by Rosamund Vallings MB BS
March 20-23rd, 2014

The day before the main scientific conference there was a joint session with those attending the patient conference, followed by professional workshops. There was a brief introduction by **Prof Jose Montoya**
(Stanford, USA) when he emphasized the importance of a multidisciplinary approach to the illness and complimented the Chronic Fatigue Initiative on their collaborative approach. Focus should be on clinical and translational research using strict methodology.

Prof Ian Lipkin (New York, USA) was the special guest speaker and his talk was entitled "Small Game Hunting". He said they were still able to use the earlier XMRV samples. He explained how one started with a hunt for "possible" microbes and then moved onto the "probable" when one could consider treatment options, followed by the "confirmed" when one looked at drugs and vaccines. He told us how emerging diseases usually came from animal sources. He explained how micro-array techniques moved onto unbiased sequencing. There are 24 million peptide micro-arrays needed to investigate the vertebrate organism. He discussed as an example Kawasaki Disease, an auto-immune condition in children. Often diseases are due to an immunological response to an environmental trigger.

He then went on to discuss the human microbiome – abnormalities of which can create gastrointestinal symptoms, which in children are sometimes predictors for autism. He is interested in this relation to autism. There maybe reduced enzyme and transporter RNA. The organism suterella is increased in autism and there may be an antibiotic cause, and a lack of the gene affecting gastrointestinal metabolism.

He then talked about his involvement with the Chronic Fatigue Initiative, who are doing multiplex assays. He is working closely with **Montoya** looking at viruses. HHV6 has been found in the plasma of 4 out of 6 cases. Looking for HHV6 in PBMCs, 13% were positive in patients and 11% positive in controls. Annellovirus was found in 75% of the samples – this may not be useful, but negative findings are as important as positive.

Looking immunologically at CFS/ME IL17 was elevated in the first 3 years and then decreased. Lower levels were found in the CFS/ME patients' cerebrospinal fluid. They are doing RNA

sequencing looking at biomarkers in 100 cases compared to 100 controls. This equates to 117million reads divided into 3 "libraries". But there is a need to control for library effects, as the "library" construction reflected the results – ie processing may be different.

They are also looking for chemicals produced by bacteria which may have systemic effects. He confirmed that there was zero evidence now for XMRV. Contamination is always a problem and contaminants must be eliminated prior to any analysis.

8 professional workshops were then held throughout the first day. I attended the immunology workshop outlined below.

PLENARY ADDRESS

The main scientific conference opened with the plenary address by **Dr Noel Rose** (Johns Hopkins, USA) with his discussion on how we recognize auto-immune disease. The main complaint in all auto-immune diseases is fatigue. Auto-immunity is an immune response to the normal antigens of the host. Auto immune disease is disease caused or significantly promoted by auto-immunity – with production of self-reactive T and B cells. We are in fact developing lymphocytes and are "auto-immune" all the time, but we have mechanisms which recognize and control. Disease occurs as a result of an uncontrolled response. Having antibodies is normal and not "disease". A cause of disease by auto-immunity is hard to prove, but may be promoted by existing disease or make it worse.

There is prevalence of 80 diseases affecting every organ system. These diseases are among the 10 leading causes of death in women under 65. These diseases equate to up to 23 million people in the USA. They are diseases which share common features, are expensive and lifelong and for which there are no cures. They are diseases which are on the rise. Systems affected most commonly include blood, connective tissue, endocrine, neuroloic, gastrointestinal, skin, kidneys and heart.

Evidence for auto-immune disease includes:

a) Direct transmission eg via serum or maternal/foetal (lupus).
b) Indirect transmission when the antigen is defined and introduced to replicate the disease

using animal models

c) Circumstantial – no evidence of causation, but much evidence of disease eg auto-antibodies, clustering, sex bias (75% female), HLA association, response to immunosuppression.

Some diseases look auto-immune but there is no evidence of self-reactive T cells. They are more likely "auto-inflammatory" (caused by an innate immune response).

A combination of genetic predisposition, environmental factors and endocrine effect lead to auto-immune disease.

Genetic predisposition includes:

   - Evidence from family history. There may be evidence of multiple auto-immune diseases in a family. There is 30% identical twin concordance.
   - Major histocompatibility complex
   - Immunoregulatory genes. There are gene changes due to insertion of methyl groups etc. leading to changes in CD4, T cell and B cell changes.

