Interleukin-10 Activates The Transcription Factor C/EBP And The Interleukin-6 Gene Promoter In Human Intestinal Epithelial Cells

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Introduction:

The intestinal mucosa and enterocyte are active participants in the inflammatory response to sepsis and critical illness. IL-10 is an anti-inflammatory cytokine that regulates transcription factors involved in the inflammatory response, e.g., NF-κB. The influence of IL-10 on the activity of C/EBP, another important inflammatory transcription factor, is not known. We examined the effect of IL-10 on C/EBP DNA binding activity and IL-6 gene activity in cultured enterocytes. The IL-6 gene is regulated by multiple transcription factors, including C/EBP.

Hypothesis:

We hypothesized that IL-10 treatment would increase binding of C/EBP to the IL-6 gene, thereby increasing IL-6 production.

Methods:

Cultured Caco-2 cells, a human intestinal epithelial cell line, were treated with IL-10 (20 ng/ml), IL-1β (0.5 ng/ml) or both for 4 hours. After incubation, nuclear fractions were prepared for determination of C/EBP DNA binding activity by EMSA. Nuclear levels of the C/EBP-β and -δ isoforms were determined by Western blotting. In other experiments, cells were transfected with a luciferase reporter plasmid containing either a wild type or a C/EBP-mutated IL-6 promoter. The transfected cells were treated with IL-10 and / or IL-1β as described above.

Results:

Treatment of cells with IL-1β increased C/EBP DNA binding activity (Fig 1a). IL-10 as well activated C/EBP and also potentiated the effect of IL-1β. Nuclear levels of C/EBP-δ were increased by both treatments with the most pronounced effect seen when cells were treated with both IL-10 and IL-1β (Fig 1b). Treatment of transfected cells with IL-1β resulted in a 2-fold increase in luciferase activity. IL-10 alone had no effect on luciferase activity, but significantly potentiated the effect of IL-1β (Fig 1c). This effect of IL-10 was abolished when the C/EBP binding site in the IL-6 promoter was mutated (Fig 1d).

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Conclusions:

These results suggest that IL-10 increases C/EBP binding activity, potentiating IL-1β-induced expression of the IL-6 gene in human enterocytes. The biological role of increased

C/EBP activity and IL-6 gene transcription in the enterocyte remains to be determined, but may be related to the anti-inflammatory properties of IL-6 reported by others.