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EXPERIMENTAL ALLERGY AND IMMUNOLOGY

Allergic sensitization to pegylated interferon- α results in drug eruptions

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Keywords

chemokines; drug eruption; lymphocyte activation test; maculopapular exanthema; pegylated Interferon.

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Abstract

Background: The introduction of pegylated interferon (PEG-IFN)- α in the treatment of chronic hepatitis C has led to an increase in sustained virological response. Despite reduced immunogenicity of the pegylated form in comparison with native interferon (IFN)- α , a high frequency of adverse cutaneous reactions has been reported with pegylated IFN- α . Here, we aimed to investigate the immunological mechanisms underlying pegylated IFN- α -induced drug eruptions.

Methods: Hepatitis C patients suffering from drug eruptions in association with administration of pegylated interferons were enrolled in the study ($n = 22$). Subjects were tested for sensitivity to pegylated IFN- α_{2a} , pegylated IFN- α_{2b} , or ribavirin using intradermal, scratch, and/or patch tests, as well as lymphocyte activation tests (LATs). Skin biopsies obtained from pegylated IFN- α -associated exanthemas, as well as from localized inflammatory skin reactions at pegylated IFN- α injection sites, were analyzed for the expression of relevant chemokines by quantitative real-time PCR and immunohistochemistry.

Results: A subset of patients suffering from pegylated IFN- α -associated exanthemas displayed positive intradermal tests to PEG-IFNs but not to conventional IFN (11/22). In selected patients, this observation correlated with the presence of pegylated IFN-specific T cells (3/11). Chemokine profiles of inflammatory skin reactions at the injection sites reflected an IFN- α -signature, whereas lesional skin of exanthemas showed induction of TH2-associated chemokines.

Conclusions: Our results indicate that specific sensitizations are one cause of exanthemas under therapy with PEG-IFNs. Clinical proof-of-concept analyses demonstrate that affected patients may benefit from a switch to conventional, nonpegylated drugs, enabling IFN- α therapy continuation without drug-associated skin eruptions.

Chronic hepatitis C (CHC), resulting from hepatitis C virus (HCV) infection, is a leading cause of chronic liver disease, cir-

Abbreviations

ADR, adverse drug reaction; CHC, chronic hepatitis C; Cpm, counts per minutes; HCV, hepatitis C virus; HS, healthy skin; IFN, interferon; IT, intradermal test; LAT, lymphocyte activation test; L, lesional; n.d., not determined; NL, nonlesional; PEG-IFN, pegylated interferon; PEG, polyethylene glycol; SI, stimulation indices; SOI, site of injection.

rhosis, and hepatocellular carcinoma (1, 2). Interferon (IFN)- α remained the sole treatment option for individuals infected with HCV until ribavirin was introduced (3, 4). Later, pegylated interferons (PEG-IFNs) led to substantially improved sustained virological response rates compared to the unmodified protein (5, 6). Pegylation refers to the modification of proteins by adding polyethylene glycol (PEG), leading to enhanced delivery, prolonged pharmacologic activity, and potentially reduced immunogenicity and antigenicity (5, 7). Despite the advantages of PEG-IFN, cessation of therapy is often reported

due to significant adverse drug reactions (ADRs) occurring in up to 48% of the treated patients (8–15).

Adverse drug reaction can be divided into two categories based on underlying pathomechanisms: type A reactions, defined by unanticipated pharmacologic actions, and, type B reactions characterized by intolerance, idiosyncratic, and allergic or hypersensitivity reactions (16). The underlying pathomechanisms of ADRs caused by PEG-IFNs have remained largely elusive (8–15). The frequently observed localized inflammatory skin lesions at the site of PEG-IFN injection may reflect the pro-inflammatory properties of IFN- α . On the other hand, PEG-IFN-induced exanthemas are characterized by the development of initial lesions at the site of injection (SOI) and a subsequent dissemination. This clinical course resembles the evolution of heparin/heparinoid-induced drug eruption, a typical T cell-mediated type B ADR (17). Based on these clinical observations, we sought to identify immunological mechanisms underlying PEG-IFN-induced localized inflammatory and disseminated exanthematic drug eruptions.

Methods

Patients

Biopsies were taken, after obtaining informed consent, from localized inflammatory lesions (SOI), generalized exanthema lesions, and nonlesional skin of patients under PEG-IFN treatment. Additional biopsies were taken from positive intradermal tests. Biopsies obtained from age-matched healthy individuals served as controls. Samples were divided, snap-frozen, and stored at -80°C . This study was performed in accordance with the guidelines of the Declaration of Helsinki and was approved by the local ethics committee.

Atopy status was defined by one or more of the following criteria: (i) reported personal history of an allergic disorder; (ii) positive prick tests to common aeroallergens; (iii) serological detection of specific IgE to common aeroallergens by ImmunoCAP; and/or (iv) total serum IgE concentration of >100 kU/l in the absence of other known causes of elevated IgE. Healthy volunteers had no history of atopic disease and negative prick tests.

Cutaneous tests

Cutaneous tests were performed in accordance with the EAACI recommendations (18). Injections of 0.03–0.05 ml IFN- α_{2a} (3 Mio. I.E./0.5 ml), PEG-IFN- α_{2a} (135 μg /0.5 ml), and PEG-IFN- α_{2b} (150 μg /0.5 ml) were used for intradermal testing. Normal saline served as negative control and histamine as positive control. For patch testing, 0.03–0.05 ml of each undiluted drug on filter paper disks in Finn chambers (12 mm; Epitest Ltd., Tuusula, Finland) applied on nonlesional back skin was used. Sodium lauryl sulfate and saline served as controls.

