Leading article

Detection of persistent measles virus infection in Crohn's disease: current status of experimental work

The aetiology of Crohn's disease is unknown. Any hypothesis must take into account the continuing increase in incidence in some countries, including the UK. The increase affects the population from early teens, and specific comprehensive epidemiological studies in children and adolescents show a continuing rise in the rate of age and sex standardised incidence in Scotland.[1] This suggests an environmental trigger, which may interact with underlying genetic susceptibility. A number of such environmental triggers have been proposed, including persistent infections, transient infections in a host with abnormal mucosal immunity, particulate materials, or dietary changes. Persistent infection with the measles virus after wild-type virus infections or immunisation with live attenuated measles vaccine have been proposed as important environmental triggers based on epidemiological observations.[2 3] Measles is a single stranded RNA virus which can induce immune suppression. Measles infection is generally self limited and results in long term immunity, but rarely, persistent infection may occur after wild-type virus infection. The virus has special affinity for epithelial cells of the respiratory tract and cells of the immune system, such as lymphocytes and macrophages.[4] A number of chronic diseases have been linked with persistent measles virus infection. These include multiple sclerosis, Paget's disease, a variety of autoimmune diseases, and autism, although definite evidence is lacking in each. Inflammatory bowel disease (IBD), especially Crohn's disease, has been linked to persistent measles virus infection. Ekbom *et al* proposed perinatal exposure to wild-type measles virus may lead to development of Crohn's disease later in life.[2 3] Such case control epidemiological studies are of course prone to a variety of confounding factors, especially selection and recall biases. Epidemiologically robust data from a large controlled prospective study showed absence of a link between intrauterine exposure to measles and Crohn's disease.[5] However, epidemiological studies linking persistent measles virus infection to Crohn's disease generated a hypothesis[6] for confirmation or rejection by searching for measles virus in Crohn's affected tissue.

Another line of epidemiological evidence linking Crohn's disease with measles implicated the vaccine strain, and the safety of measles-mumps-rubella vaccination has been a topic of lively debate both in the medical and lay press. Vaccines against measles, mumps, and rubella (MMR) are produced from live attenuated viruses which have been propagated in a variety of cell substrates, such as embryonated chicken eggs and/or human diploid cells. The genomes of all three viruses consist of a single stranded RNA molecule that has negative polarity for measles and mumps and positive polarity for rubella. MMR vaccines are effective and safe. Reported side effects include aseptic meningitis and acute arthritis.[7] Aseptic meningitis was ascribed to the Urabe strain of mumps virus, which was withdrawn in 1992. Arthritis has been related to rubella vaccination but no significant association between chronic arthropathy and rubella vaccination has been found in women. Severe allergic reactions to MMR may be related to egg allergy or allergy to gelatin.[8] In 1995, the safety of measles and MMR vaccination was brought into question by the suggestion that live attenuated measles vaccine may be a risk factor for the development of IBD, especially Crohn's disease.[9] This link was postulated following epidemiological observations of the temporal link between an increasing incidence of Crohn's disease and introduction of live attenuated measles vaccine in 1968 in the UK. The MMR vaccine was introduced much later in 1988. However, the increase in the incidence of Crohn's disease started earlier than 1968 and still continues.[1] A case control study of 140 IBD patients (83 Crohn's disease) born after 1968 and 280 controls matched for age, sex, and general practitioner area provided no support for the hypothesis that measles vaccination in childhood predisposed to the later development of either IBD overall or Crohn's disease in particular.[10] In Finland, a countrywide surveillance system to detect serious adverse events was set up in 1982 after introduction of MMR vaccination. By the end of 1996, 1.8 million individuals were immunised and three million vaccine doses were consumed. The rate of serious adverse events with possible or indeterminate causal relationship with MMR vaccination was 5.3 per 100 000 vaccinees or 3.2 per 100 000 vaccine doses. The majority of serious adverse events were neurological or allergic. No association between IBD and MMR vaccination was reported. This is currently the best prospective data on the safety of MMR vaccination.[11]

Considerable interest was then generated by the report of the presence of measles virus nucleocapsid protein in intestinal tissues affected by Crohn's disease.[12] This article reviews the current state of the evidence from the experimental work regarding the question of persistent measles virus in Crohn's disease. The epidemiological evidence itself has generated a lively debate[13–17] but this article deals with the experimental work aiming to confirm or reject the hypothesis formulated from the epidemiological evidence.

