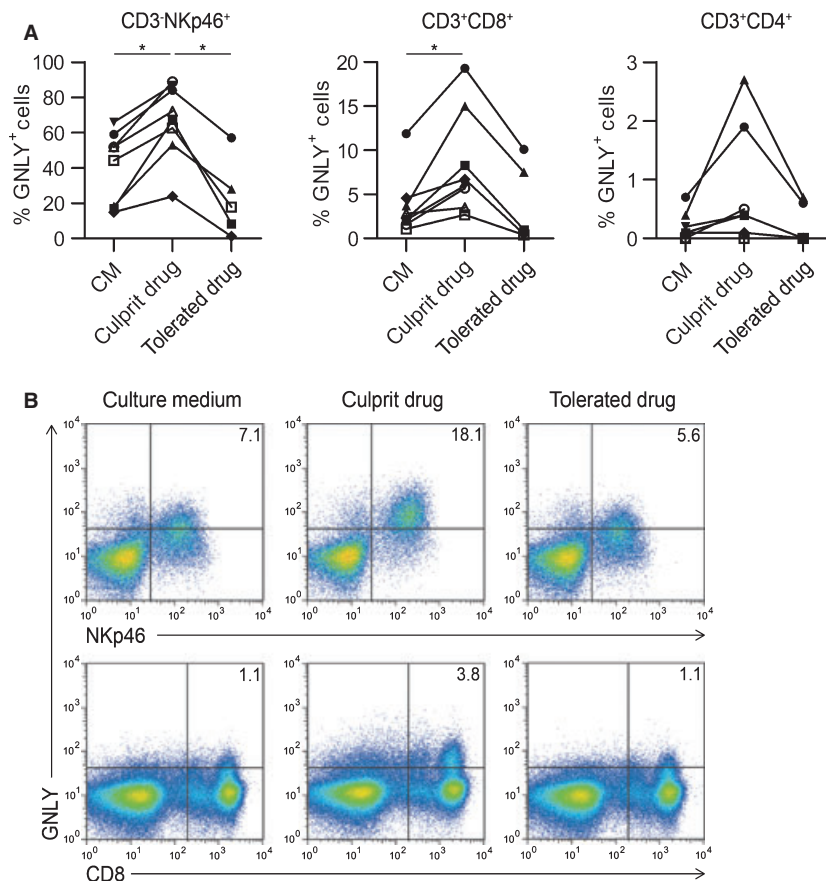


Figure 5 Upregulation of granulysin in PBMCs from drug-allergic maculopapular exanthema patients. (A) Upregulation of granulysin on CD3⁺ NKp46⁺, CD3⁺ CD8⁺, and CD3⁺ CD4⁺ after 72 h stimulation of PBMC from eight different drug-allergic donors, with culture

medium (CM), culprit drug, or tolerated drug. (B) Representative FACS plots of one donor. Statistics: Wilcoxon signed rank test, **P* < 0.05.

toward the epidermis in TEN where they express granulysin. This phenomenon seems to be specific for TEN, as the remaining cADRs failed to show substantial numbers

of NKp46⁺ cells in proximity to the epidermis. Third, NKp46⁺ cells are a main source of granulysin in various cADRs, both *in vivo* and *in vitro*, indicating their high

cytotoxic potential. Taken together, these observations indicate an important role for NKp46⁺ cells in severe cADRs and raise some intriguing questions as to the stimuli and mechanisms that lead to their activation and recruitment: On the one hand, it appears possible that NK cells are activated and recruited by cytokines released by drug-specific T cells which are currently thought to drive cADRs (6, 24). On the other hand, NK cells might also directly recognize drugs through a yet unknown mechanism. In fact, recent studies in mice demonstrate that NK cells can mediate contact hypersensitivity and develop memory of haptens in the absence of adaptive immune cells (25, 26). Alternatively, NK-cell activation may also occur via recognition of activating ligands induced by toxic drug effects on keratinocytes. In support of this, human NK cells were shown to recognize and respond to self-proteins from stressed cells via the NKp46 receptor and other activating receptors (7, 12). Also, NK-cell recruitment to inflamed skin does not require adaptive immunity, further supporting possible direct interactions between keratinocytes and NK cells (12).

In conclusion, we propose that the extent of epidermal injury in cADRs may not be uniquely attributed to the expression of granulysin, because it is expressed in multiple cADRs and it can be induced by drug tests even in mild ADRs. Drug-specific activation and recruitment of NKp46⁺ cells toward the epidermis may play a role in determining the severity of cADRs as well. Investigation into drug-specific activation and trafficking of NK cells within cutaneous compartments may therefore help to better understand the broad clinical spectrum of cADRs.

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Author contributions

All authors certify that they have participated sufficiently in the work to take public responsibility for the content, including participation in the concept, design, analysis, writing, and/or revision of the manuscript. Furthermore, each author certifies that this material or similar material has not been and will not be submitted to or published in any other publication before its appearance in *Allergy*.