Immediate readings of prick and intradermal tests were performed at 20 min and were considered positive when the diameter of the reaction was >3 mm. Delayed intradermal readings were evaluated at 24, 48, 72, 96, 120, 144 and 168 h. Erythema, infiltration and papules or vesicles were considered as positive in delayed reading. Patch tests were evaluated at 48 and 96 h.

Quantitative real-time PCR analysis

Biopsies were homogenized using a Mikro-Dismembrator U (Sartorius AG, Goettingen, Germany), and RNA was extracted with RNazol (Tel-Test, Friendswood, TX, USA). The resulting RNA was reverse transcribed, and gene expression was analyzed using quantitative real-time PCR (ABI PRISM[®] 7000 Sequence Detection System; Perkin Elmer, Waltham, MA, USA) as described previously (19).

Lymphocyte activation tests (LAT)

T cell activation was measured using BD FastImmune tests (CD69/CD3; BD Bioscience, San Jose, CA, USA). Briefly, whole blood from patients or healthy donors was stimulated for 4 h (37°C). Anti-CD2/CD2R antibodies (BD Bioscience, San Jose, CA, USA) served as positive controls. Anti-CD3-PerCP and anti-CD69-PE (50 μl) were added to each sample. Cells were analyzed by FACScan flow cytometer (BD Bioscience, San Jose, CA, USA) using CELLQuest[™]-Pro software (BD Bioscience, San Jose, CA, USA). CD3⁺ lymphocytes were displayed as two-color dot plots to measure the proportion of activated lymphocytes expressing CD69. Stimulation indices (SI) were calculated as a ratio of counts per minute (Cpm) of cells cultured in the presence vs the absence of antigen.

Histology and immunohistochemistry

Skin sections were fixed with acetone and preprocessed with H_2O_2 followed by an avidin and biotin blocking step (Vector Laboratories, Burlingame, CA, USA). Sections were stained for CCL18 (polyclonal goat IgG; R&D Systems, Minneapolis, MN, USA) and CCL22 (goat IgG; Santa Cruz Biotechnology, Inc., Dallas, TX, USA) using appropriate isotype antibodies as negative controls and the Vectastain ABC and AEC Kits (Vector Laboratories, Burlingame, CA, USA). Sections were counterstained with hematoxylin.

Cell culture and treatment of cell lines

Human primary epidermal keratinocytes, dermal fibroblasts, and dermal microvascular endothelial cells (Lonza, Walkersville, MD, USA) were cultured in specialized media (KGM-2, FGM-2, and EGM-2) as previously described (20, 21). Cells were treated with IFN- α (100, 500, or 1000 units/ml; R&D Systems, Minneapolis, MN, USA) or left untreated.

Statistical analysis

Unpaired Mann–Whitney *U*-tests were performed using GraphPad Prism 5.03 (GraphPad Software Inc., La Jolla, CA, USA). *P* values <0.05 were considered statistically significant.

Results

Characterization of patients

A total of 216 CHC patients and 55 patients with chronic hepatitis B (total *n* = 271), treated with PEG-IFNs, were evaluated. Among these, 22 patients (8.1%) developed drug eruptions and underwent additional allergological examinations. The time span between the first drug administration and appearance of the symptoms ranged from 2 weeks to 24 months. Initially, patients displayed localized inflammatory skin lesions at the SOI and subsequently developed generalized pruritic maculopapular exanthemas (Fig. 1). Histological analyses of skin specimens were consistent with the clinical diagnosis of drug eruption showing perivascular inflammation with or without spongiosis. The infiltrate was

mainly composed of lymphocytes; in some cases, eosinophils, histiocytes, or neutrophils were also observed. Atopy status was assessed by means of self-reported atopic disease, clinical examinations, total IgE levels, and allergen-specific IgE. Notably, all patients showing a positive LAT to PEG-IFNs had a negative atopy status.

Cutaneous tests

Patients with PEG-IFN-induced drug eruptions (*n* = 22) as well as healthy individuals (*n* = 3) underwent intradermal tests with conventional IFN- α_{2a} , PEG-IFN- α_{2a} , and PEG-IFN- α_{2b} (Table 1). After 20 min, no intradermal reactions to IFN- α_{2a} , PEG-IFN- α_{2a} , or PEG-IFN- α_{2b} were observed. Healthy controls did not display any positive skin reaction but exhibited adverse effects, including flu-like symptoms and myalgia, nausea, vomiting, or reversible paresthesia. Conversely, 11 of the 22 patients with drug eruptions (50%) showed delayed positive reactions to PEG-IFN- α_{2b} . Six of these patients (6/11) also demonstrated hypersensitivity to PEG-IFN- α_{2a} , whereas one patient (1/11) exhibited a reaction to all tested IFNs. Skin scratch tests did not indicate

