

manner the LPS induction of TNF production by human peripheral blood mononuclear cells (figure 1). These peptide sequence seem to correspond to the LPS binding sites of the proteins as previously proposed for BPI, LBP and LALF (7), similarly to positive charge oligopeptides based on the structure of PMB that were already shown to bind LPS and reduced mortality in animals (8). This result will permit us the design of stabilized peptides or hybrid proteins with potential therapeutic properties in neutralizing endotoxin. In the purpose to obtain IL-2 analogues from a collection of random IL-2 mutants we have first cloned the hIL-2A<sub>125</sub> fused to the pelB signal peptide from *E. carotova* in a phagemid vector. The phage pIII/hIL-2 hybrid protein was assembled on otherwise wild-type phage capsids after been targeted to the periplasm of *E. coli*. The hIL-2 hybrid protein was exposed on phages maintain biologi-

cal activity as tested in a CTLL-2 cell proliferation assay. Using mutD5 mutagenesis we will obtain a phage-exposed IL-2 mutant collection from which virions will be initially picked on IL-2R subunit expressing cell lines and further selected in proper biological assays according to desired functional features.

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## THE ROLE OF PKR IN dsRNA AND INTERFERON SIGNAL TRANSDUCTION

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The signal transduction pathway involved in the induction of interferon (IFN) genes by viruses or double-stranded RNA (dsRNA) is not well understood. One of the factors necessary for transcriptional activation of the IFN-beta gene is Nuclear Factor-kappa B (NF-kB), the activation of which is triggered by dsRNA.

It has previously been suggested that the dsRNA activated p68 kinase may act as an inducer receptor, transducing the signal from dsRNA to NF-kB through phosphorylation of the inhibitor Ikb. We show that PKR directly phosphorylates the inhibitor of NF-kB. We show that PKR directly phosphorylates the inhibitor of NF-kB, Ikb-alpha (MAD-3) thereby activating NF-kB DNA binding activity *in vitro*.

Analysis of Ikb-alpha protein from dsRNA-treated mouse macrophages indicates a dsRNA-dependent alternate phosphoform of Ikb-alpha. In cells transfected with a mutant (Lys296-Arg) PKR, dsRNA induction of a kB-dependent promoter construct is blocked support-

ing a signal transducing role for PKR. Further evidence for such a role was obtained by specifically ablating PKR mRNA in cells and demonstrating that these cells were deficient in dsRNA activation of NF-kB (in collaboration with R. Silverman). Furthermore, in cells derived from mice in which the PKR gene was homozygously deleted, dsRNA induction of the IFN-beta gene is impaired and NF-kB activation is deficient (in collaboration with C. Weissmann).

Taken together these results show that PKR plays a central role in the signal-transduction pathway involved in the induction of IFN-beta gene transcription. dsRNA also activates the transcription of a number of other genes which may also utilize PKR as a signal transducer. Using a combination of genetic ablation and expression of mutant PKR molecules we are presently identifying novel dsRNA activated, PKR-mediated regulators of transcription.