

The 5th Dancing Eye Syndrome/Opsoclonus-Myoclonus Syndrome International Workshop

The meeting was opened by Peter Beverley who welcomed delegates to the 5th International Workshop. In common with previous workshops there was international representation bringing expertise in pediatric neurology, pediatric oncology, immunology, neuroimmunology and other disciplines. Basic scientists, clinical scientists and family representatives were able to hear about the most recent scientific and therapeutic advances made in DES/OMS and to exchange experiences with each other and with those working in this research area.

Session 1: Neuroimmunology

A working hypothesis to explain the aetiology of DES/OMS is that children are exposed to an infection, tumour or perhaps some other agent that leads to an immune response. This immune response directed at an antigen, perhaps expressed by the neuroblastoma, then recognises this unknown antigen in regions of the brain that are involved in producing the clinical symptoms. A significant proportion of children who develop DES/OMS are found on examination to have a neuroblastoma. The neuroblastomas (NBs) appear to be relatively benign and do not give rise to oncological problems: they are controlled or even rapidly eliminated by the immune system. **Vito Pistoia** described how most NBs are invaded by lymphoid cells but this invasion is more profound in the NBs of DES/OMS subjects. The tumours express chemokines such as CXCL12 and CXCL13 known to recruit B-cells and other leucocytes. The first steps in the initiation of the immune response are of course difficult to study *in vivo* and the relative roles of professional antigen-presenting cells such as dendritic cells and B-cells is unknown. The evidence suggests that DES/OMS likely involves an antibody-mediated disease and a recurring theme is to identify the antigen against which the immune response is targeted. Pistoia described a novel method for the generation of B-cell lines that might aid in the identification of the antigen but to date this has not been successful.

An important question is to discover where the B-cells that have been activated to respond to a CNS antigen then reside and secrete antibody. Are they present in compartments of the central nervous system (CNS) where they secrete their antibodies into the cerebrospinal fluid, or are they in a peripheral compartment and the antibody then has to cross the blood-brain barrier (BBB)? **Franz Blaes** described a family of growth factors known to be important for B-cell function: BAFF and APRIL. BAFF is important in the survival of B-cells and is known to be over-expressed in patients with autoimmune disease and furthermore genetically modified mice over-expressing BAFF develop autoimmune disease. Over-expression of BAFF has been reported in the cerebrospinal fluid of children with DES/OMS. Blaes reported that BAFF levels were increased in the cerebrospinal fluid of DES/OMS subjects but this did not appear to be a consequence of disturbed or damaged BBB. He also reported that the binding of antibodies from the CSF of DES/OMS subjects to cerebellar granule cells *in vitro* was correlated with the levels of BAFF. It is possible that BAFF secretion into the cerebrospinal fluid is important for the survival of B-cells in this compartment but this

remains to be proven. If BAFF is critical in this role this perhaps offers another therapeutic target to modify the immune response in DES/OMS.

If DES/OMS is indeed an immune antibody-mediated insult on the CNS then it is likely that we can learn much about potential mechanisms from other antibody-mediated insults on the brain. **Beth Lang** reviewed studies and some of the key underlying methodological issues carried out in Oxford on antibody-mediated diseases of the CNS. There are well known diseases of the adult CNS associated with tumours of breast and lung leading to an antibody mediated assault on the CNS. Evidence from therapeutic interventions highlighted how important it was to establish whether antibodies were indeed pathogenic or not. Lang described a case of DES/OMS where antibodies gave rise to intracellular staining of cerebellar Purkinje cells and it seems likely that unless the antigen is on the cell surface anti-neuronal antibodies they will not be pathogenic. She described the methods required to detect the presence of either antibodies to N-methyl-d-aspartate receptor(NMDAR) subunits or antibodies to voltage gated potassium channels (VGKC) in human serum.

Session 2: Other neuroinflammatory disorders.

The next day the theme of learning from other inflammatory disorders of the CNS was continued. **Angela Vincent** described how antibodies to VGKC and the NMDA-R are associated with specific neurological syndromes and the effects of treatment. Antibodies to VGKC are found in patients with limbic encephalitis. Antibodies to the NMDA-R are associated with tumours and patients exhibit facial grimacing, other involuntary movements and may show altered states of consciousness. In both cases, patients respond rapidly to immunomodulatory therapy, usually plasma exchange and immunosuppressive agents. A good outcome depends on early treatment while patients who are treated later do not respond so well, even though specific antibody titres are often lower at this stage. Studies of patients with antibodies against VGKC and the glutamate NMDA-R illustrate several outstanding issues relating to the mechanisms of brain dysfunction associated with such specific antibodies.

