377P THE ROLE OF THE Bcl-2 FAMILY OF PROTEINS IN REGULATING NEURONAL SURVIVAL

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Bcl-2 is a 26kDa intracellular protein that plays an important role in regulating cell survival in the immune system. To investigate if Bcl-2 is involved in regulating neuronal survival in the developing nervous system we have used three approaches: Bcl-2 has been overexpressed in cultured neurons by microinjecting Bcl-2 expression vectors, the expression of endogenous Bcl-2 has been reduced by treating cultured neurons to antisense bcl-2 oligonucleotides and the survival of neurons of *bcl-2* null mice has been studied both *in vitro* and *in vivo*.

Overexpression of bcl-2 prevented the death of neurons deprived of neurotrophins (NGF, BDNF and NT3) but did not prevent the death of neurons deprived of other neurotrophic factors like CNTF. Antisense *bcl-2* prevented the long-term survival response of neurons to neurotrophins, but did not affect the survival response of the same neurons to CNTF. These findings suggest that Bcl-2 is required for the continued maintenance of embryonic neurons by neurotrophins and suggest that alternative, Bcl-2-independent, survival mechanisms operate in neurons exposed to CNTF.

In vitro studies of neurons from *bcl-2* null mutant animals suggest that the role of bcl-2 in regulating neuronal survival changes with age. During the mid-fetal period when naturally occurring cell death is taking place in sensory neuron populations, sensory neurons from *bcl-2*$^{-/-}$ animals do not survive as long in culture with neurotrophins as wild type neurons. By late fetal stages, however, Bcl-2-deficient neurons survived with neurotrophins as well as wild type neurons. There were significantly fewer neurons in the trigeminal ganglia of *bcl-2* null embryos than wild type embryos at both early and late fetal periods.

These results suggest that endogenous Bcl-2 is required for the sustained survival response of sensory neurons to neurotrophins at particular stages of embryonic development and that its absence leads to reduced numbers of neurons at these stages *in vivo*. In addition, our studies of Bcl-2 deficient neurons suggest that Bcl-2 plays a role in influencing neuronal maturation: at early developmental stages, Bcl-2-deficient neurons are less mature than wild type neurons.

Bcl-2 is the founder member of a family of homologous proteins that includes Bcl-x and Bax. Whereas increased expression of Bcl-2 or Bcl-x prevents the death of certain cytokinedependent cell lines following cytokine withdrawal, Bax overexpression of Bax increases cell death. Accordingly, overexpression of Bcl-X rescues neurotrophin-deprived neurons in culture and overexpression of Bax suppresses the survival effect of neurotrophins on cultured mouse sensory neurons.

Neurons from mid-fetal bax$^{-/-}$ mice die at the same rate as neurons from wild type mice in the absence of neurotrophins. However, during the late fetal stages, Bax-deficient neurons survive better than wild type neurons in the absence of neurotrophins, and postnatal Bax-deficient neurons survive for a very long time without neurotrophins. Paradoxically, late fetal and postnatal Bax-deficient neurons survive better in the presence of neurotrophins than wild type neurons.

These results suggest that Bax has positive and negative effects on the survival of developing neurons at late, but not early, stages of neuronal development.

378P OXIDATIVE STRESS

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Oxygen- and nitrogen-centred free radicals (such as nitric oxide, superoxide and hydroxyl radical) and other reactive oxygen/nitrogen species (such as H_2O_2 and peroxynitrite) are formed *in vivo*, both by accidents of chemistry and for useful metabolic purposes such as phagocyte killing of foreign organisms and intercellular communication.

Overproduction of these "reactive oxygen and nitrogen species" (ROS/RNS) can lead to tissue injury, which may contribute to the pathology of several human diseases. Injury mechanisms include elevated damage to DNA, lipids and proteins and interference with cell Ca^{2+} metabolism.

Evidence for damage by ROS/RNS is particularly strong for atherosclerosis and chronic inflammatory diseases (such as rheumatoid arthritis and inflammatory bowel disease) but it is growing for the neurodegenerative diseases. For example, depletion of antioxidants and increased oxidative damage to lipids, proteins and DNA have been measured in Parkinson's disease. Protein and DNA damage is also increased in Alzheimer's disease.

The deleterious effects of ROS/RNS are opposed by a balanced and co-ordinated system of antioxidant defences, some of which are made in the human body and others obtained from the diet. The evidence for a preventative role of some diet-derived antioxidants against cardiovascular disease and certain types of cancer is very strong (e.g. vitamins E and C) but more data are needed on the *in vivo* protective significance of plant phenolics and carotenoids, as well as other dietary plant pigments. The question of antioxidant nutrients and neurodegenerative diseases has scarcely been explored.

