Crohn's Disease: Pathogenesis and Persistent Measles Virus Infection

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The Inflammatory Bowel Disease Study Group at the Royal Free Hospital School of Medicine has tested the hypothesis that the primary pathological abnormality in Crohn's disease is in the mesenteric blood supply. Early morphological studies involved arterial perfusion-fixation and either resin casting and scanning electron microscopy or vascular immunostaining of resected intestine affected by Crohn's disease. Granulomatous and lymphocytic damage to intramural blood vessels, even in macroscopically normal areas, was observed. We put forward possible mechanisms by which a chronic ischemic process might account for many of the idiosyncracies of Crohn's disease. It was proposed that persistent viral infection of the mesenteric microvascular endothelium might underly this vasculitic process; based on certain behavioral characteristics of measles virus, including its tropism for the submucosal endothelium of the intestine, this agent was investigated further. This report reviews the preliminary evidence from both epidemiological and basic scientific data for persistent measles virus in the intestine of patients with Crohn's disease. Possible mechanisms for virus persistence and subsequent reactivation are discussed. In conclusion, we believe that Crohn's disease may be a chronic granulomatous vasculitis in reaction to a persistent infection with measles virus within the vascular endothelium. This granulomatous inflammation, perhaps aggravated by either a hypercoagulable state or mechanical stress, results in the clinical features of Crohn's disease.

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 as yet unidentified antigen(s).[3]

In 1987, based on 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 were clues to suggest that microvascular injury might be involved in the pathogenesis of intestinal inflammation; in biopsy specimens of patients with inflammatory bowel disease, mucosal capillary thrombi can be found[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 found only occasionally in routinely processed histological sections and was considered to be of little pathological significance.[6,7] In parallel with this apparent dismissal of the potential significance of granulomatous 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. This is somewhat surprising because the granuloma represents a localizing reaction to persistent and potentially causative antigen; therefore, the tissue relationships of this lesion assumed great importance in progressing our own 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 may have evolved from blood vessels that contain foreign antigen, clarifying the interrelationship of these two tissue elements was a priority. This was aided by overcoming vascular artifacts 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 clot obscuring the relationship of blood vessels to foci of inflammation. Immuno-

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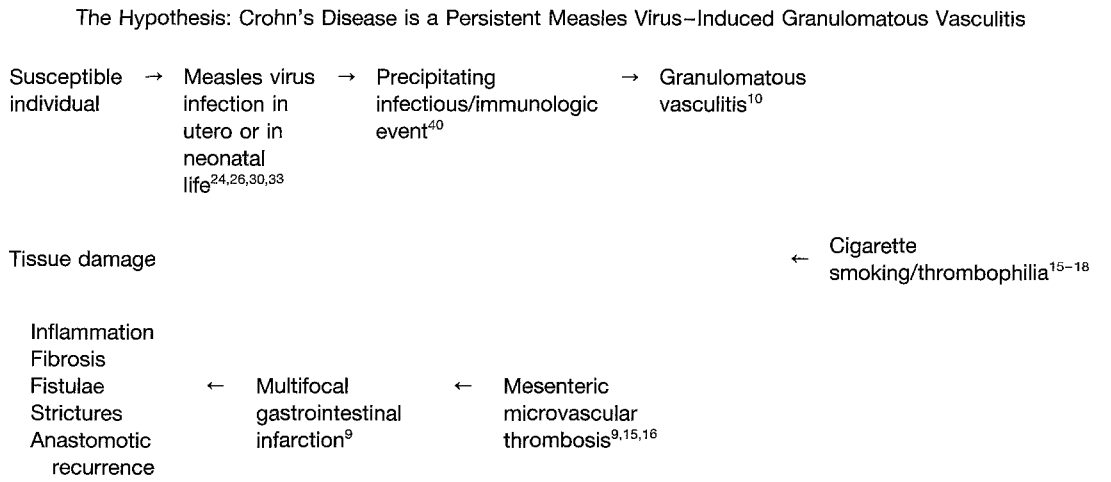

Figure 1. Proposed sequence of pathological events in Crohn's disease.

