Granulomatous vasculitis and persistent measles virus infection in Crohn's disease

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AJ WAKEFIELD. Granulomatous vasculitis and persistent measles virus infection in Crohn's disease. Can J Gastroenterol 1994;8(2):70-74. Based upon the recent observation of vasculitis in Crohn's disease, a process that is more widespread than was recognized previously, the author investigated the possibility that this mechanism may provide an explanation for some of the clinical and histological idiosyncracies of this condition. In addition, it was suggested that the mesenteric microvascular endothelium may be a source of the persistent antigen that is responsible for ongoing cellular immunity in Crohn's disease. This review discusses some of the studies designed to test these hypotheses, and discusses recent evidence for the presence of a measles-like virus in the endothelium in inflammatory foci, which may be relevant in the etiology of Crohn's disease.

Key Words: *Crohn's disease, Granulomatous vasculitis, Measles virus*

Vasculite granulomateuse et infection persistante au virus de la rougeole dans la maladie de Crohn

RÉSUMÉ : Sur la base d'un cas récent de vasculite dans la maladie de Crohn, processus beaucoup plus répandu qu'on ne l'a d'abord reconnu, l'auteur a étudié la possibilité que ce mécanisme puisse expliquer quelques-unes des idiosyncrasies cliniques et histologiques propres à cette maladie. De plus, il a été suggéré que l'endothélium microvasculaire mésentérique puisse être une source d'antigènes persistants, responsables de l'immunité cellulaire continue dans la maladie de Crohn. Cet article passe en revue certaines des études conçues pour vérifier ces hypothèses et présente les résultats récents attestant de la présence d'un virus semblable à celui de la rougeole dans l'endothélium des foyers inflammatoires qui pourraient jouer un rôle dans l'étiologie de la maladie de Crohn.

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THE PREVAILING PERCEPTION OF Crohn's disease (CD) as a primary mucosal pathology, either as an epithelial abnormality or an aberrant response of the mucosal immune system, fails to explain many of the idiosyncracies of this condition. In the absence of a plausible pathogenic basis for skip lesions, transmural inflammation, panenteric distribution of disease and anastomotic recurrence, and the deleterious effects of smoking, many have avoided these issues in favour of more elaborate hypotheses.

It is reasonable to assume that CD is characterized by a persistent cell-mediated immune response to an, as yet, unidentified antigen or antigens (1). The capacity of cells both within the lamina propria and stimulated epithelial cells to present class II antigens (2) has, in the minds of many, reasserted the central role of the mucosa in the development of CD. The abundance of luminal antigen seems to suggest that we need go no further in identifying the source of the stimulus for persisting cellular immunity; all that remains is to isolate and characterize the antigens responsible.

Before taking this final step into the darkness of the intestinal lumen, we

should reflect upon CD's phenotypic idiosyncracies when considering the underlying pathogenic mechanism – without a mechanism and, by extension, an appropriate source of persistent antigen, we are not in a position to seek the nature and origin of that antigen.

Clues as to the tissue localization of this putative antigen are present in CD in the form of the granuloma. This reaction localizes to persistent antigen, and in those diseases in which an etiological agent has been identified (including tuberculosis, paratuberculosis and schistosomiasis), the granuloma localizes to the causative antigen. Therefore the granuloma provides a logical starting point when considering both the mechanism and the cause of CD.

TISSUE RELATIONSHIPS OF THE GRANULOMA IN CD

The granuloma is an early histological feature in the evolution of intestinal inflammation in CD; these hallmark lesions occur principally in a submucosal location and can be present prior to mucosal inflammation or ulceration (3-5), an observation that argues strongly for the priority of this lesion in CD evolution. Although described previously, granulomatous vasculitis in CD has been reported as an occasional feature of no pathological significance (6,7).

For a variety of reasons, the relationship of the vasculature to foci of inflammation, such as granulomas, may be obscured in immersion-fixed, hematoxylin and eosin stained tissue sections. First, vessels collapse in the absence of an intraluminal hydrostatic pressure. Furthermore, advanced granulomatous vasculitis could be expected to destroy the vessel and to occlude the lumen of the affected vessels, obscuring the apparent vascular origin of the lesion. This is suggested by the observation that extensive vascular disruption in 'primary' granulomatous vasculitides can make it difficult to identify the vessel wherein the granuloma originated (8).