In order to facilitate studies of the role of ROS/RNS in human disease and of the therapeutic effects of antioxidants, we have developed several "biomarkers" of damage to DNA, proteins and lipids by ROS/RNS. The use of these biomarkers will be illustrated in the above diseases, and data will be presented about the mechanism of elevated oxidative DNA damage in Parkinson's disease..

These biomarkers can also be applied to nutritional studies, e.g. determining the optimal dietary intake of antioxidant nutrients.

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379P BRAIN INFLAMMATION AND NEURODEGENERATION

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Recent studies indicate that the brain can exhibit many of the classical inflammatory responses to disease and injury, although these may differ quantitatively and temporally from inflammation in peripheral tissues.

Brain responses in acute and chronic neurodegenerative diseases include oedema, pain, neutrophil and macrophage invasion, glial activation and expression of a considerable array of inflammatory or immune molecules including prostanoids, complement, acute phase proteins, MHC antigens, cytokines and growth factors. The most important questions now relate to the functional roles of these events and molecules in the pathology of neurodegenerative diseases, and the potential value of therapeutic intervention.

Invasion into the brain of circulating white cells is relatively rare, but probably contributes significantly to the pathogenesis of ischaemic brain damage and multiple sclerosis. Inhibition of neutrophil invasion is neuroprotective in experimental cerebral ischaemia and is now under clinical trials. Similarly, a number of anti-inflammatory drugs are being assessed in acute and chronic neurodegenerative conditions, including Alzheimer's.

Almost all cytokines and growth factors studied to date are overexpressed in the brain in experimental and clinical neurodegenerative diseases. Direct intervention studies reveal that, at least in experimental animals, pro-inflammatory cytokines (IL-1, IL-8 and TNFα) participate directly in acute ischaemic, traumatic and excitotoxic brain damage, since blocking their action markedly reduces subsequent brain damage. In contrast, some of the anti-inflammatory cytokines (eg IL-1ra, TGFβ) and numerous neurotrophins can inhibit neurodegeneration.

The mechanisms of action of many of these factors are unknown, although IL-1 appears to influence degeneration of neurones irrespective of subtype, probably through actions on other cell types such as glia and endothelial cells. In contrast, neutrophins may have selective actions on specific neuronal subtypes.

Other endogenous anti-inflammatory molecules have also been implicated in neurodegeneration. Lipocortin-1, a putative mediator of glucocorticoid action, is induced by experimental brain damage and markedly inhibits subsequent neuronal death, probably through actions on cytokine (IL-1) and/or corticotrophin releasing factor activities.

The realisation that the inflammatory processes can be and are activated in the brain, and apparently participate directly in several forms of neurodegeneration, has opened new avenues for potential drug development which are only just being realised.

380P THE PATHOPHYSIOLOGY OF ALZHEIMER'S DISEASE

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Scientific achievements over the last decade have given rise to a series of simple concepts which both help us understand the disease process and allow us to design new types of therapy. Alzheimer's disease (AD) has a complex pattern of epidemiology with age, genetics and environment factors acting and interacting to cause or increase the risk of disease.

Subsequent studies have shown that amyloid protein precursor (APP) is present in synaptic terminals and may play a role in neuronal communication. In addition, APP has a role in the processes of nerve cell repair and regeneration.

A variant of the ApoE gene on chromosome 19 (ApoE4) is known to be associated with an increased risk of sporadic AD. ApoE is a protein which also plays a role in repair/regeneration of nerve cell connections. ApoE4 also binds avidly to the 5-amyloid protein fragments and enhances their ability to form plaques.

At the end of ten years we have a simple concept to explain a complex disease. With age or when the brain is damaged, nerve cells make more APP as part of the process of repair. ApoE also has a role in this process. Too much APP (Downs Syndrome)/ altered processing of APP (APP mutations) or and ApoE4 genotype make plaque formation likely. Once plaques are formed they cause microglial activation and cause a cascade of events leading to an inflammatory state. This inflammation destroys synapses, terminals and nerve cells and leads, eventually to dementia.

Understanding the process driving the disease allows us to formulate clear strategies for therapeutic intervention. Reducing APP expression, blocking the processing which leads to β-amyloid protein fragments, blocking plaque formation and reducing the inflammatory response are all approaches to therapy.

381P CLINICAL FEATURES OF DEGENERATIVE DISEASES

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In this presentation I will summarise the clinical features of Alzheimer's Disease and other forms of dementia.

Current methods of management, including drug therapy, will be outlined, with reference to their limitations. It will be emphasised that dementia, except in its earlier stages, is much more than a memory disorder and that new therapeutic approaches should recognise this fact.

