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Notes and Insights

Verteporfin protects against Th17 cell-mediated EAE independently of YAP inhibition

The Hippo signaling pathway, comprising the two transcriptional co-activators YAP and TAZ, is essential for maintaining tissue homeostasis [1]. The core kinase components of canonical Hippo signaling inhibit the YAP activation and hyperproliferation of cells by phosphorylating this protein. The phosphorylated YAP can be either degraded by the proteasome or sequestered in the cytoplasm by the proteins from the 14-3-3 family. Only by switching off the Hippo pathway, YAP/TAZ co-factors can translocate to the nucleus and regulate the expression of target genes by binding to TEA domain (TEAD) family transcription factors. In the past few years, it was shown that the effector molecules of the Hippo pathway are involved in regulating the function of immune cells. Particularly, TAZ has been demonstrated to play an essential role for differentiation of Th17 cells. Animals with T cell-specific genetic deletion of TAZ were protected from Th17-driven EAE, a mouse model of MS [2]. Unlike the Th17-specific role of TAZ, several modulatory effects on T lymphocytes have been suggested for another Hippo transcriptional co-activator, YAP [3]. Recent studies have shown that T cell-specific YAP expression supports the tumor growth by promoting differentiation of the immunosuppressive regulatory T cells (Tregs) and by suppressing the activation of $CD4^+$ and $CD8^+$ T lymphocytes [4, 5]. Moreover, in T cells, YAP acts as a mechanosensor being able to regulate the T cell activation and metabolism through sensing mechanical changes in the microenvironment [6].

Naïve $CD4^+$ T cells are able to differentiate into various helper T cell subpopulations including Th1 and Th17 cells, as well as $Foxp3^+$ Tregs. Our results demonstrate that YAP is expressed in all examined effector $CD4^+$ T cell subsets. Even in the non-polarized Th0 population (stimulated only with anti-CD3 and anti-CD28 Abs), both YAP protein and mRNA expression was induced upon *in vitro* activation (Supporting Information Fig. S1A and B). Thus, we concluded that the T cell-specific YAP expression is dependent on activation of T cell receptor (TCR) and CD28 signaling, regardless of examined T cell subtypes. In contrast, we observed a Th17 cell-specific expression of TAZ (Supporting Information Fig. S1A and B), which was previously shown to act as a co-activator of RORγt, a master transcription factor of this T cell subtype [7]. These data suggest that YAP might regulate many aspects of T cell function independently of TAZ. To examine the consequences of YAP inhibition on the functional activity of effector T cells, in the following experiment, we investigated the impact of three structurally unrelated pharmacologic YAP inhibitors on the differentiation of Th17 cells. Th17 cells generated from YAP-deficient $CD4^+$ T lymphocytes were used as control cells. Remarkably, in contrast to K-975 and Peptide -17, that increased the frequency of $IL\text{-}17^+$ T cells, verteporfin strongly suppressed the differentiation of Th17 cells (Fig. 1A and B). This observation was surprising as all three YAP inhibitors promote their inhibitory effect by disrupting protein-protein interactions between YAP/TAZ and TEAD. Furthermore, YAP-deficient $CD4^+$ T cells cultured under Th17-polarizing conditions displayed elevated levels of IL-17A (Fig. 1C). These data are in accordance to results obtained previously in YAP-depleted T cells [5], suggesting that YAP may play an inhibitory role during differentiation of Th17 cells. As pharmacological inhibitors can also cause off-target effects, this might be a possible explanation for the contradictory findings. Verteporfin was previously shown to inhibit not only YAP, but also the autophagosome formation and GLK-induced AhR-RORγt interaction [8, 9], which might be essential for initiation of IL-17A production in Th17 cells. We were not able to observe any impact of verteporfin on the proliferation rate of Th17 cells or on the expression of RORγt but a strong enhancement in CD69 expression, contrary to K-975 and Peptide -17 treatment (Supporting Information Fig. S2A-C).

