

VACCINES AND AUTISM**Enterocolitis, autism and measles virus**

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We review some gastrointestinal, immunopathological and virological observations in a subset of children with developmental disorders investigated by us over the last 4 years.

Gastrointestinal symptoms in these children, often starting around the same time as the characteristic developmental regression, include chronic constipation with overflow, pain, bloating, and oesophageal reflux associated with nocturnal waking and distress. Gastrointestinal and behavioural symptoms appear to be provoked by foods such as grains and dairy products; withholding these foods produces symptomatic improvements. In a systematic analysis of 385 unselected autistic-spectrum children, Melmed and Schneider identified clinically significant gastrointestinal symptoms in 46%, compared with 10% of 97 developmentally normal paediatric controls.¹ In this population, symptoms may be underestimated and require careful attention. An autistic child in pain may be distressed, aggressive, and self injurious; our early findings that bowel clearance often abrogated these behavioural symptoms provided such a clue. Intestinal permeability, measured by urinary excretion of inert sugars after oral dosing—a non-invasive marker of epithelial integrity—is elevated in some children with autism, even in the absence of symptoms.² Thus reliance upon overt symptomatology may substantially underestimate the proportion of autistic children with possible gastrointestinal pathology.

There is compelling evidence that many children with autism and gut symptoms have organic mucosal pathology. We have evaluated over 180 children by ileo-colonoscopy and biopsy. Systematic analyses of the first 60 children^{3,4} and in-depth immunohistochemical characterisation of a subsequent 21 children⁵ with appropriate controls, have been described. Endoscopic features include ileal lymphoid nodular hyperplasia (LNH) and macroscopic evidence of colitis. Histopathological findings in the colonic mucosa include infiltration of the lamina propria and crypt epithelium by acute and chronic inflammatory cells, with excess eosinophils and apoptotic debris within the upper lamina propria, and crypt abscess formation in more advanced lesions. The lesion consists of a patchy mild to moderate pan-colitis.

Upper gastrointestinal pathology, including reflux oesphagitis, chronic gastritis, chronic duodenitis with Paneth cell hyperplasia, reduced brush-border hydrolyase activity and abnormal pancreatico-biliary secretory responses, is also present in these children at a surprisingly high rate. Horvath *et al* have reported their findings in the upper gastrointestinal tract in 36 autistic children whose symptoms included chronic diarrhoea, gaseousness, abdominal discomfort and distension.⁶ Our findings are largely in accordance with this. In order to characterise the intestinal pathology further we undertook quantitative immunohistochemistry of the mucosal lesions in the large and small intestine. Comparison with appropriate controls, including colonic tissue from cognitively normal children with ileal LNH, Crohn's disease or ulcerative colitis, and duodenal tissue from children with cerebral palsy or coeliac disease, reveals a subtle but characteristic pathology. The colonic lesion represents a novel lymphocytic colitis, with moderately dense infiltration of both T cells and plasma cells, disproportionate to the inflammation seen on routine histological examination. Both CD8+ cells and $\gamma\delta$ T cells are present at high density. Crypt cell proliferation was substantially increased and the epithelial basement membrane thicker than in either normal or disease control groups. Absence of colonic epithelial HLA-DR expression in autistic children suggests a T_H2-skewed response.

Studies of the corresponding small intestinal lesion also indicated a distinct cell-mediated immunopathology, in which lamina propria CD3 and CD8 T cell density exceeded controls including coeliac disease.⁷ Specifically, IgG co-localised with complement C1q at the epithelial basolateral membrane and epithelial proliferation was markedly increased. This was not seen in either normal controls or in cerebral palsy. These changes are consistent with an autoimmune pathology and indicate a specific and possibly important lesion.

Many affected children suffer from recurrent infections, particularly of the upper respiratory tract, with a high prevalence of dietary allergy, eczema and adenotonsillar hypertrophy. Immune profiling frequently reveals lymphopenia, affecting both CD4+ and CD8+ populations. Consistent with allergic predisposition, IgA is usually in the lower quartile of the normal range and a raised IgG1 and low IgG2 and 4, and raised IgE are common. The majority of children assessed showed unresponsiveness for all common recall antigens on cutaneous delayed-hypersensitivity testing, in contrast with age-matched controls. There is a history of organ-specific autoimmunity in first-degree family members in 30–50% of cases, as reported elsewhere.⁸