Measles serology

The hypothesis that persistent measles virus infection may result in Crohn's disease prompted serological studies for measles antibody. Sera from 14 affected members of two French families with a high incidence of Crohn's disease and from unaffected family members as well as healthy age and sex matched controls were tested for measles IgG and IgM antibodies.[18] Commercially available indirect enzyme immunoassay kits (Whittaker Bioproducts Inc., Walkersville, Maryland, USA) were used to detect measles IgM and IgG antibodies. There were no differences in measles

Abbreviations used in this paper: IBD, inflammatory bowel disease; PCR, polymerase chain reaction; RT, reverse transcriptase; SSPE, subacute sclerosing panencephalitis; NIBSC, National Institute for Biological Standards and Control; MMR, measles-mumps-rubella vaccination; pfu, plaque forming units.

Leading articles express the views of the author and not those of the editor and the editorial board

IgM or IgG antibody levels between patients and controls, or between patients and unaffected family members. The authors concluded that their data did not support the hypothesis that persistent measles virus infection causes Crohn's disease. Fisher and colleagues[19] reported that the median measles virus IgG antibody titre in Crohn's disease patients determined by an inhouse complement fixation test was actually lower than the median titre in controls. There was also a trend towards lower median measles virus IgM antibody titre determined by a commercial ELISA in Crohn's disease patients compared with controls. In a study from the National Institute for Biological Standards and Control (NIBSC), all patients with Crohn's disease, ulcerative colitis, and non-IBD controls had detectable levels of serum neutralisation antibodies against measles virus.[20] The average antibody titre for all patients and controls (n=30) was 1:197,[20] suggesting that all patients had either been exposed to measles wild-type or vaccine strains previously. The antibody titres of the non-IBD control group were similar to the titres of the Crohn's disease and ulcerative colitis groups. A characteristic feature of serological studies in subacute sclerosing panencephalitis (SSPE) cases, where measles persistence is well established, is the significantly elevated concentrations of measles specific antibodies both in serum and cerebrospinal fluid.[21–23] Such an increase in antibody concentrations has not been detected in serum samples from IBD patients by any research group. On serological grounds therefore it is unlikely that Crohn's disease shares with SSPE the characteristics of measles virus infection in early life followed by a latent illness with persistent measles virus particles in the target organ.

Non-polymerase chain reaction (PCR) based methods

The initial methods that demonstrated the presence of measles virus in Crohn's affected tissues included immunocytochemistry, in situ hybridisation, and transmission electron microscopy.[12 24] The specificity and methodology used in these studies are open to question. Non-specific reactivity of measles antibody can be problematic and the measles nucleocapsid-like particles may resemble normal cellular structures. Positive detection of measles virus in Crohn's tissue by transmission electron microscopy, immunogold staining, and in situ hybridisation may be related to lack of specificity of immune staining because of cross reactivity or non-specific adherence. Evidence for such cross reaction has been provided recently.[25] Colonic mucosa from 20 Crohn's disease, 20 ulcerative colitis, 11 "non-IBD" colitis, and nine non-inflamed control patients were immunohistochemically stained with the antimeasles monoclonal antibody 4F12. Measles related antigen was present not only in colons affected with Crohn's disease but also in colons affected with ulcerative colitis and in non-IBD colitis. It was reported by the authors that the measles related antigen was not derived from measles virus but was an as yet unidentified human protein.[26] Confirmation of measles virus detection by non-PCR based methods has eluded other teams. There is ambiguity in discriminating virus-like particles from normal cellular structures which is relevant to reports of immunogold electron microscopy detection of measles virus in intestine,[27 28] and paramyxovirus could not be detected by electron microscopy consistently.[29] Immunocytochemistry for a panel of bacterial and viral organisms failed to detect evidence of measles antigen in Crohn's affected tissues.[30] Intestines and mesenteric lymph nodes of 21 patients from two French families with a high frequency of Crohn's disease and patients from Connecticut were studied by Liu and colleagues.[30] Control tissues were from ulcerative colitis or indeterminate colitis or a miscellaneous group of surgically resected specimens. Primary antibodies were against the measles virus nucleocapsid protein, polyclonal (from the Royal Free Hospital group) and monoclonal (from Karolinska Institute). Positive control tissues were from virus infected HEP-2 cell from Bion Inc. (Park Ridge, Illinois, USA) and from SSPE brain. In all cases studied (16 patients with Crohn's disease, eight patients with ulcerative colitis, and 10 normal controls) by indirect and labelled streptavidin-biotin-peroxidase immunocytochemical techniques, tissues were non-specifically labelled by the polyclonal measles nucleocapsid protein antibody. The immunolabelling occurred in the nuclei of macrophages, fibroblasts, giant cells, smooth muscle cells, and nerves, and the labelled cells were distributed in the lamina propria, submucosa, muscle layers, subserosa, and granulomas (in Crohn's affected tissues). In contrast with the results obtained with the polyclonal antibody, none of the Crohn's disease, ulcerative colitis, or control tissues were labelled by monoclonal measles nucleocapsid protein antibody although highly specific staining with this antibody was observed in SSPE control brain tissue and in cell cultures containing measles virus. The polyclonal measles antibody was not affinity purified and therefore it probably cross reacted with normal cellular components.[30] Other studies have raised concerns about the specificity of measles virus anti-M protein monoclonal antibody, which labelled antigen in Crohn's disease affected tissues by immunofluorescence staining.[31] Overall, the immunohistochemical techniques do not provide confirmatory evidence for persistent measles virus infection in Crohn's disease and are contradictory. Therefore, investigators have tried to identify measles virus RNA in affected tissues using PCR amplification.