The author has recently examined 485 granulomas in 15 specimens of resected CD and shown that at least 85% of granulomas are directly involved in vascular injury (5). These studies were facilitated greatly by the combination of perfusion-fixation and immunostaining for vascular structures. Granulomas were seen to arise from within the walls of blood vessels and, in many cases, from within the vessel lumen, in intimate contact with the vascular endothelium (Figure 1), suggesting the presence of a previously unrecognized macrophage-endothelial cell interaction in CD. In addition, computerized three-dimensional reconstruction of serial sections of CD has confirmed that granulomas follow the course of the affected blood vessels, rather than coincidentally being adjacent to these vessels in a single plane of section (5). Granulomatous vasculitis – and venulitis in particular – is an integral part of the spectrum of vascular inflammation that the author believes is central to the pathogenesis of CD. Similar observations in sarcoidosis suggest that this process may be more common than was thought previously (9). The consequence of this vascular lesion in CD is likely to be ischemia of the dependent tissues. In terms of a mechanism, therefore, can this process explain some of the idiosyncracies of CD?

SKIP LESIONS AND MICROVASCULAR INJURY

Discontinuous inflammation and associated ulceration are characteristic features of CD; inflamed, ulcerated mucosa may occur immediately adjacent to mucosa of normal histological appearances (10,11). In an attempt to explain this phenomenon by extrapolation of the vascular hypothesis, the author's group selectively embolized the submucosal microvascular plexus of ferret intestine (12). Microspheres (27 to 90 μm in diameter) were injected into the arterial arcade of a defined loop of intestine, causing focal inflammation and ulceration 24 to 72 h later. This model did not set out to reproduce the chronic damage of CD but it did reproduce the juxtaposition of inflamed and ulcerated mucosa alongside histologically normal mucosa. In CD, repeated episodes of focal submucosal vascular occlusion could cause the characteristic patchy mucosal damage.

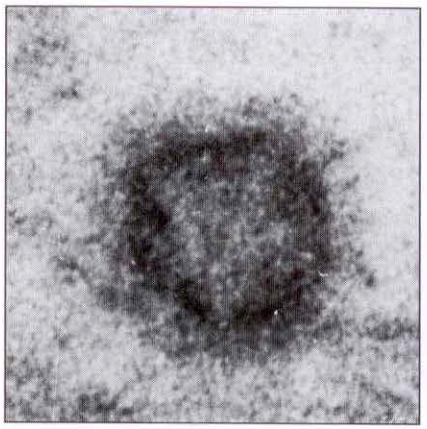

Figure 1) *Crohn's disease: intraluminal granuloma in a perfusion-fixed submucosal vessel. The granuloma adheres to the endothelium and there is no generalized inflammation associated with this lesion. Hematoxylin and eosin* ×400

ANASTOMOTIC RECURRENCE OF CD

The author's group believes that occult residual granulomatous vasculitis is the mechanism for anastomotic recurrence. The capacity of the bowel to heal after a focal, intramural vascular injury depends largely upon the integrity of the collateral plexus formed by the submucosal microvasculature (12). Thus, for any point in the bowel wall, submucosal collateral vessels from either side may preserve the circulation to that point, but division of the bowel before anastomosis will halve this collateral supply. Tissue perfusion at an anastomosis will be compromised further by the local pressure of a suture line. This devascularization is of little consequence when normal intestine is anastomosed. If, however, there is a vasculitis in the macroscopically normal bowel used to make the anastomosis, the reduced capacity of the already compromised collateral circulation may be sufficient to induce focal tissue ischemia and anastomotic recurrence. Similarly, subacute obstruction of the bowel, by increasing intraliminal pressure, will cause a profound decrease in perfusion of the mucosal vasculature (13).

