CYTOTOXIC INDUCERS IN CROHN'S DISEASE AND ULCERATIVE COLITIS

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The concept that infectious agents might be causative factors for Crohn's disease (CD) was first proposed by Crohn et al (1) and for ulcerative colitis (UC) by Bargen (2). Further impetus to research in this area was provided by the reports of Mitchell and Rees (3) and Cave et al (4) that small (less than 220nm) transmissible agents from human tissues induced changes similar to IBD in mice and rabbits.

Models for IBD

It is of interest that in veterinary medicine, inflammatory bowel diseases similar to those seen in humans have been described. In some cases the cause can be traced to viruses (5), in others to bacteria (6) and in still others to a combination of viruses with bacterial flora (7). The question can be raised as to whether or not such pathogenetic mechanisms are operative in humans.

Feline infectious peritonitis (FIP) is a transmissible disease of cats caused by a coronavirus, a pleomorphic RNA virus, 75nm in diameter, with radiating spikelike projections of its outer envelope (8,9). The virus is heat and ether labile, and phenol resistant. The progressive, nonresponsive, febrile peritonitis caused by this agent was first reported in 1953, and the virus has subsequently been identified as the FIP virus. The peritonitis is serofibrinous, accompanied by large volumes of transudate, and characterized by miliary granulomas of the serosal surfaces of viscera, particularly liver and intestines. In recent years it has been recognized that the virus can affect other systems, and produce granulomatous disease of the lungs, central nervous system, the eyes,

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kidneys, liver, or visceral lymph nodes. In this infection, differences in the immune state of the host can result in markedly different disease manifestations (10).

There are a number of veterinary diseases which affect the colon and/or small intestine and produce diseases which resemble the inflammatory bowel diseases of man. Many of the causative organisms have not been described or even sought in human diseases. Among those widely different veterinary pathogens are: the soil algae, Prototheca, which causes an IBD of the large and small intestine of dogs complete with extra-colonic manifestations such as arthritis, iritis, and dermatitis (11); an anaerobe, a species of the genus Clostridium, which produces an ulcerative enteritis of birds (12); the parvoviruses, including the small, stable DNA cryptotrophic virus which causes feline infectious enteritis; an anaerobic spirochete which interacts with one or more gram negative obligate anaerobes to produce an ulcerative colitis of swine (13); and the Mycobacterium johne which produces granulomatous inflammation of the intestine in ruminants (14). Some other interesting diseases which are probably caused by an infectious agent because of favorable responses to antibiotics include canine colitis, which histologically bears a strong resemblance to human ulcerative colitis (15), and canine granulomatous enteritis which resembles Crohn's disease including the presence of granulomas and giant cells (16).

Animal Transmission Studies

As early as 1935, Mones and Sanjaun (17) inoculated crude filtrates obtained from intestinal tissue of patients with inflammatory bowel diseases into rabbits, causing changes similar to those found in UC (see Table 1). Mitchell and Rees (3) transmitted granulomas to the foot pads of mice inoculated with filtrates prepared from CD tissue, but others failed to reproduce this work (18). Cave and associates (19) then reported their ability to inoculate rabbits intraserosally with tissue filtrates from patients with CD or UC and transmit inflammatory changes suggestive of organ specific

TABLE 1

ANIMAL TRANSMISSION STUDIES

1935	Mones & Sanjuan:	Transmitted ulcerative colitis to rabbits
1970	Mitchell & Rees:	Transmitted granulomas to mouse footpads
1973	Bolten, et al:	No transmission to mouse footpads
1973	Cave, et al:	Transmitted granulomas to rabbit ileum
1976	Cave, et al:	Transmitted ulceration to rabbit colon mucosa
1976 1977	Taub, et al: and Donelly, et al:	Control tissues produce granulomas in mouse footpads
1980	Das, et al:	CD filtrates produced lymphomas and splenic antigen in athymic mice
1980	Cohen, et al:	Transmitted granulomas to rabbit ileum
1980	IBD Research Group:	No transmission to rabbit ileum or colon

inflammatory bowel disease. Simonowitz et al (20), in attempting to confirm this work, were not able to transmit granulomas, but observed an inflammatory process in the bowel wall of rabbits inoculated with CD material. We have been unable to confirm this work (21a).

