

## Invest in ME conference London, June 1<sup>st</sup> 2012

The conference was preceded by a 2-day ME/CFS Clinical Autoimmune Working Group meeting. I was privileged to attend this meeting of 20 invited prominent researchers and clinicians. Research into ME/CFS was presented and discussed with a view to ongoing international collaboration. The main conference then followed the next day, and was held in Birdcage Walk, just behind Buckingham Palace, providing an exciting prelude to the Queen's Jubilee weekend. Security was paramount, and the conference was opened by **Dr Ian Gibson** after we had had to clear the building for a fire alarm!

The keynote speaker was **Professor Don Staines** (Gold Coast, Australia). He presented autoimmunity as a plausible hypothesis in the aetiology of ME/CFS. He discussed the research programme being undertaken at Bond University over the past 8 years.

He began by asking the question "Is ME an autoimmune disorder?" He described autoimmunity as a reaction of the body to "self- antigens" involving B and T cells. B cells are antibody-producing and CD20 is a potential biomarker. T cells need major histocompatibility (MHC) recognition. Innate immune responses may be triggered. (eg NK cells, macrophages etc). He then discussed the putative autoimmune targets: ME/CFS is often associated with infections such as campylobacter or conditions such as Guillain Barré Syndrome. Multiple Sclerosis (MS) is associated with Epstein Barr Virus. Molecular mimicry may occur (an autoimmune response to an organism or antigen similar to endogenous structures). In Sjogren's syndrome and myasthenia gravis (MG) acetylcholine receptors are autoimmune targets (eg GPCR). Vaso-active neuropeptides(VNs) – related to glucagon, secretin, insulin – are a super family of small peptide or protein-like molecules, and potential activators of adenylate cyclase (AC), which converts ATP to cyclic adenosine monophosphate (cAMP). VNs of particular interest and relevance to ME/CFS were discussed: PACAP, VIP and CGRP. They are widely distributed in the CNS. Data does support the notion of VN dysfunction in ME/CFS and symptoms do match the putative targets. VPAC2R receptors are dysregulated. There are links to FOXP3 and cAMP metabolism, and the state of receptors is crucial for transmission. cAMP is a very important neurotransmitter involved in the transmission of ATP to AMP. cAMP is a secondary messenger and has a key role in cell metabolism. It acts through CREB and ICER proteins and these have a role in CNS neuroplasticity, involving cognition, memory etc.

Further discussion then focused on blood-brain-barrier (BBB) and blood-spinal-barrier (BSB) function. The BBB keeps unwanted substances out of the CNS with pericytes involved. Vasodilation controls the entry of immune cells. Interleukin-1 $\beta$  and TNF $\alpha$  are toxic to BBB and BSB and are antagonized by cAMP. GPCRs may be inhibitory or stimulatory. Autoimmunity can lead to loss of organ function. PACAP and VIP are regulatory co-transmitters. Skeletal muscle and autonomic transmission maybe implicated. They also act on the acetylcholine system. Various other functions may include: regulation of cardiac firing; an anti-apoptosis role in neuronal cells; insulin control; hypoxia regulation; glutamate metabolism

Recent developments in purinergic signaling also contribute to understanding of pathomechanisms involving VNs in relation to ME/CFS. ATP signals cellular stress and negatively regulates AC. ATP may also have extracellular effects. There are also roles for VNs in Tregs (regulatory T cells) and NK cells. Tregs have an important contributory role.

Another question asked was: Are there abnormalities in mRNA and microRNA?

Discussion then followed as to whether symptoms of ME/CFS relate to neurotransmitter function, Purinergic signalling is involved in neuropathic pain. VN function is involved in neurohormonal

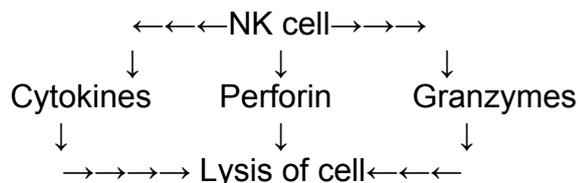
function, immunoregulation, and cardiotropic regulation. Pathomechanisms indicate derangements in the VN function. Abnormalities have been shown on MRI, showing disorders of micro-circulation, which are associated with onset of illness, loss of CNS function and illness severity.