Osborne et al (14) recently adapted the model described by Hudson (12) to study anastomotic healing in the presence of a compromised submucosal collateral circulation. Following emboliza-

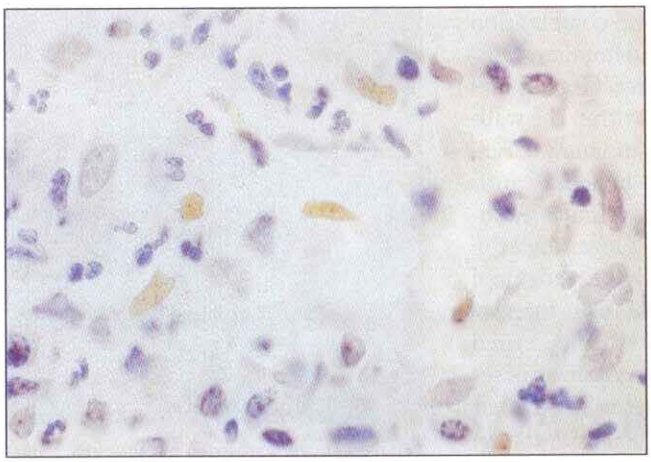

Figure 2) *Crohn's disease. Focus of granulomatous mucosal inflammation. Nuclear immunostaining with measles virus polyclonal antibody (x1000)*

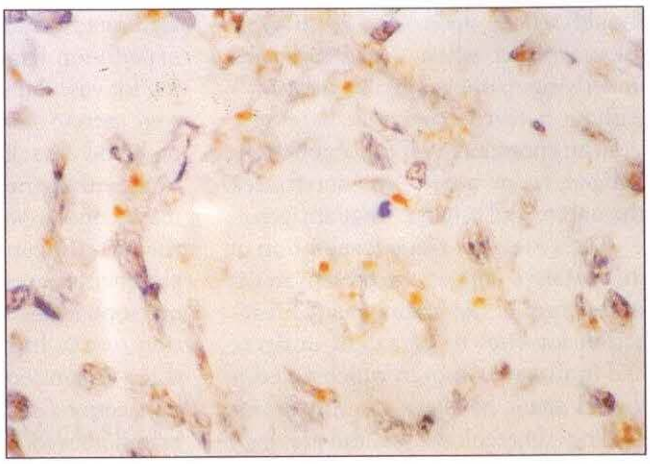

Figure 3) *Crohn's disease. Submucosal lymphoid aggregate. In situ hybridization for measles virus genomic RNA. Characteristic nucleolar pattern of hybridization (39) is seen (x400)*

tion of the intestine, which produced a self-limiting mucosal injury, an anastomosis was created through the embolized loop of intestine 72 h following the initial procedure. At two weeks there was macroscopic and microscopic inflammation in the embolized limb of the anastomosis with a combination of transmural inflammation, mucosal ulceration and occasional granuloma formation; these features were not seen in controls who received either anastomosis or microspheres alone, and the results suggest that the superimposition of two self-limiting ischemic insults can produce chronic inflammation at an anastomosis.

COLONIC CD: DIVERSION OF THE FECAL STREAM AND RESTING THE GUT

Patients with severe colonic CD often enjoy a remission following a diverting ileostomy, but relapse of the disease usually follows restoration of the fecal stream (15-17). This sequence has prompted the hypothesis that some constituent of the fecal stream is responsible for either initiating or provoking CD (17,18).

The bloodflow of the intestinal mucosa increases greatly in response to the metabolic demand placed on the mucosa by digestion and absorption (19). In CD, stenotic vascular lesions in the bowel wall would place a rate-limiting influence upon the hyperemic response to mucosal activity. This situation is analogous to intermittent claudication or exercise-induced angina – ischemia or infarction will occur when the demand for blood exceeds supply.

Fecal diversion rests the excluded bowel, helping reduce mucosal bloodflow and oxygen demand. Provided with a respite, the intestinal ischemic damage eases and healing dominates. This phenomenon may also help to explain, at least in part, the ameliorating effect of a proximal diverting ileostomy upon anastomotic recurrence (20). However, reinstitution of the fecal stream is likely to induce relapse due to further ischemic damage. This may also explain the transient clinical benefit of an elemental or polymeric diet (21).

