ASSESSING THE BIOLOGICAL AND THERAPEUTIC CHARACTERISTICS OF LAP-mIL-10

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Interleukin-10 (IL-10) is a key anti-inflammatory cytokine. The use of cytokines in the treatment of inflammatory diseases is an area of increasing interest, but one which is complicated by the high levels of cytokine which must be administered systemically in order to elicit an effect locally. Recent work has looked at the possibility of producing latent cytokines targeted specifically to disease sites, with the aim of increasing the half-life of the cytokine and making it active at sites of inflammation. This has been achieved through the construction of fusion proteins using the latency-associated peptide (LAP) of transforming growth factor (TGF)-beta 1 to render the cytokine latent. The construct contains a matrix metalloproteinase (MMP) cleavage site between the cytokine and the LAP, allowing the cytokine to be released locally to sites of inflammation with increased MMP activity. To characterise biochemically and by biological assay whether the LAP-mIL-10 construct was latent, as previously demonstrated for LAP-mIFN-beta [1]. Latency shall be assessed using the IL-10 dependent D36 cell line. LAP-mIL-10 supernatant was collected from transient transfections on 293T cells. Samples were left untreated or cleaved with recombinant MMP1. Samples were prepared for analysis with the Celltiter-Glo® Luminescent Cell Viability Assay, using the D36 IL-10 dependent cell line, and for western blotting, probing with anti-LAP or anti-IL-10 antibodies.

RESULTS: The latency of LAP-mIL-10 was confirmed via the cell viability assay and western blotting. The results also show that the uncleaved LAP-mIL-10 unexpectedly produced a significant level of D36 cell proliferation. A control experiment with 293T supernatant alone demonstrated that the supernatant was producing a background level of D36 proliferation, consistent with that seen in the uncleaved LAP-mIL-10. As with IFN-beta, IL-10 encapsulated within the LAP of TGF-beta 1 is rendered latent *in vitro*. Work with LAP-mIL-10 can now be advanced into animal models, with a view to the use of the LAP-mIL-10 construct in the treatment of inflammatory diseases.

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