Invest in ME

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9th Invest in ME International ME Conference

BIOMEDICAL RESEARCH INTO ME

On 30th May, 2014 I was privileged to attend the 9th Invest in ME conference in London. The conference opened with a brief trailer about the film "Perversely Dark" – a film produced in Norway about 2 young people with ME/CFS. This presented a moving preview of a "must see movie" with English subtitles.

The main conference was then opened by **Dr Ian Gibson**.

The first speaker was **Prof Jonathan Edwards (London)** who spoke about the lessons learnt for ME from his lifelong study of Rheumatoid Arthritis (RA). He felt he was looking from the "outside". He likened the developing studies in ME to how things had evolved with RA from 1974. The tools needed are: 1. Reproducible bio findings to build on an explanation of symptoms, and 2. A theoretical framework to build upon. By 1974, a lot had been learnt: genetic markers, an association with smoking and presence of antibodies in most patients. (Rheumatoid factors and/or anticitrulline). Inflammation is mediated by the cytokine TNF.

He then asked what factors cause disease. These include: internal genetic, environmental and internal stochastic (random) – an internally driven mutation. He discussed the antigen/antibody/B cell/T cell links. The immune complex Fc gamma IIIa expresses in many tissues and leads to release of TNF. There do not have to be external triggers necessarily, but there can be self-perpetuating auto-reactive B cells. ost auto-immunity arises by chance production of subversive B cells. The logical treatment is to remove all current B cells and start again. e.g. Rituximab – treatment with this was begun in 1998 for RA. Not all patients got better, but 2/3 had a good response – many eventually relapsed, therefore this is not the whole problem.

Lessons for ME: The mechanism may be subtle. Genetic clues are like gold-dust. (e.g. NK receptors). Specific auto-antibodies make things easier, but evidence for a general immune mechanism may do. There may be no specific infective trigger, but for some with ME, maybe there is. A cytokine pathway helps. There will be several "ME diseases"- just like RA. One can be surprised by what can be achieved.

In the Q&A session, he was asked if Rituximab was safe in the presence of infection. The consensus was that it should not be a problem in general, but that it should not be used in those with hepatitis C.

Angela Vincent (Oxford, UK) spoke about the searches for antibodies in neurological diseases and posed the question as to whether they could be similar to what may be happening with ME. She talked first about the classical auto-immune disease Myasthenia Gravis (MG) This is characterised by weakness and fatigue and is due to

an acetylcholine defect. Acetylcholine is produced at the axon to the axon receptor, this causing muscle fibre contraction. In MG there are not enough receptors as a result of antibodies to the protein receptors. The antibodies are made by the white blood cells (specifically the B cells) and are measureable in the circulation. The tips of all antibodies are what are different between them. They bind to protein on human cells and cause auto-immune disease. Antibodies can be transferred to mice and this will cause the same disease in the mice. Patients can improve with immunotherapies such as plasma exchange, steroids and immunoglobulins. The genes may be different in early and late onset disease. Another protein important in MG is MuSK.

Antibodies to VGKC (complex proteins, associated with peripheral CNS diseases) cause illnesses with many symptoms similar to ME. Acquired neuromyotonia was described. This is associated with a lot of twitching, muscle pains etc. and is due to an auto-immune potassium channel defect. Potassium channel proteins regulate nerve depolarisation and neurotransmitter release. Another disease described was Morvan's syndrome. This is a CNS disorder with major sleep problems- there is low melatonin production. Symptoms continue even at rest. Symptoms improve with plasma exchange and immune suppression. Limbic encephalitis another auto-immune neurological disease is associated with extreme short term amnesia. Plasma sodium may be low. The antibody LGI1 is associated with memory loss and seizures and is common in limbic encephalitis.

Other diseases were described including one associated with an ovarian terratoma, resulting in an encephalopathy. In another NMDAR antibodies are driven by infection eg HSV encephalitis. Antivirals can sometimes help, but patients tend to relapse. Other conditions with auto-immune probability include narcolepsy, Tourette's, autism and PANDAS. However, the relevance of antibodies in these conditions is not yet established, and some findings may be entirely incidental.

