

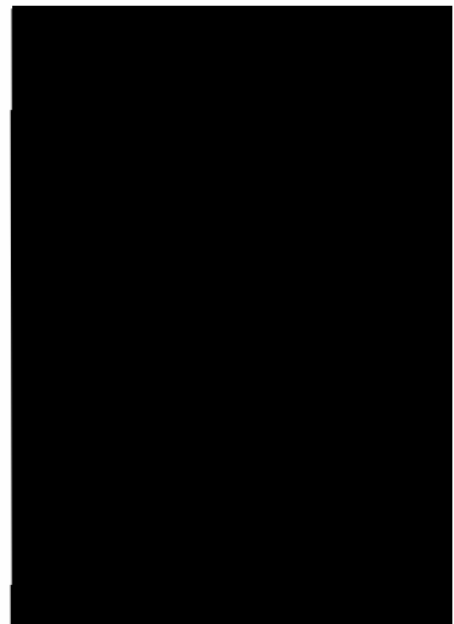
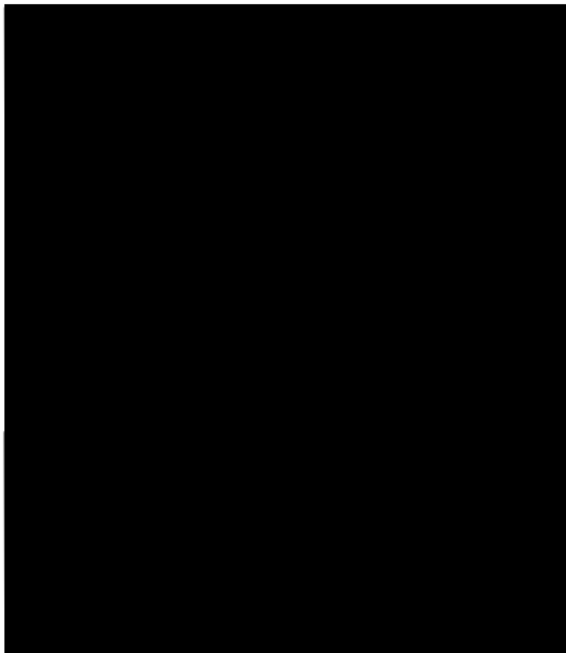
MRC: IN STRICT CONFIDENCE

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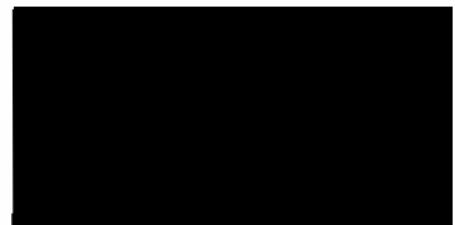
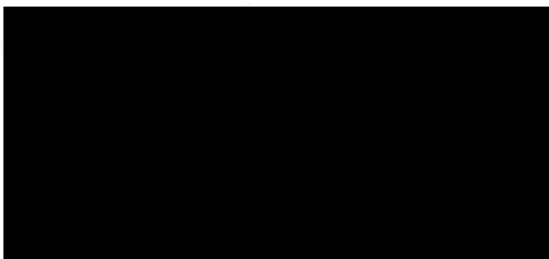
MRC *Ad hoc* Meeting to examine evidence relating measles or measles vaccine to chronic gastrointestinal inflammation

Report of the meeting held on 23 March 1998 at the Royal College of Surgeons,
Lincoln's Inn Fields, London

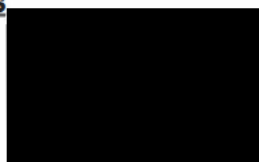
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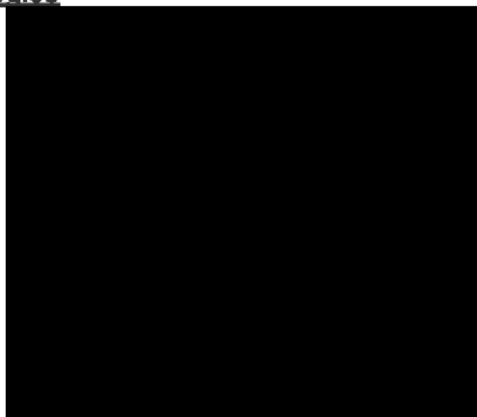
Observers



MRC Officers



Apologies



1. Background

- 1.1 In January 1998, following the publication of a number of papers reporting an association between measles or measles vaccine and chronic gastrointestinal inflammation, and earlier discussions at the Department of Health's Joint Committee on Vaccination and Immunisation, the Government's Chief Medical Officer, Professor Sir Kenneth Calman, asked the Medical Research Council to hold an *ad hoc* meeting on the 23 March 1998 to examine published as well as some pre-publication data from researchers at the Royal Free Hospital School of Medicine (RFHSM) to assess the evidence for a relationship between measles or measles vaccine and chronic gastrointestinal inflammation.
- 1.2 Vaccination and vaccine safety are issues of major concern to the public, their elected representatives and all health care workers. The UK Department of Health (DH) monitors these issues both directly, through the aegis of the Joint Committee on Vaccination and Immunisation (JCVI) and, within the UK, via the Medicines Control Agency (MCA), the National Institute of Biological Standards and Control (NIBSC) and the Public Health Laboratory Service (PHLS), and internationally, by links to the World Health Organisation (WHO).