PCR based methods

Measles specific PCR enables amplification of measles virus RNA to allow definitive molecular characterisation. Several laboratories developed their own RNA based reverse transcription (RT) PCR assays to detect measles virus genomic RNA in tissues derived from Crohn's disease patients. Most groups used resected materials which provide large amounts of tissues involving all layers of the intestine but often represented end stage disease after intensive medical therapy, including immunosuppressives. The sensitivity limits of these RT-PCR assays varied considerably between laboratories,[20 32–35] but a N, F gene specific RT-PCR nested PCR is reported to have an assay sensitivity of a single genome copy.[33]

The NIBSC evaluated both a two step approach (RT followed by PCR and nested PCR) and a single step approach (both RT and primary PCR step combined using EZ RTth RNA PCR kit).[20] The latter was at least 100- to 1000-fold more sensitive than the two step approach. The NIBSC assay was able to identify a measles virus genome sequence in control samples that had measles virus corresponding to as little as 5.5×10^{-3} plaque forming units (pfu). Measles N gene primers used in this study were highly sensitive and could amplify target sequence from all known strains of measles virus. Partially homogenised brain material from a patient with SSPE was examined as a positive control, and measles specific nucleic acid was detected from a sample equivalent to 18 cells. In contrast, no tissue sample from IBD patients derived from endoscopic biopsies was positive for measles specific nucleic acid. Biopsies from all control patients were also negative. A total of 93 colonoscopic biopsies were examined and both steroid naïve and steroid treated patients were included. About one million cells were examined per biopsy. All

Table 1 Summary of polymerase chain reaction (PCR) studies performed to identify measles virus in inflammatory bowel disease

Study group	*Diagostic groups*	*Tissue source*	*Experimental procedure*	*Assay sensitivity*	*Positive control*	*Measles virus presence*
Royal Free Hospital, London, UK[35]	CD, UC, IC, controls	Resected material + PBMC	Hybrid capture + N, H gene specific RT-PCR	10^4 RNA molecules	SSPE (brain); vaccine recipient + SSPE (PBMC)	−ve
NIBSC, UK[20]	CD, UC, IC, controls	Biopsies + lymphocytes	N gene specific RT-PCR nested PCR	5.5×10^{-3} pfu	SSPE (brain); wild-type measles virus strain (94/31825) in Vero cells	−ve
Hirosaki University, Japan[33]	CD, UC, controls	Resected intestine	N, F gene specific RT-PCR nested PCR	Single viral genomic RNA	SSPE (brain); Vero E6 cells infected with Toyoshima strain of measles virus	−ve
Akita and Osaka University, Japan[32]	CD, UC, controls	Resected intestine + biopsies	N, M, F, H gene specific RT-PCR	Not reported	Not reported	−ve
Tokyo Medical University, Kitasato Institute Tokyo, Japan; Royal Free Hospital, London, UK[37]	CD, UC, controls	PBMC	F, H gene specific RT-PCR nested PCR	Not reported	SSPE (brain); SSPE (PBMC), vaccine and wild strains	+ve
NIBSC, UK[34]	CD (affected, unaffected, lymph nodes)	Resection specimen (affected and unaffected intestine lymph nodes)	N, M, H gene specific RT-PCR nested PCR	N: 5.5×10^{-2}– 5.5×10^{-3} pfu M: 5.5×10^{-3} pfu H: 5.5×10^{-1}–5.5×10^{-2} pfu	SSPE (brain); wild-type measles virus strain (94/31825) in Vero cells	−ve

