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Complex immunomodulation by pomegranate-derived ellagitannins

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Purpose: Pomegranate peel extract (PoPEx) has been shown to have antioxidant and anti-inflammatory properties, but its effect on the adaptive immune system has not been sufficiently investigated. This study aimed to examine the immunomodulatory effects of PoPEx and its main ellagitanin constituents using in vitro models on human immune cells.
Methods: PoPEx was prepared from pomegranate peel powder by using 50% ethanol. The extract was dominantly composed of punicallagin (PG), punicalin (PN), ellagic acid (EA), and gallic acid. The extract and individual ellagitannins were tested in the culture of human peripheral blood mononuclear cells (PBMC) and co-culture of monocyte-derived dendritic cells (MoDCs) and T-cells. Cytotoxicity, autophagy, proliferation, marker expression, and cytokine production were assayed.
Results: The treatment of PBMC with PoPEx (range 6.25-400 μg/mL) resulted in cytotoxicity at concentrations of 100 μg/mL and higher, due to the induction of apoptosis and oxidative stress, whereas autophagy was reduced. Cytotoxicity of ellagitanins was obtained with lower concentrations such that IC50 values (μg/mL) were: EA (7.56), PG (38.52), and PN (69.95). Both PoPEx and ellagitanins inhibited PHA-induced proliferation of PBMC at non-cytotoxic concentrations which was followed by a dose-dependent inhibitory effect on the production of Th1 (IFN-γ), Th17 (IL-17A, IL-17F, and IL-22), Th9 (IL-9), and proinflammatory cytokines (TNF-α and IL-6) in culture supernatants. Lower concentrations of PoPEx upregulated Th2 (IL-5 and IL-13) cytokine production in contrast to ellagitanins. Lower concentrations of PoPEx and EA stimulated the production of IL-10 and increased the frequency of CD4+CD25hiFoxp3+ cells. Both PoPEx and all three ellagitannins inhibited differentiation and maturation of MoDCs, inhibited their potency to induce proliferation of alloreactive T-cells and their Th1 and Th17 polarization properties. All components were able to induce the expression of tolerogenic markers (ILT3, ILT4, and IDO1) on MoDCs upon induction of their maturation with LPS and IFN-γ which was accompanied by an increased frequency of Tregs and Tr1 cells. PG and EA were more potent than PN.
Conclusion: PoPEx exerted potent anti-inflammatory and immunoregulatory effects in vitro. The immunomodulatory effect of the extract is very complex, probably associated with the induction of tolerogenic DCs, and was dominantly attributed to ellagitannins.