2. Structure of the Meeting

- 2.1 Those presenting had been assured of the confidentiality of their data and, as far as was possible, given the potential public health impact of the outcome, were assured of the confidentiality of the discussions relating to those data. The meeting was not, therefore, open to the public, but it was considered necessary to make a public statement as soon as practicable upon its conclusion. The meeting had been arranged in full consultation with the researchers and their agreement had been sought on the structure of the agenda. Selection of reviewers was the responsibility of the MRC, seeking advice from the scientific community as appropriate. Relevant Government Departments and Non Government Agencies, in this instance the UK Departments of Health, the UK Medicines Control Agency and the World Health Organisation, were invited as observers. A report of the meeting would be presented to the Chief Medical Officer and the MRC Research Boards.

2.2 Submitted papers

Following approaches to a number of scientists three full pre-publication manuscripts were made available to the Council and thence to those attending the meeting:

• **Absence of detectable measles virus genome sequence in inflammatory bowel disease tissues and peripheral blood lymphocytes**

Afzal, M.A., Armitage, E., Begley, J., Bentley, M. L., Minor, P. D., Ghosh, S. and A. Ferguson.

• **Are concurrent measles and mumps infections in childhood in Great Britain a risk for inflammatory bowel disease**

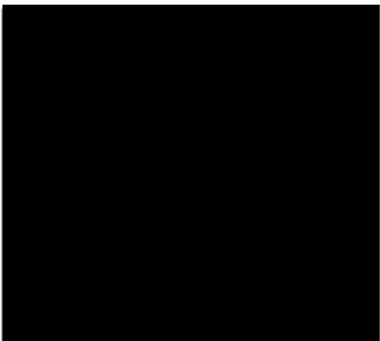
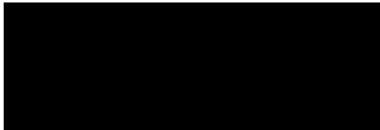


Montgomery, S. M., Morris, D. L., Pounder, R. E. and A. J. Wakefield.

• **Exposure to concurrent measles and mumps epidemics during childhood is a risk for inflammatory bowel disease.**



Montgomery, S. M., Wakefield, A. J., Bjornsson, S., Johannsson, J. H., Pounder, R. E. and B. Thjodleifsson.

2.3 Programme and presentations

The agenda was agreed in discussion with Mr Wakefield and colleagues from the RFHSM Inflammatory Bowel Disease Study Group (IBDSG).

	Welcome and Introduction
	Gastroenterology
	Infant origins of inflammatory bowel disease
	Paramyxoviruses
	Pathogenesis and aetiology of inflammatory bowel disease (immunological markers)
General Discussion	
	Epidemiology background
	Patterns of measles virus exposure and inflammatory bowel disease
General Discussion	
	A new syndrome: ileal lymphoid nodular hyperplasia, non-specific colitis and pervasive developmental disorder in children
	Key findings in autism
General Discussion	
Concluding session	
*Short talk	

3. Welcome and Introduction

- 3.1 The meeting was opened by  who welcomed and thanked all those attending, and drew participants' attention to the fact that this was an MRC meeting. The Chairman,  reminded members of the need for confidentiality and, noting that there was a large amount of data to consider, asked participants to keep all questions, except those relating to the factual content of talks, for the main discussion sessions.

