

VACCINES AND AUTISM

Detection of measles virus in children with ileo-colonic lymphoid nodular hyperplasia, enterocolitis and developmental disorder

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A novel form of inflammatory bowel disease has been reported in a cohort of children with developmental disorder, manifesting predominantly as regressive autism (affected children). The intestinal pathology includes ileo-colonic lymphoid nodular hyperplasia (LNH) and a subtle enterocolitis. Clinical and pathological aspects of this disorder have previously been reported.^{1–3}

In ileal lymphoid tissues of affected children, the presence of reactive follicular hyperplasia may reflect antigenic presence at this site. We have examined a possible association between measles virus (MV) and a chronic enterocolitis in these children. We sought to detect and localise MV in intestinal tissues from affected children.

In ileal lymphoid tissue of autistic children, MV genomic RNA was associated with follicular dendritic cells and some lymphocytes within foci of reactive follicular hyperplasia. This association distinguished children with the combined features of mucosal inflammation, ileal LNH and developmental disorder from controls. In the affected children, the combination of reactive follicular hyperplasia, ileo-colitis and persistent MV infection of follicular dendritic cells suggests an integrated pathogenetic process.

The aim of this study was to examine the presence of measles virus (MV) in ileal lymphoid tissues of affected children and controls. A range of molecular techniques was used to identify, localise and quantitate viral isolates from gut biopsies of affected children which included TaqMan reverse transcription (RT) PCR, solution phase RT PCR and in-cell RT PCR.

Overall, 73 of 77 (95%) affected children (median age 6 yrs; range 3–14; 65 male) contained MV genomes in ileal lymphoid tissue compared with five of 44 (11.4%) controls ($n = 44$; median age 10 yrs; range 0–16; 31 male). Controls included: 17 children with normal ileal biopsies, three children with ileal LNH, three children with mild non-specific changes investigated for abdominal pain, and 21 children who had undergone appendicectomy for abdominal pain including appendicitis.

For samples analysed by TaqMan RT PCR, 62 of 68 (91%) affected children were positive for MV F gene compared with four of 39 (10.3%) controls. MV copy number in positive biopsies was low, and ranged from one to 300 000 copies per ng total RNA. MV quantitation was successfully performed in archival formalin-fixed paraffin-embedded ileal biopsies from affected children. Appendix lymphoid tissues from four out of 21 children were positive for measles virus.

Using standard solution phase RT-PCR on cryo-preserved ileal biopsy material from six affected children, SSPE brain and MV-infected vero cells, specific MV amplicons could be detected for the F-gene (five of six children) and H-gene (six of six children). All six children were positive for MV F- and H-genes by RT TaqMan PCR. Amplicon specificity was confirmed by Southern blot analysis using F- and H-gene specific probes. No template controls run in parallel were negative. Using in-cell RT PCR analysis, MV amplicons in MV-infected vero cells were identified as a cytoplasmic signal. In SSPE brain material, discrete, intense foci of MV amplicons were detected in grey matter using in-cell RT-PCR, both with and without dinitrophenol (DNP) labelled tyramide. Signal was not detected in similarly processed normal brain, or when MV primers were omitted on sections of SSPE brain.

Of the 34 biopsies from affected children that were examined by in-cell RT PCR, six could not be interpreted due to degradation or loss of the section during processing. Of the remaining 28 tissues, 25 (89%) were

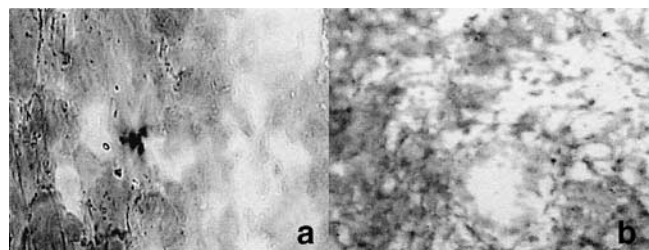


Figure 1 In-cell RT-PCR detection of MV. (a) MV N-gene in cell RT-PCR combined with ISH showing in a reactive ileal lymphoid follicle centre. Positive signal radiates from an intense central core in an apparently beaded, fibrillary pattern consistent with the morphology of a follicular dendritic cell. (b) Negative control section of (a), treated identically but omitting MV N-gene primers. No MV signal (original magnification $\times 400$). Similarly negative images were obtained when the procedure, including MV N-gene primers, was carried out on normal brain.

positive for MV; N-gene amplicons could be detected in serial sections of ileal biopsies (Figure 1a). Of the control group, one of five (20%) children with histologically normal, small and large bowel mucosa had detectable MV N-genomes, present in a distribution that was identical to that seen in biopsies from affected children. No MV signal was seen in serial control sections where primers were omitted (Figure 1b). Biopsies from 22 children were examined by a combination of in-cell RT PCR and TaqMan RT PCR. All were positive for MV and 16 of 22 were positive by both techniques.

The data confirm the presence of MV in reactive ileal lymphoid tissue. Based upon preliminary immunohistochemical findings this study focused on MV detection in mid-gut mucosal lymphoid tissue. It is interesting therefore, that MV was detected in a small proportion of controls, particularly in appendix tissue. This is consistent with the tropism of MV for intestinal lymphoid tissue,⁴ and previous evidence of MV persistence in the appendix.⁵ It is important to note, however, that the prevalence of MV persistence in intestinal lymphoid tissues of the general population is not known. A more extensive search for persistent MV genomes in the gut and elsewhere is merited, given the enhanced sensitivity of the TaqMan RT PCR method.⁶

This study has focused principally on MV; the presence of alternative infections has not been excluded. Such viruses may persist elsewhere, or exert transient effects not requiring subsequent persistence. One such transient risk for an adverse outcome to MV may be concurrent exposure to MV and another infection. This atypical pattern of exposure has been identified as a risk factor for SSPE (chicken pox & encephalitogenic enterovirus),^{7,8} and classical inflammatory bowel disease (Crohn's disease and ulcerative colitis; mumps).⁹

The presence of MV in gut mucosal follicular dendritic cells, associated with an apparent immune

mediated entero-colitis, mirrors certain aspects of human viral pathology—for example, HIV 1 infection in HIV 1 enteropathy. HIV 1, like MV, potently disrupts cellular immunity, and induces reactive follicular hyperplasia and lymphadenopathy in the early stage of infection. This is associated with expansion of the follicular dendritic cell network and trapping of HIV 1 within germinal centres. During the early latent phase of infection, HIV 1 antigens are detectable upon the surface of follicular dendritic cells in a pattern similar to that reported here for MV.^{10,11} For MV, as for other lymphotropic viruses, this location may favour the induction of immunological tolerance and failure of viral clearance.^{12,13}

The association between the presence of MV and reactive follicular hyperplasia of ileal lymphoid tissue in children with enterocolitis and regressive autism, indicates the possibility of an integrated pathogenetic process.

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