Assays are critical for identification of specific antibodies and the Oxford group use tagged recombinant molecules in immunoprecipitation assays or expressed in cell lines. Even so what appear to be anti-VGKC antibodies in acquired myotonia or Morvan's syndrome have been found to be directed against VGKC-associated proteins, not the channel itself. The role of events in the cerebrospinal fluid remain unclear, although local (within the CNS itself) synthesis of NMDA-R antibodies occurs and may be oligoclonal later in the progress of disease. Why some patients with NMDA-R antibodies relapse although they still respond to repeat treatment, is puzzling. The specificity of the brain effects suggests that some brain regions may be more accessible to blood bourn antibodies than others: for example, human immunoglobulin appears to enter the mouse hippocampus more readily than other regions. It is also not clear why the disease shifts from a cortical to subcortical pattern of damage and MRI lesions from grey to white matter.

Patricio Huerta also discussed NMDA-R antibodies. Neuropsychiatric symptoms and cognitive dysfunction are not uncommon in systemic lupus erythematosus (SLE). Many of these patients have antibodies against a short linear peptide sequence (DWEY) found in the NMDA-R subunits NR-1 and NR-2. These antibodies are neurotoxic *in vivo* in mice. It is thought that initially antibodies are present in serum and that only after the blood-brain barrier is breached can the neurotoxic antibodies enter and cause brain damage. The exact symptoms may be determined by factors that alter permeability of the BBB, since administration of LPS (to generate a systemic inflammatory response) in addition to the neurotoxic antibodies produced hippocampal damage while epinephrine in addition to the antibodies produced damage in the amygdala. In hippocampal slices *in vitro* the antibodies appear to act as agonists at the NMDA receptor leading to calcium influx, mitochondrial damage and apoptosis.

Session 3: Therapy in Neuroinflammatory Disease

The following session continued with the theme that studies in DES/OMS can learn from other neuroinflammatory disease and in particular in the context of diagnosis and therapy, the later involving the modification of the immune response.

Primary CNS vasculitis in children (cPACNS) is a rare but complex disease in children and the diagnosis is not straightforward as discussed by **Paul Brogan**. CNS vasculitis may be secondary to a number of systemic conditions (HSP, Kawasaki's, Takayatu, PAN), and various ANCA associated vasculitides. CNS vasculitis is considered to be a primary phenomenon when there is no evidence of systemic involvement, infection, or other contributing conditions. Mimics of CNS vasculitis such as ADEM, MELAS, malignancy and RPLE due to hypertension must be ruled out. Primary CNS vasculopathies may be inflammatory or not-inflammatory such as Moyamoya, fibro muscular dysplasias, and other conditions. Among the conditions presenting similarly to cPACNS, there is overlap of primary ischemic stroke, post-VZ, transient cerebral arteriopathies. CPACNS may be monophasic or polyphasic, severe, devastating or mild. Systemic testing such as ESR, CRP, and CSF testing is generally unhelpful. Even brain biopsy, thought of by some as a "gold standard" is not highly sensitive. Angiography (conventional) is the most sensitive, albeit an invasive, method of diagnosis.

Treatment involves intensive immune suppression, although there are no randomized clinical trials. Usually a 6-month induction phase, generally cyclophosphamide 500-1000 mg/m² q 3 weeks along with corticosteroids and ASA, is followed by a minimum of 1-2 years of maintenance with azathioprine, tapering of corticosteroids, and continued low dose ASA. These high-risk treatments involve risk of infection. Prognosis for progression is worse for patients presenting with neurocognitive deficits. A possible new systemic marker is circulating endothelial cells, which are elevated in both active systemic vasculitis and PACNS. Studies validating this test in cPACNS are underway. Endothelial progenitor cells, which are bone marrow derived, may play a part in repair of the damaged vasculature.

Discussion included questions about treatments in particular regarding schedules for cyclophosphamide (monthly vs q2week vs oral daily). Biologics are used for ANCA positive vasculitis as well as refractory PACNS. Anti-TNF agents probably do not have a role, but rituxamab has been used.

Multiple sclerosis (MS), the archetypal inflammatory disease of the CNS, is considered by many to be an autoimmune disease: the target cell is believed to be the oligodendrocyte, leading to demyelination but the ongoing damage also involves axons. **Alasdair Coles** described how MS commonly presents clinically as relapsing/remitting (RR-MS) disease, typically becoming secondary progressive (SP-MS) after several decades. Other forms of the disease, benign relapsing remitting (no progressive stage) and primary progressive, also occur. The secondary progressive stage with little evidence of relapses and remission involves accumulating disabilities, with the associated depression, social isolation, and cognitive problems. Treatment during the progressive phase may be "too late". The use of type I interferons reduces the relapse rate somewhat during RR-MS, but it is not clear that they affect either the onset or the severity of the progressive phases. During secondary progressive phase the type of inflammation changes to plasma cells from the meninges, which develop into lymphoid follicles. These are within the environment of the blood-brain barrier and not amenable to immunotherapy.