Christoph Schlapbach, has participated in generating, gathering, and analysis of the data for the study; wrote the majority of the original draft of the manuscript; had access to all of the raw data of the study; reviewed the pertinent raw data on which the results and conclusions of this study are based; approved the final version of this manuscript; and guarantees that all individuals who meet the Journal's authorship criteria are included as authors of this manuscript.

Anna Zawodniak, Nathalie Irla, and Jacqueline Adam, have participated in generating and gathering of the data for the study; had access to all of the raw data of the study; reviewed the pertinent raw data on which the results and conclusions of this study are based; approved the final version of this manuscript; and guarantee that all individuals who meet the Journal's authorship criteria are included as authors of this manuscript.

Robert E. Hunger, has participated in the analysis of the data; had access to all of the raw data of the study; reviewed the pertinent raw data on which the results and conclusions of this study are based; approved the final version of this manuscript; and guarantee that all individuals who meet the Journal's authorship criteria are included as authors of this manuscript.

Daniel Yerly, has participated in designing the study and in generating, gathering, and analysis of the data for the study; wrote the majority of the original draft of the manuscript; had access to all of the raw data of the study; reviewed the pertinent raw data on which the results and conclusions of this study are based; approved the final version of this manuscript; and guarantees that all individuals who meet the Journal's authorship criteria are included as authors of this manuscript.

Werner J. Pichler, and Nikhil Yawalkar, have participated in designing the study and in the analysis of the data; had access to all of the raw data of the study; reviewed the pertinent raw data on which the results and conclusions of this study are based; approved the final version of this manuscript; and guarantee that all individuals who meet the Journal's authorship criteria are included as authors of this manuscript.

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Conflict of interest

The authors have no conflict of interest to declare.

Supporting Information

Additional Supporting Information may be found in the online version of this article found at: <http://www.wileyonlinelibrary.com>

Table S1. Antibodies used in this study.

Table S2. Patient's characteristics and drugs used for experiment shown in Figure 5.

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Supplementary Table SI: Antibodies used in this study

			Antigen	Format	isotype	Clone	Source	Pretreatment
Immuno-histo-chemistry	Primary antibodies	Mouse anti-human	CD3		IgG1	F7.2.38	DakoCytomation	Boiling in TE buffer, pH 9 in a pressure cooker for 10 min.
			CD4		IgG1	4B12	Novocastra	Boiling in citrate buffer pH 6 in a pressure cooker for 10 min.
			CD8		IgG1	C8/144B	DakoCytomation	Boiling in citrate buffer pH 6 in a pressure cooker for 10 min.
			Granulysin (9kD+15kD form)		IgG2a	RF10	MBL Internat.	Boiling in citrate buffer pH 6 in a pressure cooker for 10 min.
			GranzymeB		IgG2a	GrB-7	Monosan	Boiling in citrate buffer pH 6 in a pressure cooker for 10 min.
		Goat anti-human	NKp46		polyclonal	AF1850	R&D systems	Boiling in Dako Target Retrieval Solution, pH 6 in a pressure cooker for 10 min.
	Secondary antibodies	Rabbit-anti-mouse biotinylated			polyclonal		DakoCytomation	
		Rabbit-anti-goat biotinylated			polyclonal		DakoCytomation	
		Goat anti-mouse IgG2a		FITC			Behtyl	
		Goat anti-mouse IgG1		AF594			Invitrogen	
Flow cytometry		Mouse anti-human	CD3	APC-Cy7	IgG1	SK7	BD Pharmingen	
			CD4	PerCP	IgG1	RPA-T4	BioLegend	
			CD8	PE-Cy7	IgG1	SFC121Thy2D3	BeckmanCoulter	
			CD69	PE	IgG1	FN50	BD Pharmingen	
			NKp46	AF647	IgG1	9E2	Biolegend	
			Granulysin (9kD+15kD)	AF488	IgG1	RB1	BD Pharmingen	

Supplementary Table SII: Patient's characteristics and drugs used for experiment shown in Figure 5

Patient	Clinical presentation	In vitro testing					
		Culprit drug	Final Concentration	LTT (SI)	Tolerated drug	Final Concentration	LTT (SI)
01	Maculopapular exanthema	Amoxicillin	200 µg/ml	26.0	-	-	-
02	Maculopapular exanthema	Ibuprofen	25 mM	117.0	Sulfamethoxazole	100 µg/ml	<2
03	Maculopapular exanthema	Amoxicillin	200 µg/ml	11.9	Cefuroxim	100 µg/ml	<2
04	Maculopapular exanthema	Amoxicillin	200 µg/ml	39.5	Phenprocoumonum	50 µg/ml	<2
05	Maculopapular exanthema	Sulfamethoxazole	100 µg/ml	24.0	Amoxicillin	200 µg/ml	<2
06	Maculopapular exanthema	Oxipurinol	100 µg/ml	63.9	Phenprocoumonum	50 µg/ml	<2
07	Maculopapular exanthema	Amoxicillin	200 µg/ml	5.6	-	-	-
08	Maculopapular exanthema	Sulfamethoxazole	100 µg/ml	21.0	-	-	-