Other degenerative conditions, including Parkinson's Disease, Motor Neurone Disease and other considerably rarer degenerative conditions will be discussed with liberal use of video material.

382P TRANSGENIC MODELS OF NEURODEGENERATIVE DISORDERS

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Advances in molecular genetics and molecular biology, cellular biology and embryology have given researchers unique tools for the targeted delivery and stable germline transmission of foreign genes (transgenes) into mice to model the neuropathological and behavioural hallmarks of specific human neurodegenerative diseases.

This targeted transgene expression approach has been used to model diseases that involve dominant expression of recently identified genes that are associated with the progression of the disease, for example, mutated β-amyloid protein precursor (APP) in Alzheimer's Disease (AD), or mutated prion protein in Familial Subacute Spongiform Encephalopathies, or the mutated neurofilament or superoxide dismutase enzyme in Familial Amyotrophic Lateral Sclerosis. Indeed, after many years of effort, transgenic mice have been successfully developed overexpressing the above genes. These mice show many of the neuropathological and behavioural features of the relevant disease.

In particular, several research groups have developed transgenic mice overexpressing the mutated forms of human APP under the regulation of specific promoters to drive expression into neurons. Such mice show age-related robust AD-like neuropathological features including β-amyloid deposition in the form of diffuse and neuritic plaques, reactive astrocytosis and microgliosis, dystrophic neurites and synaptic loss predominantly in the cortex and hippocampus. Such neuropathological hallmarks of the disease in the brains of these transgenic mice are accompanied by learning and memory deficits.

In parallel with this powerful approach to develop dominant expression mutants, molecular techniques have been established to disrupt or delete ("knock-out") endogenous mouse genes (which are homologues of the human genes involved in the disease process) by a process termed homologous DNA recombination in embryonic stem cells. This approach is proving particularly useful in determining if the disease process involves a dominant loss or gain of function and in helping us to understand the normal cellular function of specific genes.

A dominant "loss of function" mutation is exemplified by *Fmr1* knockout mice, which show some of the key features of the fragile X syndrome including impaired learning and macroorchidism. However, inactivation of the mouse homologue of the Huntington's Disease gene, huntingtin (Hdh) results in embryonic death in the homozygotes whereas the heterozygotes are normal. The inactivation does not mimic Huntington's Disease, suggesting that the disease involves a gain of function. Mice with a complete deletion of the APP gene show premature mortality, age-related cognitive deficits and reactive gliosis and a loss of synapses in the brain, features which are characteristic of AD. This may suggest that the disease involves a loss of APP function, although data from transgenic mice overexpressing mutated APP suggest a gain of function.

The ability to introduce stable expression of a gene into the mouse germline through transgenesis has emerged as a powerful tool in neurobiological research aiding the dissection of the molecular, neuropathological, electrophysiological and behavioural processes linked to a specific gene.

With the plethora of new genes being identified with linkage to specific diseases, eg AD, it is now possible to create multigenic transgenic mice and model the disease more precisely. Such mice will undoubtedly facilitate the identification of novel therapeutic targets and the preclinical testing of potential therapeutic agents.

383P NEW STRATEGIES FOR THE TREATMENT OF ALZHEIMER'S DISEASE

R.G. Hill, Neuroscience Research Centre, Merck Sharp and Dohme Research Laboratories, Harlow, Essex.

Much has been published on the aetiology of Alzheimer's disease (AD) but little of it has been reduced to practice. The majority of current drug candidates are cholinesterase inhibitors or muscarinic agonists, on the strength of the cholinergic hypothesis of AD (*Loudon - this symposium*). Nicotine has been been suggested anecdotally to be capable of improving memory, and the discovery of nicotinic receptor sub-types raises the possibility of effective therapy but reduced side effects. It has now been demonstrated that nicotine can enhance synaptic transmission in hippocampus by activation of α7 subunit containing receptors to increase Ca^{++} influx in terminals. ABT-418 is a synthetic nicotinic agent, with selectivity towards cytosine binding sites in cortex, which is in phase II clinical evaluation.

Other palliative therapies are being considered either on an entirely empirical basis or because AD leads to widespread neurodegeneration. The nootropics exemplified by piracetam show activity in cognition assays in laboratory animals but do not produce unequivocal improvement in AD patients. Studies on their mechanism of action showed that they were modulators of the AMPA receptor and led to the discovery of more potent agents, now called ampakines. A more subtle modulation may be gained via G-protein coupled receptors for example by agents acting at metabotropic glutamate or GABA-B receptors.