Mouse Transmission Studies

Mitchell and Rees (1970) (3) reported that they were able to induce granulomas in the foot pads of CBA mice by the injection of tissues from a patient with CD, but not by using control tissue. These granulomas evolved slowly over 3 months to 2 years and were present in normal and immunodeficient CBA mice. These findings have been partially confirmed by Taub et al (21) but not by Bolton et al (22) or Heatley et al (23) using different strains of mice. Subsequently, Mitchell and Rees extended their experiments and reported second generation passage of the histological changes. Pretreatment of the homogenates by filtration, autoclaving, irradiation and freezing has shown that the transmissible agent will pass a 220nm filter, withstands freezing, but is destroyed by autoclaving and irradiation. Cave et al (24) demonstrated similar findings in both normal and immunodeficient CBA mice and A_2G inbred mice. Frozen and 220nm filtrates were both effective at inducing granulomas given intraperitoneally or via the foot pads. Both Mitchell and Rees (4) and Cave et al (24) reported systemic spread of the granulomatous process to the intestine. This occurred late, at least 9 months after injection. Taub et al (25) suggested that the process is nonspecific since some of their controls induced similar changes. However, with control inocula these changes occurred early at 25 days and had disappeared in 150 days. Thus, this inflammatory reaction is temporally different and may have a different cause. Cohen et al (26) reported that C57Bl0/J and Balb/C mice were more susceptible to the granuloma inciting agent in CD tissues than CBA mice. He also noted transient granulomas in his control mice, but by 12 months only the CD mice showed persistence of the granulomatous reaction.

Cave et al (24) also noted that A_2G mice injected with UC tissues developed slowly evolving granulomas that could not be differentiated histologically from those induced by CD tissues. The inciting factor passed a 0.2μ filter and withstood freezing. The transmissible agents for CD and UC have been found in ileum, colon and mesenteric lymph nodes. On passage, the agent for CD has been demonstrated in ileum, lymph nodes, foot pads, liver and spleen.

Rabbit Transmission Studies

Cave et al (7) employed the New Zealand white rabbit (NZW) as an experimental animal because this animal is large enough to withstand serial biopsy of the intestine. In the initial study, frozen CD tissue homogenates induced slowly evolving granulomas in the ileum, colon, mesenteric lymph nodes and in the liver of some of the recipient rabbits. Control tissues did not incite any changes over the same 9 month period of observation. Subsequently, a larger study, using fresh CD tissue (6 patients), UC tissue (2 patients) and 5 with other diseases was initiated (27). In this study cell-free filtrates (220nm) induced a granulomatous response over 3 to 24 months in some of the recipient animals. Control animals were consistently negative. The rabbits injected with UC homogenates developed slowly evolving round cell infiltrates (28). This was predominantly mucosal in distribution, localized to the cecum and colon, but with rectal sparing. Successful passage of tissues from both the initial CD and UC rabbits followed direct injection of the bowel or IV injection using crude homogenates or cellfree filtrates. The latter route of injection revealed intestinal selectivity for the intestine of the histologic changes, which were similar to those in the first generation of animals. Furthermore, 5 of the CD donors were common to both the mouse and rabbit studies and 3 of these induced lesions in both species.

Simonowitz et al (8) used NZW rabbits and were able to induce a chronic inflammatory response in the colon, ileum and cecum of these animals. Lesions took 12 months to evolve

and were demonstrably different from control animals. They concluded that they had not transmitted chronic Crohn's disease, but they could not exclude the presence of a transmissible agent. Donnelly et al (29) again using NZW rabbits, induced a local inflammatory response with both CD and control tissue homogenates. They used a coarse homogenate. The response in an additional group of animals was abolished by preincubation of the homogenate with ampicillin. Orr et al (30) reported negative results with transmission to NZW rabbits as did Heatley et al (23). The latter group were also unsuccessful using Sprague Dawley rats and guinea pigs.

Virology

While these interesting transmission studies were being undertaken we were seeking agents in tissue filtrates from patients with CD and UC (31) (see Table 2). We reported a statistically significant serologic association of cytomegalovirus with UC. The failure of these studies to demonstrate an association between cytomegalovirus and CD was confirmed by the investigations of Roche and Huang (32), who used hybridization techniques; these investigations, however, did not evaluate the already established association of cytomegalovirus and UC.

