Hapten-Specific TH17 Cells in the Peripheral Blood of β-Lactam-Induced AGEP.

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Dear Editor

Hapten-Specific T_H17 Cells in the Peripheral Blood of β-Lactam-Induced AGEP

Acute generalized exanthematous pustolosis (AGEP) is a rare adverse drug reaction, frequently due to β-Lactams, characterized by skin eruption, nonfollicular sterile pustules, fever and neutrophilia with neutrophilic and, possibly, eosinophilic infiltrates.[1] T cell involvement has been postulated on the basis of positive *in vivo* patch tests, positive *in vitro* lymphocyte transformation test (LTT) towards the culprit drug, presence of infiltrating CD4 and CD8 lymphocytes in lesional skin and increase of neutrophil-recruiting CXCL8/IL-8-producing drug-specific T cells in the circulation.[1,2] However, as found into certain neutrophilic inflammatory processes similar to AGEP such as pustular psoriasis, regulated by the newly defined T_H17 effectors, also in AGEP a marked increase in IL-17+ cells along with IL-22 levels has been described, suggesting a downstream release of CXCL8/IL-8.[3,4] IL-17+ lymphocytes express retinoid acid related orphan receptor (ROR) γt transcription factor, activate neutrophils and, depending on the microenvironment, can acquire the ability to also produce IFN-γ or IL-4 differentiating towards a mixed phenotype.[5,6]

To disclose the possible involvement of IL-17 in AGEP, we investigated the functional phenotype of hapten-specific T cell clones (TCCs) derived from the peripheral blood of a patient with history of β-Lactam-induced AGEP. As controls, amoxicillin-specific TCCs from anaphylaxis and Stevens-Johnson syndrome (SJS) were used. Further, we studied the expression of the T_H17-related transcription factor RORγt in the skin biopsy from the amoxicillin-specific patch test of the donor with history of AGEP.

