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Dear Editor

### Hapten-Specific TH17 Cells in the Peripheral Blood of $\beta$ -Lactam-Induced AGEP

Acute generalized exanthematous pustulosis (AGEP) is a rare adverse drug reaction, frequently due to  $\beta$ -Lactams, characterized by skin eruption, nonfollicular sterile pustules, fever and neutrophilia with neutrophilic and, possibly, eosinophilic infiltrates.<sup>1</sup> 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.<sup>1,2</sup> However, as found into certain neutrophilic inflammatory processes similar to AGEP such as pustular psoriasis, regulated by the newly defined TH17 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.<sup>3,4</sup> IL-17+ lymphocytes express retinoid acid related orphan receptor (ROR)  $\gamma$ t transcription factor, activate neutrophils and, depending on the microenvironment, can acquire the ability to also produce IFN- $\gamma$  or IL-4 differentiating towards a mixed phenotype.<sup>5,6</sup>

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  $\beta$ -Lactam-induced AGEP. As controls, amoxicillin-specific TCCs from anaphylaxis and Stevens-Johnson syndrome (SJS) were used. Further, we studied the expression of the TH17-related transcription factor ROR $\gamma$ t 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  $2 \times 10^6$  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®).<sup>7</sup> Hapten-specificity was assessed by <sup>3</sup>HTdR incorporation (Perkin Elmer) under MHC-restricted conditions. Mitogenic index  $\geq 2$  was considered as positive.<sup>7</sup> IL-4, IL-5 (Becton Dickinson), IL-17, CXCL8, IL-22 (R&D System) and IFN- $\gamma$

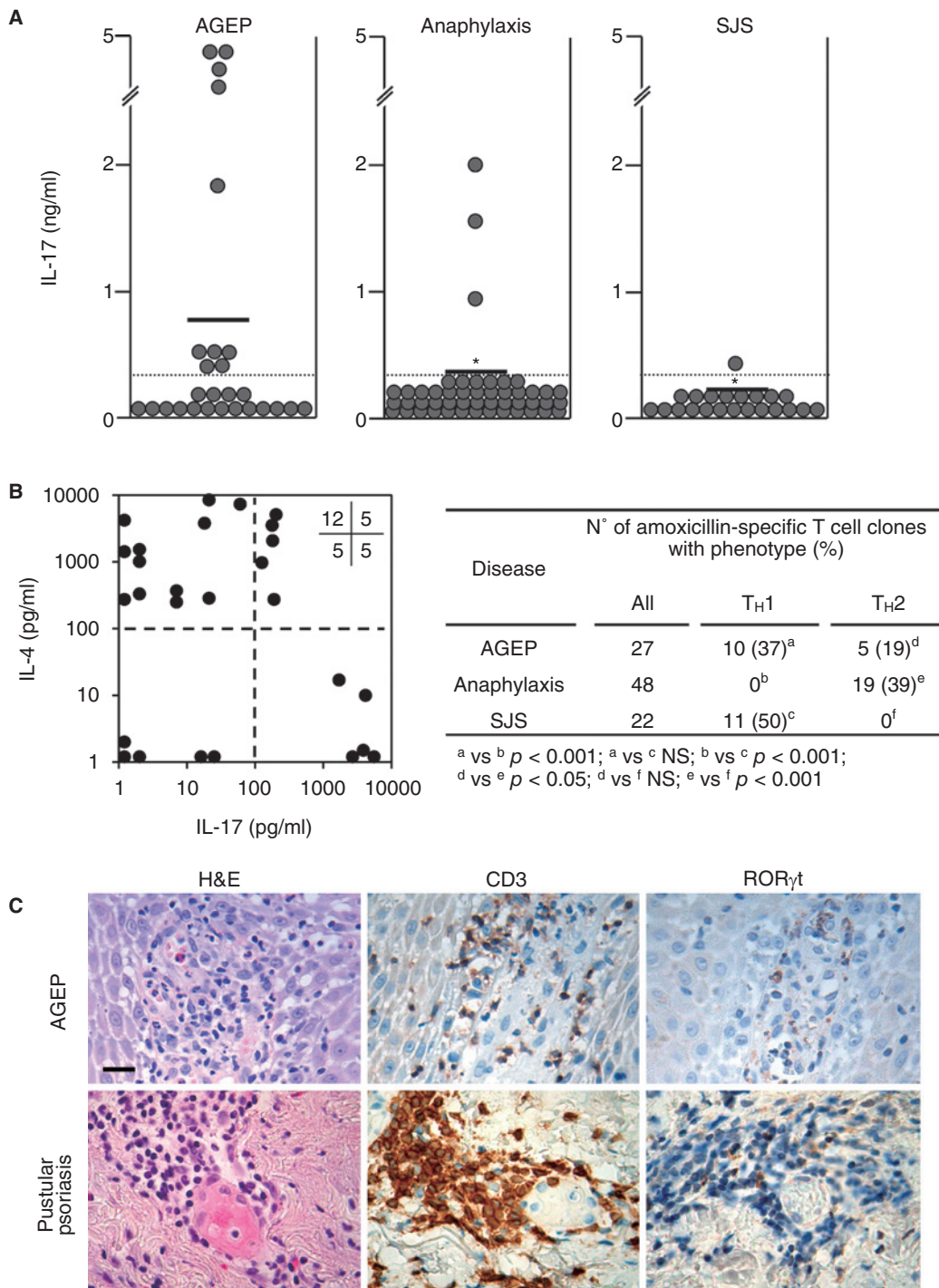
(Endogen) were measured by ELISAs into 36 h supernatants of amoxicillin-specific clones following polyclonal stimulation with PMA (20 ng/ml) (Sigma) plus 50 ng/ml anti-CD3 mAb (UCHT1, Becton Dickinson).<sup>7</sup> Formalin fixed-paraffin embedded skin specimens from the amoxicillin-positive patch test from donor L.P. and, as control, pustular psoriasis were cut into 4  $\mu$ m thick sections, stained with anti-CD3, CD4, CD8 (Dako, DK) or ROR $\gamma$ t (R&D System) mAbs and finally labelled with DAP (Dako). Statistical analysis was performed using Student's t test and  $\chi^2$  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  $\pm$  SE 0.68  $\pm$  0.7 vs 0.1  $\pm$  0.2 and 0.005  $\pm$  0.001 ng/ml, respectively, \* *p* < 0.05).