In conclusion, Prof Staines feels that a novel auto-immune mechanism may be involved implicating vaso-active neuropeptides. This hopefully will eventually lead to treatments.

**Sonya Marshall-Gradisnik** (Gold Coast, Australia) presented her work on immunological dysfunction as possible biomarkers for ME/CFS. She pointed out initially that the pathomechanism is unknown, there is no diagnostic test but there is evidence of immunological dysfunction. NK cell function is down, Treg function is implicated in ME/CFS, and research into B cells suggests an auto-immune disorder.

There is significant reduction in NK cell function coupled with significant reduction in intracellular perforin, which is responsible for NK cell lysis. Potential biomarkers include: NK phenotypes, KIR expression, decreased CD8 cell lysis function, decreased gene expression of lytic proteins of NK cells, decreased microRNA.

She then described diagrammatically the function of the NK cells.



There is significantly reduced NK cell function in ME/CFS, which is consistent over time. However the function is similarly decreased in the moderately affected group, so severity is not necessarily significant. There are 2 types of NK phenotypes: dim and bright. The dim seem unaffected in CFS, the bright very much decreased. This is consistent over time in this illness. NK cells are regulated by Tregs. One KIR receptor is associated with NK cell lysis reduction. mRNA gene expression relates to GZMA lytic protein which is significantly decreased in ME/CFS – this is a potential biomarker.

MicroRNA – there is a very small non-protein coding RNA. The mechanism of post-transcriptional gene expression is based on complimentary microRNA. Differential microRNA expression is found in NK and CD8 cells. 16 microRNA studies have been performed and significant difference was found in 9 in ME/CFS. MicroRNA regulates Treg cell function. Treg cells have been measured. FoxP3 is part of Treg function and is elevated over time in ME/CFS, as is CD39 expression. VPACR2 expression in lymphocytes is also elevated in ME/CFS.

In Norway, Mella and Fluge are investigating anti-CD20 intervention using the drug Rituximab. Results suggest that ME/CFS may be an autoimmune condition.

NK cytotoxic activity, NK phenotypes and NK proteins remain as the most consistent immunological markers. Emerging areas include: CD8 lysis, microRNA and Treg subsets. These may provide further promise for biomarker development.

**Professor Hugh Perry** (Southampton, UK) spoke on neuro-inflammation in chronic disease. He described how the symptoms of systemic inflammation affect behaviour. This is not maladaptive, but of survival value. This is an organized strategy and is what occurs in the wild, leading to homeostasis. Examples of effects on behaviour were given such as fever, leading to lethargy, leading to rest. The inflammatory response “speaks” to the brain. The key players are the macrophages in the brain known as microglia. The microglia are down-regulated by various

molecules expressed (inhibitory receptors). In the diseased brain microglia proliferate and then are activated. Perivascular macrophages communicate with the microglia, which in turn affect the neurons.

These inflammatory changes are seen in Alzheimer's disease. Systemic infection in elderly sufferers may be acute or chronic. Worsening symptoms are associated with increased microglia and cytokines in the brain. A longitudinal study was reported in 300 Alzheimer's patients with 6 months follow up of infective episodes. The more infections reported the greater the decline in cognition.

Circulating cytokines lead to increased levels of sickness behaviour. ie the microglia have been primed by infection and the symptoms of underlying brain disease worsen. It is possible microglia are primed by other events. Priming of microglia can occur as a result of prior infection weeks or months beforehand. It is likely that a past history of infection can increase responses in the brain.