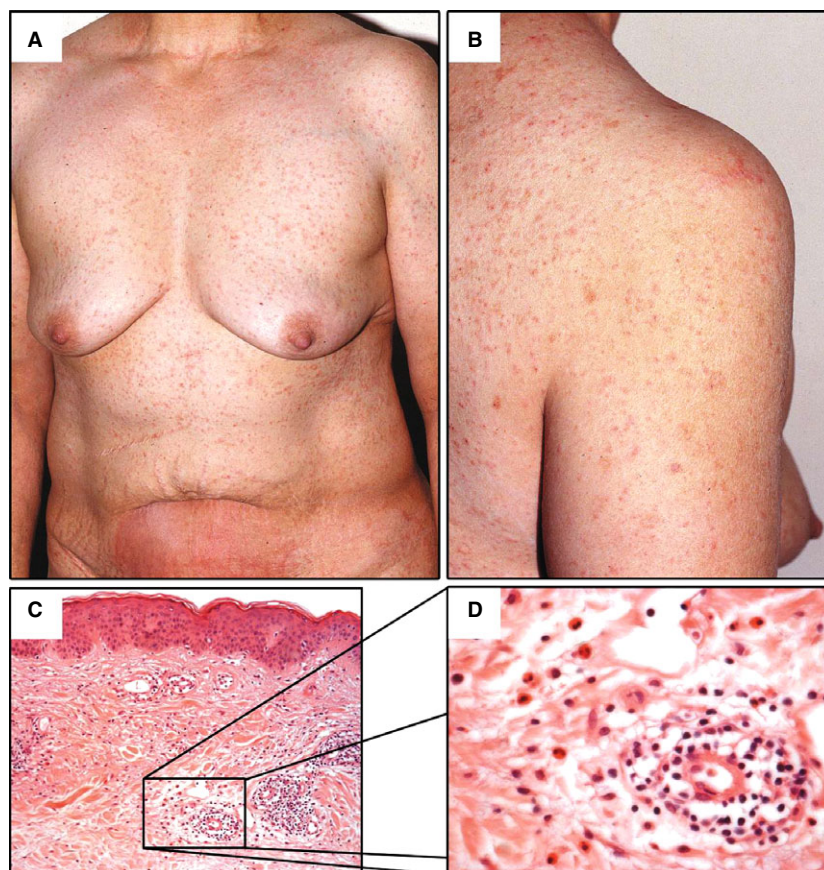


Figure 1 Clinical images from localized inflammatory skin lesions at the site of injection of pegylated interferon- α_{2b} and erythematous macules and papules scattered on the trunk and extremities at week 8

(A, B). Histological examination showing a perivascular and interstitial dermatitis (C). The inflammatory infiltrate composed of lymphocytes and eosinophils (D). (Original magnification $\times 100$ in C; $\times 400$ in D).

Table 1 Administrated drugs and test results

Administrated drug	Onset after initiation of treatment	<i>In vitro</i> tests			<i>In vivo</i> tests			
		SI in LAT [100 ng/ μ l]			Intradermal tests			Scratch
		Peginterferon- α_{2b}	Peginterferon- α_{2a}	Interferon- α_{2a}	Peginterferon- α_{2b}	Peginterferon- α_{2a}	Interferon- α_{2a}	Ribavirin
1. Peginterferon- α_{2b}	2 months	11.1	2.2	2.4	D3	D3	Negative	Negative
2. Peginterferon- α_{2b}	10 months	2.0	4.5	1.3	D6	D6	Negative	Negative
3. Peginterferon- α_{2b}	4 months	1.0	2.8	1.1	D1	Negative	Negative	Negative
4. Peginterferon- α_{2b}	2 weeks	4.2	3.5	1.5	D1	D1	D1	Negative
5. Peginterferon- α_{2a}	24 months	0.9	2.5	2.1	Negative	Negative	Negative	Negative
6. Peginterferon- α_{2a}	2 months	n.d.	n.d.	n.d.	D3	Negative	Negative	Negative
7. Peginterferon- α_{2b}	3 months	n.d.	n.d.	n.d.	D3	Negative	Negative	Negative
8. Peginterferon- α_{2a}	3 weeks	n.d.	n.d.	n.d.	D7	D7	Negative	Negative
9. Peginterferon- α_{2b}	3 months	n.d.	n.d.	n.d.	D1	D1	Negative	Negative
10. Peginterferon- α_{2b}	3 months	n.d.	n.d.	n.d.	D2	D3	Negative	Negative
11. Peginterferon- α_{2b}	3 months	n.d.	n.d.	n.d.	D2	Negative	Negative	Negative
12. Peginterferon- α_{2a}	3 weeks	n.d.	n.d.	n.d.	D2	Negative	Negative	Negative
13. Interferon- α	1–2 months	n.d.	n.d.	n.d.	Negative	Negative	Negative	Negative
14. Peginterferon- α_{2b}	3 months	n.d.	n.d.	n.d.	Negative	Negative	Negative	Negative
15. Peginterferon- α_{2b}	14 months	n.d.	n.d.	n.d.	Negative	Negative	Negative	Negative
16. Peginterferon- α_{2b}	3 months	n.d.	n.d.	n.d.	Negative	Negative	Negative	Negative
17. Peginterferon- α_{2a}	2 months	n.d.	n.d.	n.d.	Negative	Negative	Negative	Negative
18. Peginterferon- α_{2a}	2 days	n.d.	n.d.	n.d.	Negative	Negative	Negative	Negative
19. Peginterferon- α_{2a}	1.5 months	n.d.	n.d.	n.d.	Negative	Negative	Negative	Negative
20. Peginterferon- α_{2b}	2 months	n.d.	n.d.	n.d.	Negative	Negative	Negative	Negative
21. Peginterferon- α_{2a}	3–4 months	n.d.	n.d.	n.d.	Negative	Negative	Negative	Negative
22. Peginterferon- α_{2a}	4 months	n.d.	n.d.	n.d.	Negative	Negative	Negative	Negative
Controls								
23. Peginterferon- α_{2b} (Hepatitis C pos. control)	–	n.d.	n.d.	n.d.	Negative	Negative	Negative	Negative
24. Interferon- α (Hepatitis C pos. control)	–	n.d.	n.d.	n.d.	Negative	Negative	Negative	Negative
25. None (healthy control)	–	n.d.	n.d.	n.d.	Negative	Negative	Negative	Negative
26. None (healthy control)	–	n.d.	n.d.	n.d.	Negative	Negative	Negative	Negative
27. None (healthy control)	–	n.d.	n.d.	n.d.	Negative	Negative	Negative	Negative
28. None (healthy control)	–	1.3	0.8	0.6	n.d.	n.d.	n.d.	n.d.
29. None (healthy control)	–	1.4	0.9	0.6	n.d.	n.d.	n.d.	n.d.