However, recent trials with Aletuzumab (Campath) are both promising and informative. This is a biologic agent that ablates lymphocyte populations for up to 5 years. A small number of patients have received this treatment in early RR-MS and it has been shown to prevent disease progression and in a small number even reverse symptoms. A potential side-effect of this treatment is that patients may develop other autoimmune disorders. It was reported that the cytokine interleukin-21 (IL-21) is increased after Aletuzumab therapy. A SNP in the IL-21 gene is associated with increased risk of autoimmunity and this may be of value in predicting which patients may develop treatment induced autoimmunity.

Alzheimer's disease (AD) accounts for 70% of progressive dementias, Lewy Body dementia for 15%, and vascular dementias for 15%. AD is characterized by plaques and tangles, with the predominant substance in AD plaques being a 1-40 amino acid peptide amyloid-beta ($A\beta$). If mice genetically engineered to develop $A\beta$ -plaques are immunized against $A\beta$ early they do not develop plaques and cognitive impairments: if immunized later in life this will lead to the clearance of the plaques. **James Nicoll** described the outcome of a trial in which the company Elan (trialAN1792) immunized AD patients with early symptoms against $A\beta$. The trial was stopped due to an approximately 5% incidence of meningoencephalitis. In patients who died postmortem analysis revealed that remarkably the plaque density was much decreased. However, despite the dramatic reduction in plaque load this did not alter the progression of the dementia. Survival was not affected by the immunization. The adverse effects of rapid removal of $A\beta$ -plaques from the brain potentially included induction of autoimmune disease, perhaps "overload" of the perivascular system involved in the systemic removal of $A\beta$, cortical

microhemorrhages due to amyloid angiopathy. New trials will attempt to avoid T-cell activation and CNS penetration, which may cause side-effects.

The discussion was generally fairly negative about an immunization strategy for AD, with multiple questioners pointing out that the previous trial was fairly definitively negative, and this approach should be considered as failed and something else completely different tried.

Session 4: Therapeutic trials in DES/OMS

Pedro de Alarcon summarized the ongoing US treatment COG trial for children with DES/OMS. The backbone of the treatment trial is the application of Prednisolone 2mg /kg/d and CP 750mg/m²/1x month x 6. After 4 weeks of treatment children are randomized into a group that receives IVIG and another group that does not receive monthly dosages of IVIG. Children with a neuroblastoma, who had no prior chemotherapy are eligible for the trial. Further treatment with steroids for up to 2 weeks is not an exclusion criteria. De Alarcon further pointed out that children, who are receiving IVIG, but have not clinically improved can be switched over to the treatment arm with IVIG. Children who already receive IVIG, but did not improve clinically, will in addition be offered ACTH. Severity is assessed according to the DES/OMS Severity rating scales. Accrual of children had been initially slow but has picked up and 32 children are at present enrolled. Therapy has been so far tolerated well. No major side-effects have occurred. One child had died after megatherapy for stage 4 neuroblastoma

On the other side of the atlantic the European Trial “The OMS/DES 2008 trial” protocol was described by **Barbara Hero**/Gudrun Schleiermacher and is nearly completed; this includes therapy guidelines, neurological assessment protocols for escalating the individual treatment steps, tests for neurocognitive assessment, and guidelines for imaging at initial presentation (e.g. MRI with 4 mm sections the areas typically involved at manifestation of OMS (neck, chest, abdomen)). Still, a burden of administrative issues have to be solved. One major step was that the Institut Curie, (Paris, France) agreed to be the international sponsor and that in many countries national coordinators have been identified. An important alteration of the protocol schedule might be to escalate to Rituximab after 3 month of treatment with cyclophosphamide (instead of after 6 months) in case of insufficient treatment response. This will be discussed in the group in the very near future. Part of the drugs will be supplied by a drug company. This company asked if it is possible to perform an ancillary study to assess the pharmacokinetics in children receiving this drug requiring serum blood samples at different time points. Funding and precise intention of this study still need to be determined and will be worked out in the details with the company. During the discussion, it was proposed to add a “ quality of life” questionnaire to the protocol, which will be realized.

