Invest in ME C11 2016

On 3rd June, 2016, I was privileged to attend the 11th Invest in ME International conference in London - the theme being "A new decade of Invest in ME research". The conference was held at a wonderful venue, at number 1, Great George St, Westminster. This provided space, and being all on one floor, was suited well to those with ME/CFS. And we could look upwards at the magnificent painted ceiling during breaks. The conference was, as always, well attended by those with ME/CFS, carers, advocates, doctors and researchers. 18 countries were represented.

The conference was opened by **Dr Ian Gibson** with a brief overview of 10 years of research, with the comment that research is now forging ahead, and we are entering a new dawn. ME/CFS is a global problem, needing much more funding and education. There needs to be action at the level of the United Nations. In Britain a Centre of Excellence is proposed.

The first speaker was **Dr Vicky Whittemore**, (Programme Director in the National Institute of Neurological Disorders at the NIH, USA). There has been 5 years of research into ME/CFS at the NIH, leading to a new vision for research in the USA. The NIH is one agency made up of 27 institutes and centres. There is intramural research within the NIH campus and extramural research going on elsewhere.

The intramural protocol for ME/CFS research is headed by Dr Avi Nath. They are planning to recruit 40 post-infectious ME/CFS patients (within 5 years of onset). There will be extensive analysis using biospecimens. Additional cohorts are envisaged.

A working group was revitalised in 2015, to develop long, intermediate and short term goals, with strategies to support and stimulate ME/CFS research. Aims include: fostering international collaboration, setting research priorities, supplementing grants etc. Research funding has been averaging $5-6 million annually over the past 8 years, and there is much room for growth. The NIH needs information, and needs to know the emerging needs and opportunities. New young researchers are to be encouraged, with patient engagement and support from academic centres. In the long term: research will be expanded, new treatments developed, with support for clinical trials and the aim should be improved quality of life for those with ME/CFS.

Professor Olli Polo (Tampere, Finland) is a pulmonary medicine specialist working in the area of sleep, apnoea and fatigue. He has seen 500+ ME/CFS patients. His main opening message was how to get GPs to recognise symptoms, take patients seriously and then to refer on. Explanation of the physical signs and symptoms is necessary to make the complex symptomatology of ME/CFS understandable. He explained the IOM report and criteria. He remarked that the various criteria tend to make this a diagnosis of exclusion, but it should be a primary diagnosis, based on symptoms. He discussed the past scepticism and focus on this being a mental health issue – or even a figment of imagination! Doctors do need to see these patients to believe in them. Identification of physical signs should support the symptom array to give diagnostic credibility as there are no biomarkers as yet. All systems are affected.

He then went on to discuss the physical signs, illustrated by a number of excellent photographs. Clinical signs are more evident in females than males. He discussed the fact that there are often "opposite" symptom types: lean v obese, hypermobile v stiff, hyponatraemic v hypernatraemic etc. He pointed out that there is a subset with Ehlers Danlos Syndrome, and quoted the work of Dr Peter Rowe, but not all ME/CFS patients have hypermobility. Many of the photographs then shown were related to the hypermobility of joints throughout the body, skin changes, varicosities, elasticity of skin and muscles, fingernail clubbing, difficulty maintaining posture etc.

There is often a pre-phase of the disease – often these patients are "Duracell people" who are interested in everything and physically very active. The search for hypermobility should be part of the evaluation

The central descending sympathetic tone pathway is abnormal, and vital functions are compromised during sleep. Normal "rescue activation" does not occur. When awake there are then haemodynamic problems – there has been no "rescue" by sleep, and symptoms (including poor brain perfusion) are then evident. Some increase in apnoea may also contribute to symptoms.

