strategy for molecular dissection of disease with applications to target discovery in human neurological disorders and cancer. A region-specific CNS tissue expression database has been constructed as a measure of disease specific to disease-relevant brain regions. This links novel target mechanisms identified in CNS tissues to database information relating to clinical, biological, image and annotation data. An integrated analysis of human cerebrospinal fluid and CNS tissue, which is being performed currently, could yield novel therapeutics or support the rationale of existing drugs.

Proteomics has also proved a valuable tool for the evaluation of heart disease. John Weekes (Imperial College School of Medicine, Heart Science Centre, Harefield, UK) described alterations in protein expression in dilated cardiomyopathy. Specific proteins are hyperubiquitinated in diseased hearts, and these proteins can be purified by affinity chromatography and then identified using 2-D PAGE/MALDI-TOF. The ubiquitin–proteosome pathway is therefore a possible target for therapeutic intervention in heart disease.

Michael Schrader (BioVision, Hannover, Germany) concluded the programme by discussing peptidomics, i.e. the analysis of peptides from biological sources and their application for the discovery of biomarkers and drug candidates. Peptides are particularly suitable for diagnostics because they result from regulatory/disease processes and soluble molecules facilitate robust sample preparation, while peptide fingerprints can be generated from a variety of different body fluids. Therapeutic advantages of using peptides include convenience of production and drug delivery.

Conclusions

This conference reflected the considerable progress that has been made in proteomics during the past few years. The technologies presented are having a considerable impact on biomarker identification and drug discovery. Although proteomics is being promoted as a separate industry, it is in fact a set of technologies, which are being increasingly used in combination with genomic technologies in the post-genomic era. The conference was an excellent overview of the state-of-art proteomic technologies and their applications, and provided a good forum for discussion between the academia and the industry.

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Current progress on new therapies for Alzheimer's disease

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The IBC's *9th Annual Conference on Alzheimer's Disease* held in Atlanta, GA, USA on 8–9 February was chaired by Sue Griffin (University of Arkansas VAMC, Little Rock, AR, USA) and featured speakers from academia, industry and government. The sessions succeeded in touching on the range of topics promised by the conference theme, namely *Gene discovery to therapeutic applications*.

The therapeutic applications discussed included most of those currently of interest for commercial development such as γ-secretase inhibitors, immunization approaches, cholesterol-modifying agents, anti-inflammatory agents and steroids. Given the publicity around the recent novel amyloid precursor protein β-secretase (BACE) discoveries, therapeutic approaches targeting β-secretase were clearly underrepresented with only one poster by Xiao Ping Shi (Merck Research Laboratories, West Point, PA, USA) devoted to the topic.

Most of the approximately 60 attendees were from pharmaceutical or biotechnology companies and they should have come away with a good understanding of the status of the early-phase clinical trials currently under way for Bristol-Myers Squibb's (BMS) γ-secretase inhibitor and Elan/AHP's β-blocker, as

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well as emerging therapeutic approaches and potential new targets. This conference report will provide an overview of the key presentations.

Searching for new targets

Rudolph E. Tanzi (Harvard Medical School, Charleston, MA, USA) led the session with a special presentation entitled *Alzheimer's disease: from genes to drugs*. After a brief review of the known Alzheimer's disease (AD)-related genes (*APP*, *PS-1*, *PS-2*) and disease modifiers (*ApoE e4*), Tanzi argued for the existence of additional major disease-modifying genes and perhaps even causative genes for AD. A brief description followed methodological approaches to identifying genes associated with a complex trait such as AD. These were broken down into two basic strategies:

- Following alleles vertically by linkage analysis within a family(s); and
- Following allele-sharing horizontally across families looking for case-control or family-based associations of the allele with the disease phenotype.

The former approach was used to identify the disease-causing familial Alzheimer's disease (FAD) mutations in *APP*, *PS-1* and *PS-2*, while the latter approach was used to identify *ApoE e4* as a major disease-modifying gene. While successful, the latter approach has also led to a bewildering number of reports on AD gene candidates that are often poorly replicable, if at all. In Tanzi's view, single, isolated reports that cannot be replicated are probably best forgotten. However, those candidate genes that receive some, but not extensive, confirmations are still of potential interest and possibly reflect linkage disequilibrium. In this case, the poorly replicated candidate gene might not be the disease-causing gene, but rather a benign gene that 'goes along for the ride'. Presence or lack of association will depend on whether recombination events have separated the pathogenic gene from the benign genes in that particular set of families.

Using this argument, Tanzi is continuing to search chromosome 12 for polymorphisms in α2-macroglobulin (α2M). His original description of an AD-associated insertion/deletion in α2M (A2M-18I) has been replicated by a few groups, but refuted by many more. Moreover, biochemical studies could not detect any functional differences between the insertion/deletion polymorphisms. Collectively, these data suggest that A2M-18I is not a major disease-modifying polymorphism. Undaunted, Tanzi has continued to sequence A2M through a collaborative venture with Neurogenetics (San Diego, CA, USA), and has now found two other mutations in α2M. In contrast to the A2M-18I polymorphism, these mutations affect dimerization of α2M leading to a biologically inactive molecule.

