Crohn's disease – The pathogenesis of a granulomatous vasculitis: A hypothesis

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AJ WAKEFIELD. Crohn's disease – The pathogenesis of a granulomatous vasculitis: A hypothesis. *Can J Gastroenterol* 1995;9(4):199-202. Dissatisfied with traditional approaches to studying the pathogenesis of Crohn's disease, the author and colleagues proposed and developed the hypothesis that Crohn's disease is a granulomatous vasculitis mediated by a persistent viral infection of the mesenteric microvascular endothelium. Employing a range of techniques, the mesenteric vascular anatomy of intestine affected by Crohn's disease was studied and the presence of a widespread multifocal vasculitis was demonstrated. Based upon certain behavioural characteristics of measles virus, including its tropism for intestinal endothelium and its ability to persist in human tissues, this agent was sought by in situ hybridization, electron microscopy and immunohistochemistry. The virus was detected in foci of granulomatous and lymphocytic inflammation. Recent epidemiological data from Sweden support the idea that early exposure to measles virus is a risk factor for the later development of Crohn's disease. These data are consistent with the possibility of a persistent measles virus enteritis in the etiology and pathogenesis of Crohn's disease and this hypothesis merits further study.

Key Words: Crohn's disease, Measles virus, Vasculitis

Maladie de Crohn : rôle hypothétique de la vasculite granulomateuse

RÉSUMÉ : Insatisfaits des approches traditionnelles utilisées pour étudier la pathogenèse de la maladie de Crohn, l'auteur et ses collègues ont avancé et mis au point une hypothèse selon laquelle la maladie de Crohn est en fait une vasculite

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CROHN'S DISEASE, CONSIDERED BY many authorities to be a disease primarily of the intestinal mucosa (1,2) is characterized both histologically and immunologically by a cell-mediated immune response to an unidentified antigen(s) (3).

In 1987, based upon macroscopic similarities between rejecting experimental intestinal allografts and Crohn's disease, the hypothesis was formulated that common pathogenetic factors may be operating in these two otherwise distinct disease entities. In allograft rejection, microvascular activation and injury are early events, initiated by host immune recognition of alloantigen on graft vascular endothelium (4). In Crohn's disease there are clues to suggest that microvascular injury may be involved in the pathogenesis of intestinal inflammation: in biopsies of patients with inflammatory bowel disease, mucosal capillary thrombi can be seen (5) and vasculitis, including granulomatous vasculitis, is a recognized feature of Crohn's disease (6,7). Regarded previously as a secondary phenomenon, however, vasculitis was seen only occasionally in routinely processed histological sections and was considered to be of little pathological significance (6,7). Parallel to this apparent dismissal of the potential significance of granu-

granulomateuse qui dépend d'une infection virale persistante de l'endothélium microvasculaire mésentérique. Au moyen de tout un éventail de techniques, l'anatomie vasculaire mésentérique de l'intestin affectée par la maladie de Crohn a été étudiée et la présence de vasculite multifocale étendue a été démontrée. Sur la base de certaines caractéristiques comportementales du virus de la rougeole, y compris son tropisme à l'endroit de l'endothélium intestinal et son aptitude à persister dans les tissus humains, cet agent a été étudié au moyen d'une hybridation *in situ*, par microscopie électronique et par immunohistochimie. Le virus a été décelé dans les foyers d'inflammation granulomateux et lymphocytaires. Les données épidémiologiques récentes d'une étude suédoise appuient le concept selon lequel une exposition précoce au virus de la rougeole constitue un facteur de risque pour l'installation subséquente de maladie de Crohn. Ces données concordent avec la possibilité de la persistance d'une entérite due au virus de la rougeole dans l'étiologie et la pathogenèse de la maladie de Crohn et cette hypothèse mérite que l'on y consacre d'autres études.

lomatous vasculitis in Crohn's disease was a disregard, with notable exceptions (8,9), for the tissue origins of the granuloma, an early and hallmark lesion of this condition. The granuloma represents a localizing reaction to persistent and potentially causative antigen. Therefore, the tissue relationships of this lesion assumed great importance in progressing understanding of Crohn's disease.