SI, stimulation index; LAT, lymphocyte activation test.

Summary indicating the administrated drug, the onset of drug eruption, and the respective test results (d: day, n.d.: not determined). Stimulation indexes calculated as the x-fold increase of the CD69 upregulation after stimulation with the drug compared to cultures without drug.

any sensitization against ribavirin. Furthermore, patch testing with IFN- α_{2a} , PEG-IFN- α_{2a} , PEG-IFN- α_{2b} , and ribavirin did not induce any skin reactions (data not shown).

Expression of chemokines and interferon regulatory factor *in vivo*

To further understand the nature of these skin reactions, we set out to analyze gene expression changes associated with PEG-IFN treatment in the following sets of samples: (i) localized inflammatory skin lesions at SOI ($n = 3$), (ii) distant to SOI in patients suffering from PEG-IFN-induced exanth-

emas ($n = 10$), (iii) nonlesional skin of patients suffering from PEG-IFN-induced exanthemas ($n = 8$), and (iv) positive intradermal tests to PEG-IFN or conventional interferons ($n = 7$). We first investigated expression of the IFN- α -inducible gene IRF7, a biomarker for the pharmacological activity of IFN. As expected, we observed a significant upregulation of IRF7 expression at the SOI of PEG-IFN and, to lesser extent, in lesional skin of the exanthemas (Fig. 2). These results indicate that PEG-IFN exerts pharmacological effects both locally and systemically. Furthermore, we investigated the expression of selected chemokines and detected an abundant induction of the IFN- α -inducible, TH1-associated

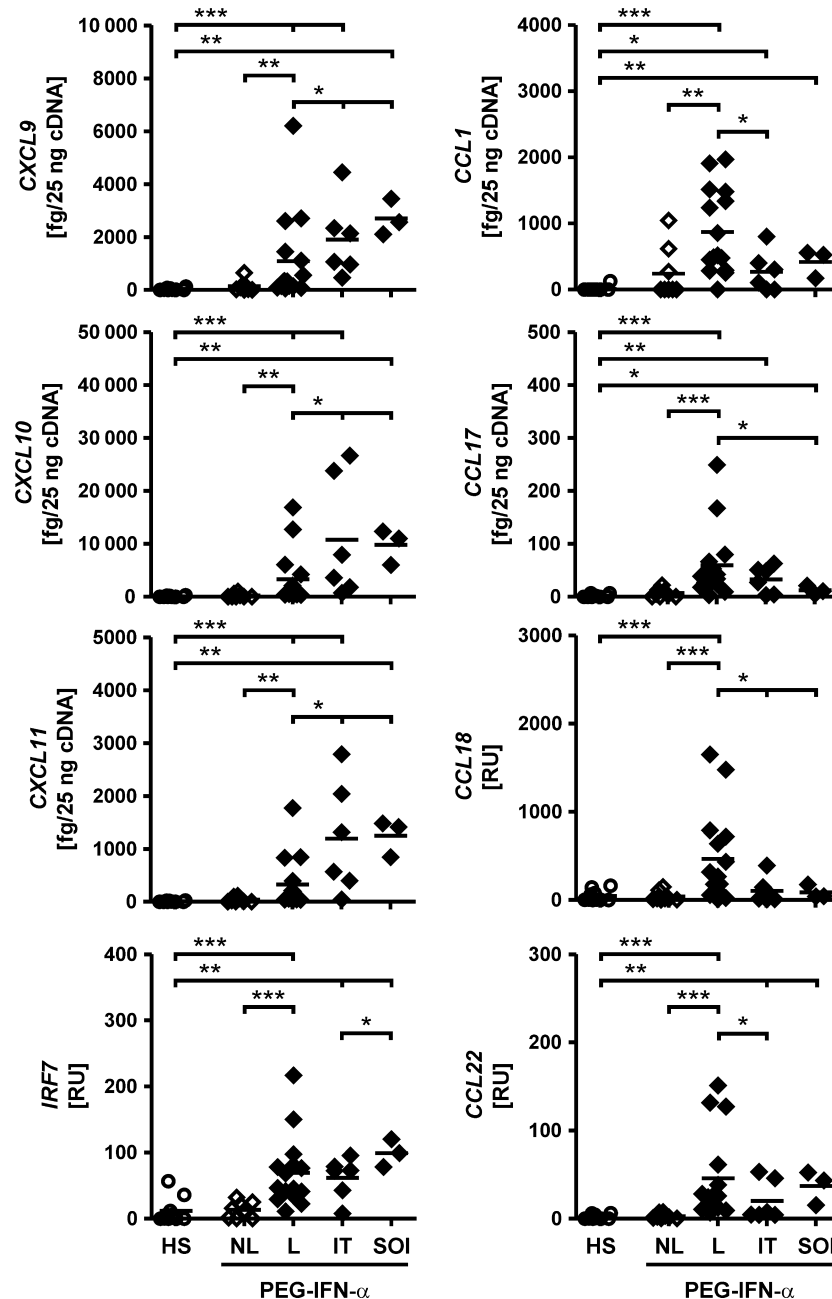


Figure 2 Quantitative RT-PCR analysis of IFN- α -inducible IRF7, CXCL9, CXCL10, CXCL11, and chemokines CCL1, CCL17, CCL18, and CCL22 in healthy skin and PEG-IFN-induced exanthemas at site

of PEG-IFN injection (SOI), lesional skin (PEG-IFN (L)), nonlesional areas (PEG-IFN (NL)), and biopsies after intradermal testing of PEG-IFNs (PEG-IFN (IT)), * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

chemokines CXCL9-11 at SOI as well as skin specimens of positive intradermal tests to pegylated or conventional IFN (Fig. 2). A similar induction was observed for PEG-IFN-induced exanthemas, albeit at lower levels.

