Royal Free Hospital School of Medicine

UNIVERSITY OF LONDON

Rowland Hill Street, London NW3 2PF

0171-794 0500 Ext. 5230/5996

Academic Department of Medicine
Direct Line: 0171 830 2105
AJW/NK
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Fax Nos: 0171 830 2867
and 0171 830 2111

Dr Michael Pegg
Chairman, Ethics Committee
Royal Free Hampstead NHS Trust

Dear Dr Pegg

Please find enclosed 18 copies of our trial entitled ***A preliminary open-label study of the effect of oral measles virus-specific dialysable lymphocyte extract transfer factor (DLE-TF_{MV}) in children with autistic enteropathy.*** We would like this to be considered by your Committee at the earliest possible opportunity.

One child who has received this treatment on a compassionate basis appears to have made substantial improvement without any noticeable adverse effects. I look forward to hearing from you.

Yours sincerely

A J Wakefield FRCS
Reader in Experimental Gastroenterology in the
Departments of Medicine and Histopathology
Hon. Consultant in Experimental Gastroenterology to the Royal Free Hampstead NHS Trust
Director, *Inflammatory Bowel Disease Study Group*

CLINICAL RESEARCH PROTOCOL

AN OPEN-LABEL STUDY OF THE EFFECT OF ORAL MEASLES VIRUS-SPECIFIC DIALYSABLE LYMPHOCYTE EXTRACT - TRANSFER FACTOR (DLE-TF_{MV}) ON CLINICAL STATUS IN CHILDREN WITH ILEO-COLONIC LYMPHOID NODULAR HYPERPLASIA, NON-SPECIFIC COLITIS AND DEVELOPMENTAL DISORDER

Short Title: DLE-TF_{MV} and AUTISTIC ENTEROPATHY

Background

(i) *Clinical study* We have investigated, in a pilot study, a consecutive series of 12 children for a new syndrome comprising ileocolonic lymphoid nodular hyperplasia, chronic non-specific colitis and regressive developmental disorder. A summary of these studies is given below. We have now completed the clinical investigation of a total of 33 children, 32 of whom show the identical syndrome.

Patients: The first 12 children (mean age 6 years, range 3-10, 11 males) were referred with a history of achievement of normal developmental milestones followed by loss of acquired skills including language, plus bowel symptoms including diarrhoea, abdominal pain and, in some cases, provocation of symptoms by certain foods. This preliminary summary describes the clinical features and aspects of the intestinal pathology in these children.

Methods Children underwent gastroenterological, neurological and developmental assessment including review of prospective developmental records. Under sedation, ileo-colonoscopy and biopsy, MRI, EEG, and lumbar puncture were performed. Barium follow-through was undertaken where possible. Chemistry, haematology and immunology profiles were examined.

Results. Onset of behavioural symptoms was associated, by the parents, with MMR vaccination in 8 of the 12 children, and with measles infection in one child and otitis media in another. All 12 children had significant intestinal pathology; this ranged from lymphoid nodular hyperplasia to aphthoid ulceration. Histology revealed patchy chronic inflammation in the colon in 11 cases and reactive ileal lymphoid hyperplasia in 7 cases, but no granulomas. One case had ileal lymphoid nodular hyperplasia alone diagnosed on barium follow-through. Behaviourally, they formed a heterogeneous diagnostic group that included autism (9/12), disintegrative psychosis (1/12) and possible post-viral/vaccinial encephalitis (2/12). All children exhibited features of severe developmental regression. Clinically, they had no focal neurological abnormalities and MRI and EEG studies were within normal limits. Abnormal laboratory tests in these children were a significantly raised urinary methylmalonic acid compared with age-matched controls ($p<0.003$), a low haemoglobin in 4/12 and a low serum IgA in 4/12. Intestinal pathogens were not identified by routine stool culture, microscopy or serology.

Conclusions of Clinical Study: We have identified significant gastrointestinal pathology in association with developmental regression in a selected group of previously, apparently normal children. In the majority there is a clear temporal association with possible environmental triggers. We are aware of some 554 children who require investigation for similar symptoms, many of whom have been, or are in the process of being referred.