staining for vascular elements and macrophages on serial sections facilitated the recognition that the great 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 (Figure 1). We have since shown that vasculitis and a chronic ischemic injury of the intestine may help to explain some of the idiosyncracies of this condition, including "skip" lesions and transmural inflammation,[12,13] aphthoid ulceration,[14] and thrombogenesis.[15–17]

Anastomotic Recurrence of Crohn's Disease

Endoscopically evident recurrence of Crohn's disease in the proximal anastomotic margin occurs rapidly after resection.[18]

It is possible that occult residual granulomatous vasculitis is the mechanism for this phenomenon. The capacity of the bowel to heal after a focal, intramural vascular injury depends largely on 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. This limited devascularization is of little consequence when normal intestine is to be anastomosed. If, however, there is vascular disease in the macroscopically normal bowel used to make anastomosis, the reduced capacity of the already compromised collateral circulation may be sufficient to induce anastomotic recurrence.[19] Subacute obstruction of the bowel by increasing intraluminal pressure will cause a further decrease in perfusion of the submucosal vasculature.

Colonic Crohn's Disease and Diversion of the Fecal Stream

Patients with severe colonic Crohn's disease often enjoy a remission after a diverting ileostomy, but relapse of disease usually follows restoration of the fecal stream.[20–23] This sequence has prompted the hypothesis that some constituent of the fecal stream is responsible for either initiating or provoking Crohn's disease.[23,24] The blood flow of the intestinal mucosa increases greatly from basal levels in response to local luminal contents[25]; blood flow increases in proportion to the metabolic demand placed on the mucosa by digestion and absorption.[25] In Crohn's disease, stenotic vascular lesions in the bowel wall would place a rate-limiting influence on the hyperemic response to mucosal activity. This situation is analogous to intermittent claudication or exercise-induced angina; ischemia or infarction occurs when the demand for blood exceeds supply.

Fecal diversion rests the bowel, and this will reduce mucosal blood flow and oxygen demand. Provided with a respite, the intestinal ischemic damage eases and healing dominates. However, reinstitution of the fecal stream is likely to induce relapse because of further ischemic damage. This phenomenon may also explain the transient beneficial effects of an elemental or polymeric diet.

Measles Virus and Microvascular Endothelium

Perhaps of greater importance in terms of the etiology of Crohn's disease was 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,[26] the hypothesis that the mesenteric microvascular endothelium is a reservoir for the persistent antigen that induces Crohn's disease seemed increasingly attractive.

The hypothesis proposes that persistent viral infection of the mesenteric endothelium is necessary for the development of Crohn's disease. Certain criteria were considered when selecting 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 that is 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, seemed to fulfill these criteria.[27] Measles virus–infected lymphocytes home in on the lymphoid tissues of the gut during primary viremia; the terminal ileum and anal canal, common sites of Crohn's disease, contain abundant lymphoid tissues. Subsequently, the virus infects the microvascular endothelium of these tissues and induces vasculitis; secondary lymphoid aggregates develop and there is necrosis of the overlying epithelium, all of which are characteristic features of Crohn's disease.[28,29] Acute measles may produce Koplick spots throughout the gut that are similar to the aphthoid ulcers of Crohn's disease.[27,30] Giant cell syncytia at the centers of secondary lymphoid follicles in the intestine are a feature of acute measles virus infection[27]; Hadfield reported similar changes in his classic description of Crohn's disease.[28] Furthermore, acute measles can produce ulceration of the gut from mouth to anal canal,[27,30] the lesional topography of Crohn's disease. Because of these histological and topographical parallels between acute measles virus infection of the gut and Crohn's disease, further study of this agent was merited.

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 that was not found in controls.[31] Transmission electron microscopy identified paramyxoviruslike nucleocapsids within endothelial cells in areas of granulomatous vasculitis; endothelial cells, apparently infected with virus, were participating actively in the inflammatory process. In situ hybridization for measles virus genomic RNA gave positive signals in the same cellular location. Hybridization signals were also strongly positive in the centers of secondary lymphoid follicles in a pattern identical to that described previously in the appendix in association with persistent measles virus infection.[32] Immunocytochemistry using both measles virus–specific monoclonal and polyclonal antibodies was positive in these lesions in the great majority of cases and was not found in intestinal tuberculosis, which was studied as a granulomatous control.