Tanzi asserts that there are major AD genes on chromosome 10 and chromosome 1. The gene on chromosome 10 shows a strong association to D10 S583, a probe that maps close to the Insulin Degrading Enzyme (IDE). This protease is thought to play a role in Aβ degradation but, to date, no mutation has been found in the *IDE* gene. The candidate gene on chromosome 1 maps close to nicastrin and is clearly distinct from *PS-2*. Nicastrin is the recently described binding partner of presenilin that modulates Aβ production. Sequencing of nicastrin is not complete and no mutations have yet been found.

Giulio M. Pasinetti (Mount Sinai School of Medicine, New York, NY, USA) discussed the changes in gene expression as a function of clinical dementia. His goal is to identify molecular indices that define the transition between different phases of dementia but with particular emphasis on the downward progression from mild cognitive impairment to frank dementia. He is using a combined genomic (chip array technology) and proteomic (powerblots) approach to catalog changes in post-mortem tissue from the extensive brain bank at Mt Sinai (New York, NY, USA). While these studies using post-mortem tissue are always subject to criticism, they do offer the advantage of mining prospective targets in the relevant tissue. Focusing on early stages of dementia can also help eliminate gene changes in late-stage disease. Although the research is still in the early stages, Pasinetti described some robust and selective changes within the synapsin gene family and suggested that altered synapse-related gene expression is a selective feature of early AD.

Patrick R. Hof (Mt Sinai School of Medicine, New York) followed up Pasinetti's talk with an elegant and complementary presentation on selective neuronal vulnerability. Using stereological methods, Hof is tracking the pattern of neuronal degeneration in the same or similar set of well-characterized post-mortem tissues from the Mt Sinai brain bank. He suggests that a pivotal step in disease progression is when the pathology jumps from the limbic cortex to the neocortex; this correlates with a movement into a Clinical Dementia Rating of 2 or mild dementia. His research with post-mortem tissues is complemented by invasive studies using aged macaques. These studies use injection of fluorescent dyes into monkeys *in vivo* to track single-cell projections, followed by immunocytochemical analyses of *ex vivo* tissue to biochemically characterize the projection neurons. Using these combined techniques, Hof has discovered that long projection neurons involved in the association pathways of the neocortex are particularly vulnerable to neurodegeneration in AD. Being able to identify these neurons and generate cell-specific transcript profiles for each neuron at various stages in the degenerative pathway would seem to offer a powerful approach to identifying potential new targets for intervention at early stages in the disease process.

γ-Secretase inhibitors

Patrick C. May (Eli Lilly and Co., Indianapolis, IN, USA) described the initial

reports of a functional γ-secretase inhibitor from Lilly's collaboration with Elan Pharmaceuticals. This molecule, DAPT {*N*-[*N*-(3,5-difluoro-phenacetyl)-L-alanyl]-*S*-phenylglycine *t*-butyl ester}, was optimized from a micromolar hit identified through a whole-cell screen for Aβ-lowering compounds. The arduous process of conducting a structure–activity relationship (SAR) study without an isolated target was conveyed in a series of SAR tables showing robust and unexpected changes in whole-cell activity following relatively benign structural changes. After optimization, this prototype molecule served as a valuable tool to complete early proof-of-principle *in vivo* efficacy studies. Administration of 100 mg kg^{-1} DAPT subcutaneously to young PDAPP mice (a transgenic mouse overexpressing human APP containing a V717F mutation) led to 30–50% reductions in total Aβ in several brain regions examined. Importantly, this level of efficacy was maintained after sub-chronic administration of a similar dose twice daily for 7 days.

Kevin M. Felsenstein (BMS, Wallingford, CT, USA) described a preclinical efficacy data package for their γ-secretase inhibitor series and provided an update on the clinical development candidate. The specific structures were not revealed, but apparently are based on the sulfonamide scaffold disclosed in a recent World Intellectual Property Organization patent application. Of some note, the pharmacology of the BMS γ-secretase inhibitor appeared somewhat different from the Lilly/Elan inhibitor, in that the BMS compound markedly inhibited (~50%) the secretion of sAPPβ and enhanced the secretion of sAPPα; the Lilly/Elan compound had minimal effects on these secreted ectodomains of APP. The inhibition of sAPPβ is suggestive of a β-secretase inhibitor, but the BMS compound showed no activity against recombinant BACE.

Oral administration of one of the Lilly/Elan inhibitors, dose undefined, resulted in acute and robust changes in brain parenchymal fluid, cerebrospinal fluid, and plasma total Aβ and Aβ 42 in young Tg2576 mice (a transgenic mouse overexpressing human APP containing a Swedish mutation). An attempt to assess efficacy of compounds in aged animals was thwarted by the high background of deposited Aβ in brain, despite attempts to resolve soluble and insoluble pools of Aβ. However, analyses in cerebrospinal fluid collected from vehicle and drug-treated animals showed the anticipated reductions in total and Aβ 42. Comparative concentration–response curves were shown for inhibition of Aβ production and inhibition of Notch cleavage. Under the assay conditions used, the BMS compound showed a 17-fold separation in the IC_{50} value for Notch cleavage versus Aβ lowering. These results led Felsenstein to conclude that significant reductions in Aβ can be achieved without marked effects on Notch signaling.

