Plenary Lectures

PI

RECENT DEVELOPMENTS IN NITRIC OXIDE RESEARCH S. Moncada, The Wellcome Research Laboratories, Langley Court, Beckenham, Kent BR3 3BS.
The formation of nitric oxide (NO) from L-arginine is now recognised as a ubiquitous pathway involved in the regulation of the cardiovascular, central and peripheral nervous systems, as well as in other homeostatic mechanisms. These physiological effects of NO are all mediated by the action of a constitutive NO synthase and subsequent activation by NO of the soluble guanylate cyclase. In addition, NO is produced in large quantities by an inducible NO synthase (iNOS) during host defence and immunological reactions. Because it has cytotoxic properties and is generated by activated macrophages, NO is likely to have a role in nonspecific immunity. Indeed, mice in which the iNOS gene has been disrupted show increased susceptibility to parasite infection as well as reduced non-specific inflammatory responses. The induction of iNOS has recently been demonstrated in human monocytes/macrophages following expression and ligation of the low affinity receptor for IgE. The induction of iNOS correlates directly with the intracellular killing of Leishmania major by these cells. Nitric oxide produced by iNOS is involved in the pathogenesis of conditions such as septic shock and perhaps also the hyperdynamic state of cirrhosis and in inflammation. Recent observations have shown that the generation of NO in human breast cancer correlates positively with tumour grade. It has also been shown that tumours from a human tumour cell line, genetically engineered to generate NO continuously, grew faster and were more vascularised than those from wild-type cells. However, the NO-generating cells grew more slowly in vitro than did the wild-type cells, suggesting that NO may have a dual pro- and anti-tumour action, depending on the local concentration of the molecule.

PII

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Gene Therapy (GT) is a novel genetic technique which holds much promise for the treatment for both inherited and acquired diseases such as cancer and AIDS. Although GT experiments in man have been going on since 1989 there are so far no published reports of long-lasting cures of any kind in man, rather these early experiments have pinpointed the inadequacies of the present vector systems and problems arising from the immune system. At the moment immunotherapy of cancer appears to be a promising application of GT. In this strategy, autologous tumor cells isolated from patients are transfected with one of several cytokine gene expression vectors, the genetically modified tumor cells are irradiated to prevent cell division and are applied as a "cancer vaccine". Preliminary results from a human phase 1 trial dealing with malignant melanomas will be presented.

PIII

COMBINATORIAL EXPLORATION OF THE DETERMINANTS OF PROTEIN FOLDING

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Two complementary mutagenesis strategies have been used to examine the relationship between protein sequence, folding, and structure. In one, we search random libraries of amino acid sequences for proteins which can fold into protease resistant structures. Proteins purified from these libraries have α-helical and β-sheet structures, are usually oligomeric, and can display cooperative thermal denaturation. In other studies, we have determined the effects of mutations produced by combinatorial and directed methods on the structure and folding of a natural protein, P22 Arc repressor. These studies reveal which side chains encode essential structural information and when during folding this information is utilized. Arc variants with enhanced stability, faster refolding kinetics, and dramatically altered surface properties will be discussed.

PIV

Nucleotide excision repair: molecular mechanisms and relevance to cancer.

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Three sun(UV)sensitive disorders; xeroderma pigmentosum (XP), Cockayne's syndrome (CS) and trichothiodystrophy (TTD), are associated with defects in the nucleotide excision repair (NER) pathway. In XP predisposition so skin cancer occurs, whereas CS and TTD are characterized by neurodysmyelination, and developmental impairment and TTD additionally by brittle hair and nails. The CS and TTD features are difficult to rationalize on the basis of defective NER. Extensive clinical heterogeneity is associated with at least two of the > 10 NER-genes: XPB and XPD. These range from XP, combined XP/CS to TTD. Three NER proteins, involved in XPB, XPD and TTDA (only one patient found to date) appeared to be simultaneously components of transcription factor TFIIH. The striking association of transcription-NER factor TFIIH with the occurrence of CS and TTD provides a link between basal transcription and the unexplained pleiotropic symptoms. We propose that TFIIH mutations affecting the NER function cause cancerprone XP features, whereas subtle alterations in the transcription function critically affect the expression of a specific set of genes. These give rise to the salient clinical features in CS and TTD, and probably a wide corollary of other related conditions. These pleiotropic disorders likely comprise a previously unrecognized class of 'transcription syndromes'.

PV

ACTIVATION AND FUNCTION OF NF-κB

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NF-κB is a transcription factor held in cytoplasm of most cells by an inhibitor, I-κB. Many forms of cellular activation lead to mobilization of NF-κB through phosphorylation of I-κB, which targets it for rapid degradation by the proteosome. We have been investigating one mode of NF-κB activation initiated by ligand binding to either the CD40 or TNFαII receptors. We have also been studying the roles of NF-κB by targeted disruption of the mouse genes encoding the factor.

CD40 and TNFRII are members of the TNF family of receptors and their ligands, CD40L and TNFα, are also structurally related. CD40 is found mainly on B cells; interaction with its ligand leads virgin B cells to mature into antibody-secreting cells. We found previously that the short cytoplasmic tail of CD40 binds to CRAF1 and that a CRAF1 dominant negative mutant can block CD40-mediated activation events. Others had found that the cytoplasmic tail of TNFRII can interact with a TRAF1/TRAF2 complex of proteins. These TRAF and CRAF proteins have a region of homology called the TRAF-C domain which interacts with the receptors; on CD40 17 amino acids of the tail, forming the TIMct, mediate the interaction. We have now found that all of the TRAF-related proteins interact through their TRAF-C domains with a 21-amino acid stretch (TIMtk) on a pioneer protein we call TANK. Transient transfection experiments have shown that TANK and TRAF2 can cooperate to activate NF-κB. TANK contains an activating N-terminal one-third and an inhibitory C-terminal two-thirds. It appears that the interaction of TANK with TRAF2 relieves an internal inhibition in the molecule. The explicit functions of the TRAF2/TANK-mediated activation remain to be determined.

Knock-outs of genes encoding the two NF-κB subunits, p65 and p50, and the IκBα inhibitor, have been carried out. They have shown that p50 plays no developmental role and a clear but minor role in B-cell activation. p65 is the more important subunit; without it cells cannot activate NF-κB-mediated gene expression. Its absence leads to embryonic hepatocyte apoptosis in an otherwise normal fetus. Fetal hematopoietic cells can be transferred to lethally-irradiated adults and will totally repopulate the blood cell compartments, indicating that p65 has no developmental role in this lineage. $p65^{-/-}$ fibroblasts die when treated with TNFα, while +/+ cells live, indicating again that NF-κB activation may be involved in countering apoptosis. $I\text{-}\kappa B^{-/-}$ animals, though born normal, die soon due to an NF-κB-mediated lethality perhaps due to uncontrolled cytokine production.