Jonas Blomberg (Uppsala, Sweden) discussed infection-induced auto-immunity in ME. His lab uses a multiplex technique, and they are able to look at hundreds of different antibodies at a time. He described how autoimmunity is avoided in the foetus by central deletion of self-specific cells in the thymus. It is normally hard for the body to distinguish friend from foe. Autoimmunity can do damage to the CNS and peripheral nerves. ME usually starts with an eliciting event leading to its clinical hallmarks – this can be bacterial or viral. He said we need to look at the comorbidities which may often also be auto-immune. There may be post-translational modifications or non-protein antigens in both microbes and humans, plus cross-reactive conserved microbial proteins. There are signs that ME patients have impaired mitochondrial function and this may relate to post exertional malaise and exhaustion. There may be impaired energy metabolism due to block by some antibodies which affect metabolism, such as IgA anti-pyruvate dehydrogenase. Many neurological diseases have an autoimmune basis e.g. MS, GBS, narcolepsy, Tourette's, PANDAS and acute disseminated encephalomyelitis. There are also many examples of post-infectious auto-immunity. Organisms involved may be: mycoplasma, chlamydia, EBV, CMV, Toxoplasmosis, Borrelia etc. There may be cross reaction between bacteria and viruses. It is better to

look for the antibodies, rather than the microbe itself. 910 antigens have been tested in their lab. It is possible to distinguish between MS and ME.

He asked the question can auto-immunity explain ME? It does need further study. Looking at the co-morbidity, several organs have auto-immune aspects, but there is increasing evidence that these co-morbidities such as IBS and FM are not auto-immune.

Mady Hornig (New York, USA) addressed her work on Pathogen Discovery. She has looked at the "3-Strike Hypothesis" – genes, environment and timing. Many microbes have been studied in many diseases. A lot of diseases have an immune-mediated pathogenesis. Koch postulated in 1890 that a specific microbe occurs and can be isolated in every disease. And Rivas in 1937 described the auto-immune response. Witebsky's criteria in 1957 showed that freshly circulating or cell-bound antibodies and their specific antigen target could be identified. Rose and Bona in 1993 showed that auto-antigen specific T cells may induce disease. In 1996 Fredericks and Relman demonstrated molecular markers.

The blood brain barrier (BBB) is a protective lining, but it may not protect the circum-ventricular organs (CVO). There are many signs suggestive of an auto-immune response in ME, and many auto-antibodies target the brain. They may break down the BBB and get access to the CVO regions. She went on to describe as an example, PANDAS – a bacterial auto-immune neuropsychiatric disorder. There may be associated OCD, anxiety and tics.

Potential pathogens implicated in ME were discussed. She described "de-discovery" of certain pathogens, such as XMRV and bornaviruses in ME as being non-implicated. She also explained that there is now no proven link between measles vaccination and autism. However in many diseases there may be severe intestinal dysbiosis and microbiota changes may be relevant. There are a number of staged strategies of ongoing ME studies looking for pathogens. Searches for DNA and RNA agents has as yet found very little. The serum is often viral free, so there is need to look at the PBMCs. When looking for pro-inflammatory cytokines, allergy-related immune signatures are more prominent. There is decrease in ecotaxin, while many cytokines are increased. Auto-immune disturbances may result from failed uptake of dietary precursors of antioxidants in the terminal ileum.

Microbiota have an important role in the tryptophan degradation pathway, and also melatonin production is affected. And auto-immune disturbances may relate to the GI tract. i.e microbes help the brain along through tryptohan and serotonin.

Carmen Scheibenbogen (Berlin, Germany) discussed the role of EBV in ME. She described how a subset have disease onset associated with EBV. Then there may be recurrent fever and nodes and the patient describes the illness as if infection is

ongoing. There may be EBV IgM and EA-IgG elevation. EBV DNA is detectable in the blood. Some patients will improve with anti-viral treatment.

EBV belongs to the Human Herpes Virus family, and the infection may be so mild as to be described like a common cold. The infection is lifelong and may be latent. The primary infection is usually in childhood and spread by saliva in 80% of cases. The illness is often severe in adolescence. There has been shown to be possible association with late onset EBV and auto-immune diseases such as MS and SLE. 98% adults carry latent infection. This can reactivate in immunodeficiency illnesses causing chronic active EBV, lymphoma etc. Diagnosis is by detecting specific antibodies (IgM early and IgG later).

She is currently looking at 2 projects:

1. Characterisation of EBV specific B and T cell response. She has found EBV specific antibodies: elevated EBV-IgM (marker for reactivation), absent EBV-EBNA antibodies in some with ME, diminished or absent EBV-specific memory B cells in many ME patients. These findings may indicate a deficient response due to late EBV infection or possibly frequent reactivation. Elevated EBV copies (EBER) was found in the blood of less that 10% of patients. There was no evidence for lytic replication.
2. EBV sero-array- looking at 5292 peptides. There was a different response in different patient cohorts. There was enhanced antibody response against EBV peptides in ME versus healthy controls.