Gudrun Schleiermacher in the first part of her talk compared the neurological outcome of children with and without neuroblastoma over the last 20 years (1988-2008) seen in 4 different centers in France. 22 children with OMS and a NB and 12 children

without a NB were included in this retrospective study. The neurological symptoms at disease-onset and at follow-up (>2 years) were assessed with the OMS symptom grading scale. One important finding was that there was no significant difference between the two groups. In the second part of her talk Schleichermacher reported the preliminary results of segmental and numerical genetic alterations in neuroblastoma tissue from different patients using a whole genomic approach the so-called array CHG-platform. The identification of numerical and segmental alteration has prognostic implications because whole chromosome copy number variations characterize NB with low risk of progression, but segmental alterations which arise from unbalanced chromosome translocations characterize neuroblastoma of poor prognosis. Material from 8 children was so far studied: 6 children had an unexpected high frequency of unfavourable segmental alterations.

Marc Tardieu presented a discussion of delegates' personal experiences of novel treatments in resistant cases. Prior to the Workshop he collected together the experience of the members of the DES/OMS study group with the treatment of children with a severe course of DES/OMS. He further reviewed the medical literature with regards to various treatment regimes and presented his personal experience in the treatment of DES/OMS with Rituximab. Only a small number of children with DES/OMS were treated with immunosuppressive drugs such as azathioprine, cyclosporin, cellcept or methotrexate. No major benefit was described in any reports, but it is important to point out that all medications were not given at the beginning but in the chronic phase of the disease. He discussed the novel immunomodulatory drugs used in subjects with MS such as Alemtuzumab (Campath directed at CD52 on T-cells), Daclizumab (anti-CD25, to inhibit T-cell activation /prolif) and the inhibitor of mTOR Sirolimus. He pointed out that the use in children is very limited but that in the future they might be considered for the treatment in children with DES/OMS. Finally, he reported that Rituximab has been used successfully in conjunction with steroids and IVIG in children (see Prazantelli et al, 2009).

Session 5: Development in clinical DES

The impact of DES/OMS on subsequent neuropsychological development was addressed in this session. **Jeremy Parr** reviewed the Autism Spectrum Disorders (ASD), these are sub-classified into Idiopathic (mostly genetic) and Secondary to other disorders (e.g. Fragile X, Tuberous sclerosis), about 15%. Among these children those with social and communication difficulties with restrictive, repetitive, stereotyped behaviour patterns do badly: those with occasional language regression – these children do particularly badly. Diagnostic criteria based on behaviour patterns give a prevalence such that about 1% of children have ASD spectrum disorders (Baird G Lancet 2006; 368: 210-5). Parr gave some historical background, including the role of parents as proposed by Bettelheim, now largely superseded by neurobiological factors as described by Rutter and including autism susceptibility genes on chromosome 11.

One case report of a DES/OMS patient and Asperger's Syndrome. De Grandis (2009) reported 3/10 identified as having social difficulties. The need for a systematic

exploration of ASD in DES/OMS was taken up by in Kate Humphreys's presentation the following day (see below).

Neuroimaging techniques have considerable potential to detect CNS pathology in patient groups and again this was an area from which DES/OMS could usefully learn. **Nathalie Boddaert** described both past and recent imaging studies in ASDs subjects including those using PET, fMRI, voxel-based morphometry (VBM) in syndromic and non-syndromic ASDs. Past data had suggested hypoplasia of the vermis but this has not been replicated; hippocampal changes had been described but the findings were inconsistent; bilateral hypoperfusion on PET, especially of the left superior temporal gyrus has also been described. Current studies using VBM report a bilateral decrease in superior temporal sulcus (closely linked to social interaction – mouth perception); on fMRI no activation of speech receptive areas when listening; MRI in 140 ASD children found 38 with non-specific white matter abnormalities, 16 with dilated Virchow-Robin spaces, 33 with signal changes in the temporal lobe. The question was raised as to whether the proportion with white matter and Virchow-Robin anomalies increased if they were compared to a mixed disease control group since these modest changes are not uncommon findings. There was some discussion of Wendy Mitchell's group study showing smaller cerebellums in DES/OMS as a group even though the changes were not individually evident: this data is not yet published.

Childhood anxiety was discussed by **Cecilia Essau**. The prevalence of anxiety in adolescents is about 10-25%, less than 50% have isolated anxiety, the rest have anxiety with 1-3 co-morbidities including depression, somatoform disorder, substance and/or alcohol abuse. In over 70% of those with anxiety and depression the anxiety precedes the depression. Risk factors for anxiety disorders consist of cognitive difficulties, female gender, perfectionism, maternal anxiety, maternal depression, and parental rearing practice, the latter especially if the individual was encouraged in avoidance responses to anxiety-provoking situations. There was discussion as to whether anxiety be formally evaluate in DES/OMS as a group, particularly as there are methods, mainly behavioural, by which children with anxiety can be helped.

