REGULATION OF MUCOSAL INFLAMMATION BY FIBROBLASTS

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av

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Avhandlingen baseras på följande delarbeten:

I **Tanya De L. Karlson, Christine V. Whiting and Paul W. Bland.** (2007) Proinflammatory cytokine synthesis by mucosal fibroblasts from mouse colitis is enhanced by interferon-γ-mediated up-regulation of CD40 signalling. Clinical & Experimental Immunology 147 (2), 313–323

II **Tanya De L. Karlson, Maria Ormestad and Paul W. Bland**
Activated fibroblasts from mouse colitis upregulate the transcription factor, C/EBPβ, which transactivates CD40-mediated proinflammatory signaling through NFκB. *Manuscript*

III **Tanya De L. Karlson and Paul W. Bland.**
Fibroblasts from normal and inflamed murine colon are equally efficient inhibitors of $CD4^+$ T cell apoptosis. *Manuscript*

IV **Christine V. Whiting, Tanya De L. Karlson, John F. Tarlton, Ian Paterson and Paul W. Bland.** Regulation of TGF-β-mediated collagen production by mesenchymal fibroblasts from murine colitis. *Manuscrip*t

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ABSTRACT

Acute inflammation in the bowel, a response of the immune system to infections or trauma, is probably a frequent but localized event, but when the barrier is repaired and the infection cleared, it is quickly followed by wound healing and resolution. However, in some individuals these mechanisms are not effective and the inflammatory bowel diseases, Crohn's disease and ulcerative colitis result. Common and important characteristics in both of these diseases are the increased accumulation of immune cells, especially non-apoptotic $CD4^+$ T cells and the activation of non-immune cells, including fibroblasts, which then become directly involved in immune responses.

The aim of this thesis was to improve our understanding of the role of the mucosal fibroblasts in intestinal inflammation by analysing the molecular signalling mechanisms underlying their inflammatory potential. Fibroblast cell lines isolated from murine normal colon tissue and from the $CD4^+CD45RB^{high}$ –transplanted SCID mouse model of colitis were used.

Fibroblasts are known to express the membrane receptor CD40 which, through interaction with its ligand (CD40L), plays a key role in inflammatory responses. We showed for the first time the existence of a subpopulation of fibroblasts isolated from inflamed tissue which, despite having lower expression of membrane CD40 compared to normal fibroblasts, were able to respond vigorously to CD40 ligation, a response that was increased by IFN-γ. This indicated that the activated fibroblasts in colitis acquire a permanently activated phenotype.

Molecular studies performed to reveal the mechanisms underlying the synergy between CD40 and IFN-γ in inflamed cells, revealed co-operation between the transcription factors CAATT/Enhancer binding protein beta (C/EBPβ) and Nuclear Factor kappa B (NFκB). Both transcription factors were expressed constitutively at higher intensity in inflamed fibroblasts, compared to normal cells, rendering inflamed mucosal fibroblasts more sensitive to CD40 ligation and IFN-γ stimulation.

Co-cultures of normal and inflamed fibroblasts with $CD4^+$ T cells showed that both fibroblast lines were equally efficient in promoting survival of $CD4^+$ T cells, thus indicating the importance of the mesenchyme in immune homeostasis in the gut.

Finally, analysis of TGF-β ligation on the fibroblast lines showed that the increased and disrupted collagen deposition which had been observed in inflamed tissue could not be explained by simple dysregulation of signalling from the TGF-βR on inflamed fibroblasts.

In conclusion, the results of this thesis suggest that mucosal fibroblasts in chronic inflammation respond to the surrounding milieu, become activated and transdifferentiate into a stable proinflammatory phenotype which may contribute to chronicity of the inflammation, and certainly influences its pathogenesis.

Key word: inflammatory bowel diseases, fibroblasts, CD40, C/EBPβ, apoptosis, TGF-β

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