By analyzing the impact of verteporfin on Treg differentiation, we observed no changes in Foxp3 expression in verteporfin-treated Tregs (Supporting Information Fig. S3A). Moreover, by examining the influence of verteporfin on Th1 differentiation, we found slightly decreased frequencies of $IFN\text{-}\gamma^+$ cells following the treatment of T lymphocytes with verteporfin (Supporting Information Fig. 3B). These results contradict previous findings that demonstrated that genetic deletion of YAP increased the expression of IFN-γ in Th1 cells [5]. Together, we show that likely off-target effects of verteporfin result in the blockade of effector T cell differentiation. The strongest inhibitory effects were observed for the cytokine IL-17A under Th17-polarizing conditions, and to lesser extent for IFN-γ in Th1 cells.

Having observed that Th17 differentiation is strongly suppressed by verteporfin, we were wondering if verteporfin-mediated effects on Th17 cells might provide a novel therapeutic approach to protect mice against Th17 cell-mediated autoimmune diseases. In myelin oligodendrocyte glycoprotein (MOG) peptide-induced EAE, which is the most commonly used experimental model for the human MS, an inflammatory demyelinating disease of the CNS is predominantly triggered through pathogenic Th17 and Th1 cells [10]. In order to investigate whether verteporfin has a

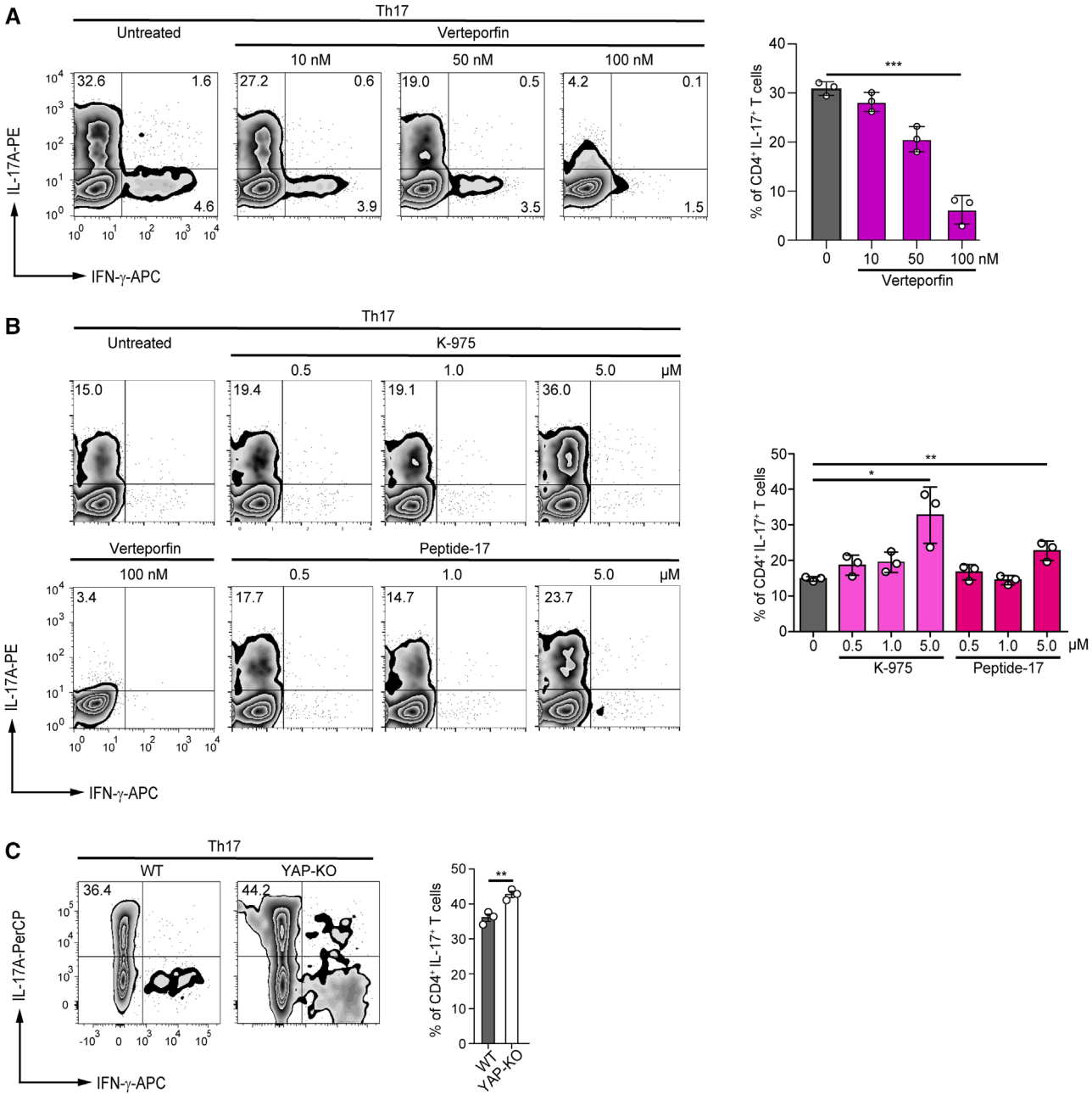