He described experiments where rats were infected with S.typhi systemically. There was increased cytokine activity and bacteria were cleared in 1-2 weeks. However, the pro-inflammatory cytokines continued to have effects on the brain with switching on of microglia. All the vascularity of the brain can be affected and effects persevere for months. The environment can have priming effects on the microglia. For example animals who were housed in a "dirty" environment had greater priming, with larger inflammatory response.

He said that ME/CFS seems like an exaggerated form of systemic inflammation with a distortion of homeostasis. Immune to brain communication may have become maladaptive. There may have been a primed innate response in the CNS, which may be the result of genetic predisposition/infection/inflammation/neurodegeneration.

**Professor Maria Fitzgerald** (London,UK) discussed pain in ME/CFS. She initially gave an overview of chronic pain, and described it as a CNS problem. Pain has a purpose and this may involve: warning, defence, escape, learning, protection, rest, healing etc. Rest is part of the healing process. Pain can be maladaptive:

- It maybe poorly related to the actual "injury" eg migraine, fibromyalgia.
- It maybe too late to be a warning eg cancer
- It maybe neuropathic eg post-surgical, post-infection
- It causes suffering, depression, anxiety, lack of mobility etc.
- It leads to a huge economic burden.

She went on to describe the physiology and pathology of pain. It is nociceptive. Persistent pain may be inflammatory or neuropathic. Inflammatory pain is usually tender, aching and accompanied by stiffness. Neuropathic pain is usually stabbing, burning or shock-like. The nature of pain is complex:

- Sensory component
- Motor/autonomic component
- Affective component
- Can alter brain function (eg anxiety)
- Affects attention
- Individual differences
- Culture, gender and age all have effects

There are multiple sites of pain processing. Pain information is modified as it travels to the brain. It can be unpredictable at every level. The nociceptors can be primed (eg by an inflammatory agent) leading to hyper-excitability.

In CFS/ME there is altered CNS processing. Fibromyalgia (FM) patients perceive greater intensity and greater temporal summation. Sensations increase in magnitude, and the CNS winds up more and more. There is alteration of endogenous pain control. FM patients lack diffuse noxious inhibitory control. Normally there is ability to inhibit pain (endogenous opioids). There is altered cortical pain processing in CFS/ME. Numerous areas of the cortex are involved. There is activation of the limbic system (anterior insular-based ganglia and cingulate cortex). The CNS is acting very differently.

Some people may have a genetic predisposition to feel more pain. Single Nucleotide Polymorphisms (SNPs) suggest genes related to pain sensitivity. Plans to incorporate genotyping are needed. Early life experiences may alter pain sensitivity in adult life – eg premature babies who have had a lot of pain exposure, may develop an altered pain threshold. “Injury “ in the first 10 days of life may promote effects that last into adulthood, leading to vulnerability, particularly if the same injury occurs, and the effects can be increasingly severe. It is not necessary to have an infection to prime the microglia, but a “wound” can lead to microglial activation.

The conclusion was that genetic determinants and early life experiences lead to nociceptor sensitisation, which is a potential cause of pain in CFS/ME.

**Dr Mario Delgado** (Granada, Spain) discussed his work with vaso-active neuropeptides. He said there are potential therapeutic opportunities. There is a need to establish a homeostatic balance. Cytokines are involved in the immune balance. The brain and immune system speak the same biochemical language. Vasoactive intestinal peptide (VIP) is found throughout the body and is produced in the CNS, the periphery and non-neural cells. It has a plethora of functions. It is involved in the pathway which activates adenylate cyclase (AC) which in turn impacts on ATP and cAMP metabolism. AC and cAMP signals are critical for neuronal survival and other brain function. VIP receptors are expressed in immune cells and have immunomodulatory activity.

