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MOLECULES MODULATING IMMUNE/INFLAMMATORY RESPONSES, DESIGN AND DEVELOPMENT OF POTENTIAL THERAPEUTICS

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INTRODUCTION

The role of different bacteria-derived agents and cytokines on the onset and development of immune and inflammatory responses has been broadly studied. Gram-negative bacteria LPS binding to inflammatory cell membranes is related to the activation of a cytokine cascade constituting a central pathogenic mechanism on sepsis, and also related with activation of HIV replication in monocytes and acute graft-vs-host diseases (1). Therefore the design of anti-LPS molecules as drugs may be useful in the profilaxis and treatment of LPS-mediated diseases. At the same time, based on some natural proteins that function as cytokine antagonists maintaining basic structural features of receptor ligands, some groups have engineered cytokines to obtain both partial agonists or antagonists. Studies based on structure-activity analysis of IL/2/IL-2R interactions, indicating that binding and activation events can be separated or modulated to elicit a subset of IL-2 responses excluding other (2), open the possibility to engineer IL-2 proteins with potential use as immunosupressing drugs (antagonist) or improved immunotherapeuticals (partial agonist). We briefly described herein preliminary results in using different approaches to obtain molecules modulating immune/inflammatory responses: (a) LPS neutralizing peptides, considering a charged cluster along the sequence of different LPS binding proteins, (b) IL-2 antagonists and partial agonists, using the phague displaying technology to expose hIL-2 and to select desired analogues obtained by random mutagenesis.

EXPERIMENTAL PROCEDURES

Peptides were synthesised using the **tea bag** method (3) and purified by RF-HPLC. Peptides were derived from: bovine BPI (KIRGKWKARKNFIK), human LPB (RVQRWKVRKSFFK), rabit CP18 (RKRLRKFRNKIKEKLKKIGQK) and anti-LPS factor from *L. poliphemus* (CHYRIKPTFRRLKWKYKGKFWC). A polymyxin B (PMB) LPS-binding peptide(CKKLFKCKTK) and a HPV/E7 peptide (DMVDTGFGAMNFADLQPNK SDVPIDI) were also used. PBMC were cultured in the presence of *P. aeruginose* LPS 20 ng/mL and equimolar concentrations of the different peptides (1.25 and 100 nM). TNF concentrations of 18-24 h PBMC cultures were determined using L929 target cells as described (4). All DNA manipulations were performed essentially as previously described (5). The biological activity of human IL-2 was tested using the IL-2 dependent murine T lymphocyte cell line CTLL-2 (6).

RESULTS AND DISCUSSION

Peptides corresponding to particular highly positive charge clusters of the sequence of different LPS-binding proteins were all able to inhibit in a dose-depending

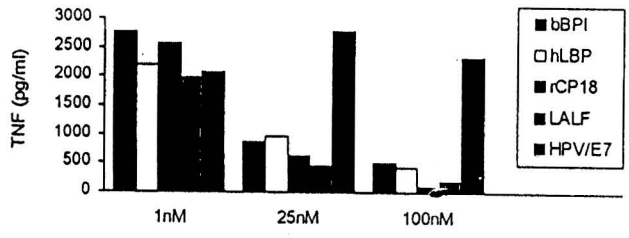

Fig. 1.-Effect of peptides on LPS induction of TNF production in PBMC

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-$2A_{125}$ 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 biological 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 prhosphorylation of the inhibitor IkB. We show that PKR directly phosphorylates the inhibitor of NK-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 supporting 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 wich 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.