CD, Crohn's disease; UC, ulcerative colitis; IC, indeterminate colitis; pfu, plaque forming unit; PBMC, peripheral blood mononuclear cells; N, nucleocapsid protein gene; M, matrix protein gene; F, fusion protein gene; H, haemagglutinin protein gene.

patients had detectable neutralisation antibodies against measles virus in serum and 5/30 patients had confirmed measles vaccination.

Thirty one peripheral blood lymphocyte preparations (20 patients with IBD, 11 non-IBD controls) were also examined by the rTth RT-PCR nested PCR method, as described above, at the NIBSC.[20] None of the amplifications of the nucleic acids extracted from the lymphocyte preparations produced DNA fragments of the size expected for measles specific sequences.[20]

Colonoscopic biopsies are superficial and may miss virus persistence in deeper layers within the granulomas. Hence full thickness resection specimens of seven Crohn's disease patients taken from macroscopically inflamed and healthy areas of the gut as well as mesenteric lymph nodes were further investigated.[34] Resected specimens had been snap frozen in liquid nitrogen immediately after removal. Despite using oligonucleotide primers corresponding to three different portions of the measles virus genome (N, M, and H gene regions, full details of primer positions and sequences reported elsewhere[34]), none of the surgically resected specimens gave any positive signals that could suggest the presence of measles virus RNA sequences in Crohn's tissue. The extraction mixtures derived from 3–4 slices of intestinal tissue had about 6–8 million cells, and that derived from 2–3 slices of mesenteric lymph node tissue had 4–6 million cells. The assay sensitivity limits for the wild-type strain of the N gene primer was 5.5×10^{-2} to 5.5×10^{-3} pfu , of the M gene primer was 5.5×10^{-3} pfu, and of the H gene primer was 5.5×10^{-1} to 5.5×10^{-2} pfu. The assay cut off for SSPE brain material was 30 cells.[34] As all samples were examined with primers specific for three different genes of the measles virus genome, the possibility of false negative results, in case one part of the virus genome is less suitable for RT-PCR amplification, is minimised.

Other groups, including the Royal Free Hospital group, failed to detect measles virus genome in Crohn's tissue, despite targeting multiple regions of the genome and different experimental approaches.[35 36] Although it is proposed that measles virus may persist in intestinal endothelial cells causing intestinal vasculitis, the Munich group[36] found no evidence of persistence of measles virus genome in 22 endothelial cell antibody positive IBD patients using a PCR assay on formalin processed colonic biopsies. The sensitivity of their PCR assay is unclear. A summary of the assay characteristics used by different research groups has recently been reported and reviewed.[7] Updated details of RT-PCR assays published by different groups are provided in table 1. The oligonucleotide primer sequences of the nucleocapsid protein gene (N), matrix protein gene (M), and haemagglutinin protein gene (H) used in the NIBSC studies are provided in table 2.

Although all attempts to detect measles virus genome in Crohn's affected tissue using RT-PCR have failed, a recent study based on a Japanese-Royal Free Hospital group collaboration have claimed detection of the presence of wild-type and vaccine-type measles virus genome in peripheral blood mononuclear cells from IBD patients (as well as patients with autism).[37] However, the NIBSC study also attempted to detect measles virus genome in lymphocyte preparations from IBD and control patients with paired tissue specimens and all lymphocyte preparations were negative. The experience with clinical specimens at NIBSC has shown that even with the most meticulous technique, cross contamination of specimens can occasionally occur.[20] PCR contamination has also been reported by the Royal Free Hospital group.[35] A range of methods and considerable experience is needed to recognise non-specific reactions which might be misinterpreted as positive if confirmatory techniques are not applied. The issue of cross contamination during PCR based diagnosis is well documented and reviewed recently in context with human

Table 2 Oligonucleotide primer sequences of three measles virus genes used in the NIBSC study