4. Presentations and Discussions

4.1 Virology

- 4.1.1 Talks were given by [REDACTED] (Gastroenterology), [REDACTED] (Infant origins of inflammatory bowel disease), [REDACTED] (Paramyxoviruses) and [REDACTED] (Pathogenesis and aetiology of inflammatory bowel disease). Mr Wakefield detailed his work on the vasculature of the intestine in Crohn's disease, outlined Professor Miyamoto's data describing measles infection in the gut and then explained his own investigations, using *in situ* hybridisation to the measles nucleocapsid gene (N-gene)¹, electronmicroscopy (EM)², and immunogold electronmicroscopy³, of the presence of viruses in granulomas from Crohn's patients. He then turned to attempts to detect viral RNA using the reverse transcriptase polymerase chain reaction (RT-PCR). All those who had tried had failed to demonstrate the presence of viral RNA, including Mr Wakefield's own laboratory; this formed the first major discussion point of the meeting.
- 4.1.2 Dr Minor opened the discussion and described work from his laboratory, using a potentially definitive RT-PCR approach, that had sought to test the inference (that measles virus was present in inflammatory bowel disease) arising from Mr Wakefield's findings. Firstly, based on published data from the RFHMS IBD Group [REDACTED]
[REDACTED]
[REDACTED] From these published results the following predictions could be made:
- i) Measles should be present in a high proportion of Crohn's disease patients, but not necessarily in all cells in a granuloma (an estimate of 15% from immunogold EM was suggested);
 - ii) RNA should be present as it had been seen by *in situ* hybridisation. In addition, it would be difficult to envisage that nucleocapsid structures and antigens could persist in the absence of persistent expression from viral RNA;
 - iii) The amount of RNA present should, from the published comparisons, be of the same order as the amount found in subacute sclerosing panencephalitis (SSPE), a persistent measles infection of the brain.
- 4.1.3 From this starting point, Dr Minor's group had attempted to isolate measles RNA from biopsy specimens from Crohn's disease patients provided by [REDACTED]. These patients all had antibodies to measles virus. Using a sensitive RT-PCR technique, which was shown not to be inhibited by any cellular components that might be present in the reaction, and which could pick up both messenger and genomic sense RNA from the measles N-gene, it was possible to detect measles RNA in SSPE brain tissue samples containing as few as 18 cells. However, with one exception, it had not been possible to detect measles RNA from any of the specimens from nine Crohn's disease patients or nine patients with ulcerative colitis. The viral genome isolated in the exceptional reaction was shown to be that of a laboratory control strain and was therefore classified as a laboratory contaminant.
- 4.1.4 This work (summarised in Afzal *et al.*⁴), in conjunction with the work reported by Mr Wakefield, raised a number of issues which the group addressed:
- a) Which tissue should be biopsied?
- This was a difficult question and there was considerable discussion. There was agreement that each patient with Crohn's disease presented a different clinical picture; moreover, the picture in each individual could change week by week. Because the primary event in Crohn's disease was unknown, there were differing views on the most relevant site from which to take biopsy specimens. [REDACTED]
[REDACTED]
[REDACTED] It was in this deep biopsy material that the RFHMS group had looked for evidence of measles virus. The presence of measles virus in these tissues had been suggested by

immunohistochemical means, but the RFHMS group had not been able to isolate measles RNA from the tissues using RT-PCR. Others contended that evidence of aphthoid ulcers correlated with disease activity and contained granulomas. The material examined by Dr Minor's laboratory had thus been taken from a range of biopsy sites, namely aphthoid ulcers, gross fibrotic ulcers, confluent colitis (where present) and uninfamed tissue. It was unclear whether any of these sites would contain material from deep in the sub-mucosal tissue, but members concluded that it would have been surprising if none of the samples contained granulomas.

b) Was SSPE tissue (as used in Dr Minor's study) the most appropriate control in RT-PCR?

It was argued that SSPE might not be a suitable control tissue because it represented a very different type of tissue with an established persistent measles infection of many cells, whereas a granuloma from gut tissue might represent an inflammatory autoimmune response to a small subset of select cells with persistent measles infection. Therefore, an alternative control, based on the injection of a known number of measles virus genomes, was suggested as more appropriate. However, participants noted, from the data suggesting the presence of measles virus, that measles might have been present in amounts similar to those seen in SSPE controls and thus some members argued that SSPE was indeed the most suitable control. Certainly, the specificity of the RT-PCR technique as performed in Dr Minor's laboratory was such that if there had been measles virus RNA in Crohn's disease samples tested, it was at a level 50,000 fold less than in SSPE. This contradicted the results obtained by other, less sensitive, techniques which had detected virus, albeit in different tissue samples.

c) Was the measles N-gene the most appropriate RNA to look for?

Both the *in situ* hybridisation work and the RT-PCR were based on the measles virus N-gene and it was suggested that over a long period of time the measles virus genome might fragment. While experts in this field agreed that the genome would indeed be likely to fragment over the years, all experience suggested that persistent infection could not be maintained in the absence of the N-gene. The N-gene was essential for replication and replication was essential to maintain persistent infection. Furthermore, members observed that it would not be possible to see viral nucleocapsid structures by immunogold EM unless those structures had been transcribed and translated from a copy of the N-gene. It was therefore agreed that the N-gene was the logical one to look for and, if it were not found, then this would be persuasive evidence against the hypothesis of persistent infection.

d) If RNA was not present, were there other explanations for the positive results observed by other means?