This is all a basis for development of diagnostic tests and treatment development.

Prof Simon Carding (Norwich, East Anglia) looked at the role for leaky gut and intestinal microbiota in the pathophysiology of ME. There has been an explosion of interest in the last 2 years. The gut is 9 metres long and has the largest collection of neural cells in the body. It could be described as our “second brain”. It is also the largest immune system in the body with a huge area of surface villae. There are multi-layers of protection. The microbiota form a protective barrier. There are 100trillion microbes in the gut ranging from bacteria to fungi to viruses. So 99% of our DNA is microbial in origin. The microbiome refers to the genes. The microbiota weigh 1 kg and have a volume of 1L. There are between 300 and 1000 species. Food is the fuel for the bacteria and 1.4L of gas is produced daily. 60% of the stool is bacteria. The food and who we are shapes our microbiota. It is strongly influenced by species and region. The microbiota originate from our mothers and there are changes with age. They are there for protective function, structural function and metabolic function. In fact the intestine is a “bioreactor”, and the microbiota are essential to providing our daily needs.

The absence of microbiota compromises our health. “Germ free” animals have various defects as a result – such as a poor immune system and susceptibility to infection. Gut microbes however can cause disease in humans: e.g. H Pylori, clostridiae and enterococci.

There is a microbiota gut/ brain axis – there is increasing evidence that the bacteria are a source of effects on brain function and disease. Stress also impacts on the microbes in the gut. The normal gut microbiota modulate brain development and behaviour. He asked the question "Is there a role involving the microbiota in ME?". There may be alterations in the intestinal barrier, leading to "leaky gut", malabsorption and inflammation. There are many possible causes of the so called leaky gut: drugs, infection, stress, antibodies, diet, neurotransmitters, cytokines, enzymes etc. Bacteria can breach a leaky barrier. There are many disease associations. In ME, IBS is common. This may be associated with auto-immune responses. Probiotics may have a potential role.

Sonya Marshall-Gradisnik (Gold Coast, Australia) updated us on the current knowledge of immunological biomarkers in ME. She initially described the different cells in the innate (dendritic and NK cells) and adaptive (NKTcells, T cells, B cells and γδT cells) immune systems. NK cell function is apoptosis by exocytosis of perforin and granzymes. There are 2 main types of NK cells: CD56dim - whose main function is lysis, and CD56bright whose main function is to produce cytokines that activate NK cells. MiRNA controls gene expression.

The aim of recent studies has been to compare changes in relation to the severity of the illness. NK lysis has been shown to be decreased markedly in severe cases compared to moderate cases and controls. KIR receptors are inhibitory. The dim phenotype KIR2DL1 is significantly reduced, and CD94dim is increased in moderate and severe cases. These are responsible for increased cell lysis. Dendritic cells are increased significantly in moderate and severe cases. This is accompanied by increased production of cytokines, which cause clinical signs and symptoms. With B cell phenotypes, there is significant increase in memory and naïve B cells, due to increased dendritic cell and cytokine production. This indicates an auto-immune response. γδT cell phenotypes are significantly decreased with reduced lysis function. iNKT cells are increased in severe cases and this leads to increased cytokines. NK cell lysis is low and there is significant reduction in adhesion markers. There is decreased migratory ability of NK cells to migrate towards the antigen to lyse. MiRNA plasma in ME – significant differences are expressed between ME cases and non-fatigued controls. Isolated WBCs in the plasma have an important role in the immune system in preventing inflammation, in T cell development and Treg function.

In conclusion, there is consistent decrease in NK lysis and the severity of the illness reflects the immune changes. There is significant loss of function in KIRs. There is significant increase in dendritic cells, iNKTcells and naïve B cell phenotypes.

She has set up a cell phone APP called clinihelp and patients can access this to record symptoms regularly.

James Baraniuk (Washington, USA) discussed his work with MRI and victims of Gulf War Illness (GWI). He has explored a model sub-group dating back to 1990-91. Many had experienced acute illness, possibly as a result of sarin exposure. There has been chronic progression of illness over 20 years. Up to 25% of Gulf War veterans

were affected. There are resulting problems with the brain, its functions and networks. i.e. “The pain is in the brain”. This results in problems with fatigue, attention, working memory, pain, anxiety, tenderness and exertional exhaustion.