He described therapeutic effects on experimental inflammation and auto-immunity, and finds there is helpful potential in a number of diseases. VIP protects from the lethality of endotoxins. It is neuroprotective in neuro-inflammation caused by brain trauma, and is therapeutic in collagen-induced arthritis. It also has potential for treating experimental autoimmune encephalomyelitis (EAE) (animal model of MS). In this condition it inhibits the inflammatory response and the Th1 response. It also induces the emergence of CD4 CD25 Tregs during EAE. Neuropeptides induce the generation of different types of Treg cells. VIP has been shown to be an important neuropeptide in immune tolerance.

He then asked several questions:

- a) Does a healthy VIP system make us more healthy?
- b) Is there a role in ME/CFS? – as many symptoms relate to the effects of VIP.
- c) Is VIP ready for the clinical situation? - need to consider: side effects, stability, non-oral administration
- d) What are the effects on immunosuppression?

Aviptadil (an analogue of VIP) is marketed for some conditions such as erectile dysfunction and the inflammation associated with sarcoid. VIP has also had some success in Guillain Barre Syndrome and ciguatera poisoning. A clinical trial is in progress looking at VIP by inhalation.

**Professor James Baraniuk** (Washington,DC, USA) discussed potential mechanisms of ME/CFS symptoms. He had designed a simple questionnaire using the Fukuda criteria and looked at

severity scores.. He had produced a 3D diagram and had divided his findings into 4 groups. The ME/CFS symptoms could be clustered, and matched the cerebral spinal fluid proteomics.

He then went on to discuss the symptoms seen in ME/CFS in general.

a) Headaches

He found the prevalence of migraine headaches to be 75-80%. 2/3 of these had no aura, and 1/3 had aura. There was an accompanying range of symptoms. There was improvement with triptans. 67% of those with migraine were found to also have fibromyalgia. There was also a high prevalence of these symptoms in Gulf War Illness. Migraine can cause abnormal cortical depolarization and abnormal pain processing, and accompanying central desensitization. He hypothesised that migraine is initiated in the brainstem.

b) Pain and tenderness

There was increased sensitivity to deep pressure in FM. There was also increased sensitivity of proprioceptive and stretch receptors on nerves innervating the joint capsules, tendons etc. There maybe loss of the anti-nociceptive system, and loss of norepinephrine release, which normally regulates responses, and initiates autonomic responses. It is as if “the light is on but no-one is home”, and the brain is in a default mode pathway.

He looked at pain dolorimetry – pressure-induced thresholds, and did frequency analyses. Central sensitization starts in the periphery – there maybe peripheral sensitisation such as by hay fever. Spinal sensitization leads to hyperalgesia and allodynia.

Microglia are the key players, and microgliosis occurs in pain. There is potential for microglial-neural biomarkers and useful therapeutics. Potential treatments discussed were: Resveratrol (in red wine), antipsychotics, tricyclic antidepressants, anti-oxidants, endocannabinoids (medical marijuana).

c) Brain fog –

He discussed the findings in the grey matter, in particular the midbrain reticular substance and periaqueductal grey matter. Changes indicated that the body’s alarm clock is damaged. There was decrease in the white matter depending on the duration of fatigue, and it was found to shrink by about 1% per year.

d) Sleep

It is likely the brainstem is involved

e) Effects of exercise

With exercise, the brain fMRI shows improvement in healthy people. He then described 2 types of people with CFS: “increasers” who on day 2 of exercise have to work very hard to improve their mental score. And “decreasers”, whose fMRI shows a lot of activation on day1, but no task activation ability on day 2. They are in default mode only.

The conclusions in this presentation were: That fatigue is the result of all symptoms, that headache is a result of myalgia and that general symptoms are due to a fundamental effect in the brainstem.

**Professor Olav Mella and Dr Øystein Fluge** (Bergen, Norway) did a joint presentation looking at B cell lymphocyte depletion in ME/CFS. They reiterated how 3 original lymphoma patients who coincidentally had ME/CFS had improved remarkably following treatment for their lymphoma with the B cell targeting drug Rituximab. In these patients all symptoms relating to their ME/CFS diminished with the treatment. B cells may have something to do with central mechanisms. The

responses however were delayed for up to 6-12 weeks, despite B cells being cleared in about 2 weeks.