Professor Carmen Scheibenbogen (Berlin, Germany) then spoke on auto-antibodies and acetylcholine receptors in ME/CFS. She explained how autoantibodies directed against neurotransmitter receptors are causing various types of auto-immune diseases. 1/3 of patients in an immunological outpatient clinic were found to possibly have ME/CFS. Immune activation (increased IgG levels, elevated ANA titres and/or T cell activation) was found, leading to likely auto-immunity and disturbance of nervous system regulation. A role of auto-antibodies is ME/CFS is suggested, and treatment with rituximab may help after 3-4 months of treatment. This drug targets the auto-antibodies. Chronic stress can also lead to auto-immune dysfunction. Japanese patients have also been found to have elevation of muscarinic acetylcholine antibodies. They suspect that auto-antibodies can activate immune cells by imitating adrenaline/acetylcholine stimulation. Various ME/CFS symptoms can be explained by overstimulation of the sympathetic/parasympathetic nervous system.

She discussed the rituximab trials in Norway – some patients had improved and some had no response. Those patients improving had normalisation of the auto-antibodies, which then tended to stay low. Other treatments discussed included high dose immunoglobulin and immune adsorption.

Dr Jo Cambridge (UCL, London) **and Fane Mensah** (PhD student) discussed B cell biology and rituximab treatment in patients with ME/CFS. Initial explanation clarified the brain/gut/immune system connections, with effects to and from the sympathetic nervous system, cytokines and auto-antibodies. Antibodies are produced by the B cells to molecules in the sympathetic nervous system. B cells are produced in the bone marrow, pass into the tissues, produce antigens which interact with T cells and form auto-antibodies.

B cells are affected by rituximab, which binds to and kills CD20 B cells. It is used immunotherapeutically in rheumatoid arthritis, lupus and lymphoma, all of which are affected by B cell function. Rituximab does not affect pre-B cells or memory B cells. In their trials, initially rituximab 1 gm is given intravenously 1-2 weeks apart. B cells are quickly killed in the blood, but decline slowly in the tissues. A blood test is performed at one month to see if the B cells are decreased, and there maybe residual memory cells. B cells start to resume from the bone marrow at approximately 6 months.

Rituximab works best in autoimmune diseases where auto-antibodies are part of the disease process. It removes the "parent" of the auto-antibodies, and stops the supply of auto-antibodies in the pro-inflammatory immune complex (such as in lupus and rheumatoid arthritis). Because of some positive outcomes in ME/CFS, does this imply that auto-antibodies are part of the disease process? The response can take months, and the time varies. Tests need to be done at baseline, depletion stage, repopulation stage and during relapse. The B cells return once the rituximab is cleared from the bone marrow. Relapse is not always associated with B cell return. In rheumatoid arthritis, 70% respond after 4 weeks, B cells are back in approximately 6 months. Some B cells can trigger a relapse at certain times.

In ME/CFS, rituximab stops the B cells differentiating into plasma cells, and stops the B cells interacting with other cells (such as T cells). Treatment protocols can be fitted to how the B cells are involved, by looking at the changes in the B cells. Interventions should be modelled specifically in the immune system changes in ME/CFS.

Fane Mensah's particular research focusses on comparing B cell phenotypes in ME/CFS patients and controls. Differences have been found in the maturation marker CD24 and in whole CD19. Functional studies are done on B cells after stimulation and culture for 5 days. He also uses "Microtacker" to look at mitochondria. There are low mitochondria in naïve compared to memory B cells. He is also investigating T cell interactions with B cells, and is using an in vitro system looking at soluble serum factors. There are other anti-B cell drugs, and trials are needed.

A question from the audience asked if there was any legal way of getting rituximab. The answer was "No, we do not yet know enough about the implications". Fluge and Mella do not want it used, because it is really their responsibility. Doctors are advised not to treat with this drug outside a clinical trial.

