LETTERS TO THE EDITOR

Immunohistochemical analysis of measles related antigen in IBD

Editor,—What is one to make of the immunohistochemical study of Iizuka et al (Gut 2000;46:163–169)1? Before addressing the possible scientific implications of their findings, let us first clarify a few points with respect to our own research. Iizuka et al state that “the measles hypothesis is based on the theory that measles antibody recognises measles virus itself and the measles virus antigen is uniquely present in Crohn’s disease.” Is this the authors’ hypothesis? It is certainly not ours. That the measles antibody used in our studies (not that used by the authors in the present study) recognises the measles virus N-protein rather than the N-protein in tissues infected with other viruses, and tissue studies incorporating in situ hybridisation (ISH) and combined ISH–reverse transcriptase-polymerase chain reaction (RT-PCR)–ISH for measles virus N-gene in serial tissue sections of infected human tissues suggest that the antibody, when used appropriately, is specific for measles virus.

Our own hypothesis relates not to the unique presence of measles in Crohn’s disease but rather to the specific localisation of the virus to the hallmark lesions of this disease—granulomas and secondary lymphoid reactions—that are themselves a likely response to persistent and potentially causative antigen(s). While the focus of our studies has been the exclusive presence of viral antigen in these foci in Crohn’s disease, Iizuka et al have assiduously avoided these pathological structures altogether. Instead, they have identified a pattern of non-specific staining in inflamed mucosal biopsies that has never before been observed in our own laboratory using the measles virus monoclonal antibody. We have particular anxieties about their statement regarding the cellular localisation of the positive signal. The authors state that, “As Wakefield and colleagues reported, positive cells comprised macrophages, lymphocytes, vascular endothelial cells, fibroblasts, and neutrophils.” This is clearly misleading. We described positive staining in “endothelium, macrophage-like cells, and occasional lymphocytes in foci of granulomatous inflammation.”1 It was not seen elsewhere in tissue sections, either in the presence or absence of inflammation. Iizuka et al have abbreviated their statement by saying that any reference to granulomatous inflammation and substituting their own findings of signal in fibroblasts and neutrophils. Moreover, the signal that they identified was predominantly cytoplasmic. The signal localisation that we reported was exclusively nuclear, except in rare syncytial giant cells where it was seen additionally in cytoplasm (fig 1). We are also concerned by the implication that we would consider a weakly positive signal to be negative thus potentially biasing the results. Signal strength in Iizuka’s studies was evidently on a continuum. In contrast, we found that it could be readily dichotomised into present (discrete and punctate; fig 1) or absent.

A crucial experiment that has been omitted from their study is application of their antibody to pristine cell lines either infected or stimulated with something other than measles virus. The indication from the observations in gut tissues is that expression of the epitope recognised by their antibody is a function of inflammation. Is this elicited by specific or non-specific processes?

It is worth emphasising the differences between the study of Iizuka et al and our own. They used a different antibody on tissues processed in an entirely different way (peroxidase/lysine/4% paraformaldehyde fixed, cryostat sections versus 10% neutral buffered formalin fixed, paraffin sections). They identified markedly different signal characteristics, both in the lineage of positive cells and the subcellular distribution (cytoplasmic versus nuclear). Most importantly, they excluded from analysis the specific foci that were identified as exclusively positive in our studies. Despite its limitations, this work may contribute to our understanding of why measles has been linked with inflammatory bowel disease (IBD). It is our hypothesis that atypical exposure to the measles virus in early life increases the risk of subsequent IBD among genetically susceptible individuals. Parallels can be drawn from the rare neurological disease subacute sclerosing panencephalitis (SSPE), a delayed sequelae to persistent measles virus infection with a long natural history where the chronic disease manifests several years after the acute measles infection. In SSPE, several atypical characteristics of acute measles infection and atypical infection may alter the characteristics of this response, increasing the risk of inappropriate immune reactivity. Measles (or at least components of measles virus) may persist at very low copy number concentrations, making it difficult to detect using conventional RT-PCR, even with hybrid capture.2 Indeed, it has been argued that such retention of viral material may be important in maintaining functional immunity.3 Therefore, two important questions are: (1) What patterns of acute infection increase the risk of inappropriate immune programing and subsequent IBD? (2) What are the likely mechanisms that may result in IBD? The answer to the first question is beginning to be answered by epidemiological studies that identify complex patterns of exposure to measles virus. This includes a close temporal relationship of measles with another para- myxovirus infection taking place during the first five or six years of life.