Although viral RNA had been seen by *in situ* hybridisation, it was agreed by all that this was a relatively insensitive and error-prone technique that required substantiation. Positive results obtained by immunogold EM and monoclonal and polyclonal antibody histochemistry were potentially of greater significance. On the first of these, it was noted that the structures observed by immunogold EM were similar in the tissues from SSPE and Crohn's disease patients and, while there was room for disagreement on the interpretation of any EM results on morphology alone, these structures might well be paramyxovirus nucleocapsids. However, the RFHSM work had omitted a number of important controls recommended by the manufacturer of the immunogold reagents and it was agreed that it was essential that these be completed to confirm the results.

Several members reported incidents of cross-reactivity between very specific monoclonal antibodies and completely unrelated molecules; for example, a monoclonal antibody to the CD57 antigen on natural killer cells of the immune system also reacted with Purkinje cells in the brain. Thus, while monoclonal antibodies were

by definition specific, it was vital to carry out all controls to eliminate the possibility of cross-reactivity. Furthermore, participants reported that one of the monoclonal antibodies used by the RFHSM group had subsequently been used by a group in Japan⁶. The Japanese had isolated and sequenced a clone from an expression library that also gave a positive result with the monoclonal antibody, and found that the sequence was unrelated to measles virus.

Turning to polyclonal antibodies, it was agreed that these were difficult to use and members cited the literature on multiple sclerosis where similar problems had been encountered, resulting in a large number of studies that could not be reproduced by anyone. The polyclonal antibody that had given positive results in the hands of Mr Wakefield's group had subsequently been shown to be non-specific in a publication by Liu *et al.*, 1995⁶. It was noted that these sorts of continuing technical problems hampered work in the whole field of putative interactions at very low levels of gene expression within the context of a specific disease.

4.1.5 Having discussed these points all members of the group agreed that the first step in resolving the issues raised by the virological data was for all those working in the field to be using the same samples and experimental protocols, and preferably the same antibodies, and that all the conceivable specificity controls should have been undertaken. All those concerned expressed willingness to exchange samples, reagents and protocols.

4.1.6 However, members noted that while there were questions about site of biopsy, the most sensitive technique, RT-PCR, had so far proved negative on all samples. This raised the vital question that if the RNA were not present, where did the protein that interacted in the immunological studies come from? In that there were potential alternative explanations for the positive results given by the immunological studies, the current balance of virological evidence had to be against the persistence of measles virus in Crohn's disease.

4.1.7 In the absence of definitive evidence for the presence of the virus, [REDACTED]

[REDACTED] the Chairman asked members if there were other reasons why measles virus should be considered a serious candidate as a causal agent in Crohn's disease. The following points were raised:

- i) There were unequivocal data that measles was an immunosuppressive agent;
 - ii) There was no doubt that measles caused protein-losing enteropathy and the presence of Warthin-Finkeldy giant cells during acute measles infection had first been recorded in a small bowel biopsy specimen more than 20 years ago;
 - iii) Wild-type measles caused SSPE, especially if contracted at an early age.
- However, it was noted that since the programme of measles vaccination had begun, the incidence of SSPE had declined, suggesting a protective effect against SSPE with vaccine virus. All evidence to date showed that SSPE was due to wild-type virus. It was thus important to remember that vaccine virus was different from wild-type virus;
- iv) Wild type measles infection caused diarrhoea, particularly in susceptible individuals - again it was important to note that this was not true of vaccine virus;
 - v) Extremely malnourished, immunosuppressed individuals had shown incidences of persistent measles virus infection with giant cells and prolonged excretion of virus associated with reduced cell-mediated immune response to measles. However, even in these vulnerable individuals, the infection had persisted for months rather than the years that would be necessary for persistence to be involved in the majority of Crohn's disease cases.

- 4.1.8 The work examined thus far had raised questions about the involvement of measles virus in inflammatory bowel disease. The virological data currently available did not support a causal role for persistence of measles virus in Crohn's disease. Furthermore, if viral RNA were to be found, it was unclear how one might differentiate between viral reactivation due to immunosuppression induced by treatment for Crohn's disease and viral activity as a causal event. Finally, no evidence implicating vaccine virus had been presented and, while the situation was unclear and could merit further research, current published evidence suggested that vaccination should, if anything, be protective.
- 4.1.9 Members cited reports in the media and from personal contacts suggesting that uptake of MMR vaccination was being adversely affected by recent publicity surrounding the publication of the results from the RFHSM IBD group. They noted that the work reported raised potentially interesting scientific questions about the possible involvement of a virus in the aetiology of inflammatory bowel disease, however, they expressed concern that the serious risk to public health that would ensue if vaccination uptake rates were to fall should not be forgotten. Members agreed that it would be important to stress this in any output from the meeting.