Much research is now focussed on the neurotoxicity of amyloid peptide (AβP), in particular of the 1-42 peptide sequence which is deposited early in AD. This peptide can be shown to be overexpressed in transgenic mice carrying the human ADD and PS-1 familial ADgenes. The mechanism by which AβP causes neurodegeneration and loss of function is unknown, hence emphasis has been given to preventing production by inhibition of secretases or by preventing aggregation.

It has been shown that anionic sulphonates will prevent splenic amyloidosis and it has been suggested that a similar strategy might be effective in AD. Dyes such as congo red and chrysamine G have been shown to block formation of amyloid fibrils, thus increasing the likelihood of of proteolysis and reducing the burden of the toxic aggregated form. It has been suggested that AβP acts via the cerebral vasculature by producing an excess of superoxide radicals which cause endothelial damage.

An altemative point of view is that the neurodegeneration in AD is related to tau-protein aggregation, and it has recently been found that the non-neuroleptic phenothiazines related to methylene blue will prevent tau aggregation. There is also evidence for inflammatory and oxidative stress components in AD (*Rothwell; Halliwell - this symposium*) suggesting the use of e.g. NSAIDs or antioxidants. Indeed, there is already some clinical evidence that patients taking chronic NSAIDs have a lower incidence of AD. The observation that HRT with oestrogens reduces the probability of developing AD in the elderly needs a mechanistic explanation and may be therapeutically important.

The development of drugs for AD is in its infancy and the next decade is sure to bring real clinical benefit to patients for whom little treatment is currently available.

384P MUSCARINIC PARTIAL AGONISTS IN THE TREATMENT OF ALZHEIMER'S DIESASE

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Degeneration of central cholinergic projections is a consistent finding in Alzheimer's disease (AD) and is correlated with a loss of cognitive function. Therapeutic approaches in AD have included investigation into ways of enhancing release of acetylcholine (ACh), inhibiting its breakdown or mimicking its effect at postsynaptic muscarinic receptors in the cerebral cortex and hippocampus. Of these, development of cholinesterase inhibitors (AChEIs) and direct acting agonists are popular approaches.

AChEIs prolong the availability of synaptic ACh, thus potentiating its activity at the post synaptic receptor. This approach depends on the integrity of presynaptic neurons, so it is likely that efficacy will diminish as the disease progresses. New compounds emerging from this approach appear to have improved safety profiles compared to early AChEIs but have yet to demonstrate long-term efficacy in the face of unremitting neurodegeneration.

Unlike cholinesterase inhibitors, the cognition-enhancing potential of muscarinic agonists is not dependent on the availability of endogenous ACh or the integrity of the presynaptic neurons. However, early studies using muscarinic full agonists did not show therapeutic benefit due to poor bioavailability and unacceptable side effects.

Following the discovery of muscarinic receptors subtypes, it was postulated that the side effects seen with such treatments were largely due to stimulation of peripheral M_2 and M_3 muscarinic receptors. It followed that development of agonists which were selective for M_1 receptors could provide cognition enhancement in the absence of other, undesirable, effects. Agonists selective for M_1 receptors have proved elusive, but muscarinic partial agonists have been found to exhibit the desired profile as a result of functional selectivity for M_1-mediated actions.

This approach has resulted in the clinical evaluation of SB 202026 and other muscarinic partial agonists. SB 202026 has shown advances over the earlier agonists tested in terms of tolerability, with improved separations between clinically effective doses and those inducing adverse effects.

Recent evidence suggests that cholinergic approaches could also bring therapeutic benefit through disease modifying actions by interfering with the production and deposition of amyloid, a central feature in the pathogenic process in AD. If disease modification is demonstrated in the clinic, cholinomimetic therapies would be expected to have utility at all stages of the disease.

385P REGULATORY ASPECTS OF DEMENTIA TRIALS

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Despite confusion over European harmonisation, there is no doubt that nowadays all trials must be carried out to the highest GCP standards, particularly in high profile new drug development for dementia-like illnesses. Study designs must be valid and data must support intended use.

The FDA have set out guidelines for the development of dementia drugs, the key points including placebo controls, avoidance of pseudospecific claims, special patient subgroups and the acceptance of preselected patients.

Trials should start in 'healthier' patients, but are also best started in institutions. There is as yet no acceptable active control. One needs more than one adequately controlled trial showing superiority in both clinician's global assessment and a performance objective test.

As new drugs are being registered, we may see a relaxation in the need for placebo controls. Regulatory strategy is another major consideration, particularly in Europe. It is still possible to get fast registration in some countries using a national system: however, we urge the use of CPMP/EMEA centralised systems for a variety of reasons.

It is vital to keep control of the clinical operating plan, as well-intentioned enthusiasts will ruin a good set of data. The UK in particular has a habit of using named patient and DDX schemes to do quick research which is not always best documented.