(ii) Laboratory studies

Background: Many physical conditions known to affect the brain can be associated with autistic disorders or aspects of autistic behaviour. Viral encephalitides can give rise to autistic disorders, particularly when they occur early in life (Wing L. The Autistic Spectrum Constable, London 1996;pp66-71). Both measles and rubella viruses have a documented association with autism and, in the context of regressive developmental disorders, including Heller's disease, both measles virus (Rutter M, et al. In: Child and Adolescent Psychiatry. 3rd edition, London pp581-5821 and, as with some of our children, the combined MMR vaccine have been causally implicated (Fudenberg HH. Biotherapy 1996;9:13-17. Gupta S. Proc DAN Conference. Chicago 1996;pp 455-461

Aims: to investigate evidence of persistent measles and rubella virus infection and to assess immunological states in children with a combination of regressive developmental disorder, ileo-colonic lymphoid nodular hyperplasia and non-specific colitis

Methods: immunohistochemistry was used to detect the two viruses and, in addition, adenovirus, mumps virus, Herpes simplex virus, human immunodeficiency virus and *Pneumocystis carinii*, using appropriate controls. Measles virus was also sought in peripheral blood mononuclear cells (PBMC), by reverse transcription PCR in 8 affected children. IgG and IgM antibody immunoreactivity for measles, mumps, rubella and CMV was measured by ELISA in serum in 22 affected children and 32 normal controls of similar age, sex and vaccination status, and cerebrospinal fluid in 6 affected children. Immunocytological profiles of PBMC were examined by flow cytometry.

Results: measles virus nucleocapsid protein antigen but no other viral antigen, was detected in tissues from 5 of 7 affected children. staining was present exclusively in follicular dendritic cells in foci of ileal follicular hyperplasia. Staining was not seen in either 9 of 10 age-matched control ileo-colonic biopsy series, ileal lymphoid follicles in 10 children with Crohn's disease, or 6 of 6 lymphnodes from adults with AIDS. One control - ulcerative colitis with ileal lymphoid nodular hyperplasia - showed identical staining in a focus of reactive follicular hyperplasia. Measles virus haemagglutinin gene, was amplified and sequenced in coded, PBMC samples from 3 of 8 affected children but from neither PBMC from 2 cases of subacute sclerosing panencephalitis (SSPE) nor control RNA samples. Serum measles IgG immunoreactivity, but neither measles IgM nor rubella, mumps or CMV antibody immunoreactivity, was significantly elevated in affected children compared with normal controls (3688 MIUnit vs. 2042 MIUnit, $p = 0.018$). Total IgG was not elevated in affected children, for whom there was no significant relationship between measles IgG and either total IgG or rubella IgG ($p = 0.4$). Virus-specific immunoglobulin was not detected in CSF. Eleven of 12 affected children had

low absolute numbers of CD4+ and CD8+ T cells, B cells, and natural killer cells. CD4:CD8 ratios were within normal limits. Further detailed virological studies are underway to confirm these data in a larger cohort of affected children.

Conclusion: the data indicate the possibility of an acquired lymphopenia and persistent measles virus infection of ileal lymphoid tissue, in children with colitis and regressive developmental disorder. The identification of a potential cause provides an opportunity for treating these children.

Principal Scientific Investigator
Dr A J Wakefield FRCS
Reader in Medicine & Histopathology
Royal Free Hospital School of Medicine
Rowland Hill Street
London NW3 2PF

Telephone No: 0171 830 2528/794 0500 x 3278/5996
Fax No: 0171-435-5803

Principal Clinical Investigator
Professor J Walker-Smith MD FRCP(Lon &Edin) FRACP
Professor of Paediatric Gastroenterology
Royal Free Hospital School of Medicine
Rowland Hill Street
London NW3 2PF

Telephone No: 0171-830-2243/794 0500 x 3999

Dr S Murch PhD MRCP
Senior Lecturer in Paediatric Gastroenterology
Royal Free Hospital School of Medicine

Telephone No. 0171 794 0500 x6235

Principal Collaborative Investigator
Dr HH Fudenberg MD
NeuroImmunoTherapeutics Research Foundation
1092 Boiling Springs Road
Spartanburg
South Carolina, 29303, USA

Telephone No: 864-591-0944
Fax No: 864-591-0622
E-mail NIT@compuserve.com

Clinical Statistics
Dr Scott Montgomery PhD
Department of Medicine
Royal Free Hospital School of Medicine

Telephone No: 0171 794 0500 x 6224