4.2 Epidemiology

- 4.2.1 For this session, the focus of the meeting moved from predominantly virological to predominantly epidemiological. Participants heard a scene-setting talk on the use and the general problems of epidemiological data from [REDACTED]. This was followed by [REDACTED] presentation (Patterns of measles virus exposure and inflammatory bowel disease), including data from the two pre-circulated confidential papers from his group.
- 4.2.2 Members noted that the published epidemiological evidence suggesting a link between measles or measles vaccinations and inflammatory bowel disease (IBD) had been extensively reviewed in the literature and attempts had been made to reproduce the results. There had been a general consensus in the literature that this previous evidence had not provided a sound epidemiological basis for suspecting either measles, or measles vaccination exposure as a factor in IBD.
- 4.2.3 [REDACTED] presented two new studies: Firstly, using the 1970 British Cohort Study (BCS70), he sought to test the hypothesis that concurrent infection represented an atypical pattern of exposure to measles virus which would prove a risk factor for later inflammatory bowel disease. The analysis had identified concurrent infection with measles and mumps before age six, or IBD in a first degree relative, as risk factors for IBD. The second study, an analysis of a population dataset from Iceland, showed that concurrent measles and mumps epidemics had become more common and this led him to suggest that concurrent infection represented a significant risk factor for Crohn's disease.
- 4.2.4 The discussion then centred around the following points:
- a) Analysis of data from the 1970 British Cohort Study (BCS70). Self-selection bias was a potential problem in the BCS70 study. The original cohort had 13,099 individuals for whom contact addresses were known, and 5,717 of the responses obtained had been considered fully satisfactory; thus under 50% of the cohort for whom addresses were available had been included in this analysis. In response to questions on this issue, [REDACTED]

[REDACTED]

Another potential confounding factor considered was the general health of the two groups; [REDACTED]

[REDACTED] There had been an excess risk of otitis media in those who had subsequently developed Crohn's disease and an increased risk of eczema amongst those who later developed ulcerative colitis⁷. [REDACTED]

[REDACTED] Given the excess risk of eczema and otitis media, members suggested that it would have been helpful if these children had been investigated for abnormalities in cell-mediated immunity or antibody production to see if this group represented a population with immune deficiency who would therefore be more likely both to contract concurrent infections and possibly to develop IBD. Birth order had also been considered as a potential confounder, and no significant influence had been seen.

Whilst being a potentially useful prospective dataset, the numbers in the BCS70 study were very small. For example, the analysis showed a total of fifteen cases of Crohn's disease; three cases had experienced concurrent epidemics and the remaining 12 cases had not. It was suggested that if one case were changed, all statistical significance would be lost. Thus members considered that these results, although statistically significant, were not sufficiently robust to allow conclusions to be drawn from them.

Members noted that neither measles nor mumps infection had been confirmed by laboratory studies and it was known that without such confirmation measles could be easily misdiagnosed. Given that numbers were small, this also raised potential questions about the validity of the findings. Another potential problem with the BCS70 dataset was that information had been collected at age 10 and there was potential for recall bias, although there was no reason to suspect that recall bias would be different between the IBD and the control group.

Members questioned how vaccination had been treated within this analysis. Had it been regarded as an infection or were vaccinees excluded from the analysis? [REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

b) The analysis of the Icelandic dataset.

Members agreed that the data did not have sufficient power to provide evidence one way or the other for concurrent exposure to measles and mumps epidemics as a risk factor for IBD. The rate of exposure to epidemics and the rate of IBD were both variables that were shown to be increasing over time and a temporal association would therefore be expected and was observed. However, members noted that this should not be interpreted as evidence that concurrent infection was a risk factor for IBD. It was noted that the average age of infection for both mumps and measles was about 5 to 6 years and that this might suggest, contrary to the hypothesis, that concurrent infection might be a more normal pattern of exposure than single exposure. In addition, exposure to concurrent mumps and measles epidemics could be expected to be more frequent than exposure to concurrent epidemics of either mumps or measles

with an unrelated virus, chicken pox for example, that exhibited a different age range for infection. This was an important confounding factor that would need to be taken into consideration.

Members recognised that the Icelandic data had certain advantages, however they considered that, in this instance, Iceland was not an ideal population to examine as numbers were small. In these circumstances definition of exposure (for this study an exposure year had been defined as one in which more than a hundred cases had been registered) could affect the results. Members suggested that findings from this study would need to be confirmed by another study and that there were a number of cohorts around the world where similar data might be sought.

Members questioned how vaccination had been treated within the analysis.

Patients born before 1960 would not have been vaccinated and the association was still observed if the data were cut off at this point. Therefore, the study did not address vaccination or its effects.

Members agreed that BCS70 was a better source of data than the Icelandic dataset. As the authors had stated, the study gave no support to the hypothesis that either primary measles infection or monovalent measles vaccine were risk factors for IBD. From these data the possibility had emerged that exposure to concurrent measles and mumps infection was associated with an increased risk of IBD, at a low level. Thus, if this association were valid, it could be said that the vast majority of cases of IBD were not related to simultaneous infection.