Gene	*Designation*	*Position*	*Sequence (5' →3')*
N	MV1/outer	1198–1218	TTAGGGCAAGAGATGGTAAGG
N	MV2/outer	1609–1630	GTTCTTCCGAGATTCCTGCCA
N	MV3/inner	1248–1268	AGCATCTGAACTCGGTATCAC
N	MV4/inner	1480–1500	AGCCCTCGCATCACTTGCTCT
M	MV13/outer	3565–3584	GCGACAGGAAGGATGAATGC
M	MV14/outer	3832–3851	GTTTGCGTTGAAAGACACTCC
M	MV15/inner	3587–3603	TATGTACATGTTTCTGC
M	MV16/inner	3811–3829	GTTGTTAGGACCTTTCTCC
H	MV-M3/outer	8106–8125	CAGTCAGTAATGATCTCAGC
H	MV-M6/outer	8676–8701	CTTGAATCTCGGTATCCACTCCAAT
H	MV-H7/inner	8147–8171	GAGCTCAAACTCGCAGCCCTTTGTC
H	MV-H4A/inner	8457–8482	ATCCTTCAATGGTGCCCACTCGGGA

The nucleotide positions are in relation to the sequence reported for the measles virus genome in the EMBL-Genbank data under accession number K01711.
N, nucleocapsid protein gene; M, matrix protein gene; H, haemagglutinin protein gene.

viral diagnosis.[38] The exquisite sensitivity of the PCR reaction has led to applications in medical diagnostics, population genetics, and forensic analysis. Obviously, the fewer the number of molecules we are trying to detect, the more is the need to guard against false positives or mistyping, a caveat well known to forensic molecular biologists.[39] The use of PCR for amplification of a few molecules of nucleic acids requires rigorous adherence to a strict set of protocols as the product of amplification serves as the substrate for generation of more product. A single PCR cycle generates very large numbers of amplifiable molecules that have the potential to contaminate subsequent amplifications of the same target sequence. To control contamination, one must prevent the transfer of nucleic acid molecules between amplified samples, and between positive and negative experimental controls. A number of precautions are generally adopted to reduce false positive rates in laboratories,[39] but false positives may still occur. Indeed, if there is any reasonable doubt about a critical experimental result, it is wisest to repeat the experiment. The negative controls may eliminate reagent contamination but cannot guarantee against sporadic contamination; it is however very unlikely that a sporadic contamination event would occur twice in exactly the same way. It is possible that the sequences reported by Kawashima and colleagues[37] have emerged as a result of cross contamination of the samples with measles virus controls or pre-existing DNA templates during nested PCR amplification. This is supported by the fact that the sequences derived from cases of IBD and autism cannot be grouped effectively in relation to their clinical histories and established phylogenetic relationships of wild-type and vaccine strains.[40] In a recent correspondence, we have presented evidence that the results of Kawashima *et al* are not internally consistent and not consistent with the findings of others.[40] Based on the sequence data provided by Kawashima *et al*, the sequence derived from a Crohn's disease patient differed by only two nucleotides from the vaccine strain while the sequences derived from cases of autism, which theoretically should be identical to the vaccine strains, differed by 3–5 nucleotides. There is also evidence to suggest the possibility that mixed DNA fragments were present in the PCR products that are usually produced by cross contamination with more than one template.[40]