It is possible that the work of Iizuka et al may provide a clue to a potential mechanism linking atypical measles infection with IBD. This group reported the presence in inflamed tissue of “measles related antigen” and speculated that this is a human protein as yet unidentified. An atypical measles infection and viral persistence could increase the risk of inappropriate immune reactivity to “measles related antigen”; therefore, molecular mimicry could be one mechanism to explain chronic inflammation in IBD patients. The presence of this protein in other inflammatory disorders of the bowel may indicate that it is produced as part of the inflammatory cascade. This may shed some light on why the onset of IBD appears to be triggered by transient enteric infections. If gut inflammation results in expression of “measles related antigen” in an individual who has been primed by an earlier atypical measles infection, this could trigger an ongoing immune response. Clearly this is highly speculative but if this group has identified a human protein that shares a high degree of similarity with measles virus N-protein, it may be a useful tool to investigate the relationship between atypical exposure to measles virus and IBD.

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Reply

Editor,—We thank Dr Wakefield and colleagues for their interest in our work and we acknowledge that we had initially pursued the same hypothesis that they proposed. However, we have come to a different conclusion through a series of studies1–3 revealing that the antiserales monoclonal antibody (Seralab, Crawley Down, Sussex, UK) that Wakefield et al used in their study1 and in fig 1 above recognises a host antigen as well as measles virus. Furthermore, we proved this cross reaction by identifying a clone with this antibody in a lambda-phaege expression library constructed from a typical Crohn’s disease patient, by subsequently obtaining a monoclonal antibody to this clone (which we found to be unrelated to measles virus), and finally, by demonstrating that this monoclonal antibody (9F12) reacted with both measles virus and the antigen used for immunisation (the host protein).

Further to our observation that no part of the measles virus genome was detected by reverse transcription-polymerase chain

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reaction, our reasons for concluding that what was stained with 4F12 (and the monoclonal antibody that we purchased from Seralab) was not the measles antigens but the host protein unrelated to the measles viruses were given in detail in our paper (Gut 2000;46:163–169) and we see no need to reiterate them here. One final word with regard to the comment of Wakefield et al on our description of molecular mimicry as a possible mechanism for pathogenesis, let us be clear that our report should not be interpreted as support for the hypothesis of measles viruses or measles vaccinon triggering Crohn’s disease.

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Survivin gene expression and prognosis in recurrent colorectal cancer

EDITORS—Sarela and colleagues (Gut 2000;46:45–50) report on the association of Survivin gene expression and prognosis in recurrent colorectal cancer. The methods described for detecting Survivin mRNA relied on reverse transcription-polymerase chain reaction (RT-PCR), an exquisitely sensitive technique that has not previously been validated for this gene. We wish to point out three areas of technical difficulty in the methodology.

(A) The fidelity of mRNA extraction and RT was tested using oligonucleotide primers for glyceraldehyde-3-phosphate dehydrogenase (GAPDH), a “housekeeping” gene. However, this may give rise to false positives by amplification of pseudogenes from contaminating genomic DNA. The β-actin primers (as described by Raff and colleagues) do not amplify genomic DNA and therefore provide absolute evidence that RT has been successful. Alternatively, this problem could be corrected either by DNase digestion of RNA before RT or by having negative RT controls for each sample.

(B) The process of RT using an oligo dT nucleotide as the RT primer results in the creation of cDNA templates for all mRNAs in the sample. This may be a problem if the gene for effector cell protease receptor 1 (EPR-1) is expressed. This gene codes for a cellular receptor of blood clotting factor Xa. The DNA sequence for this gene is highly homologous to that of Survivin and differs by only five nucleotide changes and six nucleotide insertions. The reverse primer described recognises the EPR-1 sequences (as ascertained by searching of the basic local alignment search tool of the National Cell Genebank (GeneBank Accession No. L26425). Human effector cell protease receptor-1 (EPR-1) mRNA, partial CDs). Implicit in the description is that this sequence is incomplete. Given the close similarity between the probable sequences of the two genes it is not impossible that this homology continues and could provide a recognition site for the forward primer in EPR-1. This problem has been alluded to by Mahotka and colleagues who used a sequence specific RT primer to eliminate it but was not taken into account elsewhere in work on survival in small cell lung cancers. This may explain the detection of “Survivin” mRNA in normal colorectal mucosa.

(C) The PCR primers as published are in the first and fourth exons. The amplified sequence would be expected to include the published splice variants caused by deletion of the third exon or insertion of the 2B exon, as described by Mahotka and colleagues. This would result in multiple bands detected on agarose gel. We would be interested to know whether these points were taken into account.

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Figure 1 β-Actin and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) reverse transcription-polymerase chain reaction (RT-PCR) on two colorectal cell lines, demonstrating amplification of the GaPDH pseudogene in the RT negative control.