Aronson et al (33) reported that biopsies of CD tissue and tissue from some other intestinal disorders produce a cytopathic effect (CPE) on early passage human diploid lung fibroblasts (W138). This effect could be passaged. Subsequent characterization suggests that the agents were small RNA viruses that were acid and ether stable, heat stable at 60^{o}C for one hour and labile in the presence of magnesium chloride. The agents were found to be pathogenic for newborn CBA mice. Beeken et al (34) extended these observations. Gitnick et al (35) using a different technology which included homogenization and filtration through a 0.2 micron filter showed that a cytopathic substance could be isolated from CD tissues and another material could be isolated from UC tissues (see Table 3). Both were shown to produce CPE in a rabbit ileal cell line

TABLE 2

STUDIES OF VIRUSES AND CYTOTOXINS
IN CROHN'S DISEASE AND ULCERATIVE COLITIS

1962	Schnierson et al:	No viruses found in UC or CD
1973	Farmer & Gitnick:	No specific viral serology in CD. CMV superinfection in UC
1975	Aronson, Beeken, Phillips:	Small RNA virus in CD using WI-38 tissue cultures
1976	Greenberg & Kapikian:	Mycoplasma hyorhinas and SV40 found in Aronson, et al materials
1976	Gitnick et al:	Development of sensitive tissue culture systems for CD CPE and EM studies
1976	Korsmeyer et al:	Anti RNA antibodies in IBD patients and relatives but not in matched controls
1977	Strickland et al:	Lymphocytotoxic antibodies in IBD patients and spouses but not in matched controls
1977	Whorwell, Phillips,Beeken:	Isolation of reovirus-like agent in CD
1977	Riemann et al:	EM of virus-like particles in CD
1977	Cooper et al:	CMV in UC toxic megacolon
1980	Phillpotts et al:	? Tissue factors in CD using Wl38 but no CPE in rabbit ileum or avian tissue culture
1980	IBD research group:	Confirmed UC and CD CPE in each tissue culture system
1981	McLaren and Gitnick:	Heat labile cytotoxin in CD Heat stable cytotoxin in UC and colon CA Heat labile cytotoxin in UC and colon CA

TABLE 3

CPE INDUCERS IN GASTROINTESTINAL DISEASE

	NO.	+CPE
CROHN'S DISEASE		
Ileitis	36	35
Colitis	27	27
Ileocolitis	7	7
ULCERATIVE COLITIS		
Backwash Ileitis	2	2
Colon	27	27
COLON CARCINOMA[+]	27	27
RADIATION ENTERITIS	5	2
NECROTIZING ENTEROCOLITIS	1	1
COLONIC POLYPS BENIGN	2	0
FAMILIAL POLYPOSIS BENIGN	2	1
VOLVULUS	1	0
HIRSCHSPRUNG'S DISEASE	2	0
DIVERTICULITIS	23	1
NORMAL ILEUM	5	0
NORMAL COLON	1	0

[+]Grossly normal intestinal tissue studied

and subsequently were shown to destroy RIF free chick embryo fibroblasts and Peking duck embryo fibroblasts. Neutralization tests employing human sera as well as guinea pig immune sera have suggested that these agents are not immunologically identical. Preliminary electronmicroscopic evidence also suggested differing morphologies. The fact that the cytopathic inducers could be serially passaged made it conceivable that these are either living, replicating agents of a size consistent with a virus or highly concentrated toxic materials. Whorwell et al (36) suggested that the agents were Rotaviruses; however, this was refuted serologically with temporally matched control sera. Phillpotts et al (37) using other methods than previously reported suggested that the cytopathic change was induced by low and middle molecular weight nonviable tissue factors. The nature of these factors was not identified. They did not attempt exact reproduction of the previous reports by using identical techniques used by others. Greenberg et al (38) reported finding a Mycoplasma hyorhinis contaminating some of the cultures used by Aronson et al, but were unable to find Mycoplasma in the cultures reported by Gitnick et al. Reimann et al (39) presented electronmicroscopic evidence of viruslike particles in CD tissue and Dourmashkin et al (40) described a possible precursor to the apthoid ulcer and myxovirus-like budding from the surface of epithelial cells.