In contrast, the TH2-associated chemokines CCL17 and CCL18 were significantly induced solely in exanthematic lesions distant to SOI (Fig. 2). Similar expression profiles were observed for other TH2-associated chemokines such as

CCL1 and CCL22 but without reaching statistical significance. Immunohistochemical analyses demonstrated that CCL18 protein was induced in cells with dendritic morphology within epidermal and dermal compartments (Fig. 3). Expression of CCL22 was observed in basal keratinocytes, skin-infiltrating leukocytes, and cells with dendritic morphology. CCL18 and CCL22 were absent in healthy skin (results not shown).

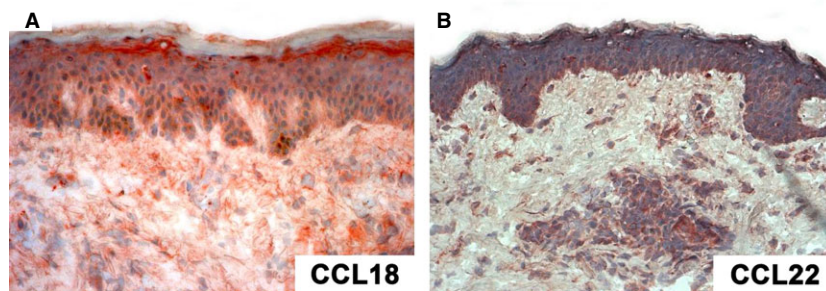


Figure 3 Immunohistochemical analysis of CCL18 (A) and CCL22 (B) in lesional skin specimens obtained from patients with drug eruptions

after administration of pegylated interferons (PEG-IFNs). (Original magnification $\times 200$).

Effect of IFN- α on structural cells of the skin *in vitro*

To rule out that upregulation of TH2-associated chemokines reflects a direct pharmacologic effect of IFN- α , we next analyzed the effects of IFN- α on structural cells of the skin *in vitro*. We previously demonstrated that IFN- α rapidly and markedly induces expression of the TH1-associated chemokines CXCL9-11 in structural cells of the skin (22). Here, we broadened our previous survey to include CCL1, CCL17, CCL18, and CCL22 (Fig. 4). IFN- α had no relevant effect on the expression of these genes, although we detected robust induction of IRF7 in all cell types.

Lymphocyte activation tests

To investigate whether the observed exanthema and localized inflammatory reactions were mediated by activation of drug-specific T cells, we performed LATs by measuring the upregulation of CD69 on CD3⁺ lymphocytes (Fig. 5). Beeler et al. (23) define a SI cutoff value of 2 to discriminate between allergic and nonallergic patients. As IFN- α represents an immunostimulatory cytokine, we first performed quality control experiments and conducted dose-response experiments with PBMCs of healthy individuals. In dose-response curves of nonallergic, nonexposed healthy controls, the SI value peaked

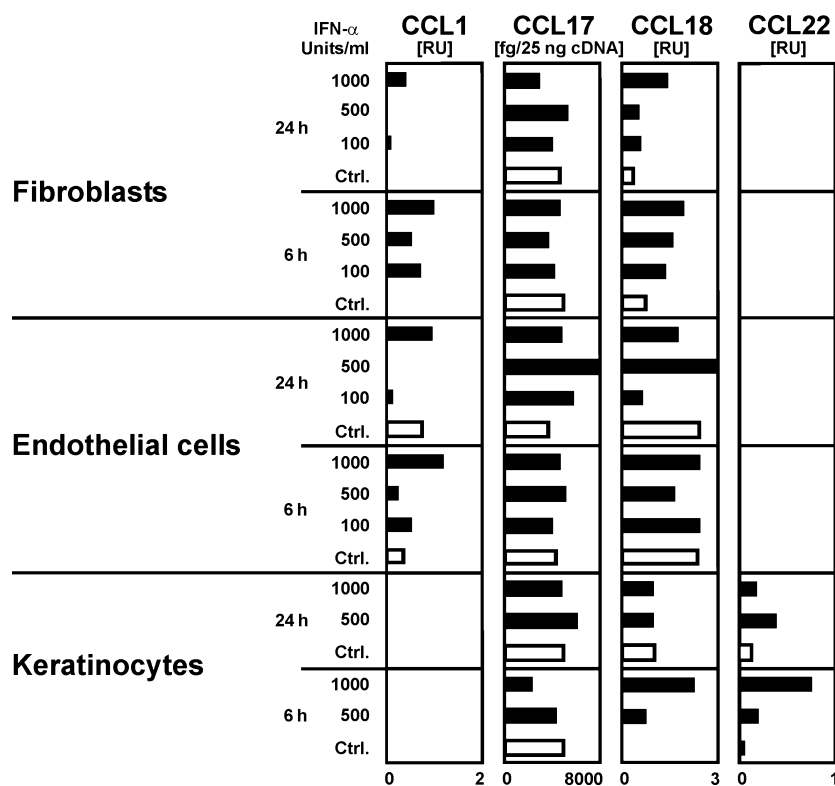


Figure 4 Quantitative RT-PCR analysis of TH2-associated chemokines CCL1, CCL17, CCL18, and CCL22, in dermal fibroblasts, dermal endothelial cells, and cultured human primary keratinocytes

stimulated with increasing doses of IFN- α . Gene expression is shown as absolute units (CCL17) or as relative units (CCL1, CCL18, and CCL22). (Representative results from three experiments).