Professor Tom Wileman (East Anglia, UK) then discussed the gut virome in ME/CFS. The gut contains billions of bacteria, and has its own immune system, which ignores our own bacteria, leading to homeostasis. It will attack "bad" bacteria. The immune system and microbiota keep in balance, but an inflammatory threshold may be reached leading to production of cytokines and interferons. This can then affect other organs, leading to disease. Many diseases are implicated. A patient may have a predisposition because of host genes (immunophenotype)

The gut also contains viruses, which are an important part of the inflammatory threshold. Some viruses live in or on the bacteria (phages). A number of small and large viruses are involved. They may kill the good as well as bad bacteria. The virome and genotype compromise the immunophenotype leading to an inflammatory threshold. The bacteria may then move into the immune system and upset the inflammatory threshold. The greater the diversity of viruses, the less the diversity of bacteria. The metabolites of bacteria maybe affected thus affecting the immune system.

There is a need to find the viruses in the gut. The technique was explained with illustration. 100mg of faeces was placed in water, centrifuged and strained to obtain just the viruses. 16 samples of those with moderate ME/CFS have been studied. Each sample gives 2 million readouts. Software then sequences the viruses. 23 different families of bacteria were found, each with different phages. The imbalance of phage population may correlate with disease. This has been shown in inflammatory bowel disease.

Professor Don Staines (Gold Coast, Australia) gave an update from the NCNED (Griffith University) entitled "Receptor identification and intracellular signalling". He described evidence for a channelopathy in B lymphocytes and Natural Killer (NK) cells. NK cells lyse and their cytotoxic activity is down. Transient Receptor Potential ion channels (TRPs) may have a major role. They are located on every system in the body. TRP receptors amplify signals, particularly to calcium – this is a "physiological treatment".

Their recently published paper reported examination of 678 Single nucleotide polymorphisms (SNPs). Eleven SNPs for TRP ion channel genes (*TRPC4*, *TRPC2*, *TRPM3*, and *TRPM8*) were identified in the ME/CFS group. Five of these SNPs were associated with *TRPM3*, while the remainder were associated with *TRPM8*, *TRPC2*, and *TRPC4* ($P<0.05$). There was a reduction of *TRPM3* receptors on B cells. This has the potential for a biomarker.

He suggested that intracellular calcium channel pathways may be affected, with impairment of calcium signalling. There is a complex pathway analysis. Calcium is allowed into the cell but needs to be outside. Protein kinases are critical in cell function. ERK 1 and 2 are impaired, leading to upregulation of inflammatory cytokines : TNFα, GM.CSF (Granulocyte-macrophage colony-stimulating factor an important hematopoietic growth factor and immune modulator), and INFα.

The conclusion was that impaired TRP receptor function and impaired calcium signalling and stores are suggestive of the pathology of ME/CFS.

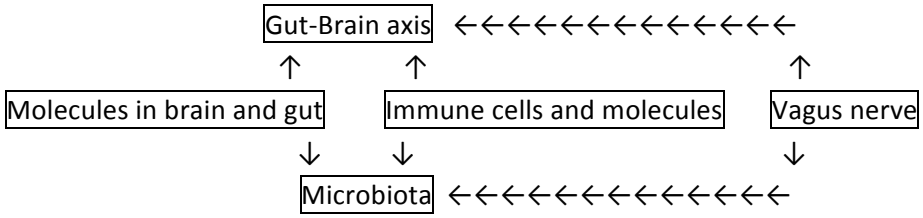
Professor Simon Carding (Norwich Research Park, UK) talked about the work of the European ME Research Group (EMERG). He stressed the need at address biomarkers, including brain imaging. Looking for cause, making a diagnosis and standardisation of samples should be included. A "together approach" is needed. Euromene is a group of researchers hoping to establish a network across Europe. Euromene and EMERG will work together. There is a need to look for opportunities for funding. They will build on current activity and feasibility, such as:

1) Infectious origin a) environment
   b) microbiome alterations

2) Clinical trials a) Rituximab
b)Bacteria based therapy.

Coming together is a beginning. Keeping together is progress. Working together is success. (Henry Ford)
The Journal of Clinical Medicine has just accepted an article by Navena Navaneetharaja on: The Role of Intestinal Microbiota and Virome in ME/CFS.
An audience question asked about role of probiotics: Answer: A cocktail of these is not usually effective. They may be helpful, but there is scant evidence.