GRANULOMATOUS VASCULITIS IN CROHN'S DISEASE

Based on the hypothesis that foci of both granulomatous and lymphocytic vasculitis in Crohn's disease evolved from blood vessels that contained foreign antigen, clarifying the interrelationship of these two tissue elements was a priority. This was aided by overcoming vascular artefacts produced by

routine immersion-fixation of tissues, and immunostaining for specific vascular and granulomatous elements in tissue sections (10,11).

Perfusion-fixation at mean arterial pressure not only produced excellent tissue preservation, but also prevented vascular collapse and blood clots obscuring the relationship of blood vessels to foci of inflammation. Immunostaining for vascular elements and macrophages on serial sections showed that the majority of granulomas in Crohn's disease arise from blood vessels – predominantly thin-walled veins. This process is associated with thrombosis, vascular occlusion and likely ischemia of the dependent tissues (Figures 1,2). The author and colleagues have since shown that vasculitis and a chronic ischemic injury of the intestine may help to explain many of the idiosyncrasies of this condition, including 'skip' lesions and transmural inflammation (12,13), aphthoid ulceration (14), anastomotic recurrence (15) and thrombogenesis (unpublished data,16-18).

Of greater importance, perhaps, was

Figure 3) Crohn's disease: submucosal microvascular endothelium containing discrete pleomorphic tubular and vesicular virus particles 70 to 100 nm diameter consistent with budding paramyxoviridae

Figure 1) Top left Focus of serosal granulomatous vasculitis in Crohn's disease (x192)

Figure 2) Bottom left Crohn's disease: intravascular mucosal granuloma surrounded by fibrin thrombus immunostained for fibrinogen (arrowed) (Dako) (x229)

Figure 4) Crohn's disease: in situ hybridization for measles virus N-gene (arrowed) in the centre of a granuloma (x262)

Figure 5) Crohn's disease: immunohistochemistry for measles virus N-protein (arrowed) in a small submucosal granuloma (x262)

the observation that many granulomas were associated intimately with pathologically altered endothelium. In view of the capacity of activated endothelium to present antigen in association with class II determinants (19), the hypothesis that the mesenteric microvascular endothelium is a reservoir for the persistent antigen that induces Crohn's disease seemed increasingly attractive. The hypothesis did not maintain that the vasculature, or indeed the endothelium, was the only site of primary antigen presentation. These elements did, however, provide a target for further detailed studies.

MEASLES VIRUS AND MICROVASCULAR ENDOTHELIUM

The hypothesis proposed that persistent viral infection of the mesenteric endothelium is necessary for the development of Crohn's disease. Certain criteria were considered in the selection of candidate viruses for further study:

enterotropism during the primary infection, the capacity to infect and persist within microvascular endothelium, and the capacity to induce a profound cellular immune response associated with giant cell formation.

Measles virus – a common, highly infectious agent recognized for its capacity to persist within, and induce chronic inflammation of, cerebral tissues – appeared to fulfill these criteria (20). A number of other viruses were studied in detail, but were not found to be implicated (21).

Subsequent detailed examination of 24 cases of Crohn's disease and 22 inflammatory and noninflammatory intestinal controls identified measles virus in the endothelium, lymphocytes and macrophages of inflammatory foci in Crohn's disease patients, but not in controls (22). Transmission electron microscopy identified paramyxovirus-like nucleocapsids within endothelial cells in areas of granulomatous vasculitis; endothelial cells, apparently

infected with virus, were participating actively in the inflammatory process (Figure 3). In situ hybridization for measles virus genomic RNA gave positive signals in the same cellular location (Figure 4). Hybridization signals were also strongly positive in the centres of secondary lymphoid follicles in a pattern identical to that described previously in the appendix in association with persistent measles virus infection (23). Immunocytochemistry using both measles virus-specific monoclonal and polyclonal antibodies was positive in these lesions in 13 of 15 cases (Figure 5) and was not seen in intestinal tuberculosis, studied as a granulomatous control.

Other groups (24,25) have obtained independent evidence, from both basic science and epidemiological studies, of a role for persistent measles virus infection in the etiology of Crohn's disease. Although much work remains to be done these data are interesting and merit further study.