Environmental factors include:

   - Viruses (eg EBV and MS), bacteria (eg B haem strep and rheumatic fever)
   - Food (eg iodine effects and thyroid)
   - Pollutants (eg mercury)

Other effects include:

   - Hormones
   - Stress
   - Drugs

Pregnancy has a profound effect on auto-immune disease – some are better, some are worse.

We should use autoantibody presence in the blood with caution. This is NOT proof of auto-immune disease. Autoimmune diseases develop very slowly and a patient may have multiple antibodies over time.

The main conference papers were then presented, and the first session covered the latest research in immunology.

IMMUNOLOGY

Susan Levine (Columbia, USA) discussed allergy related immune signatures and the duration of illness in CFS/ME. She discussed the importance of allergy mediators and links between IL4 and IL13 (both produced by Th2 cells, and IL17 cells produced by Th17 cells, IL10 produced by CD4 Th2 cells and eoxtaxin produced in asthmatics. For her study, she divided patients into groups with recent and longer lasting illness (less than or greater than 3 years). Unique immune signatures were found in short duration patients. Higher levels of cytokines associated with the allergic response were found in short duration patients compared to the longer duration patients. A Th2 shift was supported. She stressed the need for future studies to include measures of histamine levels, total and specific IgE, prostaglandin E2, tryptophan and serotonin.

Ludovic Giloteaux (New York, USA) reported on his study of plasma cytokines in patients before and after a cardiopulmonary exercise test. Tests were done 24 hours apart. Baseline VO2max was compared with healthy controls. There was a significant difference. The difference was also more significant after the 2nd test. Cytokines were measured at baseline and after exercise. There were no significant differences in cytokines between CFS/ME and healthy controls, but the chemokine IP-10 decreased significantly after exercise in the CFS/ME group. Results implied an abnormal immune response with immune suppression.

Tellah Huth (Gold Coast, Australia) presented on Natural Killer cell degranulation and lytic proteins in CFS/ME. She looked at 2 natural killer cells: CD56 and CD16, both involved in cytotoxic lysis. She explained how granzymes and perforin are involved in the secretory pathway to the target cell. In CFS/ME there is significant reduction in Granzyme B, which when elevated inhibits the pathogenic activation signals. When down there is delayed target cell apoptosis. Her results showed decrease in Granzyme B is CFS/ME patients. This may contribute to reduced NK cell activity seen in CFS/ME. Further studies are needed to determine if there is a link between dysfunctional NK cell degranulation and release of lytic proteins.

Mangalathu Rajeevan (Atlanta, USA) then discussed a genome-wide analysis of differential methylation associated with CFS/ME. His results concluded that this differentiation was identified in CFS/ME, but only TERT was correlated with a corresponding change in gene expression. There was decreased methylation in CFS/ME, and increased expression of TERT. With shorter telomere length. Telomeres (the ends of chromosomes) are shorter in CFS/ME than in controls. Short telomeres are a marker for aging.

VIROLOGY

This session started with a presentation by **John Chia** (Lomita, USA). His study was looking to see if chronic pelvic pain in ME/CFS patients was associated with chronic enterovirus infection of the ovarian tubes. Chronic pelvic pain has to have been present for at least 6 months and not necessarily associated with menstruation before a diagnosis is made. Enterovirus VP1 (enteroviral protein) was found in the tubes of most patients with ME/CFS, but not in controls. Furthermore, injection of the lysate into SCID mice caused infection. Enterovirus was also detected in the vaginal secretions of 2 women. It should be noted that as the egg travels down the tube, there is a possibility of vertical transmission.

Human parvo-virus B19 was discussed by **Santa Rasa** (Riga, Latvia) in relation to typical symptoms and markers in ME/CFS. Patients who have suffered infection with this virus can end up with the characteristic symptoms for a diagnosis of ME/CFS. A large study of 190 patients compared to controls was reviewed, and B19 genomic sequence was detected more frequently (41/190) in the patients' DNA isolated from plasma (a marker for active infection) than from whole blood (15/190) - (a marker for latent infection), and this was more frequent than in controls. Most symptoms were slightly less frequent in the patients in the latent phase of their illness, apart from muscle and joint pain, which was worse in the latent group. Activation and reactivation of this virus may be a risk factor for ME/CFS. It should be noted too that there was increased incidence of infertility.