The French Connection

Independently of our own studies, Van Kruiningen et al. were studying two French families with a high incidence of Crohn's disease that was present in nonsanguinous relations across two generations.[33] They suggested that an environmental rather than a genetic influence was operating in these cases, although an extensive search for such an agent only excluded many potential pathogens. Prompted by our observations, they concentrated their attention on the vascular endothelium and granulomas,[34] in which they identified abundant intracytoplasmic and intranuclear inclusions composed of condensations of paramyxoviruslike nucleocapsids identical to those described previously in subacute sclerosing panencephalitis.[35,36] Although these early studies lacked appropriate controls, further studies are now in progress to address this issue and characterize the particles observed.

The finding of a measleslike virus in the early and characteristic histological lesions of Crohn's disease and, in particular, the apparent localization of granulomas to endothelial cells infected with this agent suggests but does not prove a causal association.

Measles Virus and Crohn's Disease: Epidemiological Correlates

Further evidence to suggest that measles virus may be involved in the pathogenesis of Crohn's disease has come from Sweden. In 1991, Ekbom et al. reported a case-control study based on prospectively collected data; the investigators showed that perinatal virus infection, in particular with measles virus, was a strong risk factor for the development of Crohn's disease later in life (odds ratio = 18).[37] A similar phenomenon occurs in subacute sclerosing panencephalitis in which exposure to measles virus early in life is a strong risk factor for the development of encephalitis, often after a long disease-free interval.[38]

A birth cohort phenomenon in those born in Sweden during the period 1945–1954 and a cluster phenomenon by both birth date and place of residence was observed in patients with Crohn's disease.[39] It was subsequently shown that during the period 1945–1954, during which there were five measles epidemics in central Sweden, there was a statistically significant excess number of patients diagnosed with Crohn's disease born up to 3

months after each measles epidemic.[40] The same effect was not observed for ulcerative colitis.

Further epidemiological studies are currently underway that may help to clarify the strength of this association. However, the data are interesting and do suggest that exposure to measles virus in utero or neonatally predisposes to the later development of Crohn's disease.

Measles Virus: Persistence and Progression

It is well recognized that exposure to certain viruses in utero can result in delayed sequelae[41] and that exposure to measles virus early in life provides an opportunity for viral persistence[38]; the host and virus-related events that confer persistence are currently subjects of great research interest.

To produce persistent viral infection, many strategies have evolved to avoid host immunity. Immunologic tolerance is achieved by lymphocytic choriomeningitis virus when it infects mice with an immature immune system during the neonatal period. It is suggested that viral antigens delete immature T cells with complimentary receptors, much as self-antigens delete immature self-specific T cells[42]; as a result, the mice become persistently infected. Recent evidence suggests that tolerance may also be induced by exhaustion of mature T cells; large numbers of viruses produce an antigenic load that induces a brief and intense immune response culminating in the exhaustion and deletion of cytotoxic T lymphocytes.[43] Either of these mechanisms may be relevant to measles virus persistence in cerebral and intestinal tissues. In addition, measles virus exploits a number of molecular strategies, reviewed by Schneider-Schaulies and Liebert[44] and Oldstone,[45] that may prevent host immune recognition and result in persistent infection.

The events in later life that may lead to breakdown of immunologic tolerance and chronic inflammation in susceptible individuals, often following a long disease-free interval, are poorly understood. Intercurrent infection is a recognized precursor of both subacute sclerosing panencephalitis[38] and Crohn's disease.[46] Recent interest has centered on the capacity of certain viruses to interact with each other, possibly either directly or through a modification of the host immune response to infection. In the context of virus persistence, reexposure to a different strain of the same virus may be relevant; it is recognized that severe clinical disease can be induced in cattle persistently infected with noncytopathic bovine diarrhea virus after superinfection with a cytopathic strain of the same virus.[47] Persistent infection can be induced experimentally by exposing the bovine fetus to noncytopathic bovine diarrhea virus[47]; severe enteritis is achieved in these animals postpartum by inoculation with cytopathic bovine diarrhea virus. This "two-hit" phenomenon provides a model that may be relevant to a number of diseases that are associated with viral persistence in humans, although there is no evidence for this at present. We recently identified persistence of herpesviral DNA in a large proportion of both inflamed and noninflamed intestinal tissues.[48] This family of viruses is noted for its capacity either to induce serological reactivation of coexistent herpesviral infection or to modulate the host immune response to intercurrent viral infection,[49–51] including measles.[52] It is tempting to speculate that a state of coinfection with both a herpesvirus and measles could lead to either persistence or reactivation of persistent measles virus in the gut.

