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NF-kB is Involved in Platelet-Activating Factor (PAF)-Mediated Tumor Necrosis Factor (TNF)-α Gene Expression

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Tumor necrosis factor (TNF)-α and platelet-activating factor (PAF) are important proinflammatory cytokines involved in a variety of biological conditions. They stimulate the release of each other via positive feedback and share many *in vivo* biological activities. We have shown that, in several different experimental systems, the release of PAF in response to various stimuli precedes the induction of TNF-α gene expression, indicating that PAF is an initial triggering molecule for TNF-α expression. However, the molecular mechanism responsible for this has yet to be determined. It has been shown that TNF-α promoter contains consensus sequences for nuclear factors, such as SP-1, CRE, C/EBP, and NF-kB. Of these, NF-kB is believed to be of primary importance in inducible TNF-α transcription. Based on this information, we have investigated whether NF-kB might be involved in PAF-mediated TNF-α expression. Treatment of macrophage cell lines, RAW 264.7 and J774A.1, with either LPS (0.1-1$\mu\ell$/mℓ) or PAF (0.1-1$\mu\ell$/mℓ) resulted in NF-kB mobilization, TNF-α gene transcription and TNF-α protein production. The same effects of LPS and PAF were also seen *in vivo*. Pretreatment of PAF antagonist, BN50739, before LPS injection blocked *in vivo* NF-kB mobilization, TNF-α gene expression, and TNF-α protein production. The data strongly suggest that NF-kB is involved in PAF-mediated TNF-α expression. (HRC-96-0102)

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INTRODUCTION OF MUTATED TGF-β1 cDNA CONFERS MACROPHAGE TO SECRETE AN ACTIVE FORM OF TGF-β1

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Transforming growth factor-β1 (TGF-β1) is a multifunctional immunoregulatory molecule which is often secreted as a biologically latent form. Macrophage is known to be one of the major immune cell populations to secrete TGF-β1. It is difficult to evaluate the autocrine and paracrine effect *in vitro* because endogenous TGF-β1 is secreted not only in small amounts but in an inactive form. To circumvent these problems, a macrophage cell line, P388D1 was stably transfected with mutated TGF-β1 cDNA under the control of the metallothionein promoter. P388D1 cells were transfected and a clone, PK-19, was selected where TGF-β1 transcripts induced by $ZnSO_4$ treatment was detected by RT-PCR. This clone secreted 14.5 ng/ml of TGF-β1. In additon, a very high proportion of the TGF-β1 (83%) was secreted in an active form. On the other hand, P388D1 transfected with wild type of TGF-β1 cDNA constitutively secreted a latent form of TGF-β1. The results from the present study indicate that macrophages transfected with mutated TGF-β1 directly secrete recombinant TGF-β1 in an active form. We are currently examining the role of this transfectant cell line in B cell differentiation.