As TH17 cells can easily shift to other phenotypes depending on the cytokine milieu<sup>5,6</sup> and mixed phenotypes might explain the presence of activated eosinophils in AGEP,<sup>1</sup> 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- $\gamma$ , thus showing a TH2/TH17 (5/10 clones) or TH1/TH17 phenotype (5/10). None of the clones from anaphylactic shock exhibited the TH1 phenotype, most being TH2 (19/48, 39%), whereas 5/27 clones from AGEP produced IL-4 (and IL-5) exclusively (19%), a large number being TH1 (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  $\pm$  SE 7.03  $\pm$  2.22 vs 4.0  $\pm$  2.7 ng/ml; *p* < 0.05) irrespectively of IL-17 release, thus confirming previous data<sup>2</sup> and further supporting the central role of these cells in the neutrophil accumulation.<sup>8</sup> IL-22 production was highly variable among clones (not shown).

To confirm the relevance of IL-17+ lymphocytes in AGEP, the expression of the TH17-related transcription factor ROR was evaluated in the skin. As shown in Figure 1C, ROR $\gamma$ t+ 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<sup>9</sup> used as positive control (10  $\pm$  2/5 HPF and 4  $\pm$  2/5 HPF, respectively).

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



**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 ( $\pm 5$  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<sub>H</sub>1 and T<sub>H</sub>2 as producing IFN- $\gamma$  or IL-4 alone, respectively (Table). (C) Immunohistochemistry of skin specimens from the amoxicillin-positive patch test of AGEP and pustular psoriasis. Original magnification  $\times 40$ , scale bar 20  $\mu$ m.

volvement of IL-17 was recently proposed on the basis of increased serum levels or production after PBMC polyclonal activation.<sup>3,4</sup> 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 TH17 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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Lucia Fili<sup>1</sup>, Elisa Cardilicchia<sup>1</sup>, Maurizio G Severino<sup>2</sup>, Sergio Testi<sup>2</sup>, Andrea Matucci<sup>1</sup>,

Alessandra Vultaggio<sup>1</sup>, Milena Paglierani<sup>3</sup>,

Daniela Massi<sup>3</sup>, Enrico Maggi<sup>1</sup> and Paola Parronchi<sup>1</sup>

<sup>1</sup>Center of High Excellence, Transfer and Education DENOTHE, <sup>2</sup>Nuovo Ospedale San Giovanni di Dio

and <sup>3</sup>Department of Surgery and Translational Medicine (DCMT), University of Florence, Florence, Italy

Email: paola.parronchi@unifi.it

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