John Chia (Lomita, USA) then spoke again about the pathogenesis of chronic enterovirus infection in ME/CFS looking at stomach tissues. Patients with enterovirus in the gastro-intestinal tract usually have functional dyspepsia, and irritable bowel symptoms in the lower G.I. tract. 82% and 63% of the stomach biopsies stained positive for VP1 and dsRNA respectively (significantly higher than controls). 24 mice were injected with the VP1+,RNA+ stomach biopsies , and 66% of their spleen biopsies tested positive for VP1. Other organs were also involved. Only one control specimen tested positive.Enteroviral dsRNA was frequently demonstrated in the stomach biopsies, and as it may play a central role in pathogenesis, it should be targeted for anti-viral therapy. (It was noted that there are over 150 enteroviruses and

the Chia lab can only test for 11.)

TREATMENT STUDIES

Treatment of Orthostatic Intolerance (O.I) using midodrine was addressed by **Nicole Baldwin** (Salt Lake City, USA). She described O.I. as treatable with midodrine and there are 2 FDA trials happening. Hours of vertical activity (HVA) was used as a measure to assess effectiveness. A pilot study of 23 patients was undertaken, but 12 never started the medication, 5 did not continue (lack of compliance) and 6 ended up taking it at the full therapeutic dose (10mg 3-4 hourly during hours of being upright). Those taking the full dose showed a small but meaningfully useful increase in HVA. Those who continued the medication tended to be those who were worse at baseline. There were improved symptom scores particularly for headaches. Lack of compliance was due to side effects and rebound effects. The drug has a short half life, so frequent dosing is needed. The benefits were small but significant and outweighed the side effects.

A presentation then followed by **Tian-fang Wang** (Beijing, China) – the first time we had had attendees from mainland China. She talked about the intervention effect of Baduanjin on the fatigue state in fatigue-predominant sub-health. Baduanjin is a set of qigong exercises. She showed a video to begin with to demonstrate the technique. The exercises were very gentle and simple, and were undertaken for 2 periods of 15 minutes each day. 129 subjects were assessed (62 in the treatment group and 52 controls). Symptom scores declined significantly in the treated group.

Takakazu Oka (Kyushu, Japan) then discussed the use of isometric yoga in CFS/ME. The technique consists of 6 slow and simple poses with associated breathing exercises. It is done in a sitting position (mainly because space is very limited in the hospital. The study had to be designed to avoid post-exertional malaise, bearing in mind also that these patients are deconditioned. The technique also needed to be "mentally" simple. The exercises lasted 20 minutes, and the study was done over 8 weeks. Of the 24 patients studied (in 2 groups – one using yoga and one with conventional pharmacotherapy), one yoga patient complained of dizziness. No-one's fatigue was exacerbated by the yoga. All said their bodies became lighter and warmer and severity of pain decreased. DHEA-S increased and prolactin levels decreased significantly in the yoga group.

Fred Friedberg (New York, USA) spoke of the results of a randomized trial looking at home-based self-management for severe CFS/ME. The 137 patients studied were mostly disabled and unemployed. They were assigned to 3 categories: 1. Wait list, 2. Home

self-management using web diaries and activity monitors and 3. Home self management using paper diaries and step counters. Fatigue, functioning and depression improved in both of the 2 self management strategies, compared to wait-list controls, and were not significantly different from each other. There is therefore a role for home self management activities in generating improved outcomes.

CHRONIC FATIGUE INITIATIVE

Over lunch on that day, we had a group presentation from the Chronic Fatigue Initiative (CFI). This was introduced by **Scott Carlsson**. Several sites are involved and with a strong group of research scientists and clinicians. Nancy Klimas talked about the already 200 strong patient database with 200 controls for obtaining biosamples. A detailed assessment is done and there will be a biorepository for blood, urine, tears, saliva and rectal swabs. Very sick debilitated patients are included. Already it is evident that acute onset patients have a higher level of inflammatory symptoms. Females are more severe than males.

Gail Ironson talked about subgrouping with 4000 variables giving 18 factors associated with the main sets of symptoms. Symptom sets include: fatigue, neuroinflammation, gastrointestinal, FM, POTS, hyperlipidaemia, hypertensive, fever, NMH, increased weight. Other factors include: age/total cholesterol ratio, stress, unsteadiness/dizziness, past infection, asthma, abnormal potassium, no evidence of recent infection, hypothyroidism.

Dana March discussed clinical epidemiological aspects and the aims of the study. There are 4 main sites. They need 1000 completed surveys. They will look at changes over time. 60% patients are of sudden onset. Fibromyalgia is the commonest co-morbid condition. Deaths have been reviewed and so far cancers account for 37.8%, heart disease for 18.9% and suicide for 18.9%. Rate of cancer is higher than norms. Helpful treatments include: self-help 65%, traditional medicine 53% and alternative medicine 17%.