Measles Vaccination and Crohn's Disease

In the United Kingdom, the introduction of live attenuated measles vaccination in 1968 saw a dramatic decrease in the incidence of subacute sclerosing panencephalitis.[38] In contrast, many countries have reported an increase in the incidence of Crohn's disease; for example, in Scotland, the rate of Crohn's disease in children has increased sharply during the last two decades.[53] However, it should be borne in mind that the peak onset for subacute sclerosing panencephalitis is between 5 and 15 years of age, whereas it is between 20 and 30 years of age for Crohn's disease. A steady decrease in the incidence of Crohn's disease might be anticipated if the association between measles virus and Crohn's disease is one of causation. If the current upward trend in the incidence of Crohn's disease in children continues and the association holds, the role of vaccination will need to be reviewed.

Conclusions

If Crohn's disease has an infectious etiology, there are certain behavioral features of measles virus plus epidemiological and basic scientific data derived from independent studies of affected tissues to implicate this agent in its pathogenesis. These are not enough. In this neo-Kochasian era, elements of the relevant gene need to be isolated consistently from affected tissues; they need to be sequenced and compared with known sequences of this virus. Measles-specific immunohistochemical and gene hybridization signals need to be shown to colocalize with putative viral particles at the ultrastructural level, and a Crohn's disease–specific response of intestinal immune cells to measles virus should be evident.

Assuming for the present that measles virus is a com-

ponent cause of Crohn's disease, it is apparent from our studies that persistence of measles virus in the intestine is not inevitably associated with chronic inflammation. Other factors (e.g., a genetic predisposition, smoking, or a thrombotic tendency[15–17,54]) may influence the progression to loss of immunologic tolerance and subsequent tissue damage. These factors require further detailed study.

The tools and techniques necessary to investigate this hypothetical sequence of pathological events are available; whether measles is right or wrong, our long-suffering patients with Crohn's disease deserve an expeditious conclusion.