Figure 1. Impact verteporfin on differentiation of murine Th17 cells. (**A–C**) Murine CD4 positive T cells were polarized toward Th17 cells in absence or presence of different YAP inhibitors. The frequencies of IL-17A$^+$ Th17 cells were analyzed by flow cytometry after 3 days of treatment with increasing concentrations of verteporfin (A and B), K-975 or Peptide-17 (B), respectively. Th17 cells in (**B**) were polarized under suboptimal Th17 conditions. The histograms are colored according to the treatment, verteporfin (purple), K-975 (rose), Peptide-17 (magenta), and untreated (gray). (C) WT and YAP-deficient Th17 were analyzed and frequencies of IL-17A$^+$ Th17 cells determined. Results are represented as the mean ± SEM of three independent experiments, $^*p < 0.05$, $^{***}p < 0.001$ (data were analyzed using the Student's *t*-test).

therapeutic capacity to ameliorate autoimmune T cell responses, we induced EAE in mice and treated them intraperitoneally with verteporfin. Remarkably, the administration of verteporfin led to significantly lower incidence and severity of disease as compared to the control group of mice (Fig. 2A). Consequently, the treatment of mice with verteporfin efficiently reduced the number and frequency of pathogenic T cells in the CNS (Fig. 2B-D). Of note, while we did not observe any difference in Treg frequencies in the draining lymph nodes during the priming phase, the Treg infiltration in the CNS was reduced upon verteporfin treatment suggesting a Treg-independent role in suppression of inflammatory T cells (Supporting Information Fig. S4A–C). Thus, by targeting pathogenic T cell populations, verteporfin strongly suppressed CNS autoimmunity.