Mady Hornig is involved in pathogen discovery. She is collecting faecal samples to look at the microbiome. Repeat immunological profiling will be used for longitudinal analysis. She will be looking at spinal fluid etc.

Overall future directions of the CFI will include: metabolomics, proteomics, the entire microbiome, sequencing PBMCs and longitudinal analysis of immune signatures.

Tony Komaroff summarized this meeting by saying collection of data is 85% of the "sweat" and analysis about 15%. He reiterated how in such a short period of time, so much has already been achieved.

The afternoon session was interactive with case presentations by **Drs C Lapp, L Bateman, R Vallings and D Peterson**   ,
chaired by
Dr Klimas
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CASE DEFINITIONS

Leonard Jason (Chicago, USA) led this discussion and compared the various case definitons and their evolution over the past 25 years. The main variation in the definitions is the number of symptoms required for making a diagnosis. Case definitions that do not require the cardinal symptoms may not accurately identify the illness. However defintions requiring larger numbers of symptoms may include more psychiatric morbidity. Current consensus based definitions may lack construct validity, resulting in inaccurate diagnosis of CFS/ME.

A study to determine whether immunological, physical and social functioning varies in CFS/ME was presented by **Sharni Hardcastle**, (Gold Coast, Australia). Findings suggest that the ICC case definition identifies a group within the 1994 Fukuda defined patients with more severe impairment to their physical and social functioning. The ICC may also be more effective at identifying differences in the immune system found in CFS/ME. The presence of immune abnormalities combined with clear clinical measures could potentially serve as an important diagnostic tool in this illness.

Leonard Jason (Chicago, USA) then looked at diagnostic and criterion issues. Studies included looking at the prevalence of chronic fatigue in the general population. In order to progress the search for biologic markers and effective treatments, essential features of the illness might be empirically identified, so that individuals included in samples have the same underlying illness. Advanced statistical techniques, such as data mining are promising methods for identifying these features.

PUBLIC HEALTH RESEARCH

Nicolette Carlo-Stella (Pavia, Italy) looked at the differential diagnosis of CFS/ME and fatiguing illnesses from a community-based sample. 89 patients were self referred complaining of post-exertional neuro-immune exhaustion to a specialized CFS/ME clinic. 40% of them were diagnosed as suffering from CFS/ME while 60% were not. The non-CFS/ME patients were suffering from a variety of illnesses: rheumatological 43%, endocrine 4.6%, psychiatric 5.6%, gastrointestinal 4.5%, haematological cancer 2.3% and bladder cancer 1.1%. Caution is therefor warranted before making a final diagnosis of CFS/ME.

Increasing medical knowledge about CFS/ME and related diseases was addressed by **Tina Tidmore**
(Alabama, USA). The aim was also to set up a speciality centre. A local politician showed interest as he had a personal interest in Lyme disease. As a result a CME course was set up for medical professionals. The new Primer for clinicians was distributed. Further CME on Fibromyalgia and Tick-borne diseases was planned. Efforts continue to recruit physicians to run a specialized clinic. The long time needed for patient visits can be a barrier, as insurance companies do not re-imburse. First steps in creating a centre are to attract a physician willing to work in this somewhat difficult area.

Family aggregation studies in CFS/ME were discussed by **Jesus Castro-Marrero** (Barcelona, Spain). The objective of their study was to know the family history and familial aggregation of Spanish CFS/ME patients. 1140 CFS/ME patients were included in the DNA databank. 36% patients had first degree relatives with CFS/ME and/or related conditions, such as fibromyalgia, immune-mediated diseases or rheumatological conditions.

Eliana Laerdo and **Erinna Bowman** (London, UK) talked about 2 years' experience with the UK CFS/ME biobank. This is based at the London School of Hygiene and Tropical Medicine. They first discussed how the lay-scientific partnership had shaped and developed the biobank. A consultation process involved discussion between those with CFS/ME and carers, clinicians, researchers and experts in the filed of human tissue banks. Those with CFS/ME were overwhelmingly willing to contribute with blood donations. A robust protocol has been developed. Samples are collected from patients with CFS/ME and controls (without fatigue or with MS). Several diagnostic criteria are being used to provide comparison. Regular follow-ups are planned. Blood samples need to be collected and processed within 6 hours. The biobank infrastructure and procedures were presented. The samples will provide an open resource for research, with the potential to lead to biomarker discovery and work that could translate into treatment. Collaboration with other international biobanks is planned.