MULTIFOCAL GASTROINTESTINAL INFARCTION AND APHTHOID ULCERATION

It has been argued that aphthoid ulceration in CD is not a feature of mucosal ischemia (22). However, in cases of Wegener's granulomatosis or Behçet's disease, both of which are idiopathic necrotizing vasculitides involving the intestine, aphthoid ulceration of the mucosa (including the oral mucosa) is a prominent feature (23,24). It is likely that these diseases, which produce occlusive vascular inflammation in affected segments of intestine, produce focal ulceration by causing mucosal ischemia; it is interesting that anastomotic recurrence is also a feature of Behçet's disease (24). The authors group has proposed that ulceration in CD represents focal infarction of the mucosa that may progress, depending on the location and severity of the underlying vascular disease, to penetrating ulceration and fistulation (25). To accept that this damage is due to 'multifocal gastrointestinal infarction' needs a reappraisal of the concepts of infarction (22). 'Classical' intestinal infarction involves fibrosis, mucosal atrophy and hemosiderin deposition – all features of large mesenteric vessel occlusion. But this definition precludes a spectrum of tissue injury that may result from chronic damage at the level of the microvasculature of the bowel wall. Infarction of the mucosa will not be immediately apparent, not only because of the bowel's ability to shed dead tissue into the lumen, but also because of its remarkable capacity for repair and regeneration without sequelae. Mucosal ulceration may be seen in a variety of other conditions in which ischemia is implicated – for example, pseudomembranous colitis, pigbel and necrotizing enterocolitis (26-28). These appearances are not those of classical infarction but, if infarction is defined as 'tissue necrosis due to interruption of its blood supply', these conditions qualify and so, the author suggests, does CD.

By examining macroscopically normal, noninflamed areas of bowel in patients with CD, Sankey and colleagues

(29) identified subtle mucosal changes that would normally be obscured in areas of active inflammation. Using a combination of perfusion-fixation at mean arterial pressure and immunohistochemical techniques, she was able to examine small mucosal capillaries in detail. Focal, early mucosal changes appear to be associated with damage to small capillaries. This was manifest as disruption of the capillary basement membranes or the vascular endothelium with hemorrhage and trails of fibrinogen in the surrounding lamina propria. These were very much more common in patients with CD (50%) than with ulcerative colitis (15%) or in controls (15%). This vascular damage preceded both the focal accumulation of inflammatory cells and necrosis of the overlying epithelium and cannot, therefore, be a consequence of inflammation.

The mucosal changes described by Sankey et al occurred at all levels of the mucosa, both in areas close to, and distant from, Peyer's patches. The relation between these mucosal changes and the classical 'aphthoid' ulcers occurring over Peyer's patches is unclear. The centres of lymphoid follicles are supplied by end arterioles. It has been suggested that as a consequence, they are particularly prone to ischemia (30); thus, Peyer's patches may provide a favoured site for this kind of ulceration. Alternatively, many 'aphthoid' ulcers overlie lymphoid aggregates rather than true follicles; it is possible that progression and extension of the early features that were described would be compatible with the ultimate development of an 'aphthoid' ulcer.

SYSTEMIC THROMBOGENESIS, SMOKING AND CD

Independent risk factors for systemic thrombogenesis and an increased incidence of thrombotic disease include smoking, elevated plasma levels of lipoprotein(a) (31) and factor VIIc (32). The author's group recently showed that only six of 49 patients with CD did not have one or more of these additional risk factors for thrombosis (unpublished data). It is tempting to postulate that a primary mesenteric microvascular injury and a systemic thrombotic tendency combine to precipitate clinically active CD.

MESENTERIC MICROVASCULAR ENDOTHELIUM AND MEASLES VIRUS

The observation of granulomatous vasculitis in CD, and the possibility that many of the idiosyncracies of this condition might be explained by a compromised intramural circulation, focused attention upon the mesenteric microvasculature. Vascular endothelium plays a central role in both immunity and hemostasis (33); furthermore, it can harbour both productive and persistent viral infections (34,35). There was sufficient epidemiological evidence of an infectious etiology for CD to progress this hypothesis in search of a source for persistent antigen. Since CD is neither vertically nor horizontally transmissible, it is likely that the putative infection is nonproductive and therefore cell-associated. If so, is it possible that the microvascular endothelium of the gut is the source of this infection and, thus, the persistent antigenic stimulus that drives cellular immunity in CD?

It was hypothesized that measles virus could be the etiological agent in CD. Measles virus-infected lymphocytes 'home' to the lymphoid tissues of the gut during the primary viremia; subsequently, the virus infects the microvascular endothelium of these tissues and induces vasculitis – secondary transmural lymphoid follicles develop in the gut wall and there is necrosis of the overlying epithelium (36), all of which are characteristic features of CD (3,4,11). Thus, measles can produce Koplik's spots throughout the gut that are similar to the aphthoid ulcers characteristic of CD (36-38). Giant cell syncytia at the centre of secondary lymphoid follicles in the intestine are a prominent feature of measles virus infection (36). Hadfield (3) reported similar changes in his classical histological description of CD. Acute endothelial cell infection by measles virus occurs principally in the submucosal microvasculature throughout the entire length of the gastrointestinal tract (36), the lesional topography of CD (3,5).