A further larger control study was undertaken. Patients in the study group were infused with 2 infusions of Rituximab 2 weeks apart, and the controls were similarly infused, but with normal saline. These patients were then followed every 2 weeks for 12 months. In all patients there was a strong family history of autoimmune disease. Side effects were infrequent – in 2 treated patients, their psoriasis worsened. 2 patients felt “unease” and some insomnia. Several patients had normalization of a previously abnormal menstrual cycle. There was a positive response in 67% of patients. There was a 13% response in the placebo group. Again there was delay in response and after the effects wore off the response declined but was re-instated at the 2<sup>nd</sup> infusion. Some patients however continued to improve but there was not a consistent pattern. BAFF (B cell activating factor) was lower in CFS patients than controls after treatment. BAFF is increased in auto-immune disease. BAFF was again increased at 3-6 and 8 month follow ups. This is the expected normal feedback mechanism.

Psychological symptoms did not change indicating this was therefore not a psychological illness.

There are ongoing studies. 1) 26 patients to be observed for 15 months. This is a subjective study without controls. And 2) A severe group of 6 patients. Plasma exchange may be given before treatment with Rituximab. Patients will be given 2 infusions 2 weeks apart, with maintenance therapy at 3-6-10 and 15 months. Follow up will be for 3 years. Non responders will be given etanercept weekly subcutaneously for a year. They are also looking at a study on genetic predisposition in 3 families. As yet there is no clear candidate as a plausible target for an autoimmune process. Over the past 3 years they have looked for a specific autoantibody, but have found none so far. Something else may be triggering a pro-inflammatory state. They reiterated that patients should not be treated with Rituximab outside clinical trials.

The next presentation was by **Professor Indre Ljungar** (Stockholm, Sweden). Her focus was on a one year experience of a standardized team-based assessment of suspected ME/CFS. The aim of this ME/CFS project was to improve diagnosis, transfer clinical knowledge to primary care, to establish rehabilitation methods and to conduct research. The project was established in 2010 and used a team based approach such as used in other diseases. A multidisciplinary team was involved consisting of a doctor, nurse, psychologist, physiotherapist, occupational therapist and social worker. Diagnosis was based on the Fukuda and Canadian 2003 case definitions. Every patient had a full medical workup, and a wide range of tests were used. Included were blood and urine analysis, polysomnography, 3T brain MRI. Also included were 2-day activity scores, sleep questionnaire, WAIS, SF36 etc.

In the past year 101 patient visits were referred for evaluation, and ME/CFS was suspected in 55%. Of these 33 fulfilled the criteria for ME/CFS. Of the others, diagnoses included psychiatric illness, sleep disorders, neuropsychiatric disorders, other physical illnesses, fibromyalgia, idiopathic fatigue etc. The importance of multi-disciplinary team assessment, coupled with thorough medical investigation was stressed, as symptoms are very complex and there is considerable overlap with other disorders. This approach helps to create a homogenous group for potential research. Subjective symptoms can be tested objectively and reliable diagnosis helps in recommending treatment.

**Dr Daniel Peterson** (Incline Village, Nevada, USA) gave a clinical research update. 6000 articles have now been published on ME/CFS. The greatest challenge is the disease itself, and there is a lack of biomarkers. In clinical research, IT and translational medicine are becoming more widely used. Translational research leads to multidisciplinary collaboration, accountability and standards, shared data and data integration and common goals drive the advancement of applied science.

The benefits are: larger samples, decreased cost, more controls, increased value of results, decreased time lines, and the whole approach is more manageable for clinicians and researchers, many of whom may only work part-time. The disadvantages are that technology is forever playing catch-up and there is almost too much information generated.