Many types of MRI are now available: Voxel-based, diffusion tensor imaging, BOLD (measures oxygenation of blood in brain), fMRI (at rest and during tasks), functional connectivity, structural connectivity, cerebral blood flow and concentrations of brain chemicals. He has been interested in looking at cortical thickness, and grey and white matter intensities and lucencies. He has looked at the white matter volume and fatigue duration. The white matter is decreased in ME. There is shrinkage of 1% per year. There is also volume loss in the superior cerebellar peducles, pons and medulla in GWI. Also some brain stem atrophy.

Using BOLD and fMRI, these measures are blood oxygenation level dependant and the areas that are functioning can be identified. Brain blood flow is regulated from the neuron, which releases glutamate, affecting NMDA, which then activates astrocytes to produce D-serine, which releases nitric oxide, which relaxes the arterioles and thus increases the blood flow. The fMRI shows differences in GWI leading to more errors.

He then discussed resting state brain networks. When the mind is wandering, it is rehearsing and/or debriefing. A default network starts. When you stop a task, there is functional connectivity. Patterns of resting state networks may be indicative of specific diseases. He also talked about the differences in easy and difficult tasks, and also what happens when you switch tasks. Different parts of the brain function depending on difficulty. You may go into default mode if you need to switch tasks.

If a person has post-exertional malaise, there are differences in cognitive function identified also. In a 2 day exercise test, healthy controls experienced some fatigue and switched to default mode, but those with GWI found symptoms came on, and there was basal ganglia activation for cognitive compensation. In a second test everything went down and additional brain areas were recruited. The GWI patients could be divided into phenotypes according to their response to exercise. There were START and STOPP groups. In the former exercise caused autonomic dysfunction. In the STOPP group there was phantom perception.

He finished by saying that these new brain scanning studies will redefine psychiatry. For example, in depression the amygdala has a central role and has dysfunction, while the cerebellum is shown to be involved in emotional processing and fibres have connection with amygdala and areas of atrophy.

Julia Newton (Newcastle, UK) focused on the Autonomic Nervous System (ANS)and its relationship to ME. She explained that there is overlap between the ANS and many diseases associated with fatigue. The experience of fatigue is the same in many diseases. She described the ANS, and said that dysautonomia in ME is likely. The fatigue in 89% of those with ME may be due to Orthostatic Intolerance (OI).

There are objective measures which can be done such as BP, HRV, tilt-table testing (HUT). Neurally mediated hypotension and POTS can thus be diagnosed. Mechanisms are upstream to the brain and downstream to the vascular system. The valsalva manoeuvre is used in some studies, particularly those associated with cognitive performance. Measurements can be taken using MR spectroscopy associated with 2 minutes of exercise. Acid accumulates in the muscles in ME. The intracellular pH has been measured in cultured muscle cells from ME patients and controls, and there was significant difference with increase in acid in those with ME after exercise. The liver is very involved in BP control, and liver volume can be measured while performing 15 seconds of valsalva. The liver volume changes dramatically. In ME there may be problems with liver volume. Using cardiac MRI, 1/3 ME patients had a significant PCr/ATP value of less than 1.6. There was exaggerated torsion of the left ventricle during pumping. These measures confirmed that there were autonomic abnormalities in ME, with associated brain, cardiac and muscle abnormalities. However there were similar findings in other fatigue related diseases. Fatigue is common and can relate to very specific physiological abnormalities. Symptoms are suggestive of ANS dysfunction. The dysfunction also correlates with fatigue severity.

Maureen Hanson (New York,USA) – discussed markers of post-exertional malaise. She pointed out that exercise does not usually exacerbate symptoms in healthy people or in most other diseases. In ME exercise causes worsening of symptoms. CPET using a bike with resistance showed on a 2nd test 24 hours later that CPET values could not be reproduced in ME patients. In other diseases patients can usually reproduce their base response 24 hours later (e.g. heart failure, end stage renal disease). There is therefore something odd going on in ME. Other studies have also shown that having the 2nd test is important. There is a need to prove that it is not just a matter of the ME patients not trying on the 2nd test.

Resting exercise rate= CO2 exhaled/O2 consumed. This will equate to equal to or greater than 1:1 with maximum effort. At rest 0.8 is typical. As effort increases, muscles release CO2 and more oxygen is consumed. VO2 max equates to the level of anaerobic physical fitness. VO2 is the volume of oxygen consumed per minute. At ventilatory threshold, (VT) anaerobic metabolism begins. Patients cannot wilfully alter the amount of oxygen they inhale or the amount of CO2 they exhale.