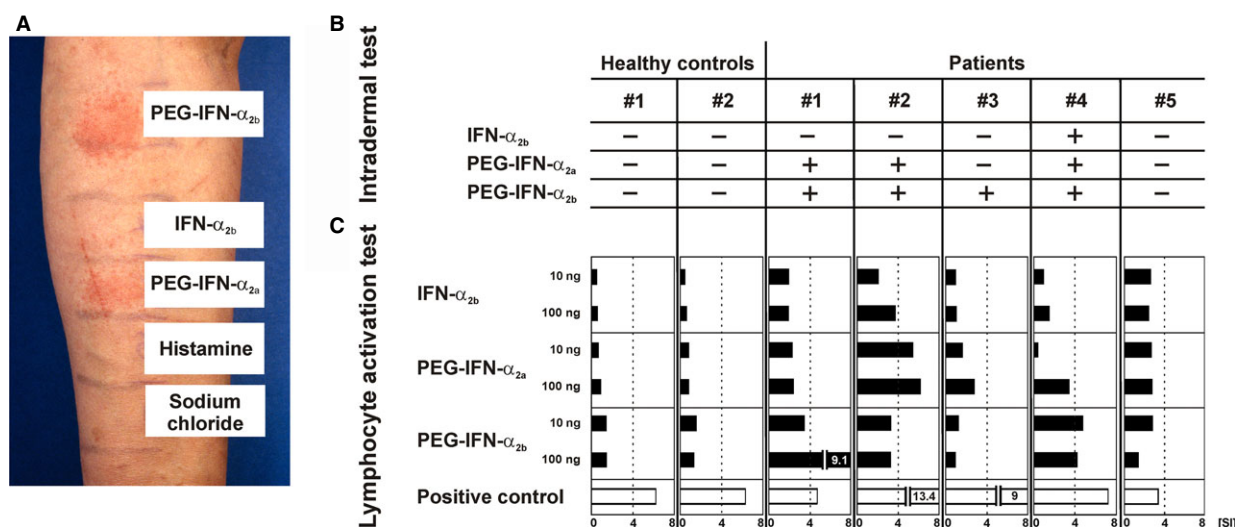


Figure 5 Representative findings of intradermal tests of interferon- α_{2a} , peginterferon- α_{2a} , and peginterferon- α_{2b} (A). Results of cutaneous tests of two healthy controls and five patients with

drug eruptions after administration of pegylated interferons (PEG-IFNs) (B). Corresponding results of lymphocyte activation tests (C).

at 3.1 in response to 1000 ng/ml PEG-IFN- α_{2b} (data not shown). However, in these individuals, no SI > 2 was observed at lower concentrations (10–100 ng/ml). Hence, to avoid false-positive results, we classified tested PEG-IFNs and IFN as positive, only when the SI was > 4 in response to 100 ng/ml, as recommended by Pichler et al. (24).

We then performed LATs with blood cells from five patients with PEG-IFN-associated ADRs, four of which displayed positive results in the intradermal tests (+ID). LATs were positive (SI > 4) in response to pegylated or conventional IFN in three of four +ID patients and negative for the single –ID patient (Fig. 5).

Taken together, we detected drug-specific T cells to PEG-IFN in 3 of 5 (60%) of the tested patients.

Discussion

The long-term use of PEG-IFN is hampered by the development of ADRs (8–15). We found ADRs to PEG-IFN in 22 of 271 patients (8.1%), which consisted of exanthema in the vast majority of the cases (Table 1). Withdrawal of PEG-IFN and topical treatment with glucocorticoids resulted in resolution of the lesions, suggesting that PEG-IFN was the causative agent. Moreover, a subset of these patients was switched without any further ADR to a combination therapy of nonpegylated IFN and ribavirin.

Our clinical observations are in accordance with publications by other groups (10, 12, 25). Veldt et al. (10) reported a patient with a drug eruption under therapy with PEG-IFN and ribavirin that tolerated subsequent therapy with conventional IFN and ribavirin. Cottoni et al. (12) observed ADRs using PEG-IFNs in combination with amantadine and/or oral ribavirin, necessitating a termination of the treatment. Finally, Li et al. presented a CHC-positive woman tolerating native but not pegylated IFN (10, 12, 25).

To characterize the PEG-IFN-related ADRs, we conducted a series of tests on patients with treatment-associated exanthema. In intradermal tests, 11 of 22 patients (50%) exhibited a positive reaction to PEG-IFN (Table 1). Only one of these 11 patients showed a positive reaction to conventional IFN. With regard to intradermal tests of PEG-IFN, healthy controls showed systemic reactions to the drug (flu-like symptoms) but no cutaneous symptoms. Hence, positive results in the intradermal tests were suggestive of sensitivity to PEG-IFN.

Chemokines play a central role in inflammation by recruiting specific leukocyte subsets to the site of irritation. Additionally, certain chemokine expression profiles are associated with distinct reactions, including ADRs. Increased expression of TH1- and TH2-associated chemokines has been reported in drug-induced exanthema (26, 27). As T cells are likely involved in PEG-IFN-associated ADRs, we chose to focus on whether distinct patterns of TH1 (e.g., CXCL9, CXCL10 and CXCL11) or TH2 (CCL1, CCL17, CCL18, and CCL22)-associated chemokines were present in skin samples from PEG-IFN-sensitive patients. In addition, we have previously shown that IFN- α is a potent inducer of the CXCR3 ligands CXCL9, CXCL10, and CXCL11 *in vitro* (22). Here, we show that IFN also elicits an upregulation of CXCL9, CXCL10, and CXCL11 at the site of PEG-IFN injection, concomitant with an upregulation of the IFN response gene IRF7 (Fig. 2). These chemokines and IRF7 are also upregulated, to a lesser extent, in lesional skin of patients with exanthema. Thus, we detected an IFN- α -induced signature, indicating a direct pharmacological ADR associated with PEG-IFN.