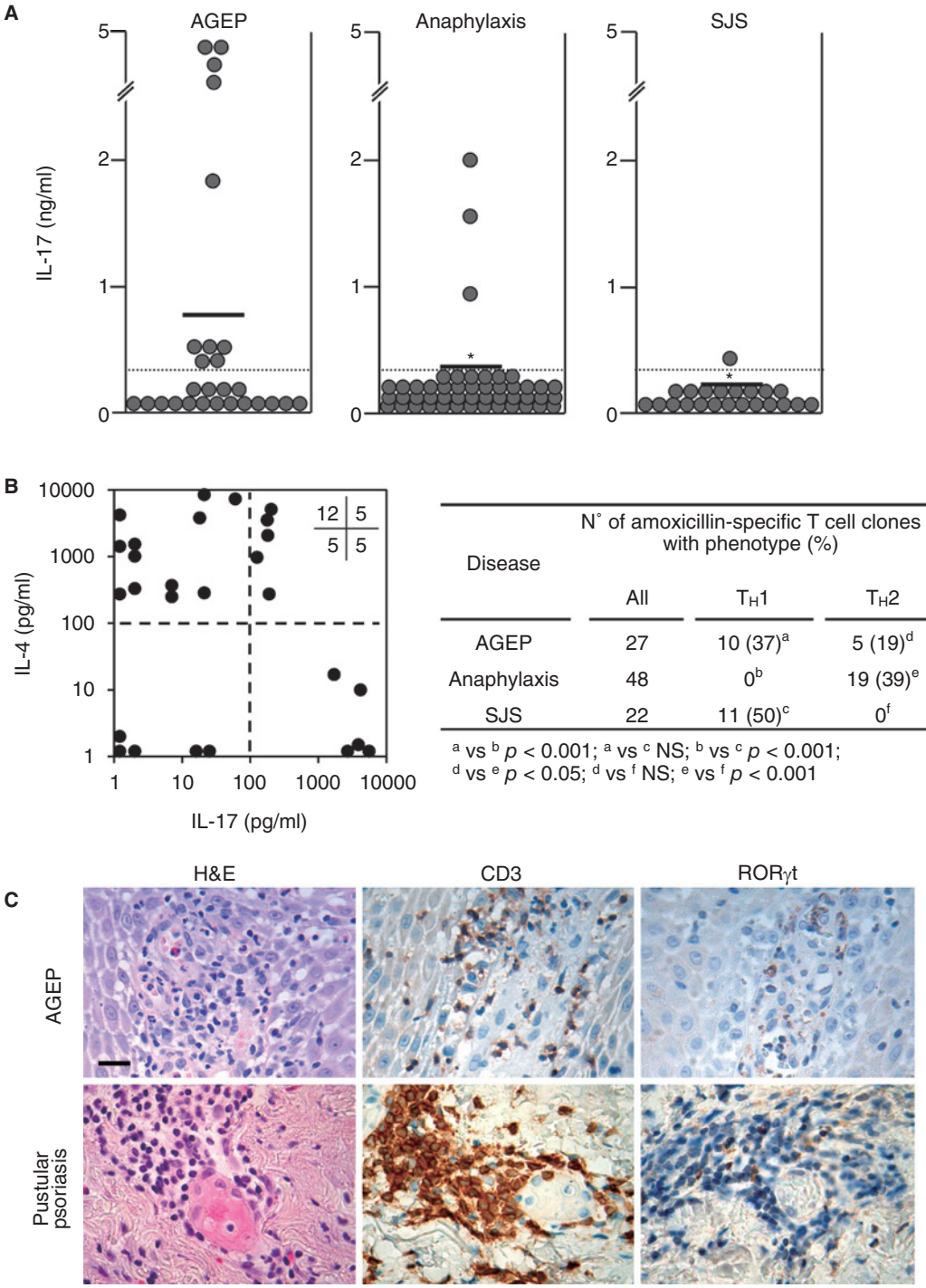
L.P. was a 55 yr woman referring AGEP. F.A. was a 62 yr woman referring anaphylaxis. P.M. was a 35 yr man referring Stevens-Johnson syndrome (SJS). The different clinical pictures were all due to the oral intake of amoxicillin. All the patients exhibited positive LTT towards amoxicillin. L.P. and F.A. also showed amoxicilloyl-specific serum IgE. Informed written consent was obtained in accordance with the ethical standards of the responsible regional Committee. T cell clones were obtained under limiting dilution (0.3 cell/well) from hapten-specific T cell lines generated stimulating 2×10^6 PBMCs with amoxicillin (0.5 mg/ml) for 6 days in complete RPMI 1640 containing 5% autologous serum and expanded with rIL-2 (20 U/ml) (Novartis Co., Proleukin®).[7] Hapten-specificity was assessed by ^{3}HTdR incorporation (Perkin Elmer) under MHC-restricted conditions. Mitogenic index ≥2 was considered as positive.[7] IL-4, IL-5 (Becton Dickinson), IL-17, CXCL8, IL-22 (R&D System) and IFN-γ (Endogen) were measured by ELISAs into 36 h supernatants of amoxicillin-specific clones following polyclonal stimulation with PMA (20 ng/ml) (Sigma) plus 50 ng/ml anti-CD3 mAb (UCHT1, Becton Dickinson).[7] Formalin fixed-paraffin embedded skin specimens from the amoxicillin-positive patch test from donor L.P. and, as control, pustular psoriasis were cut into 4 μm thick sections, stained with anti-CD3, CD4, CD8 (Dako, DK) or RORγt (R&D System) mAbs and finally labelled with DAP (Dako). Statistical analysis was performed using Student's t test and χ^2 with p values < 0.05 as significant.

Twenty-seven amoxicillin-specific T-cell clones were obtained from the peripheral blood of donor L.P. with history of AGEP. From donor F.A. (anaphylaxis) and P.M. (SJS), 48 and 22 hapten-specific clones were derived, respectively. Ten amoxicillin-specific clones derived from the peripheral blood of donor L.P. produced IL-17 (37%) whereas 3 IL-17+ specific clones were obtained from donor F.A. (6%) and only 1 (4.5%) from donor P.M. ($p < 0.01$) (Fig. 1 A). Hapten-specific clones from AGEP also produced significantly higher levels of IL-17 in comparison with TCCs from anaphylactic shock and SJS (mean ± SE 0.68 ± 0.7 vs 0.1 ± 0.2 and 0.005 ± 0.001 ng/ml, respectively, $^* p < 0.05$).