Molecular mimicry

As persistence of measles virus genomic sequences in Crohn's tissue has not been demonstrated by a number of researchers using state of the art techniques, we now have to re-examine the original immunohistochemical demonstration of signal for measles virus N protein using monoclonal antibody immunohistochemistry (Serelab, Crawley Down, Sussex, UK). Iizuka *et al* have presented evidence that this monoclonal antibody recognises a host antigen as well as measles virus.[25] The host antigen recognised by the monoclonal antibody was unrelated to the measles virus.[41] Serum neutralisation antibodies against measles virus is ubiquitous, both in IBD patients and in non-IBD controls.[20] The measles related antigen has not been identified, as no homologous proteins have been found in the protein databases. However, the nucleotide sequence of the positive clone was 99% homologous with the human gene AA449055 deposited in DNA databases. A Southern blot analysis confirmed the human origin of the measles related antigen.[26] The functional role of the measles related antigen is unclear but it may be associated with a subpopulation of macrophages. Molecular mimicry, the concept that antigenic determinants of micro-organisms resemble antigenic determinants of the host, is frequently cited as a plausible mechanism to account for the association of infection and autoimmune disease. Although an impressive number of such mimicry have been reported, there are no clear examples of a human disease caused by molecular mimicry.[42] Epitope homology of a viral and self determinant is not in itself adequate evidence for mimicry as a pathogenetic mechanism.[43] The mimicking determinant must also be capable of inducing disease in the absence of replicative virus, and there is no such evidence in Crohn's disease. Molecular mimicry at the T cell level may be important in the development of systemic autoimmunity.[44] Studies in multiple sclerosis found no evidence of a multiple sclerosis specific pattern of T cell responses to recombinant measles virus structural proteins[45] but similar studies have not been performed in Crohn's disease. Liu and colleagues[30] found that almost all immunolabelling by polyclonal measles virus antibody occurs in the nucleus, and measles virus phosphoprotein is known to have immunological cross reactivity with an intermediate filament protein of human cells, probably vimentin.[46] Antimeasles monoclonal antibodies may also cross react with mouse anterior pituitary, gastric mucosa, salivary gland, and neurones in the brain.[47] A comparison of the temporal profile of the epidemiology of measles and Crohn's disease makes it unlikely that molecular mimicry might be a plausible hypothesis to explain the onset of Crohn's disease after an outbreak of measles. The widespread use of measles vaccine in children older than one year in developed countries has greatly reduced the incidence of measles in all age groups, including infants under one year. Exposure to wild measles virus in infancy when the infant is immunologically immature (as suggested by Van damme and colleagues[48]) in developed countries (including Scotland) has therefore dramatically reduced over a period in which the incidence of juvenile onset Crohn's disease has increased, especially in the 12–16 year old age group.[1] Prior to measles immunisation, 30% of measles cases in urban Africa occurred in infants under one year.[49] This burden of wild measles infection should have resulted in an epidemic of Crohn's disease in the survivors long ago.[50]

Summary

Epidemiological studies may generate a hypothesis regarding the aetiology of chronic diseases that may be tested directly. In the case of persistent infections, demonstration of a putative organism by a sensitive and specific test provides essential evidence in favour of such a hypothesis. The measles virus hypothesis had generated considerable excitement, attracted resources, and spurred Crohn's disease researchers to perform corroborative tests. It also however had undesirable effects on uptake of immunisation, a potential public health hazard.[51 52] As a number of well conducted studies to demonstrate measles virus genome in Crohn's disease tissues using state of the art RT-PCR based assays have proved to be negative, it must be concluded that the persistent measles virus infection hypothesis in the aetiology of Crohn's disease lacks confirmatory evidence. There is now enough experimental evidence to conclude that failure to detect measles virus genome in IBD tissues is not due to the inefficiencies of the PCR based detection systems but to the absence of measles virus particles. The challenge facing the medical profession is to convince the public that there is no evidence of measles virus persistence in the intestine of Crohn's disease patients.

S GHOSH
E ARMITAGE

Gastrointestinal Unit, Department of Medical Sciences, University of Edinburgh, Western General Hospital, Edinburgh EH4 2XU, UK

D WILSON

Paediatric Gastroenterology and Nutrition, Royal Hospital for Sick Children, Edinburgh, UK and Department of Child Life and Health, University of Edinburgh, UK

P D MINOR
M A AFZAL

Division of Virology, National Institute for Biological Standards and Control, South Mimms, Potters Bar, Hertfordshire EN6 3QG, UK

Correspondence to: Dr S Ghosh, Western General Hospital, Edinburgh EH4 2XU, UK. sg@srv0.med.ed.ac.uk.

Supported in part by the Wellcome Trust.