Professor Mady Hornig (Columbia, USA) discussed the programme at Columbia University. She pointed out that there are many microbial factors in brain disorders. There is gastrointestinal comorbidity in brain conditions, including a subset with ME/CFS, and often sleep disorders. Microbiota are complex and involve the whole body. Normal microbiota modulate brain development and behaviour. Many things can cause disruption, from pregnancy to birth and infancy. Then follows environmental effects, genetic development and issues such as antibiotics. Serotoninergic effects continue over a lifetime. She then described several important pathways:



Metabolism involves the gut → blood →brain.
There is an innate immune response to viral and bacterial challenges.
Cytokines → Brain→ sympathetic activity → POTS etc.
Work underway was then discussed.
Their team uses a staged strategy for pathogen disarray in immune-mediated brain disorders. They have looked at DNA and RNA agents using multiplexed immunoassay. They are mapping the host response to exercise challenge – a tricultural study. They are looking at normal microbiota too. Autoimmunity is modulated by intestinal microbes, so autoimmune responses may relate to microflora composition of the gut. There is a mechanism described for eating and anorexic disorders too.
There are distinct plasma immune signatures for ME/CFS present early in the course of the illness.
They are also looking at the cytokine network analysis of the cerebrospinal fluid in ME/CFS. They are trying to tie all this back to the microbiota. Processes are important as is regulation - keeping things well balanced.
There is much progress in learning of all these mechanisms. A quote from Einstein probably sums up the progress: Comment from student: "The questions in the exam are the same as last year?" Einstein's reply "But this year the answers are all different".

Professor Maureen Hansen (New York, USA) leads a large team looking at biomarkers for ME/CFS. She asked the question as to why we need a biomarker. She provided 4 answers:

1. The need for a diagnostic test to distinguish ME/CFS from other illnesses
2. The need for objective measures for the effect of interventions and drug therapies
3. Selection of participants for research
4. Information that can be used to identify underlying causes of ME/CFS and its major symptoms.

Potential biomarkers include:

1. Altered NK cell activity
2. 2-day cardio-pulmonary exercise tests

3. Abnormal brain imaging
4. New biomarkers which need replication.

She then discussed the human microbiota – telling us that there are as many microbiota cells (mainly in the intestine) as human cells. They provide protection against pathogens. She cited an important article in Science (April 2016) – "Microbiota at Work". Gut microbiota abnormalities are associated with disease (eg diabetes, Crohn's disease). The question is "Is this a cause or a consequence?" Associated factors include diet, health status, genotype, age etc.

Gastrointestinal symptoms are common in ME/CFS. One study of 38 female (30 controls) and 11 males (9 controls) was described. 32/47 patients suffered intestinal discomfort compared to 8/39 controls.

Some bacteria have lipopolysaccharides on the surface and these can be a marker of inflammation when in the bloodstream. They are significantly elevated in ME/CFS compared to healthy controls. sCD14 levels are also down in ME/CFS. These findings indicate damage to the gut. They have also looked at DNA sequencing of microbiomes. This can reveal the family and sometimes the genus. Operational taxonomic limits are used to cluster the DNA sequences. This technique can be used to separate animals with different types of diets. However this did not separate patients and controls, so other methods are needed.
Bacterial diversity was looked at – the more sequencing, the more species are discovered. Controls show more diversity than patients. Do probiotics help? Again the answer was that she did not know, and this needs further investigation.

She also looked at a computational method to identify ME/CFS. 53% of patients were identified in this way. The limitations of these studies are:

1. That the data are inadequate to identify particular strains of bacteria
2. They do not reveal what beneficial pathogens are present
3. No indication of whether viruses are present

None of these findings can be explained by psychological theories!