References

1. Price A, Talbot IC, Thompson H, Williams GT. Pathogenesis of Crohn's disease (letter). Lancet 1990;1:804.
2. Morson BC. The earliest histological lesion of Crohn's disease. Proc R Soc Med 1972;65:71–72.
3. 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 ed. New York: Churchill-Livingstone, 1990:127–146.
4. Bishop GA, Waugh GA, Landers DV, Krensky AM, Hall BM. Microvascular destruction in renal transplant rejection. Transplantation 1989;48:408–414.
5. Dhillon AP, Anthony A, Sim R, Wakefield AJ, Sankey EA, Hudson M, Allison MC, Pounder RE. Mucosal capillary thrombi in rectal biopsies. Histopathology 1992;21:127–133.
6. Knutson H, Lunderquist A. Vascular changes in Crohn's disease. Am J Roentgenol 1968;103:380–385.
7. Geller SA, Cohen A. Arterial inflammatory infiltration in Crohn's disease. Arch Pathol Lab Med 1983;107:473–477.
8. Lockhart-Mummery HE, Morson BC. Crohn's disease of the large intestine. Gut 1964;5:493–509.
9. Morson BC. Pathology of Crohn's disease. In: Brooke BB, ed. Clinics in gastroenterology. Crohn's disease. London: Saunders, 1972:265–277.
10. Wakefield AJ, Sawyerr AM, Dhillon AP, Pittilo AP, Rowles PM, Lewis AAM, Pounder RE. Pathogenesis of Crohn's disease: multifocal gastrointestinal infarction. Lancet 1989;1:1057–1062.
11. Wakefield AJ, Sankey EA, Dhillon AP, Sawyerr AM, More L, Sim R, Pittilo M, Rowles PM, Hudson M, Lewis AAM, Pounder RE. Granulomatous vasculitis in Crohn's disease. Gastroenterology 1991;100:1279–1287.
12. Hudson M, Piasecki C, Sankey EA, Sim R, Wakefield AJ, More LJ, Sawyerr AM, Dhillon AP, Pounder RE. A ferret model of acute multifocal gastrointestinal infarction. Gastroenterology 1992;102:1591–1596.
13. Hudson M, Piasecki C, Wakefield AJ, Sankey EA, Dhillon AP, Osborne M, Sim R, Pounder RE. A vascular hypersensitivity model of acute multifocal intestinal infarction. Dig Dis Sci 1994;39:534–539.
14. Sankey EA, Dhillon AP, Wakefield AJ, Sim R, More L, Hudson M, Sawyerr AM, Pounder RE. Early mucosal changes in Crohn's disease. Gut 1993;34:375–381.
15. Hudson M, Wakefield AJ, Hutton RA, Sankey EA, Dhillon AP, More L, Sim R, Pounder RE. Factor XIIIA subunit in Crohn's disease. Gut 1993;34:75–79.
16. Hudson M, Hutton RA, Wakefield AJ, Sawyerr AM, Pounder RE. Evidence for activation of coagulation in Crohn's disease. Blood Coagul Fibrinolysis 1992;3:773–778.
17. Wakefield AJ, Sawyerr AM, Hudson M, Dhillon AP, Pounder RE. Smoking, the oral contraceptive pill and Crohn's disease. Dig Dis Sci 1991;36:1147–1150.
18. Rutgeerts P, Goboes K, Vantrappen G, Beyls J, Rowemans R, Hiele M. Predictability of the postoperative course of Crohn's disease. Gastroenterology 1990;99:956–963.
19. Osborne MJ, Hudson M, Piasecki C, Dhillon AP, Lewis AAM, Pounder RE, Wakefield AJ. Crohn's disease and anastomotic recurrence: microvascular ischaemia and anastomotic healing in an animal model. Br J Surg 1993;80:226–229.
20. Melbrath DC. Diverting ileostomy or colostomy in the management of Crohn's disease of the colon. Arch Surg 1971;103:308–310.
21. Kettlewell MGW, Jewell DP. Split ileostomy and ileocolostomy for Crohn's disease of the colon and ulcerative colitis: a 20 year survey. Gut 1983;24:106–113.
22. Harper PH, Lee ECG, Kettlewell MGW, Bennett MK, Jewell DP. Role of the faecal stream in the maintenance of Crohn's colitis. Gut 1985;26:279–284.
23. Winslett MC, Keighley MRB. Faecal challenge as a predictor of the effect of restoring intestinal continuity in defunctioned Crohn's colitis (abstr). Gut 1988;29:A1475.
24. Rutgeerts P, Goboes K, Peters M, Hiele M, Penrick F, Aerts R, Keeremans R, Vantrappen G. Effect of faecal stream diversion on recurrence of Crohn's disease in the neoterminal ileum. Lancet 1991;338:771–774.
25. Granger DN, Kvietys PR, Parks DA, Beroit JN. Intestinal blood flow: relation to function (abstr). Surg Dig Dis 1983;1:217.
26. Petty RG, Pearson JD. Endothelium—the axis of vascular health and disease. J Royal Coll Phys Surg 1989;23:92–102.
27. Norby E, Oxman MN. Measles virus. In: Fields BN, ed. Virology. New York: Raven, 1990:1013–1044.
28. Hadfield G. The primary histological lesion of regional ileitis. Lancet 1939;2:773–775.
29. Dourmashkin RR, Davies H, Well S. Epithelial patchy necrosis in Crohn's disease. Hum Pathol 1983;14:643–648.
30. Corbett EH. The visceral lesions in measles with a report of Koplick spots in colon. Am J Pathol 1945;21:905.
31. Wakefield AJ, Pittilo RM, Sim R, Cosby SL, Stephenson JR, Dhillon AP, Pounder RE. Evidence of persistent measles virus infection in Crohn's disease. J Med Virol 1993;39:345–353.
32. 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. Br Med J 1986;293:523–524.
33. Van Kruiningen HJ, Colombel TF, Cartun RW, Whistlock RH, Koopmans M, Kangro HO, Hoogkamp-Korstanje AA, Lecomte-Houke M, Devred M, Paris JC, Cotort A. An in-depth study of Crohn's disease in two French families. Gastroenterology 1993;104:351–360.
34. Knibbs DR, Van Kruiningen HJ, Colombel JF, Cotort A. Ultrastructural evidence of paramyxovirus in two French families with Crohn's disease (abstr). Gastroenterology 1993;104:A726.
35. Horta-Barbosa M, Hamilton R, Wittig B, Fucillo DA. Subacute sclerosing encephalitis: isolation of measles virus from a brain biopsy. Nature 1961;221:974.
36. Connolly J, Allen I, Hurwitz L, Millar J. Measles virus antibody and antigen in subacute sclerosing panencephalitis. Lancet 1967;1:542–545.
37. 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;132:1111–1119.
38. Miller C, Farringdon CP, Harbert K. The epidemiology of subacute