1 Armitage E, Drummond H, Ghosh S, *et al.* Incidence of juvenile-onset Crohn's disease in Scotland. *Lancet* 1999;**353**:1496–7.
2 Ekbom A, Wakefield AJ, Zack M, *et al.* Perinatal measles infection and subsequent Crohn's disease. *Lancet* 1994;**344**:508–10.
3 Ekbom A, Daszak P, Kraaz W, *et al.* Crohn's disease after in-utero measles virus exposure. *Lancet* 1996;**348**:515–17.
4 Casali P, Nakamura M, McChesney MB. Immunosuppression by measles virus. In: Spector S, Bendinelli M, Friedman H, eds. *Virus induced immunosuppression*. New York: Plenum Press, 1989:345–73.
5 Jones P, Fine P, Piracha S. Crohn's disease and measles. *Lancet* 1997;**349**:473.
6 Wakefield AJ, Ekbom A, Dhillon AP, *et al.* Crohn's disease: Pathogenesis and persistent measles virus infection. *Gastroenterology* 1995;**108**:911–16.
7 Afzal MA, Minor PD, Schild GC. Clinical safety issues of measles, mumps and rubella vaccines. *Bull World Health Organ* 2000;**78**:199–204.
8 Patja A, Makinen-Kiljunen S, Davidkin I, *et al.* Allergic reactions to measles-mumps-rubella vaccination. *Pediatrics* 2001;**107**:E27.
9 Thompson NP, Montgomery SM, Pounder RE, *et al.* Is measles vaccination a risk factor for inflammatory bowel disease? *Lancet* 1995;**345**:1071–4.
10 Fenney M, Ciegg A, Winwood P, *et al.* A case-control study of measles vaccination and inflammatory bowel disease. The East Dorset Gastroenterology Group. *Lancet* 1997;**350**:764–6.
11 Patja A, Davidkin I, Kurki T, *et al.* Serious adverse events after measles-mumps-rubella vaccination during a fourteen year prospective follow-up. *Pediatr Infect Dis J* 2000;**19**:1127–34.
12 Wakefield AJ, Pittilo RM, Sim R, *et al.* Evidence of persistent measles virus infection in Crohn's disease. *J Med Virol* 1993;**39**:345–53.
13 Pardi DS, Tremaine WJ, Sandborn WJ, *et al.* Perinatal exposure to measles virus is not associated with the development of inflammatory bowel disease. *Inflamm Bowel Dis* 1999;**5**:104–6.
14 Montgomery SM, Morris DL, Pounder RE, *et al.* Paramyxovirus infections in childhood and subsequent inflammatory bowel disease. *Gastroenterology* 1999;**116**:796–803.
15 Thompson NP, Montgomery SM, Wadsworth ME, *et al.* Early determinants of inflammatory bowel disease: use of two nation longitudinal birth cohorts. *Eur J Gastroenterol Hepatol* 2000;**12**:25–30.
16 Pardi DS, Tremaine WJ, Sandborn WJ, *et al.* Early measles virus infection is associated with the development of inflammatory bowel disease. *Am J Gastroenterol* 2000;**95**:1480–5.
17 Morris DL, Montgomery SM, Thompson NP, *et al.* Measles vaccination and inflammatory bowel disease: a national British Cohort Study. *Am J Gastroenterol* 2000;**95**:3507–12.
18 Van Kruiningen HJ, Mayo DR, Vanopdenbosch E, *et al.* Virus serology in familial Crohn disease. *Scand J Gastroenterol* 2000;**35**:403–7.
19 Fisher NC, Yee L, Nightingale P, *et al.* Measles virus serology in Crohn's disease. *Gut* 1997;**41**:66–9.
20 Afzal MA, Armitage E, Begley J, *et al.* Absence of measles virus genome sequence in inflammatory bowel disease tissues and peripheral blood lymphocytes. *J Med Virol* 1998;**55**:243–9.
21 Connolly JH, Allen IV, Hurwitz LJ, *et al.* Measles virus antibody and antigen in subacute sclerosing panencephalitis. *Lancet* 1967;**i**:542–4.
22 Jabbour JT, Sever JL. Serum measles antibody titers in patients with subacute sclerosing panencephalitis. *J Pediatr* 1968;**73**:905–7.
23 Rima BK. Paramyxoviruses and their role in disease. *Semin Arthritis Rheum* 1994;**23**:230–1.
24 Wakefield AJ, Sim R, Akbar AN, *et al.* In situ immune responses in Crohn's disease: A comparison with acute and persistent measles virus infection. *J Med Virol* 1997;**51**:90–100.
25 Iizuka M, Chiba M, Yukawa M, *et al.* Immunohistochemical analysis of the distribution of measles related antigen in the intestinal mucosa in inflammatory bowel disease. *Gut* 2000;**46**:163–9.
26 Iizuka M, Masamune O. Measles vaccination and inflammatory bowel disease. *Lancet* 1997;**350**:1775.
27 Daszak P, Purcell M, Lewin J, *et al.* Detection and comparative analysis of persistent measles virus infection in Crohn's disease by immunogold electron microscopy. *J Clin Pathol* 1997;**50**:299–304.