ME patients showed a 25% decrease on VO2 max on second day. In patients who also have dysautonomia, the BP does not go up and they have to stop. Sub groups have been detected also in the 2nd CPET, which may correlate with signalling molecules in the blood. There are changes in chemokines and cytokines. 10 cytokines were measured and 5 were decreased markedly. A pilot study compared metabolites in ME patients and found 52 significant differences between before CPET1 and after CPET2. There was reduction in several acylcarnitines after exercise. 300 polar metabolites were examined and 83 differed significantly. Most were higher in controls than patients. Acetyl-carnosine was 2-fold lower in patients than controls.

In conclusion: ME patients cannot reproduce their performance on a 2nd CPET. The abnormal responses can affect the autonomic or physiological responses to exercise. Both cytokines and plasma metabolites are altered compared to controls.

Amolak Bansal (Surrey, UK) discussed diagnosis and treatment of ME within the NHS. His initial comments mentioned that eventually the current exclusion criteria may go on to be included, and that if anything the new ICC can make things more complicated. His team use the Sutton CFS/ME scoring system, needing 8 out of 13 points to make a diagnosis. He noted in particular that if a patient can manage 4 glasses of alcohol in one sitting he is unlikely to have ME (usually there is extreme sensitivity to alcohol). When comparing ME to depression, there is more motivation coupled with often adverse reactions to anti-depressants. Conditions which can mimic ME include: joint hypermobility, hypothyroidism, Addison's disease, gluten sensitivity, Sjogren's syndrome, primary sleep disorders, cardiac disease, Parkinson's disease, persistent anxiety and depression.

When examining an ME patient there is an abnormality in the pupils. Holding the light there, there will be constriction – dilation and then further constriction. Other signs include increased respiratory rate and cold peripheries. Vitamin D should be checked as there is risk of osteoporosis. There is little or no evidence of fungal infection.

Treatment plan should include: stress management, gentle exercise and sensible diet. B12 injections can help with cognitive symptoms. Other supplements of use include: magnesium, L-carnitine, CoQ10 and D-Ribose. Naltrexone and nimodipine help some patients. Hormones such as thyroid, growth hormone, glucocorticoids and oestrogen may be appropriate for some people. Other treatment options to consider include: immunotherapy, antivirals, antibiotics, ampligen and anti-B cell therapy. Betablockers can be useful for anxiety.

Andreas Kogelnik (California,USA) went on to discuss the diagnosis and treatment of ME in the USA. He stressed that this is not a psychiatric disease. He outlined the many activities of the Open Medicine Institute. They are gathering very big data. As ME is a multi-system disease, many different methodologies are needed. For some patients there are three and a half billion data points. But a huge amount of time would be needed to analyse it all. He then outlined some of their current studies. These include:

1. Proteomics – 64 patients in 4 subgroups looking at autoantibody arrays. Already EBV is featuring prominently.
2. Genetics – MTHFR – so far mutations are disproportionately represented in ME.
3. Large multisite ME study
4. Gene expression profiling

5. Functional gene classes
6. Viral studies
7. Exercise testing
8. Treatment pilots which include: antivirals, IV immunoglobulin, rituximab, metabolic pathways.

Julian Blanco (Barcelona,Spain) gave an external view of ME research strategies. He compared the number of papers written for HIV with those for ME. There were many more for HIV. Research priorities tend to look at other fields – such as cancer, AIDS, neurodegenerative diseases and cardiovascular diseases. ME is a social problem with lower visibility, an economic problem (but there is more data on other diseases) and a scientific challenge with no clear target. The situation needs to be reversed. It needs more money, social visibility, and pressure on policy makers. The latter should include epidemiological data and economic impact. Biomedical research can offer: genomics, proteomics, imaging cell function (flow cytometry), B cell function and systems biology. These can all help to unravel the complexities. There is need for well defined, large study populations. Hard clinical work is also needed. The required logistics include sample storage, data management and multi-disciplinary approaches.

Regarding treatment, the example of rituximab should be followed. There should be no treatment without clinical basis, and treatment should be done in a clinical trial setting. It is the patients who are moving the treatment ahead. His concluding words were: “Dialogue between science and society has never been more important”.

The conference was closed by Dr Ian Gibson who reiterated these comments.

I must thank ANZMES and Invest in ME for making it possible for me to attend this worthwhile event. Things are moving forward rapidly, and while much work lies ahead, the new directions and science have become increasingly exciting.

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