Effects of the tryptophan metabolite picolinic acid on $CD4^+$ T cells

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ABSTRACT:
The tryptophan metabolite picolinic acid (PA) is one of the key metabolites, which is formed in several steps through the kynurenine pathway. The first and rate-limiting enzyme of the kynurenine pathway is indoleamine 2,3-dioxygenase (IDO). The up-regulation of IDO and subsequently the generation of tryptophan metabolites are important mechanisms of immune tolerance but also associated with various diseases, such as HIV, autoimmune diseases, neurological disorders and tumours. In this work, we could show that PA suppressed CD4+ T cell proliferation and metabolic activity in a dose-dependent manner. In contrast to other tryptophan metabolites such as kynurenine, PA had no significant effect on T cell viability. T-cell effector functions which were dependent on protein synthesis such as cytokine secretion and cell surface marker up-regulation were not or only weakly inhibited by PA. On the molecular level, we could not find any effect of PA on major signalling pathways of T-cell activation such as the MAPK, mTOR, NFAT, NFκB and AP-1 signalling pathways. In accordance with our functional observatins, T-cells exposed to PA presented with a reduced phosphorylation of c-Myc at Ser62, which is an indicator of c-Myc activation. Since this activation is mainly mediated by the MAPK ERK, it is possible that PA acts as an inhibitor of ERK activity. This hypothesis will be tested in future studies.

1 INTRODUCTION

$CD4^+$ T cells play a pivotal role in initiating and maintaining diverse immune responses. T helper cells are important producers of a broad range of cytokines, which govern the immune responses to eliminate microbial pathogens, such as bacteria, viruses and fungi. At the same time, aberrant T cell responses may be involved in autoimmune disease, allergic diseases and tumour development [1]. Consequently, a complex network of immune-regulatory factors exists, which serves to limit excessive or misdirect immune activation. Tryptophan metabolism, which is mainly regulated by the expression of the indoleamine 2,3-dioxygenase (IDO) constitutes such a tolerogenic mechanism by the depletion of tryptophan and the accumulation of tryptophan metabolites. In this degradation of tryptophan the key metabolites are: L-kynurenine (KYN), 3-hydroxykynurenine (3-OH-KYN), 3-hydroxyanthranilic acid (3-OH-AA), quinolinic acid (QA) and picolinic acid (PA) [2]. Inappropriate up-regulation of the kynurenine pathway is also associated with various diseases, including infectious diseases, such as HIV, autoimmune diseases, neurological disorders and tumours [3]. In 2002 *Terness et al*. showed that kynurenines, such as L-kynurenine, 3-hydroxykynurenine and 3-hydroxyanthranilic acid suppress allogeneic T cell proliferation *in vitro* and have highly pro-apoptotic effects on T cells [4]. However, their study did not investigate the effects of PA on T cell activation. Thus, in order to fully understand the underlying principle of immune regulation by IDO and tryptophan metabolites, it is necessary to define the effects of PA on T-cell activation. Consequently, in this project we aimed to describe potential immune-modulatory potency PA on $CD4^+$ T cell. For this purpose, a broad range of activation induced effector functions including apoptosis, proliferation, metabolism, cytokines production and secretion and expression of cell surface markers in PA-exposed T-cells was analysed. Furthermore, the influence of PA on major intracellular signalling pathways of T-cell activation was assessed.

2 METHODS

$CD4^+$ T cells were isolated from peripheral blood mononuclear cells from healthy donors, followed by magnetic cell sorting (MACS®) via depletion of non-target cells. For all experiments, $CD4^+$ T cells were cultured in 96 and 24-well flat bottom plates. In order to analyse the effects of picolinic acid, $CD4^+$ T cells were pre-incubated with four different concentrations (2000µM, 1000µM, 500µm and 250µM) for one hour. Then, T cells were activated with agonistic anti-CD3/anti-CD28 monoclonal antibodies coated onto microbeads. T-cells activated in PA-free medium served as reference. Different analysis methods were used to assess the respective activation-induced effector functions. The proliferation rates were measured by thymidine incorporation and by flow cytometry using a fluorescent cell proliferation dye. For measurement of apoptosis rates we used annexin V-FITC/propidium iodide staining. Cytokine secretion levels were analysed by multiplex analysis from supernatants, intracellular flow cytometry and quantitative PCR. Up-regulation of the cell surface activation markers CD25, CD69, CD71 and CD98hc were investigated by multi-color flow cytometry. Metabolic parameters such as oxidative respiration and anaerobic glycolysis rates were measured on a Seahorse XF24 Flux Analyser. To gain insights into the mechanisms of PA-dependent inhibition of $CD4^+$ T lymphocyte proliferation, we examined the effects of PA on intracellular signalling pathways. Therefore, we measured signalling protein phosphorylation of the cell cycle regulator c-Myc, the MAP-kinases ERK and p-p38 and the mTOR downstream target S6RP by intracellular flow cytometry. Small molecule inhibitors with a well-defined mechanism of actions such as the protein kinase C inhibitor GÖ6983, the mTOR pathway rapamycin and the calcineurin inhibitor cyclosporine A were included as controls into these assays. Furthermore, we analysed the molecular effects of PA using Jurkat reporter cell lines harbouring either an NFAT, NFkB or AP-1 reporter construct.

3 RESULTS

Our experiments could show that the proliferation of $CD4^+$ T lymphocytes was inhibited by PA in a dose-dependent manner. In contrast to other tryptophan metabolites, PA had no significant influence on T cell apoptosis. IL-13, IL-17 and IFN-γ levels were dose-dependently decreased by PA in supernatants, although no influence on transcriptional levels was found. IL-2, IL-4 and IL-10 secretion were not impaired by PA. Similarly, PA did not influence early up-regulation of cell surface activation markers in $CD4^+$ T cells after 24 hours. All metabolic parameters of oxidative respiration and anaerobic glycolysis were attenuated by PA. Activity of the NFAT, NFκB and AP-1 promoter in Jurkat T cells were not affected by PA. In addition, we found out that PA had no significant effect on the phosphorylation of the signalling proteins p-ERK, p-p38 and p-S6RP. However, our analyses indicated that Ser62 phosphorylation of c-Myc, which is an indicator of c-Myc activation, was significantly inhibited in T-cells exposed to PA.

4 DISCUSSION

In conclusion, we show for the first time a comprehensive overview about the functional effects of PA on T cells. As described above, PA does not induce apoptosis in T cells in contrast to more upstream tryptophan metabolites. Furthermore, PA selectively inhibits activation-induced T cell proliferation and concomitant metabolic activity, while protein production is left intact. These effects are specifically mediated by the inhibition of c-Myc activation.

Finally, the unique immunomodulatory properties of PA may also be harnessed for therapeutic properties in situations where pathological immune responses need to be dampened but not fully abrogated.

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