He went on to describe several new projects:

- a) XMRV/MLV – funded by NIH at 5 sites, using a centralized lab. Results will be published at end of June 2012. Samples have been established, so may be open to further research.
- b) Chronic Fatigue Initiative pathogen discovery project to establish a biobank. There are 40 patients and 40 healthy controls at 5 sites. Samples of blood, saliva, urine, faeces and tears will be taken. This will help to establish subsets, which is particularly important for pathogen discovery. A centralized biobank will be established and samples kept at -80°C.
- c) Collaborative research using cerebro-spinal fluid. The goal is to look for aberrations, pathogens and markers. 30 cases are being investigated, evaluating cytokines and microRNA in the spinal fluid
- d) Simmaron/Bond – molecular and cellular investigation to identify NK function.
- e) CFIDS funded research projects – have established the Research Institute without Walls for translational research, and are funding 5 studies:
  - 1) Brain imaging looking at blood markers after exertion.
  - 2) Cognitive improvement looking at treatment of unrefreshing sleep and effectiveness of drugs
  - 3) Epigenetic markers –(heritable changes in gene expression)
  - 4) Brain fog, orthostatic challenges and therapeutic approaches.
  - 5) Autonomic dysfunction looking at neuromuscular strain/central sensitization.
- f) CASA (collection, aggregation, storage and analysis) – a collaborative effort between the NIH, CDC and clinicians and researchers comparing studies of ME/CFS. Using many different questionnaires is not helpful, and a consensus document is needed using standardized questionnaires. Results are otherwise divergent and often not useful. The goals therefore are to establish research standards and determine appropriate tools.
- g) The Open Medicine Institute headed by Dr Andreas Kojelnik.

**Dr Andreas Kojelnik** (California, USA) is Medical Director of the Open Medicine Institute (OMI) – a community-based research clinic focused on chronic infectious diseases, neuro-immune disease and immunology. He feels there is much optimism now in that treatments are beginning to emerge, mainstream science is getting interested and technologies are advancing our understanding. Disease acceptance in 2012 is far better than in 1992, and he pointed out that many other diseases have been through a similar history, with understanding evolving slowly. We are in a different era now, with genetic profiling becoming useful, and accurate in diagnosis and treatment. In ME/CFS there is a syndromic research conundrum. There is a lot of overlap, definitions are not mature, there is scarcity of biomarkers, treatments are not standardized and outcome data is limited.

He described how the OMI works by creating a network between biotechnology, informatics, social networking and biosampling all linking into clinical medicine and research. There is need for convergence of these disciplines. A large network gains: longitudinal controls, plenty of samples, a lot of pilot treatments, control over protocols and standards, control over lab measurements and standards and options to use the best of the best.

He went on to describe research progress in the past year:

- a) Positive studies with Rituximab
- b) XMRV studies “debunked”
- c) CDC funding clinical networks

- d) Ampligen study published
- e) Other molecular studies showing progress: eg spinal fluid in ME/CFS vs Lyme disease, blood findings in depression vs ME/CFS/FM

He then outlined the work being undertaken at the OMI:

#### Diagnostic studies

- a) Viral flora – sequencing and quantitation
- b) Antibody/antigen array
- c) Cytokine arrays
- d) Deep sequencing

#### Treatment studies:

- a) Valganciclovir and other antivirals (NB long delays in response)
- b) Anti-inflammatories
- c) Rituximab
- d) Rifaximin (antibiotic) etc

#### Aetiology studies

- a) Immunological studies
- b) Infections: bacterial, viral
- c) Metabolic derangements (VO<sub>2</sub> max, mitochondria etc)
- d) Environmental factors (heavy metals, diet etc)

Today, much collaboration is occurring internationally between academia, industry and government. The OMI is very involved and now has a biobank of 10,000 patients.

This conference was organized by Richard and Pia Simpson for Invest in ME, and I must thank them for producing a wonderful event. Thanks must also go to the Alison Hunter Memorial Foundation and ANZMES for enabling me to attend.

Rosamund Vallings MNZM, MBBS