Recent detailed analysis of 24 cases of intestine affected by CD using a combination ultrastructure, immunohistochemistry (Figure 2) and in situ hybridization (Figure 3) suggests that measles virus may play an etiological role in this condition (38,39), and that intestinal microvascular endothelial cells may be the principal reservoir of infection (infected cells were often observed specifically in foci of granulomatous vascular injury). A profound cellular immune response to apparently low levels of endothelial cell-associated virus, with the consequent vascular compromise that is seen, might account for the extensive tissue injury in CD. Identical intranuclear paramyxovirus inclusions in CD have been observed recently by others (40). There is an urgent need to confirm these observations at a molecular level and provide amplificants for viral sequencing studies.

A possible role for measles virus in CD has received recent support from epidemiological studies undertaken in Sweden: Ekbom et al (41) observed that perinatal measles virus infection was a strong risk factor for the development of CD later in life, a phenomenon that exhibits similarities to subacute sclerosing panencephalitis.

CONCLUSIONS

In early nonulcerated CD there is evidence of granulomatous and lymphocytic vasculitis: the combination of this lesion with either a systemic prothrombotic environment or mechanical devascularization of the intestine, before anastomosis, may induce mucosal ischemia and ulceration. We hypothesised that the mesenteric microvascular endothelium may harbour the antigenic stimulus for vasculitis in CD, and early morphological and epidemiological data suggest that this stimulus may be persistent measles virus infection. A great deal more work needs to be undertaken to confirm or refute these early etiological observations before any firm conclusions can be reached.