REFERENCES

- Price A, Talbot IC, Thompson H, Williams GT. Pathogenesis of Crohn's disease. *Lancet* 1990;ii:804.
- Morson BC. The earliest histological lesion of Crohn's disease. *Proc R Soc Med* 1972;65:71-2.
- Jewell DP, Snook JA. Immunology of ulcerative colitis and Crohn's disease. In: Allan RN, Keighley MRB, Alexander-Williams J, Hawkins C, eds. *Inflammatory Bowel Diseases*, 2nd edn. Edinburgh: Churchill-Livingstone, 1990;12:127-46.
- Bishop GA, Waugh GA, Landers DV, Krensky AM, Hall BM. Microvascular destruction in renal transplant rejection. *Transplantation* 1989;48:408-14.
- Dhillon AP, Anthony A, Sim R, et al. Mucosal capillary thrombi in rectal biopsies. *Histopathology* 1992;21:127-33.
- Knutson H, Lunderquist A. Vascular changes in Crohn's disease. *Am J Roentgenol* 1968;103:380-5.
- Geller SA, Cohen A. Arterial inflammatory infiltration in Crohn's disease. *Arch Pathol Lab Med* 1983;107:473-7.
- Lockhart-Mummery HE, Morson BC. Crohn's disease of the large intestine. *Gut* 1964;5:493-509.
- Morson BC. Pathology of Crohn's disease. In: Brooke BB, ed. *Clinics in Gastroenterology*, 1(2); Crohn's Disease. London: WB Saunders, 1972:265-77.
- Wakefield AJ, Sawyerr AM, Dhillon AP, et al. Pathogenesis of Crohn's disease: multifocal gastrointestinal infarction. *Lancet* 1989;ii:1057-62.
- Wakefield AJ, Sankey EA, Dhillon AP, et al. Granulomatous vasculitis in Crohn's disease. *Gastroenterology* 1990;100:1279-87.

12. Hudson M, Piasecki C, Sankey EA, et al. A ferret model of acute multifocal gastrointestinal infarction. *Gastroenterology* 1992;102:1591-6.
 13. Hudson M, Piasecki C, Wakefield AJ, et al. A vascular hypersensitivity model of acute multifocal intestinal infarction. *Dig Dis Sci* 1994;39:534-9.
 14. Sankey EA, Dhillon AP, Wakefield AJ, et al. Early mucosal changes in Crohn's disease. *Gut* 1993;34:375-81.
 15. 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.
 16. Hudson M, Wakefield AJ, Hutton RA, et al. Factor XIIIa subunit in Crohn's disease. *Gut* 1993;34:75-9.
 17. Hudson M, Hutton RA, Wakefield AJ, Sawyerr AM, Pounder RE. Evidence for activation of coagulation in Crohn's disease. *Blood Coagul Fibrinolysis* 1992;3:773-8.
 18. Wakefield AJ, Sawyerr AM, Hudson M, Dhillon AP, Pounder RE. Smoking, the oral contraceptive pill and Crohn's disease. *Dig Dis Sci* 1991;36:1147-50.
 19. Petty RG, Pearson JD. Endothelium – the axis of vascular health and disease. *J R Coll Physicians* 1989;23:92-102.
 20. Norby E, Oxman MN. Measles virus. In: Fields BN, ed. *Virology*. New York: Raven Press, 1990;37:1013-44.
 21. Wakefield AJ, Fox JD, Sawyerr AM, et al. Detection of herpesvirus DNA in the large intestine of patients with ulcerative colitis and Crohn's disease using the nested polymerase chain reaction. *J Med Virol* 1992;38:183-90.
 22. 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.
 23. Fournier JG, Lebon P, Bouteille M, Goutiers F, Rozenblatt S. Subacute sclerosing panencephalitis: detection of measles virus RNA in appendix lymphoid tissue before clinical signs. *BMJ* 1986;293:523-4.
 24. 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.
 25. Ekbom A, Wakefield AJ, Zack M, Adami HO. The role of perinatal measles infection in the aetiology of Crohn's disease: a population based epidemiological study. *Lancet* 1994;344:508-10.
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