4.2.5 Members noted that the virological evidence discussed earlier in the day had suggested that measles virus would be present in a high proportion of Crohn's cases. Furthermore, the earlier discussion had considered measles virus alone as the 'cause' of Crohn's disease in a large number of cases. In contrast, the epidemiological evidence suggested that the vast majority of IBD was not related to simultaneous infection and that neither primary measles infection nor monovalent measles vaccination was a specific risk factor for IBD. Thus, it was suggested that if measles were a factor at all, it would involve a complex interaction and thus would be an attributable risk in only a small number of cases.

4.2.6 Conclusions: After discussion, participants agreed that:

- i) For the reasons detailed in paragraph 4.2.4, the data from the Icelandic study did not provide useful evidence for or against the hypothesis that measles or measles vaccination played a role in IBD;
- ii) If further analysis of this type were warranted, the hypothesis should be tested with a different cohort;
- iii) The BCS70 data had the advantage that they had been collected prospectively; however, the number of IBD cases was very small.
- iv) The study suggested that there might be a small proportion of IBD cases related in some way to exposure to two paramyxovirus infections within the same year. However, the relationship would only be valid if it were assumed that there had been no selection bias, no recall bias, no prior undiagnosed health problems amongst the group identified, and that misdiagnosis of infection or gut condition had not occurred;
- v) The epidemiological data were not compatible with a simple causal model for measles virus or measles vaccination;
- vi) None of the data presented implicated measles vaccine virus. Published data suggested that vaccination should be protective.

4.2.7 In summary, there was currently no evidence relating measles vaccine or MMR to inflammatory bowel disease.

4.3 Autism

4.3.1 [REDACTED] opened the third session with a presentation and update on work that had been published in the Lancet⁸. The published work had described 12 children with ileal lymphoid hyperplasia, non-specific colitis and pervasive developmental disorder. Since publication, the RFHSM IBD Group had examined a further 39 children who had been referred to them. [REDACTED]

[REDACTED] In addition, unpublished data from two groups in the USA were briefly presented. These case series of measles serology, in 16 and 38 autistic children respectively, had led the authors to suggest an association between MMR and autism. Subsequently, [REDACTED] summarised some key empirical findings in the field of autism. [REDACTED]

4.3.2 The following questions were raised:

a) Did the developmental disorder in the children examined by the RFHSM Group constitute a new syndrome?

[REDACTED] explained that he had examined all the children himself. [REDACTED]

[REDACTED] Two cases had been classified as post-viral encephalitis and these could not be confirmed without referral to a neurologist. [REDACTED]

b) What was the relationship of the children's symptoms to Crohn's disease? Members said that it was common to find lymphoid ileal hyperplasia in children presenting with ill-defined gastrointestinal problems and that this normally resolved over time without developing into more serious disease. It was suggested that the inflammation seen in some of the children was slight and would have been passed as normal in adults. [REDACTED]

[REDACTED] Other experts noted that there were also signs of focal inflammation (more usually consistent with a pattern of Crohn's disease than microscopic colitis), and it was noted that aphthoid ulcers had been seen in two cases.

Dr Fombonne described two studies he had recently undertaken, which would soon be published in the Lancet⁹. The first was of the Maudsley Hospital data set with about 900 autistic children (200 born in or after 1987 and thus likely to have received MMR) where the World Health Organization's International Classification of Diseases (ICD), 9th revision (ICD-9) disorders (including Crohn's disease and ulcerative colitis) had been recorded. Two cases of Crohn's or ulcerative colitis had been recorded in the non-autistic psychiatric controls, but no cases of inflammatory bowel disease in any of the autistic children. Similarly, in a large epidemiological sample of 6000 children of

whom 174 had autism there had been two cases of inflammatory bowel disease in the non-autistic children, but none in children with autism. [REDACTED]

[REDACTED] Finally, Dr Fombonne noted that he had recently conducted a review¹⁰ of 23 epidemiological surveys of autism, with a total of over 1500 subjects, and that there had been no report of an association with measles or mumps virus infections or with Crohn's disease or ulcerative colitis in these surveys. [REDACTED]

Members asked if the children had diarrhoea or if they were constipated, as it was not unusual for parents to report diarrhoea and for children to be incontinent, when the real problem was chronic constipation with overflow. [REDACTED]

[REDACTED] Dr Murch added that, as published in the Lancet¹¹, many parents had noticed a behavioural improvement in the children following bowel clearance in preparation for colonoscopy and that this improvement could be maintained if constipation was prevented. Further cognitive improvement could be attained in response to combined treatment with aminosalicylates and anti-constipation medication.

c) How were the patients selected?

Members were interested in how the children had come to be referred to the RFHMS team, as this had a bearing on the issue of bias in the generation of the case series. [REDACTED]

d) What were the children's immunological status?