28 Lewin J, Dhillon AP, Sim R, *et al.* Persistent measles virus infection of the intestine: confirmation by immunogold electron microscopy. *Gut* 1995;**36**: 564–9.
29 Minor PD. Measles vaccination as a risk factor for inflammatory bowel disease. *Lancet* 1995;**345**:1362–3.
30 Liu Y, van Kruiningen HJ, West AB, *et al.* Immunocytochemical evidence of *Listeria, Escherichia coli*, and *Streptococcus* antigens in Crohn's disease. *Gastroenterology* 1995;**108**:1396–404.
31 Miyamoto H, Tanaka T, Kitamoto N, *et al.* Detection of immunoreactive antigen, with a monoclonal antibody to measles virus, in tissue from a patient with Crohn's disease. *J Gastroenterol* 1995;**30**:28–33.
32 Iizuka M, Nakagomi O, Chiba M, *et al.* Absence of measles virus in Crohn's disease. *Lancet* 1995;**345**:199.
33 Haga Y, Funakoshi O, Kuroe K, *et al.* Absence of measles viral genomic sequence in intestinal tissues from Crohn's disease by nested polymerase chain reaction. *Gut* 1996;**38**:211–15.
34 Afzal MA, Armitage E, Ghosh S, *et al.* Further evidence of the absence of measles virus genome sequence in full thickness intestinal specimens from patients with Crohn's disease. *J Med Virol* 2000;**62**:377–82.
35 Chadwick N, Bruce IJ, Schepelmann S, *et al.* Measles virus RNA is not detected in inflammatory bowel disease using hybrid capture and reverse transcription followed by polymerase chain reaction. *J Med Virol* 1998;**55**: 305–11.
36 Folwaczny C, Loeschke K, Schhnettler D, *et al.* Endothelial cell autoantibodies are a marker of disease susceptibility in inflammatory bowel disease but apparently not linked to persistent measles infection. *Clin Immunol* 2000;**95**:197–202.
37 Kawashima H, Mori T, Kashiwagi Y, *et al.* Detection and sequencing of measles virus from peripheral mononuclear cells from patients with inflammatory bowel disease and autism. *Dig Dis Sci* 2000;**45**:723–9.
38 Clewley JP. *The polymerase chain reaction (PCR) for human viral diagnosis*. London: CRC Press, 1995.
39 Kwok S, Higuchi R. Avoiding false positives with PCR. *Nature* 1989;**339**:237–8.
40 Afzal MA, Minor PD, Ghosh S, *et al.* Measles virus persistence in specimens of inflammatory bowel disease and autism cases. *Dig Dis Sci* 2001;**46**:658–60.
41 Iizuka M, Watanabe S, Chiba M. Immunohistochemical analysis of measles related antigen in IBD. *Gut* 2001;**48**:136–7.
42 Rose NR, Mackay IR. Molecular mimicry: a critical look at exemplary instances in human diseases. *Cell Mol Life Sci* 2000;**57**:542–51.
43 Lawson CM. Evidence for mimicry by viral antigens in animal models of autoimmune disease including myocarditis. *Cell Mol Life Sci* 2000;**57**:552–60.
44 Farris AD, Keech CL, Gordon TP, *et al.* Epitope mimics and determinant spreading: pathways to autoimmunity. *Cell Mol Life Sci* 2000;**57**:569–78.
45 Pette M, Liebert UG, Gobel U, *et al.* Measles virus-directed responses of CD4+ T lymphocytes in MS patients and healthy individuals. *Neurology* 1993;**43**:2019–25.
46 Fujinami RS, Oldstone MBA, Wroblewska Z, *et al.* Molecular mimicry in virus infection: crossreaction of measles virus phosphoprotein or of herpes simplex virus protein with human intermediate filaments. *Proc Natl Acad Sci USA* 1983;**80**:2346–50.
47 Srinivasappa J, Saegusa J, Prabhakar BS, *et al.* Molecular mimicry: frequency of reactivity of monoclonal antiviral antibodies with normal tissues. *J Virol* 1986;**57**:397–401.
48 Van damme W, Lynen L, Kegels G, *et al.* Measles vaccination and inflammatory bowel disease. *Lancet* 1997;**350**:1174–5.
49 Taylor WR, Kalisa R, Ma-Disu M, *et al.* Measles control in urban Africa complicated by high incidence of measles in the first year of life. *Am J Epidemiol* 1988;**127**:788–94.
50 Miller E, Goldblatt D, Cutts F. Measles vaccination and inflammatory bowel disease. *Lancet* 1998;**351**:755–6.
51 MMR vaccine coverage falls after adverse publicity. *Communicable Disease Report Weekly* 1998;**8**(5).
52 MMR vaccine coverage falls in the United Kingdom. *Communicable Disease Report Weekly* 1999;**9**(5).