Professor Elise Oltra (Valencia, Spain) presented work on molecular biomarkers of ME/CFS. She said that biomarkers are needed for accurate diagnosis, and are essential tools for accurate diagnosis. She has chosen to work with microRNAs, which are more stable, once identified are easy to implement for diagnosis and have been unexplored for the possibility of ME/CFS. They regulate gene expression by controlling target translation and/or degradation.

Already microRNA has been identified significantly for fibromyalgia (FM). Genome-wide expression profiling of microRNA was assessed on PBMCs of 11 FM patients and 10 controls. All patients were diagnosed with chronic fatigue using the Multi-dimensional Fatigue Inventory (MFI). Results were displayed on a gene expression omnibus. There was an inhibitory tendency in the patient group, but no useful upregulating microRNAs.

Professor James Baraniuk (Washington USA) – went through the various definitions and criteria for making a diagnosis of ME/CFS. Early description and criteria for a diagnosis of "Fibrositis" (1843) closely matches our ME/CFS defintion today. In the 1990s the FM definition was stripped down to include 11/18 tender points, when the examiner's thumbnail blanches with a pressure of 4kg, and the result is positive if pain is felt. In 2010 this was thought to be a very difficult assessment and could be linked to catastrophising. He then discussed the scale for "Catastrophising". When this assessment scale was used in ME/CFS, GWI and FM, results were positive with GWI being the highest.

Further discussion of the previous ME/CFS diagnostic criteria followed, including the acute onset encephalomyelitis (Royal Free epidemic), !994 Fukuda definition (CDC viral epidemiology analysis), 2002 Carruthers definition (emphasising post exertional malaise and autonomic dysfunction) and the 2015 IOM report renaming the condition Systemic Exercise Intolerance Disorder (SEID).

Returning to the pressure point analysis, dolorimetry is now being used, but false positives can occur. The analysis is more likely to be positive in FM. But this form of analysis is less useful for men. Women tend to be "more tender" .

He described the 2-day exertional exhaustion testing being used. This involves pre-test blood analysis and MRI, then the exercise on day 1, followed by further blood test. On day 2, exercise test was followed by MRI and lumbar puncture. Results from day 1 showed good muscle function, on day 2 there was bad muscle function. He described testing for exercise induced POTS. The patient lies down for 5 minutes, then stands. 25% had tachycardia. One patient studied had severe hypotension, but this was reversed by drinking gatorade (electrolyte drink). It is probable that exercise dysregulates the autonomic nervous system causing these symptoms.

Professor Ron Davis (California, USA) – discussed the Big Data Approach. He was on the IOM panel and mentioned also that he has a son with ME/CFS. His team is looking for biomarkers. Assessments are being made with blood, urine, saliva and stool. They are looking widely and the cost per patient is $70,000. It is possible they could look more intensely at the severely ill, as these patients may have larger molecular signatures. Then those results could be applied to the less severely ill.

He has already looked at 25 severely ill patients (and 10 controls) and done a wide range of tests including 10 tests on sequencing and metabolomics. 7 immunology tests will be added in. Much expertise is needed as new "devices" are developed. They are particularly keen to do more with metabolomics and services have been donated by "Metabolon". The next pilot will do 3 patients compared to 43 healthy controls (precision medicine) and this will mainly be done on serum. This will lead to a massive amount of data. A lot of often very big differences are being found in ME/CFS. He provided a list of 24 top substrate outliers.

Tryptophan metabolism was discussed. This is upregulated by infection and affects melatonin production. Melatonin controls sleep onset.

He concluded by saying that responses in research are not the same for mice and humans, and often mice are used in research. They are really not suitable for validating a drug.

Dr Ian Gibson wound up the conference with reference to the latest findings as discussed through the day. He took more questions and discussion points from the floor. He thanked the many talented speakers. The conclusion was that much exciting research is now forthcoming and there has been a tremendous advance over even the past year, with much hope on the horizon.

I must thank Invest in ME and ANZMEs for making it possible for me to attend this very exciting day.

Rosamund Vallings

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