**Vidofludimus induces p53-mediated apoptosis in activated T cells and inhibits IL-17A and IL-17F expression decoupled from lymphocyte proliferation**

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**Introduction**

- Vidofludimus is a novel oral immunomodulator with substantial activity in various autoimmune models that is currently clinically developed for inflammatory bowel disease (IBD). Induction of apoptosis in mucosal T cells from IBD patients is a relevant therapeutic mechanism since several effective IBD drugs like azathioprine, infliximab, or sulfasalazine were shown to induce apoptosis in activated T cells (Tiede et al., 2003; Atreya et al., 2011; Doering et al., 2004), whereas etanercept was not able to induce apoptosis in these cells and offers no clinical benefit in Crohn’s disease (Van den Brande et al., 2003; Sandborn et al., 2001).

- The cytokine interleukin-17 (IL-17) is involved in many inflammatory diseases and increased IL-17 levels were found in inflamed gut mucosa and serum of IBD patients (Fujino et al., 2003). Especially, IL-17F plays a crucial role in the pathogenesis of IBD (Yang et al., 2008). Vidofludimus has been described to inhibit IL-17 in vitro and in vivo in IBD models (Fitzpatrick et al., 2010).

- In this study the apoptosis inducing capacity of vidofludimus and its inhibitory effect on IL-17F expression by activated T cells in relation to its effect on cell proliferation have been investigated.

**Results**

- Vidofludimus is a potent inducer of apoptosis in PHA activated T cells and Jurkat cells, comparable to reference compound 6-MP (active metabolite of azathioprine, Fig. 1A and 1B). The active metabolite of lefunomide (AA71726) was less active, particularly in Jurkat cells (Fig. 1B and 1C). G1/S arrest (Fig. 1D and 1E) and strong accumulation of p53 (Fig. 1F) by vidofludimus preceded apoptosis induction.

- Vidofludimus inhibits production of IL-17AA/AF and IL-17FF in a dose-dependent fashion with IC₅₀ values between 3 and 10 µM (Fig. 2A). This correlates well with reduced gene expression patterns for IL-17 subtypes 20 h after PHA stimulation (Fig. 2B).

- This effect on cytokine expression is decoupled from its effect on T cell proliferation as significant inhibition of IL-17F production is observed already 24 h after stimulation whereas inhibition of T cell proliferation by vidofludimus as judged by BrdU incorporation is not detectable earlier than 48 h after activation (Fig. 2C: representative results for IL-17FF 24 h and 48 h after stimulation; and Fig. 2D: summary for IL-17FF and IL-17AA after 24 h).

**Methods**

- PBMC isolated from blood of healthy volunteers, stimulated with phytohaemagglutinin (PHA) and treated as indicated were analyzed for cell cycle arrest (PI staining, flow cytometry), cell proliferation (BrdU cell proliferation ELISA, Roche) and IL-17 secretion (ELISA, LumineX) after 48 hours.

- Apoptosis induction (AnnexinV/PI staining) was determined by flow cytometry after 72 hours. P53 accumulation was measured at indicated time points using R&D ELISA. IL-17A and IL-17F gene expression was analysed by qPCR 20 hours after stimulation using QuantTec-Assays from Qiagen in one cycle (BioRad).

- BrdU-based intracellular incorporation and intracellular IL-17 were measured using FITC BrdU Flow and Cytofix/Cytoperm Fixation/Permeabilization Kits from BD, and fluorescence labelled IL-17 antibodies from ebioscience.

- In Jurkat cells, apoptosis induction was determined by AnnexinV/PI staining 72 hours after treatment.

**Conclusions**

- Vidofludimus has been shown to strongly induce cell cycle arrest, p53 accumulation and subsequent apoptosis in activated T cells and, thus, possesses crucial features of an effective IBD drug.

- In addition, vidofludimus inhibits production of both IL-17A and IL-17F and, therefore, is capable of inhibiting this important family of pro-inflammatory cytokines, especially IL-17F which has been described to be of particular relevance for IBD.

- We could demonstrate that inhibition of IL-17 family cytokines by vidofludimus is decoupled from its effects on T cell proliferation. This observation has been confirmed by experiments in a TNBS-induced rat colitis model where the additional administration of uridine to vidofludimus had an influence on most macroscopic and histological colitis parameters while IL-17 and STAT3 expression was not affected by the addition of uridine (data presented elsewhere).

- The combination of all these effects apparently contributes substantially to the beneficial effects of vidofludimus observed in IBD patients as previously reported.