Aggregation as a bottleneck for IL-10 production in *Nicotiana benthamiana*

LOTTE WESTERHOF, RUUD WILBERS and ARJEN SCHOTS

Wageningen University, Laboratory of Molecular Recognition and Antibody Technology, Droevedaalsesteeg 1, 6708 PB Wageningen, The Netherlands

ABSTRACT

For a long time inteleukin-10 (IL-10) has been suggested one of the most promising immunosuppressive molecules. This signaling molecule is mainly used by the immune system to down regulate or prevent immune responses. Produced by monocytes IL-10 inactivates macrophages, disables antigen presentation by dendritic cells and inhibits production of pro-inflammatory cytokines by T cells. But, as with all suggested biopharmaceuticals, its success depends on the ability of the biotechnology industry to produce this molecule cheaply in large quantities. The human IL-10 (hIL-10) gene encodes a 160 amino acid (aa) monomeric protein preceded by an 18aa signal peptide for secretion. The mature protein consists of six alpha helices (A-F) with two internal disulfide bridges. Helices E and F intertwine with helices A-D of another monomer forming a non-covalent homodimer of 37kDa, which is the biologically active form of IL-10. Human and mouse IL-10 (mIL-10) (homology 73%) share a potential glycosylation site which is not glycosylated. mIL-10 has yet another site that is glycosylated, which results in the inability of mIL-10 to trigger a response from human cells. However, hIL-10 can trigger responses from mouse cells. Nowadays, for research purposes IL-10 produced in many different expression systems including *Escherichia coli*, insect cells, Chinese hamster ovarian cells and human cells. Human, mouse and viral IL-10 have also been produced in plants, but accumulation levels are not economically interesting yet. We transiently expressed human and mouse IL-10 in *Nicotiana benthamiana* and found both proteins to be biologically active. Accumulation levels were comparable with levels reported in literature, and the human and mouse proteins could now be compared in the same experimental setup. The difference in accumulation was significant whereby mIL-10 accumulated 28-fold higher then hIL-10. This may be due to the glycosylation event occurring on mIL-10 conferring stability. This implication was supported by the fact that when a tromine-HIS6-KDEL sequence was added accumulation of hIL-10 increased 21 fold and mIL-10 2,3 fold reducing the difference between them to 2,6 fold. Furthermore, as could also be seen on western blots in literature, we found multimeric forms of IL-10 besides the monomeric and biologically active dimeric form. However, none of the previous reports on IL-10 production in plants discusses this phenomenon. We continued to investigate this aggregation and by the use of IL-10-GFP fusions determined it already occurs *in planta* causing golgi-like vesicles to form. By introducing a flexible GS linker between helices D and E, a stable monomeric form of IL-10 was created, previously used for structure-function studies. Expression of this stable monomer in plants increased human IL-10 accumulation 8 fold, suggesting that a crucial limiting factor of accumulation is aggregation by domain swapping.