sclerosing panencephalitis in England and Wales 1970–1989. Int J Epidemiol 1992;21:998–1006.
39. Ekbom A, Zack M, Adami HO, Helmick HO. Is there clustering of inflammatory bowel disease at birth? Am J Epidemiol 1991; 134:876–886.
40. 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;344:508–510.
41. Fine PM, Dedelsrein AM, Snowman J, Clarkson JA, Evans SM. Long term effects of exposure to viral infections in utero. BMJ 1985;290:509–512.
42. Pircher H, Burki K, Lang R, Hengartner H, Zinkernagel RM. Tolerance induction in double specific T-cell receptor transgenic mice varies with antigen. Nature 1989;342:559–561.
43. Moskophidis D, Lechner F, Pircher H, Zinkernagel RM. Virus persistence in acutely infected immunocompetent mice by exhaustion of antiviral cytotoxic effector T cells. Nature 1993;362:758–761.
44. Schneider-Schaulies S, Liebert NG. Pathogenetic aspects of persistent measles virus infections in brain tissue. The neurosciences. Volume 3. Philadelphia: Saunders, 1991:149–155.
45. Oldstone MBA. Molecular anatomy of viral persistence. J Virol 1991;65:6381–6386.
46. Kangro HO, Chong SKF, Hardiman A, Heath RB, Walker-Smith JA. A prospective study of viral and mycoplasma infections in chronic inflammatory bowel disease. Gastroenterology 1990;98:549–553.
47. Barton JR, Gillon S, Ferguson A. Incidence of Crohn's disease in Scottish children between 1968 and 1983. Gut 1989;30:618–622.
48. Wakefield AJ, Fox JD, Sawyerr AM, Taylor JE, Sweenie CH, Smith M, Emery VC, Hudson M, Tedder RS, Pounder RE. 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;38:183–190.
49. Kirchesch H, Mertens T, Burkhardt N, Kruppenbacher JP, Hoffken A, Eggers HJ. Seroconversion against-human herpesvirus 6 (and other herpesviruses) and clinical illness. Lancet 1988;2:273–274.
50. Linde A, Dahl H, Warren B, Friedell E, Salahuddin Z, Biberfeld P. IgG antibodies to human herpesvirus 6 in children and adults both in primary Epstein-Barr virus and cytomegalovirus infections. J Virol Methods 1988;21:117–123.
51. Neiderman J, Lin C, Kaplan M. Clinical and serological features of human herpesvirus 6 infection in 3 adults. Lancet 1988;2:817–819.
52. Suga S, Yoshikawa T, Asano Y. Simultaneous infection with human herpesvirus 6 and measles virus in infants. J Med Virol 1990;31:306–311.
53. Bolin SR, McClarkin AW, Cutlip RC, Coria MF. Severe clinical disease induced in cattle persistently infected with non-cytopathic bovine viral diarrhoea virus by superinfection with cytopathic bovine viral diarrhoea virus. Am J Vet Res 1985;46:573–576.
54. Orholm M, Iselins L, Sorensen TIA, Binde V. Investigation of inheritance of chronic inflammatory bowel diseases by complex segregation analysis. Br Med J 1993;306:20–24.

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