Margaret Parlor (Ontario, Canada) presented the results quantifying and interpreting a broad

range of health characteristics associated with CFS/ME, FM, and Environmental Sensitivities (ES)/MCS. This was to support health care planning in the area. Results were taken from the 2005 and 2010 Canadian Community Health Study (CCHS). 411,500 Canadians have been diagnosed with CFS/ME, 439,000 with FM and 800,500 with ES/MCS. This equates to a prevalence of 4.9% in 2010, which had increased from 4.2% in 2005. These illnesses were shown to have significant impact on individuals and families with many unmet healthcare needs. The information has been forwarded to health system planners, along with clinical and academic information.

The group headed by **Dana March** (New York, USA) determined the natural course of CFS/ME in a multi-site epidemiology study, compared to other studies undertaken. 4 clinical sites were chosen and patients had had to have been in treatment for at least 5 years. The study was conducted by telephone interview by trained interviewers. The survey comprised mainly middle aged educated white women, born in the USA. Approximately 1/3 had experienced at least one remission. Viral type symptoms showed the most improvement over time. Many reported co-morbid conditions such as FM, depression, anxiety and hypothyroidism. Relatively few were engaged in work, school or equivalent activity. There was some variation across sites. The odds for permanent remission seemed low.

Lucinda Bateman (Salt Lake City, USA) examined the types of treatment which alter the course of CFS/ME in another similar multi-site epidemiology study. The most effective treatments were self-help strategies (65.2%) such as rest, diet and exercise, followed by traditional medicine (53.3%) such as prescription drugs and vitamins. A small percentage reported benefits from alternative/complementary medicine (16.9%) such as herbal remedies, massage, acupuncture. There was variation between sites.

The prevalence of CFS/ME and co-morbid and consequent conditions was examined by **Salima Darakjy**
(New York, USA). 84% of the CFS/ME patients reported at least one significant diagnosis after CFS/ME onset. These included FM, depression, anxiety and hypothyroidism. 16.4% reported malignancies, which is 4 times the prevalence in the normal adult US population. Further research is warranted looking at whether some CFS/ME patients are most at risk of cancers particularly in relation to shared pathophysiological mechanisms.

PROVOCATION STUDIES

Betsy Keller (New York, USA) talked about the superior ability of a 2-day CPET (cardiopulmonary exercise test) protocol to detect functional impairment in ME/CFS compared

to either a single CPET, submaximal exercise test or a VO2 prediction equation. The single CPET failed to identify functional impairment in 20% patients, and diminished functional capacity due to post-exertional malaise (PEM) could not be detected, yet occurred in 55% of subjects. Two serial CPET tests however did identify functional impairment in 98% subjects. Submaximal exercise tests were poor predictors of VO2 max in CFS/ME as was using the predicted VO2 equation. The results demonstrated the superiority of using two serial CPETs to delineate functional impairment in CFS/ME.

J Mark Van Ness (Stockton, USA) showed diminished pulmonary ventilation in CFS/ME patients during graded exercise tests. He postulated that this could be due to deconditioning. A second study showed that post-exertional effects seem to reduce the ventilator response further in CF/MES. These effects may be due to diminished ventilator muscle function during PEM or reduced ventilator neural "drive".

David Patrick (Vancouver, Canada) addressed the issue of whether screening for mitochondrial dysfunction and other metabolic disorders was important in CFS/ME. CPET often leads to symptom flare, and so alternative approaches were discussed. Changes in haemoglobin and oxyhaemoglobin in wrist extensors using grip tests were measured. Response to the test was compared between cases and controls by calculating the area under the curve of HHb over time. Submaximal exercise procedure did not distinguish between cases and controls, but HHb levels did vary between the cases and a subset of 19% met criteria that prompted further testing for mitochondrial disease. 3 cases had particularly low levels of HHb and 2 had adverse physical reactions. There was a greater level of self-assessed exertion in the ME/CFS group. Even submaximal testing was intolerable for a subset of ME/CFS patients and we should be mindful of using exercise for testing or treatment.

Oxygen extraction and lactate were found to be low in patients assessed by **Ruud Vermeulen** (Amsterdam, Holland). This would indicate a metabolic cause for exercise intolerance in the majority of 178 women with CFS/ME studied. The low lactate after exercise in the majority of these patients suggests that physical impairment in this group is caused by downregulation of carbohydrate metabolism.