In response to questions [REDACTED]

In the recent investigation, they had found that total IgG levels had not been raised, IgG against mumps, rubella and cytomegalovirus had not been raised, but there had been a doubling of mean IgG level against measles virus. Cerebrospinal-fluid profiles had been normal and negative for all viral antibodies. C-reactive protein (CRP) levels and erythrocyte sedimentation rate (ESR) were, with one exception, normal suggesting that the raised IgG titre against measles was not simply an aspect of the acute phase response. Members expressed surprise at seeing normal levels of CRP in very sick children, but it was explained that while CRP level was raised in Crohn's disease, it was possible to be very ill with ulcerative colitis in the absence of a raised CRP. Turning to the immunocytochemistry, which had suggested the presence of measles virus in biopsy samples from five of the autistic children and in one control sample from a child with ulcerative colitis, members noted that a completely different antibody had been used to that used in the studies presented in the morning session. [REDACTED]

The published work had suggested that some of the children had a transient IgA deficiency. This was questioned by members as only one of the patients was technically IgA deficient by the protein reference range for the UK. However, it was noted that those children within the normal reference range were all in the lower quartile for IgA response; this was highly predictive for allergy. 15 of the 30 children had low or low normal IgA. Members commented that there were problems in relating blood levels of IgA to mucosal IgA production, but that it would be useful to examine autistic children who had not been exposed to MMR to see if mild immune abnormalities were generally present in autism.

e) Was vitamin B12 involved?

Members noted that urinary methylmalonic acid (MMA) excretion was raised in all eight children tested and that this could be a marker of vitamin B12 deficiency. However, the significance of this finding was unclear. [REDACTED]

f) Was there evidence for association of these patients' symptoms with MMR?

Members concluded that there was a potentially interesting association between autism or pervasive development disorder and bowel abnormalities, the significance of which was unclear. However, it required a very large leap to associate this with MMR vaccination. Participants noted that parents had reported an association with MMR, but recalled that a previous investigation examining brain tumours in men had recorded wives' belief that the origins of these could be traced to head injuries. Thus there was a basic human tendency to ascribe blame to a specific incident or event. The onset or first measurable symptoms of autism were often observed in the second year of life and this was coincidentally the timing of MMR vaccination. It was not unreasonable that parents should associate the two events; however, there was currently no evidence that the two events were related in any causal way. It was thought surprising that other countries that had implemented MMR vaccination programmes earlier than the UK (for instance in North America and Scandinavia) had not reported a similar association. This issue could be pursued further on an international basis.

5. Concluding remarks

The Chairman thanked the members of the RFHSM IBD Group for making their data available prior to publication, and for attending the meeting and permitting their unpublished work to be subjected to peer review. He also thanked [REDACTED] who had likewise made pre-publication data available.

6. Conclusions and Recommendations

6.1 Virology

- Mr Wakefield's initial work on the vasculature of the intestine was considered elegant and impressive;
- It was noted that Crohn's disease or ulcerative colitis affected 100,000 or more people in Britain and were the primary cause of death in over 300 people a year (with 25 of these being under 50);
- Sample site within bowel tissue was agreed to be important. Putative measles virus had been identified in granulomas and these would be the preferred site for seeking evidence of persistent measles virus infection. The granulomas were predominantly found deep in the mucosa;
- Molecular data implicating the presence of paramyxovirus nucleocapsid protein using immunogold electron microscopy and monoclonal and polyclonal antibody histochemistry could not be confirmed by the more sensitive technique of RT-PCR;
- Experiments with immunogold electron microscopy had omitted important controls; these ought to be done before drawing conclusions from this work;
- The histochemistry work had potential to give false positives; there were published studies showing cross-reactivity between monoclonal antibodies and molecules unrelated to those to which the antibody had been raised. Without confirmatory studies this work could not be said to provide evidence for measles infection in the gut wall;
- The failure of RT-PCR to demonstrate the presence of viral genome was unlikely to be explained by fragmentation of the viral genome; in particular the measles N-gene which was essential for viral replication would need to be intact for persistent infection;
- The negative results from RT-PCR were incompatible with the levels of nucleocapsid protein predicted from the immunogold and immunocytochemistry studies;
- Those working in the field should be using the same samples and experimental protocols, and preferably the same antibodies, to see if the results could be replicated;
- All conceivable specificity controls should be undertaken;
- The ability of measles virus to produce immunosuppression meant that it would not need to remain in tissues to produce cellular aberrations; however, this would relegate its significance to equivalence with a large number of other viruses that might be hypothesised to play a role in the induction of an auto-immune response;
- It had been reported that, while wild measles virus caused subacute sclerosing panencephalitis (SSPE), vaccination with attenuated measles virus appeared to protect against SSPE and while wild virus causes diarrhoea the vaccine virus did not. Therefore, vaccine virus appeared to be less biologically plausible as a causal agent of disease than wild virus;
- Data on the prevalence of inflammatory bowel disease were poor; those on time trends were particularly unreliable;
- Crohn's disease was a serious and debilitating recurrent disease and any further research that might shed light on its origins, incidence or potential treatments would be welcomed;