Gudrun Schleiermacher described her analysis of the neurological outcomes in DES/OMS with and without neuroblastoma and the tumour genetics. The data from this retrospective study of children from 1988-2008 included 22 NB-positive and 12 NB-negative and showed that the neurological outcome was similar for both groups. Array-CGH that compares the copy number of segments in two different genomes was carried on 8 NBs associated with DES/OMS. Six cases had unfavourable tumour genetics but did well oncologically.

Two particular outcomes of this session were firstly, the importance of exploring the behavioural/neuropsychiatric disorders in children with DES/OMS, rather than simply leaving assessment at the level of the DES/OMS diagnosis, since there are treatments available that may ameliorate these problems. Secondly, the genetics study although based on small numbers, suggest that it is the immune response in DES/OMS that controls the NB rather than the NBs being intrinsically more benign

Session 6: Neuropsychiatric disorders in children

The importance of neuropsychological problems in children with DES/OMS means that there is much that can be learnt by a study of the difficulties faced by these children with those suffering from other neurological diseases that also give rise to neuropsychological problems. The similarities and differences are likely to be informative with regard to interventions and treatments that may be helpful.

Dr Kate Humphreys presented findings from her study of neuropsychological functioning in individuals with DES/OMS. 40 individuals were evaluated, 70% of them being male. Of the 40, 16 had a history of neuroblastoma, 16 had a history of infection, and for the remaining 4 there was no identifiable trigger. Kate reviewed previous work suggesting attentional problems, self-injury, obsessions and compulsions, depression with poor mood regulation, and social difficulties. Her data found low rates of autism and impulsiveness, but similar rates of compulsiveness, aggression, low mood, anxiety and hyperactivity to individuals with other causes for their developmental difficulties. She concluded that there is evidence for a subtle neuropsychiatric profile of special developmental needs and emphasised that treatment needs to be adapted to these issues.

Professor Eric Taylor reviewed the field of attentional deficits and associated impulsiveness and distractibility. He defined ADHD as a disorder of dysregulation that results in distractibility, forgetfulness, difficulties in sustaining effort and erratic behaviour. The substantial literature supporting beneficial, swift and effective responses to stimulant medication was touched on. The condition shows very high heritability of underactive dopamine brain systems. Medications seem to work by making the individual more sensitive to cues in the environment. There is a need to distinguish between ADHD behaviours and neuropsychology deficits, for example executive function difficulties and working memory problems. Delay aversion and impaired response inhibition are important as are difficulties with task switching and cognitive flexibility more generally.

Professor Jeremy Turk reviewed the area of obsessive and compulsive phenomena. There is an overlap of these features with autism spectrum conditions and with obsessional personality traits. A degree of obsessionality is healthy and useful but, as for most psychological traits, excessive amounts can be seriously debilitating, requiring treatment. Occasionally obsessive-compulsive disorder results from a bacterial streptococcal throat infection which results in antibodies that cross-react with the basal ganglia resulting in Paediatric Autoimmune NeuroPsychiatric Disorder Associated with Streptococcus (PANDAS). In these circumstances treatment is antibiotics instead of the usual cognitive-behavioural psychotherapeutic approaches and if necessary anti-obsessive medications such as the selective serotonin reuptake inhibitors.

Session 7: Family experience

Mandy Caunter gave a very moving and evocative account of her daughter Ellie's illness from a very personal prospective and demonstrated that it is necessary to treat the "family unit" as well as the patient. When asked what she lacked during Ellie's illness, Mandy stated that she felt she needed a consultant or expert "on-tap" in order to answer the questions about her daughter's disease and the medication she was receiving. She was unable to get this information from her local GP or hospital staff as none of them had ever seen a case before. For this reason Mandy found contact with the Dancing Eye Support Group family network, with the knowledge that they had about DES/OMS, particularly useful.

Mark Gorman, Joslin Murphy and Tony Tzoubris from the US DES/OMS Support group spoke of their attempts to set up a US network and gain funding from the National Institute of Health (NIH). Mark Gorman related how he came to the last meeting and was motivated to work on DES/OMS. Together they have set up an inaugural American DES/OMS workshop that will be a satellite meeting of the next American Academy of Neurology (AAN) meeting which will take place in Toronto, Canada from the 10th to 17th April 2010. The DES/OMS meeting will take place on the first day (10th April). They have already invited clinicians, researchers, patient advocates and NIH program staff from Europe, the US, and Canada and Tony extended an invitation to all those present to come to the meeting and pleaded that people should think "outside the box" to find a cure for DES/OMS.