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REFERENCES

1. Fiocchi C. Immunology of inflammatory bowel disease. Curr Opin Gastroenterol 1991;7:654-61.
2. Scott H, Solheim BG, Brandtzaeg P, Thorsby E. HLA-DR-like antigens in the epithelium of the human small intestine. Scand J Immunol 1980;12:77-82.
3. Hadfield G. The primary histological lesion of regional ileitis. Lancet 1939;ii:773-5.
4. McGovern VJ, Goulston SJM. Crohn's disease of the colon. Gut 1968;9:164-76.
5. Wakefield AJ, Sankey EA, Dhillon AP, et al. A granulomatous vasculitis in Crohn's disease. Gastroenterology 1990;100:1279-87.
6. Knutson H, Lunderquist A. Vascular changes in Crohn's disease. Am J Roentol 1968;103:380-5.
7. Geller SA, Cohen A. Arterial inflammatory infiltration in Crohn's disease. Arch Pathol Lab Med 1983;107:473-7.
8. Bariety J, Jaquot C. The vasculitis syndromes in small and medium sized vessels. In: Camilleri JP, ed. Diseases of the Arterial Wall. Berlin: Springer-Verlag, 1989;27:424-8.
9. Takemura T, Matsui Y, Saiki S, Mikani R. Pulmonary vascular involvement in sarcoidosis. Hum Pathol 1993;23:1216-23.
10. Thompston H. Histopathology of Crohn's disease. In: Allan RN, ed. Inflammatory Bowel Diseases, 2nd edn. London: Churchill Livingstone, 1990;22:263-85.
11. Morson BC. The early histological lesion of Crohn's disease. Proc Sco Med 1972;65:71-2.
12. Hudson M, Piasecki C, Sankey EA, et al. A ferret model of acute multifocal gastrointestinal infarction. Gastroenterology 1992;102:1591-6.
13. Ohman N. Studies of small intestinal obstruction III. Circulatory effects of artificial small bowel distension. Acta Chir Scand 1975;141:536-44.
14. Osborne MJ, Hudson M, Piasecki C, et al. Crohn's disease and anastomotic recurrence: Microvascular ischaemia and anastomotic healing in an animal model. Br J Surg 1993;80:226-9.
15. Melbrath DC. Diverting ileostomy or colostomy in the management of Crohn's disease of the colon. Arch Surg 1971;103:308-10.
16. Kettlewell MGW, Jewell DP. Split ileostomy and ileocolostomy for Crohn's disease of the colon and ulcerative colitis: A 20 year survey. Gut 1983;24:106-13.
17. Harper PH, Lee ECG, Kettlewell MGW, Bennett MK, Jewell DP. Role of the faecal stream in the maintenance of Crohn's colitis. Gut 1985;26:279-84.
18. Winslett MC, Keighley MRB. Faecal challenge as a predictor of the effect of resuming intestinal continuity in defuctioned Crohn's colitis. Gut 1988;29:A1475.
19. Grange DN, Kvietys PR, Parks DA, Beroit JN. Intestinal blood flow: Relation to function. Surg Dig Dis 1983;1:217.
20. Rutgeerts P, Goboes K, Peters M, et al. Effect of faecal stream diversion on recurrence of Crohn's disease in the neoterminal ileum. Lancet 1991;338:771-4.
21. Raouf AH, Hildrey V, Daniel J, et al. Enteral feeding as sole treatment for Crohn's disease: Controlled trial of whole protein versus amino acid based feed and a case study of dietary challenge. Gut 1991;32:702-7.
22. Price AB, Talbot IC, Thompson H, Williams GT. Pathogenesis of Crohn's disease. Lancet 1990;i:804. (Lett)
23. Sokol RJ, Farrell MK, McAdams AJ. An unusual case of Wegener's granulomatosis mimicking inflammatory bowel disease. Gastroenterology 1984;87:426-32.
24. Kasahara Y, Tanaka S, Nishino M, Umemura H, Shiraha S, Kuyama T. Intestinal involvement in Behçet's disease. Dis Colon Rectum 1981;24:103-6.
25. Wakefield AJ, Sawyerr AM, Dhillon AP, et al. Pathogenesis of Crohn's disease: Multifocal gastrointestinal infarction. Lancet 1989;ii:1057-62.
26. Price AB, Davies DR. Pseudomembranous colitis. J Clin Path 1977;30:1-12.
27. Devitt PG, Stamp GWH. Acute clostridial enteritis – or pig-bel? Gut 1983;24:678-9.
28. Kliegman RM, Farnaroff AA. Necrotizing enterocolitis. N Engl J Med 1984;310:1093-103.
29. Sankey EA, Dhillon AP, Wakefield AJ, et al. Early mucosal changes in Crohn's disease. Gut 1993;34:375-81.
30. Kojima M, Takahashi K. Pathological study on the changes of lymphatic tissues in shock with special reference to secondary nodule lesions. Acta Path Jpn 1971;21:387-403.
31. Scott J. Lipoprotein(A): Thrombotic and atherogenic. Br Med J 1991;305:663-4.
32. Meade TW, Brozovic M, Chakvabarti RR, et al. Haemostatic function and ischaemic heart disease. Lancet 1986;ii:535-7.
33. Petty RG, Pearson JD. Endothelium – the axis of vascular health and disease. J R Coll Physicians 1989;23:92-102.
34. Beilke M. Vascular endothelium in immunology and infectious disease. Rev Infect Dis 1989;11:273.
35. Kirk J, Zhou A-L, McQuaid S, Cosby SL, Allen IV. Cerebral endothelial cell infection by measles virus in subacute sclerosing panencephalitis: Ultrastructural and in situ hybridisation evidence. Neuropathol Appl Neurobiol 1992;17:289-97.
36. Norby E, Oxman MN. Measles virus. In: Fields BN, ed. Virology. New York: Raven Press, 1990;37:1013-44.
37. Corbett EH. The visceral lesions in measles with a report of Koplick spots in colon. Am J Pathol 1945;21:905.
38. Simon M, Ballon HC. Appendiceal lesions in the prodromal stage of measles. Am J Pathol 1984;18:796-804.
39. 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.
40. Knibbs DR, Van Kruiningen HJ, Colombel JF, Cotort A. Ultrastructural evidence of paramyxovirus in two French families with Crohn's disease. Gastroenterology 1993;104:A726.
41. Ekbom A, Adami HO, Hernick CG, Jonzon A, Zack MM. Perinatal risk factors for inflammatory bowel disease: A case control study. Am J Epidemiol 1990;132:1111-9.
42. Kipps A, Dick G, Moodie JW. Measles and the cerebral nervous system. Lancet 1983;ii:1406-10.