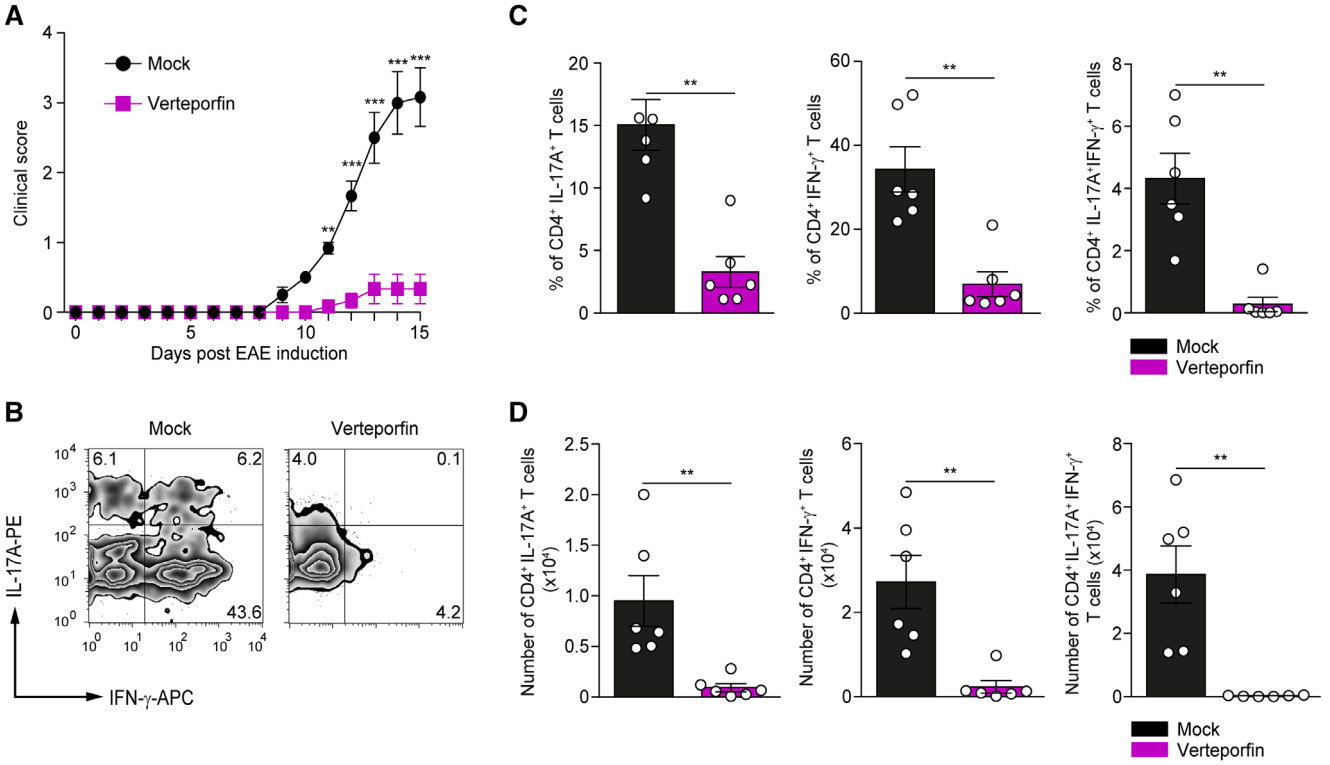

Figure 2. *In vivo* treatment of mice with verteporfin ameliorates the course of EAE. (A) Mean clinical scores (± SEM) of EAE induced in WT control mice and WT mice treated on alternate days with verteporfin (i.p. injection, 100 mg/ kg) over the course of the experiment. Data are pooled from two individual experiments, each experiment was performed with 3 mice per group. (B–D) The percentages (B and C) and absolute cell numbers (D) of IL-17A$^+$, IFN-γ^+, and IL-17A$^+$IFN-γ^+ Th cells in the CNS of mice on day 15 following EAE induction and treatment with verteporfin. The cells were gated on CD4$^+$ gate and analyzed with flow cytometry. Data (C and D) are merged from two independent experiments ($n = 3$ mice per group) and shown as the mean ± SEM, $^{**}p = 0.001$–0.01 (results were analyzed using the Student's *t* test).

Considering verteprofin's unique effect on CD69 upregulation, its involvement in acquisition of effector or regulatory phenotypes as well as the egress of effector T cells from lymph nodes and thus their migration might provide hints to elucidate the mode of action. These results indicate that the small molecule verteporfin, which is a FDA-approved drug frequently used in photodynamic therapy, is a promising therapeutic candidate for treatment of Th17 cell-driven immunopathology.

In summary, so far, it has been reported that the genetic deletion of YAP leads to enhanced activation of T cells, suggesting immunomodulatory activity of this protein [5]. We here report that a YAP-independent activity of verteporfin, which is a known suppressor of the YAP/TEAD complex, reduces development of pathogenic Th17 cells, resulting in a protection against Th17-mediated autoimmunity in mice. Further, our data emphasize the importance to validate the target-specificity of pharmacologic inhibitors in genetic models. The use of T cell-specific YAP- and TAZ-deficient animals in combination with verteporfin administration would gain fundamental insights into the role of YAP in Th17 differentiation and verteporfin's mode of action for the design of novel drugs targeting autoimmunity.

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Abbreviations: **TEAD**: TEA domain · **Treg**: regulatory T cell

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