ADVANCES in PAEDIATRIC PRACTICE

Almost a whole morning was devoted to paediatric research. Diagnosis and management of paediatric CFS/ME in Australia was discussed by **Sarah Knight** (Melbourne, Australia). An online survey was sent to registered paediatricians. She pointed out that there are more challenges in adolescence and most with this illness miss on average a year of school. 39% of

the National Network of Paediatricians see patients with CFS/ME. A large number of comorbidities were diagnosed. There was wide variation is diagnostic and management practices, which may reflect a lack of knowledge of diagnostic criteria and a paucity of management guidelines.

Peter Rowe (Baltimore, USA) presented work on the impaired range of motion (ROM) in adolescent CFS/ME. 55 patients and 48 matched "flexible" controls were studied. Impaired ROM was more common in CFS/ME than controls. There was wide overlap with Ehlers Danlos Syndrome (EDS) with a number of postural abnormalities such as slumping. There was abnormal range of movements in the limbs and spine, which were worse for 1-2 days after longitudinal strain therapy in a subset, but there was eventually overall improvement in CFS/ME symptoms after the therapy.

Tracking post-infectious fatigue in a clinic using routine lab testing was addressed by **Gordon Broderick**
(Florida, USA). 301 adolescents following diagnosis on infectious mononucleosis were studied and followed over 24 months. Incidence of CFS/ME at 6, 12 and 24 months was 13%, 7% and 4% respectively. Standard lab tests were performed at each point. 13 patients (all female) had a diagnosis of CFS/ME at 24 months. At 6 months, ACTH was lower and glucose was higher in those that were still ill at 24 months. Estradiol was lower and T4 was higher at 12 months. At 24 months neutrophils were above normal and basophils decreased. These results help to support early assessment of those who may go on to develop CFS/ME over time.

A further presentation by **Peter Rowe** (Baltimore, USA) concerned the prevalence of delayed milk-protein hypersensitivity in young adults with CFS/ME. Children who were "picky" eaters were associated with non-IgE mediated food allergies. Characteristic symptoms were epigastric pain and other gastro-intestinal symptoms. There was no mast cell activiation. There was a higher than expected prevalence in those with CFS/ME. Strict dietary avoidance led to clinical and histological response. The diet needs to be rigid, and supplements with multivitamins and calcium is needed.

Peter Rowe then looked at the impact of CFS/ME in adolescence. Self-reported quality of life is significantly lower for adolescents and young adults with CFS/ME compared to healthy controls, and lower than scores reported for adolescents with other chronic health conditions, such as cystic fibrosis, epilepsy, diabetes and renal transplants, and comparable to scores for those with fibromyalgia and paraplegic cerebral palsy.

Katherine Rowe (Melbourne, Australia) had looked at what young people find helpful in managing their chronic illness. She had studied 788 people over 18 years. 1200 questionnaires were returned. An individualized plan of their own allowed them control over their lives again and was very important. Included in this should be a pleasurable out-of-home activity each week. Not being believed was the most difficult thing to handle. "Quacks" saying they could cure the illness was very upsetting and a lot of money was wasted by parents in this way. Loss of social contacts often led to depression, but this is a healthy response. SSRIs were used. Engagement in education was the best predictor of functional outcome.

Her further paper looked at whether functional outcomes were associated with depression at presentation. Average duration of illness prior to first visit was 15 months. 25% were significantly depressed compared to base rate of depression in adolescents of 20%. Not being believed, difficulty remaining engaged with school and severity of symptoms were contributing and perpetuating factors. Functional ratings at follow up were less good than the non-depressed group, but the overall duration of the illness was not significantly different.

Japanese are one of the most sleep-deprived groups of people, and **Seiki Tajima** (Hyogo, Japan) talked about sleep education carried out since 2007, to help prevent school non-attendence in junior high school and to help prevent risk of paediatric CFS/ME. Teachers and lifestyle educators were involved. 83% of patients (66) with school non-attendence fitted the criteria for CFS/ME. Incidence of school non-attendence decreased year by year, with all of the group attending regularly by 2012.

ADVANCES in BRAIN RESEARCH

Neuroscience of fatigue and CFS/ME using PET was presented by **Yasuyoshi Watanabe** (Osaka, Japan). PET molecular and functional imaging is used to demonstrate brain dysfunction. Results of large studies in Japan showed reduced binding potential of 5HTT in CFS/ME patients, and this correlated with the pain scores. PET scanning could also be used for differential diagnosis. There were correlations between gastrointestinal symptoms and binding potential of 5HTT, and PET studies on neuroinflammation corresponded to activated microglia. fMRIs showed changes or reversible atrophy in the prefrontal cortex and a mirror system of fatigue (MEG). There were also changes in circulation from acute to chronic phases. Watanabe stressed now the need to look more at what is happening in healthy people.