- The balance of current virological data did not support the involvement of persistent measles virus infection in the aetiology of Crohn's disease and ulcerative colitis

6.2

Epidemiology


- Previously published epidemiological evidence for the involvement of measles or measles vaccination as a cause of Crohn's disease or ulcerative colitis had been extensively reviewed in the literature with the general consensus that it did not provide a sound epidemiological basis for any such association;
- The unpublished papers available to the participants did not address measles vaccination;
- The Icelandic study was examining two rates that were known to increase with age – exposure to viral epidemics and incidence of inflammatory bowel disease – and it was thus not surprising that a temporal association between the two could be seen. This association did not provide any evidence of a causal link;
- Both measles and mumps viruses infected children at about age five. Thus an apparent epidemiological association between these viruses was not surprising. Furthermore, this evidence might suggest that two paramyxovirus infections in the same year was a usual rather than atypical pattern of exposure;
- The Icelandic dataset was very small (and thus definition of exposure rate could significantly affect results), retrospective and, therefore, not an ideal dataset to examine the hypothesis put forward;
- The analysis of the British Cohort Study 1970 suffered from attrition in numbers and risk of bias in terms of the response rate;
- The relevant information in the BCS70 study had been obtained at 10 years and the diagnosis of measles or mumps had not been confirmed in the laboratory, thus raising the possibility of significant recall bias;
- **It was possible that those in BCS70 with inflammatory bowel disease represented a group with a prior health problem (for example a host immune deficiency) and it would be desirable to investigate this possibility;**
- The BCS70 study suggested that only a very small proportion of inflammatory bowel disease (IBD) could be related to measles or mumps infection; thus if laboratory studies demonstrate the presence of measles or mumps virus in the gut wall of people with IBD, it was unlikely that there was a causal connection in the majority of IBD cases.
- **There would be considerable advantage in supplying independent groups with the raw epidemiological data for further analysis in order to attempt to resolve this issue;**

6.3 Autism

- An association between autism or autism spectrum disorder and bowel abnormalities had been described; its significance for pathogenesis and treatment was not clear;
- There was currently no evidence to indicate a causal link between MMR vaccination and autism; the frequent first appearance of autistic symptoms in the second year of life at a time when children would receive MMR vaccination suggested that a temporal association between the two would be expected;
- The absence of similar observations from countries with longer experience of MMR vaccination programmes was surprising;
- **Further research, probably on an international basis in order to include a sufficiently large number of patients, would be needed to settle the question of any possible association between autism and MMR vaccination.**

7. Follow-up Action

Participants were informed that the MRC would hold a press conference on Tuesday 24 March 1998 to report the main conclusions from the meeting: a) that the balance of available virological and epidemiological evidence was against the persistence of measles in Crohn's disease; b) that there was no correlation between measles or mumps infection alone and Crohn's disease or ulcerative colitis; and c) that there was no current evidence linking bowel disease or autism with MMR vaccine and there was thus no reason, arising from the work considered, for a change in the current MMR vaccination policy¹. The outcome of the meeting would be reported orally to Council on Thursday 26 March 1998. In view of the degree of public concern and the importance of the issue to public health, Council would be asked to consider setting up an Expert Subgroup to steer and monitor research in this area.



¹ Wakefield, Sim, Akbar, Pounder and Dhillon, 1997, *J Med Virol* 51: 90-100

² Wakefield, Pittilo, Sim *et al.*, 1993, *J Med Virol* 39: 345-353

³ Lewin, Dhillon, Sim, Pounder and Wakefield, 1995, *Gut* 36: 564-569

⁴ Afzal, Armitage, Begley *et al.*, 1998, *Lancet* 351: 646-647

⁵ Iizuka and Masamune, 1997, *Lancet* 350: 1775

⁶ Liu, Van-Kruiningen, West *et al.*, 1995, *Gastroenterology* 108:1396-1404

⁷ Montgomery, Morris, Thompson *et al.*, 1997, *Gut* 40: (suppl 1):A22

⁸ Wakefield, Murch, Anthony *et al.*, 1998, *Lancet* 351: 637-641

⁹ Fombonne, 1998, *Lancet* 351: 955

¹⁰ Fombonne, 1998, in: *Autism and pervasive developmental disorders: Monographs in Child Psychiatry no2*. Volkmar (Ed) Cambridge University Press, 32-62

¹¹ Murch, Thompson and Walker-Smith, 1998, *Lancet* 351: 908