The cause of developmental regression in these chil-

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dren is not known. Many parents report a temporal relationship with exposure to MMR vaccine. While these observations clearly cannot confirm a causal link, there are precedents for a role for measles virus (MV) in childhood developmental disorders including autism, disintegrative disorder and developmental regression.⁹ More recently, Singh *et al* linked atypical serological responses to MV and myelin basic protein to autism.¹⁰ Parental reports implicate the combined MMR vaccine in their child's regression, raising the possibility of a compound effect of the concurrent exposure. MV exhibits interference phenomena with other viruses including, those in MMR¹¹—that may modify the immune response and, theoretically, increase the risk of persistent infection. The possibility of a compound risk for mucosal immunopathology with MMR is consistent with the data showing that close temporal exposure to measles and mumps is a risk factor for later inflammatory bowel disease.⁹ Although causality should not be inferred, it is important to consider why some children might potentially react aberrantly to a vaccine when the majority do not. Possible risk factors reported to us include vaccination during an infection or following recent antibiotic use, a history of atopy including food allergy, exposure to multiple vaccines concurrently, and a strong family history of autoimmune disease.

In virological studies, MV anti-nucleocapsid (N) protein monoclonal and polyclonal antibodies were characterised for specificity in immunoblot and Western blot analyses, immunocytochemistry on MV, mumps and rubella infected and uninfected Vero cells, and MV-infected and normal brain tissue. Antibodies were subsequently applied to intestinal tissues from autistic children and control tissues from developmentally normal children. A characteristic pattern of MV staining was observed in tissues of autistic children, that was consistent with the follicular dendritic cell (FDC) matrix and some lymphocytes in ileal lymph follicles. Flow cytometric analysis of single cell suspensions derived from ileal lymphoid biopsies in 22 affected children showed populations of MV+ lymphocytes (CD3+CD45+) and FDC (CD14+CD21+). CD3+ intraepithelial lymphocytes from the same biopsies were negative. Isotype controls for MV monoclonal antibodies, and CMV monoclonal antibody labelling, were also negative in these lymphocyte and FDC populations. Mean percentage of MV+ cell populations was significantly increased compared with developmentally normal controls. Serum IgG immunoreactivity was significantly elevated in affected children compared with age-matched controls; mumps, rubella and CMV IgG antibody titres were not elevated. From this, and the accompanying molecular data, we conclude that there is persistence of at least some components of MV in affected children.

Future studies need to focus upon investigating cellular immune responses to MV in such children and appropriate controls. Although almost all studies of the immune response to MMR vaccine have concentrated upon serology, it is clear that in children with congenital immunodeficiencies, T cell responses are critical in

regulating infection. As familial predisposition to autoimmunity is common in regressive autism, and the intestinal lesion has the hallmarks of autoimmunity, an important study is that by Griffin and colleagues in a rhesus macaque model of vaccine-associated autoimmunity.¹² Use of an insufficiently immunogenic monovalent measles vaccine led to a modest serological response but without generation of MV-specific cytotoxic T cells. Animals remained well until re-challenged with wild-type MV, when autoimmune pneumonitis was triggered, indicating that a failed cellular immune response to measles vaccine is a potential cause of autoimmunity. These findings strongly suggest that proper assessment of any potential link between MMR vaccination and autism should include study of T cell responses to monovalent and trivalent vaccines, comparing children with and without a family history of autoimmunity.

The nature of any pathogenetic interaction between the gut lesion and the cognitive impairment is not known, although autoimmunity and gut-mediated toxic encephalopathy are plausible mechanisms. Toxic gut-brain encephalopathies are seen in patients with failure of hepatic detoxification mechanisms. They are also seen in intestinal pathologies such as infantile intussusception, short bowel syndrome and coeliac disease. Presence of urinary opioids of dietary origin in some affected children and their clinical response to exclusion of dietary opioid substrate may be clues to a toxic encephalopathy. Further studies clearly need to validate this and attempt separation of patient groups within the autistic spectrum.

For the children described above we hypothesise that the root problem lies in aberrant early immune programming, particularly within the mucosal immune system. Immunological immaturity may permit the generation of inappropriate or inadequate cytotoxicity in the face of atypical viral exposures. Factors that modify maturation of T helper cell effector function, including vaccines, toxins or natural infections (or lack thereof), may prolong dysregulated T cell function or delay maturation and thus impair antiviral responses. Inappropriate early conditioning of the mucosal immune system, in which faecal flora plays an obligatory role, may allow inappropriate persistence of agents which home to gut-associated lymphoid tissue. The immunomodulatory nature of MV suggests that persistent expression within mucosal lymphoid tissue may affect mucosal tolerance mechanisms and alter risk for autoimmunity and inflammation.

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