Marcie and Mark Zinn (California, USA) then discussed their work on EEG peak alpha frequency associated with CFS/ME, as disabling fatigue and cognitive impairment are cardinal symptoms. The first presentation was to evaluate the relationship between EEG peak alpha

frequency in CFS/ME compared to matched controls. Findings were consistent with reduced efficiency of thalamo-cortical connections in CFS/ME patients. And this correlated with reduced ability to coordinate cognitive symptoms. There may be prognostic value to facilitate the evaluation of CFS/ME as part of the diagnostic regimen. The second presentation looked at cortical hypoactivation during resting EEG in those with CFS/ME, and suggested central nervous system pathology. Exact low resolution electromagnetic tomography (ELORETA) could be applied to the understanding of brain fog. There were elevated delta sources in CFS/ME, with the highest percentage of voxel activations mainly in the left hemisphere regions of interest. Implications of abnormal delta sources can include speech difficulties, as Broca's area is involved, and a possible indication of limbic encephalitis. No patients with psychiatric comorbidity were included in these studies.

Susan Cockshell (Adelaide, Australia) had investigated cognitive functioning in CFS/ME, using meta-analysis of previous studies and using that information to study a group of those with CFS/ME. Out of 4000 abstracts, 50 reported cognitive symptoms. She then studied 50 patients and matched controls. The main cognitive deficit found in those with CFS/ME appears to be a slowing in information processing speed. All were motivated to perform well. After testing it took 2-3 days for the CFS/ME patients to recover from the resultant fatigue.

REVISION OF IACFS/ME PHYSICIANS' PRIMER

This panel session highlighted the changes that have been made to the new edition of this manual for physicians. Most of the authoring committee were present and **Ken Friedman** (Vermont, USA) summarized the changes. Particular important changes included a section on the Severely Ill, and a comprehensive index.

There was much discussion from the floor including a plea from **Dr Lily Chu** (California, USA) to include more issues relating to mortality in the next revision.

The conference was summarized by **Dr Anthony Komaroff** and that summary is available on line.

WORKSHOPS

8 professional workshops were then held throughout the first day. I attended the immunology workshop outlined below:

IMMUNOLOGY WORKSHOP
Dr Dan Peterson chaired this and introduced the session with a brief discussion about our body defences, such as the skin, mucus membranes, stomach enzymes, bacterial toxins in GI tract, phagocytes and natural killer (NK) cells. He explained the innate and adaptive immune systems which interact via the cytokines. A number of presentations by immunology experts then followed.

1. **Sonya Marshall-Gradisnik** (Gold Coast, Australia) who gave an overview of her work and the establishment of the $150million building attached to Griffith University – the National Centre for Neuro-immunology and Emerging Diseases. She discussed the role of NK cells, residing in the immune system. They influence the adaptive immune response and kill abnormal cells. An envelope in the cell contains granzymes, which pass through a protein via perforin pores. The abnormal cell is lysed through a cell-death pathway. LAMP (membrane proteins) encapsulate granzymes and perforin. NK cells bind to target cells. Chemokine receptors (KIRs) sneak out infected cells. There are 2 types of KIRs (killer immunoglobulin-like receptors): inhibitory and activating. MicroRNAs control the function of the NK cells. There are 2 NK phenotypes –" dim" and "bright".

The current status of CFS/ME is that there is no diagnostic test, pathology is unknown but there is consistency of NK cell lysis. A snapshot analysis of moderately ill CFS/ME patients was done looking at lytic proteins, to see if lysis and phenotypes are consistent over time. NK cells are found to be down in function over time, and there is a significant reduction in "bright" over time. The question was asked "Do lytic proteins play a role in CFS/ME?" Granzyme B decreases over time. Impaired degranulation is suggested. There was significant reduction in lysis in the severely ill groups. There were significant differences between severely ill and controls in NK phenotypes (CD158b+ and CD158a/h+) with reduced activation. A number of microRNAs were significantly reduced on NK cells, which influence efficiency.

2. **Sharni Hardcastle** (Gold Coast, Australia) looked at their severe cohort. And the adaptive immune system. There were alterations in T-regulatory cells and increased B-cell activation. There were some cytotoxic cross-over cells. In the severely ill patients there was an increase in plasma dendritic cells, increased naïve and plasma B-cells, decrease in transitory and regulating B-cells and a decrease in ꝺ1 phenotypes. Regulatory NKT cells were increased in the severe patients. CD8a and CD4 were changed in the severely ill.