In contrast to TH1-associated CXCR3 ligands, the TH2-associated chemokines CCL1, CCL17, CCL18, and CCL22 show a prominent upregulation within generalized exanthema but not at injection sites or in intradermal tests (Fig. 2). At

the SOI, the high transient concentration of IFN is likely to exert immediate effects on the local tissue cells that dominate immunomodulatory effects of the infiltrating TH2 cells. Notably, these TH2-associated chemokines are not regulated by IFN- α *in vitro* (Fig. 4). This differential expression of TH2 chemokines indicates that at least two molecular and/or cellular mechanisms are involved: (i) a delayed-type reaction via drug-specific T cells as well as (ii) direct pharmacological effects of (PEG-) IFN. The link to pegylation is further supported by results from the LAT experiments in which we detected PEG-IFN-specific T cells in a subset of patients (Fig. 5). Importantly, Tough et al. (28) did not observe an upregulation of CD69 in T lymphocytes poststimulation with type I IFNs.

Notably, we detected PEG-IFN-specific T cells but no specific T cells against conventional IFN. PEGs are considered to be inert, and pegylation of proteins is used to decrease immunogenicity and antigenicity (5, 7). Nevertheless, allergic contact dermatitis against PEG, an integral part of many cosmetics, has been described (29, 30). Furthermore, several studies have reported the development of antibodies against pegylated liposomes in humans as well as in animal models, suggesting that PEG moieties represent potential antigenic epitopes (31–35). The mechanism of T cell sensitization by PEG-IFNs remains unclear. It is possible that either the PEG molecule itself or the linkage between PEG and IFN resulting in a formation of new antigenic epitopes is causative. However, further experiments are required to clarify the exact nature of the elucidating antigen. Of note, a limitation of the present study is that neither PEG-specific T cell clones were generated nor other pegylated proteins were used as controls.

Although PEG-specific hypersensitivities are rare, these findings suggest that sensitizations against PEG may result in

a considerable clinical importance. This concept of PEG-specific T cells and hence the presence of a type B ADR in patients receiving PEG-IFNs is supported by the following findings: (i) the clinical observation showing that conventional, nonpegylated IFNs are tolerated, whereas PEG-IFNs are not, (ii) intradermal tests presenting positive results for PEG-IFNs and seldom for conventional, nonpegylated IFN, (iii) LATs detecting drug-specific T cells, and (iv) the induction of TH2-associated chemokines (CCL1, CCL17, CCL18, CCL22) in exanthematic lesions but not at sites of PEG-IFN administration.

The differentiation between pharmacological type A reactions and allergy-induced type B reactions has direct therapeutic implications. The identification of drug-specific T cells in patients may grant these patients the option to continue antiviral treatment using conventional IFN.

Author contributions

S.M. and P.A.G. performed and analyzed most of the experiments in this study and participated in their design. A.K. performed and analyzed LATs. U.W. performed cell culture experiments. E.B. performed histological stainings, microscopy, and analyses of histological data. S.K. and P.H. participated in real-time PCR analyses. T.G., A.E., S.M., and B.H. collected and characterized clinical samples and participated in the expression analyses. S.M., P.A.G., A.K., and B.H. wrote the manuscript. A.Z., D.H., A.E., and B.H. supervised the study and were involved in the design, drafting, and revising the manuscript.

Conflict of interest

The authors declare that there is no conflict of interest.

References

- Liang TJ, Rehmann B, Seeff LB, Hoofnagle JH. Pathogenesis, natural history, treatment, and prevention of hepatitis C. *Ann Intern Med* 2000;**132**:296–305.
- Cohen J. The scientific challenge of hepatitis C. *Science* 1999;**285**:26–30.
- Davis GL, Balart LA, Schiff ER, Lindsay K, Bodenheimer HC Jr, Perrillo RP et al. Treatment of chronic hepatitis C with recombinant interferon α . A multicenter randomized, controlled trial. Hepatitis Interventional Therapy Group. *N Engl J Med* 1989;**321**:1501–1506.
- McHutchison JG, Gordon SC, Schiff ER, Shiffman ML, Lee WM, Rustgi VK et al. Interferon α -2b alone or in combination with ribavirin as initial treatment for chronic hepatitis C. Hepatitis Interventional Therapy Group. *N Engl J Med* 1998;**339**:1485–1492.
- Youngster S, Wang YS, Grace M, Bausch J, Borden R, Wyss DF. Structure, biology, and therapeutic implications of pegylated interferon α -2b. *Curr Pharm Des* 2002;**8**:2139–2157.
- Manns MP, McHutchison JG, Gordon SC, Rustgi VK, Shiffman M, Reindollar R et al. Peginterferon α -2b plus ribavirin compared with interferon α -2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. *Lancet* 2001;**358**:958–965.
- Harris JM, Martin NE, Modi M. Pegylation: a novel process for modifying pharmacokinetics. *Clin Pharmacokinet* 2001;**40**:539–551.
- Meller S, Erhardt A, Auci A, Neumann NJ, Homey B. Drug-induced exanthema caused by pegylated interferon- α 2b. *Hautarzt* 2003;**54**:992–993.
- Hashimoto Y, Kanto H, Itoh M. Adverse skin reactions due to pegylated interferon α 2b plus ribavirin combination therapy in a patient with chronic hepatitis C virus. *J Dermatol* 2007;**34**:577–582.
- Veldt BJ, Schalm SW, Janssen HL. Severe allergic eczema due to pegylated α -interferon may abate after switching to daily conventional α -interferon. *J Clin Gastroenterol* 2007;**41**:432.
- Milkiewicz P, Yim C, Pache I, Heathcote J. Diffuse skin reaction in patient with hepatitis B, treated with two different formulations of pegylated interferon. *Can J Gastroenterol* 2005;**19**:677–678.
- Cottoni F, Bolognini S, Deplano A, Garruciu G, Manzoni NE, Careddu GF et al. Skin reaction in antiviral therapy for chronic hepatitis C: a role for polyethylene glycol interferon? *Acta Derm Venereol* 2004;**84**:120–123.
- Kawada K, Maeda N, Kobayashi S, Sowa J, Tsuruta D, Kawada N. Injection site with generalized rash caused by pegylated interferon α 2a injection. *Dermatology* 2006;**212**:82–83.
- Tavakoli-Tabasi S, Bagree A. A longitudinal cohort study of mucocutaneous drug eruptions during interferon and ribavirin treatment of hepatitis C. *J Clin Gastroenterol* 2012;**46**:162–167.