The BMS γ-secretase clinical development compound has passed successfully through a Phase I single ascending dose safety study in normal volunteers and sporadic AD patients. This study was primarily designed to assess pharmacokinetics, safety and tolerability, but some samples for Aβ analyses were also taken to assess the compound's pharmacodynamics. According to Felsenstein, the pharmacokinetics of the compound looked quite good and could support once-daily dosing. The development candidate is scheduled to enter a 30-day multiple ascending dose safety study in normal and sporadic Alzheimer's patients within the second quarter of 2001.

Immunization approaches to AD therapeutics

Frederique Bard (Elan Pharmaceuticals, San Fransciso, CA, USA) reviewed Elan's preclinical studies in PDAPP mice subjected to either active immunization protocols (Aβ peptides plus adjuvant) or passive immunization protocols (systemic injection of polyclonal or monoclonal antibodies directed against Aβ peptide). The results of both these studies have been highly publicized and will not be recapitulated here in detail. Suffice to say that immunization of young mice markedly decreases subsequent Aβ deposition, while immunization of older, plaque-bearing mice blocks further accrual of Aβ as well as facilitates clearance of at least some forms of pre-existing Aβ deposits. Mechanism of action studies continue to support Elan's contention that plaque prevention/clearance is effected by antibody Fc-mediated microglial clearance. Using an *ex vivo* phagocytosis assay, Bard demonstrated that F(ab′)2 fragments clearly bind to Aβ deposits but do not promote Aβ clearance.

Bard closed with a brief update of the status of Elan's initial clinical trials with AN1792 (Aβ 1–42), which are principally designed to look at safety and tolerability. The single-dose escalation study has been completed and a multi-dose escalation study is either well under way or just completed. In both studies, the immunizations with AN1792 were well tolerated. Bard mentioned that, in addition to active immunization clinical trials, Elan were also planning passive immunization clinical trials using human monoclonal antibodies to the Aβ peptide.

Cynthia A. Lemere (Harvard Medical School, Boston, MA, USA) presented data on a related active immunization approach in which the immunogen (a mix of Aβ 40 and Aβ 42) is administered via alternative routes including nasal mucosa. Significant titers of antibodies were achieved that, when mapped to overlapping 15-mers of Aβ, were primarily directed towards the N-terminal 15 amino acids of Aβ. Interestingly, different strains of mice showed marked differences in the titers generated against Aβ, a variable that should be noted as various APP transgenic mice on different background strains are subjected to these immunization protocols. Finally, a significant

increase in titers was achieved by inclusion of a modified heat-labile enterotoxin (LT) that has been used previously as a mucosal adjuvant. Preliminary data with the PS/APP transgenic mice subjected to repeated nasal administration looked promising.

Thomas Wisniewski (NYU School of Medicine, New York, NY, USA) expressed concern about immunizing humans with a potentially toxic peptide. To address his concern, he engineered out the toxicity of the Aβ peptide by adding polylysine (K6) or poly-aspartate (D6) to the N- or C-terminus of Aβ. One peptide homologue, K6–Aβ 1–30 failed to undergo any detectable fibrillization on incubation for 2 weeks and was also non-toxic to neuronal cell cultures. Immunization of 10-month-old TG2576 transgenic mice with this non-toxic, non-fibrillar peptide produced significant antibody titers (again to the first 16 residues of Aβ) and attenuated the amyloid burden in 18-month-old mice. Wisniewski indicated that his laboratory is attempting to apply this same active immunization approach to the treatment of prion diseases such as Creutzfeld–Jacob Disease.

Atorvastatin clinical trial

Larry Sparks (Sun Heath Research Institute, Sun City, AZ, USA) reviewed his pioneering research on Aβ accumulation in cholesterol-fed rabbits. While not a perfect phenocopy of AD, cholesterol-fed male rabbits share several pathological markers with AD. Sparks went on to review some of the more recent literature showing cholesterol effects on APP processing. He also offered some intriguing data that Aβ peptide kills neurons by inhibiting cholesterol synthesis and, similarly, that high doses of statins can kill neurons via an apoptotic cascade.

Based on these data, Sparks believes that statins that poorly cross the blood–brain barrier will be more efficacious in clinical trials for AD. Accordingly, he has initiated a 120-patient, double-blind, placebo-controlled clinical trial with atorvastatin, an HMG-Co A reductase inhibitor with poor CNS penetrability. Subjects with mild-to-moderate AD (mini-mental status exam scores of 12–28) will be randomized to one of two groups receiving placebo or a daily dose of atorvastatin. Enrollment, which started in October 2000, is approximately one-third complete.

Summary

Overall, this conference achieved its stated goal of delivering the latest scientific discoveries and updates on clinical trials for therapies for AD. For the most part, the speakers were excellent and provided high-quality handouts. From my vantage, most delegates left the meeting more informed in the field than before and looking towards IBC's *10th Annual Conference on Alzheimer's Disease*.