3. **Nancy Klimas** talked about cytotoxic T cells and the immune system in CFS/ME. This is part

of the acquired system. NK cells evolve first and the acquired immune system develops over time. 5% of the immune system is in the blood, the rest is everywhere else, particularly in the gut. The cells need to be close together (eg in lymph nodes) to be efficient. There are more glial cells (white blood cells) than neurons in the brain. There is duality of the immune system: a) humoral (NK cells) and b) cell-mediated (T cells). T cells only recognize antigen associated with MHC (major histocompatibility complex) molecules on the cell. This is very specific. Perforin and granzymes poke holes in the cells leading to death. Networks of communication may get severed. Ways to reconnect need to be modelled. Perforin has been found to be down in T cells.

Biomarkers can be treatment targets. eg Neuropeptide-Y connects the immune system and the nervous system. IN CFS/ME there is high NPY and low CD26.

4. **Paula Waziry** (Miami, USA) discussed gene regulation. There are variable symptoms in CFS/ME. Viral reactivation may occur due to genetic, environmental and epigenetic effects on gene expression. PBMCs are involved (B and T). Mononuclear cells have a nucleus! And everything is regulated at many levels by gene expression. Data shows decreased levels of human microRNAs in CFS/ME. In EBV there may be impaired NPC function, but interferon will "undo" the reaction.

5. **Konstance Knox** (Wisconsin, USA) had looked at B cell function and the pathogenesis of CFS/ME. B cells are produced in the marrow and travel to lymphoid organs. They are part of the adaptive immune system. 2-5% of serum IgM/IgG is not directed at specific pathogens. They are naturally occurring antibodies. Work has been done on mice (Jay Levy).

Regulatory B cells (0.5% of B cells): a) secrete IL10 (anti-inflammatory) which restores Th1/Th2 balance and b) secrete TGFβ1 which induces apoptosis. B cells receive and present the antigen. She mentioned the rituxin studies. The drug is directed at CD20. She questioned whether there may be an increased risk of lymphoma in CFS/ME. The B cell may be a very important component of CFS/ME.

6. **David Baewer** (Wisconsin, USA) Talked about the role of serology in the diagnosis of the herpes virus, and how to order diagnostic studies. There are limitations in serology in CFS/ME, but there is a body of literature and a need to rule out a herpes diagnosis in CFS/ME. There are billions of herpes viruses in our systems, and we will all have viral antibodies. Positive IgM equates to active infection leading to latency for life. Reactivation to IgM response is rare.

Therefore IgM will be of little use. Immune dysregulation means tests are unreliable. Molecular testing for herpes is more reliable. Qualitative tests for virus DNA is 85% positive in the population. Virus replication needs to be tested using reverse transcriptase PCR. Late transcript viral RNA is more reliable. The virus actively transcribes active gene products. There may be a smouldering herpes infection going on.

7. **Troy Querec** (Atlanta, USA) discussed cytotoxicity in a multicenter trial looking at impaired NK cells. e.g. CD107a functional assay. He stressed the point that shipping time decreases cell viability, and discussed methods of shipping. Impaired NK function is a promising biomarker. Functional assays are not always available. A pilot study is planned to assess design variables.

8. **Isabel Barao-Sylvestre** (Nevada, USA) had looked at NK cells and cytokines. Cytokines are LMW proteins. There is no single organ source. Lymphokines come from lymphocytes, chemokines are functional, interleukins are messengers between leucocytes. There are chains of cytokine action. They are potentially useful markers. She talked about therapeutic antibodies being used in auto-immune diseases, and IgG subclasses, with deficiencies in some patients in CFS/ME.

9. **Mary Ann Fletcher** (Miami, USA) – was described as having much wisdom after 35 years of research into this illness. In 1990 she found reduced NK function with elevated pro-inflammatory cytokines. These findings have been confirmed around the world and are potential biomarkers. Age of sample, technique used and identified diagnosis of the patient are all important factors. Their team is ready to go ahead.

10. **Dennis Mangan** (California, USA) is a communications expert, which seems apt in the field of immunology . He describes communication as having a sender and receiver with a message flowing between them. Modification and sensitization is possible.

11. **Beth Unger** (Atlanta, USA) describing herself as a "non-immunologist" summed up the workshop. She described it as a very complex situation. There needs to be very good laboratories. With a focus on methods, there are excellent prospects. A lab has the potential to do almost "anything" if it is deemed important enough. The immune system seems crucial to understanding this illness.

POSTER PRESENTATIONS

89 posters were displayed and will be summarized later.