15. Fried MW. Side effects of therapy of hepatitis C and their management. *Hepatology* 2002;**36**(5 Suppl 1):S237–S244.
16. Posadas SJ, Pichler WJ. Delayed drug hypersensitivity reactions – new concepts. *Clin Exp Allergy* 2007;**37**:989–999.
17. Schindewolf M, Lindhoff-Last E, Ludwig RJ, Boehncke WH. Heparin-induced skin lesions. *Lancet* 2012;**380**:1867–1879.
18. Brockow K, Romano A, Blanca M, Ring J, Pichler W, Demoly P. General considerations for skin test procedures in the diagnosis of drug hypersensitivity. *Allergy* 2002;**57**:45–51.
19. Homey B, Wang W, Soto H, Buchanan ME, Wiesenborn A, Catron D et al. Cutting edge: the orphan chemokine receptor G protein-coupled receptor-2 (GPR-2, CCR10) binds the skin-associated chemokine CCL27 (CTACK/ALP/ILC). *J Immunol* 2000;**164**:3465–3470.
20. Homey B, Dieu-Nosjean MC, Wiesenborn A, Massacrier C, Pin JJ, Oldham E et al. Up-regulation of macrophage inflammatory protein-3 alpha/CCL20 and CC chemokine receptor 6 in psoriasis. PG – 6621–32. *J Immunol* 2000;**164**:6621–6632.
21. Pivarcsi A, Muller A, Hippe A, Rieker J, van Lierop A, Steinhoff M et al. Tumor immune escape by the loss of homeostatic chemokine expression. *Proc Natl Acad Sci U S A* 2007;**104**:19055–19060.
22. Meller S, Winterberg F, Gilliet M, Muller A, Lauceviciute I, Rieker J et al. Ultraviolet radiation-induced injury, chemokines, and leukocyte recruitment: an amplification cycle triggering cutaneous lupus erythematosus. *Arthritis Rheum* 2005;**52**:1504–1516.
23. Beeler A, Zaccaria L, Kawabata T, Gerber BO, Pichler WJ. CD69 upregulation on T cells as an in vitro marker for delayed-type drug hypersensitivity. *Allergy* 2008;**63**:181–188.
24. Pichler WJ, Tilch J. The lymphocyte transformation test in the diagnosis of drug hypersensitivity. *Allergy* 2004;**59**:809–820.
25. Li Z, Ji F, Zheng Y, An J, Peng Z. Pegylated interferon, but not conventional interferon therapy induced severe skin lesions. *Ann Hepatol* 2012;**11**:570–571.
26. Fernandez TD, Mayorga C, Torres MJ, Cornejo-Garcia JA, Lopez S, Chaves P et al. Cytokine and chemokine expression in the skin from patients with maculopapular exanthema to drugs. *Allergy* 2008;**63**:712–719.
27. Tapia B, Morel E, Martin-Diaz MA, Diaz R, Alves-Ferreira J, Rubio P et al. Up-regulation of CCL17, CCL22 and CCR4 in drug-induced maculopapular exanthema. *Clin Exp Allergy* 2007;**37**:704–713.
28. Tough DF, Borrow P, Sprent J. Induction of bystander T cell proliferation by viruses and type I interferon in vivo. *Science* 1996;**272**:1947–1950.
29. Fisher AA. Immediate and delayed allergic contact reactions to polyethylene glycol. *Contact Dermatitis* 1978;**4**:135–138.
30. Hannuksela M, Pirila V, Salo OP. Skin reactions to propylene glycol. *Contact Dermatitis* 1975;**1**:112–116.
31. Judge A, McClintock K, Phelps JR, MacLachlan I. Hypersensitivity and loss of disease site targeting caused by antibody responses to PEGylated liposomes. *Mol Ther* 2006;**13**:328–337.
32. Richter AW, Akerblom E. Antibodies against polyethylene glycol produced in animals by immunization with monomethoxy polyethylene glycol modified proteins. *Int Arch Allergy Appl Immunol* 1983;**70**:124–131.
33. Richter AW, Akerblom E. Polyethylene glycol reactive antibodies in man: titer distribution in allergic patients treated with monomethoxy polyethylene glycol modified allergens or placebo, and in healthy blood donors. *Int Arch Allergy Appl Immunol* 1984;**74**:36–39.
34. Garay RP, El-Gewely R, Armstrong JK, Garratty G, Richette P. Antibodies against polyethylene glycol in healthy subjects and in patients treated with PEG-conjugated agents. *Expert Opin Drug Deliv* 2012;**9**:1319–1323.
35. Shimizu T, Ichihara M, Yoshioka Y, Ishida T, Nakagawa S, Kiwada H. Intravenous administration of polyethylene glycol-coated (PEGylated) proteins and PEGylated adenovirus elicits an anti-PEG immunoglobulin M response. *Biol Pharm Bull* 2012;**35**:1336–1342.