As T_H17 cells can easily shift to other phenotypes depending on the cytokine milieu[5,6] and mixed phenotypes might explain the presence of activated eosinophils in AGEP,[1] we looked at other cytokines co-produced by amoxicillin-specific TCCs. Interestingly, in AGEP all the IL-17+ clones also released high amounts of IL-4 (and IL-5) or IFN-γ, thus showing a T_H2/T_H17 (5/10 clones) or T_H1/T_H17 phenotype (5/10). None of the clones from anaphylactic shock exhibited the T_H1 phenotype, most being T_H2 (19/48, 39%), whereas 5/27 clones from AGEP produced IL-4 (and IL-5) exclusively (19%), a large number being T_H1 (10/27, 37%) similarly to SJS (11/22, 50%) (Fig. 1B). Significantly higher amounts of CXCL8 were produced by drug-specific clones from AGEP than from anaphylaxis (mean ± SE 7.03 ± 2.22 vs 4.0 ± 2.7 ng/ml; $p < 0.05$) irrespectively of IL-17 release, thus confirming previous data[2] and further supporting the central role of these cells in the neutrophil accumulation.[8] IL-22 production was highly variable among clones (not shown).

To confirm the relevance of IL-17+ lymphocytes in AGEP, the expression of the T_H17-related transcription factor ROR was evaluated in the skin. As shown in Figure 1C, RORγt+ cells were found, along with a robust CD3+ cell infiltrate, in the skin biopsy of the amoxicillin-induced patch test of patient L.P. at high numbers as pustular psoriasis itself[9] used as positive control (10 ± 2/5 HPF and 4 ± 2/5 HPF, respectively).

In conclusion, although CXCL8-producing T cells were claimed to mediate drug-induced AGEP, the in-



Disease	N° of amoxicillin-specific T cell clones with phenotype (%)		
	All	T_H1	T_H2
AGEP	27	10 (37)[a]	5 (19)[d]
Anaphylaxis	48	0[b]	19 (39)[e]
SJS	22	11 (50)[c]	0[f]

[a] vs [b] $p < 0.001$; [a] vs [c] NS; [b] vs [c] $p < 0.001$; [d] vs [e] $p < 0.05$; [d] vs [f] NS; [e] vs [f] $p < 0.001$

Fig. 1 (**A**) IL-17 production from polyclonally stimulated amoxicillin-specific TCCs. Straight lines: mean production (ng/ml) by all the TCCs. *$p < 0.05$. (**B**) IL-17 and IL-4 production from polyclonally stimulated amoxicillin-specific clones of AGEP (total 27). Dotted lines represent the mean cytokine concentration (±5 SD) produced by stimulated irradiated feeder cells (plot). Hapten-specific T cell clones derived from AGEP, anaphylactic shock and Stevens-Johnson syndrome (SJS) were categorized as T_H1 and T_H2 as producing IFN-γ or IL-4 alone, respectively (Table). (**C**) Immunohistochemistry of skin specimens from the amoxicillin-positive patch test of AGEP and pustular psoriasis. Original magnification ×40, scale bar 20 μm.

volvement of IL-17 was recently proposed on the basis of increased serum levels or production after PBMC polyclonal activation.[3,4] Despite our data come from a single patient due to the rarity of the disease thus representing a concern to provide a more general pathogenic mechanism, our study suggests the possible role of hapten-specific T_H17 cells in AGEP which was not provided so far. The simultaneous production of IFN-γ or, more interestingly, IL-4 (and IL-5) together with IL-17 by amoxicillin-specific clones may also explain the presence of neutrophils, activated eosinophils and culprit drug-specific circulating IgE, thus combining apparently contrasting findings.

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