Studies by Strickland et al (41) have identified high levels of antibodies to synthetic double stranded RNA and lymphocytotoxic antibodies in the sera of patients with either CD or UC and their unaffected spouses. These studies also revealed high levels of circulating interferon in patients with IBD. Cellular mechanisms of tissue damage in IBD are suggested by several studies which have investigated the in vitro cytotoxic effect of IBD peripheral blood lymphocytes (PBL) to colon epithelial cells in short term culture (42). The cell responsible for this effect is reported to have the characterics of a K-cell (43). Recent studies of circulating nonspecific K-cell activity in IBD have yielded conflicting

results. Increased K-cell activity in CD using a plaque assay was reported by Eckhardt et al (44). In contrast, Britton et al (45) using an antibody dependent cellular cytotoxicity (ADCC) assay with a mouse lymphoma line as a nonspecific target reported insignificant differences between PBL from CD and normal subjects. The possible role of K-cells in the bowel damage of IBD has been further clouded by reports that this cell is absent in lymphoid populations derived from the intestine, both in IBD and in control subjects. The study by Britton et al (45) clearly showed enhanced K-cell activity in mesenteric lymph node cells from patients with CD when compared to disease control mesenteric node cells. Recent work by Chiba et al (46) indicates that a major reason for the apparent lack of K-cell activity reported by other investigators in intestinally derived lymphoid cells is selective deflection of this subpopulation during the prolonged (18 hour) process of lymphoid cell isolation from the gut.

Effector lymphocytes that are responsible for in vitro cytotoxicity against virus infected target cells in humans may include both T-cells (47-49) and K-cells (50-53). Further, interferon has been shown to substantially enhance spontaneous cell mediated cytotoxicity (SCMC) activity against virus infected targets (54). In addition to being potentially important mechanisms of resistance to recovery from viral infection such reactions may also be important in the genesis of chronic tissue damage. For example, the development of chronic hepatitis in patients with hepatitis B infection has been linked to such cytotoxic effects (55). Similar mechanisms could be postulated as contributing to the chronic intestinal damage in IBD.

Thus transmissible cytopathic agents have been described in CD and UC. Although several laboratories have reported the existence of such agents, others have been unable to isolate them. Many problems have existed in extending the initial reports. Among these have been the inability to cultivate the cytopathic materials to high enough titer to

allow proper characterization. The low titer of the cytopathic materials also precluded obtaining adequate electron microscopic photos of these putative agents. Recently one group suggested that the transmissible materials could not be found in tissue culture utilizing modifications of tissue culture systems initially reported but utilizing other systems a cytopathic toxic factor could be identified (37). Subsequently we reported that following inoculation of tissue culture with filtrates from CD and UC 0.2 micron filtrates a transient cytopathic effect (CPE) could be seen in rabbit ileum tissue culture. After this, the tissue culture system recovered and then a definite and extensive CPE developed. In contrast, in chick embryo tissue culture within 48 hours of inoculation extensive CPE developed leading to destruction of the cell sheath and no late CPE inducer could be demonstrated. We further reported that the factor responsible for the early transient CPE in rabbit ileum tissue culture and the early severe CPE in chick embryo tissue culture, was a nonviable toxin unrelated to C. difficile or other clostridial toxins and unrelated to E. coli toxin. However, this finding did not necessarily explain the late extensive CPE which developed in rabbit ileum tissue culture and which was the basis for initial reports of the transmissible CPE inducer derived from CD or UC materials.

Progress in the control of Crohn's disease and ulcerative colitis is unlikely until a better understanding of the pathogenesis of these diseases is achieved. In our ongoing studies seeking the role if any, of infectious agents in the pathogenesis of CD and UC we have recently described the presence of cytotoxins capable of destroying colon, ileal and chick embryo cells while sparing 16 other tissue culture systems (56). These cytotoxins may be of bacterial, viral or tissue origin. If these cytotoxic substances were of tissue origin the narrow range of cell cytotoxicity would be difficult to explain. The selective cytotoxicity for colon, ileum and chick embryo cells while sparing other tissue culture systems would not be characteristic of a general cell toxin. They

are not immunologically related to nor do they have the physical and chemical properties of C.difficile or E.coli toxins.

The inducers of cytopathology which have now been partially characterized and isolated from CD, UC and some control speciments may represent the effector system responsible for the final development of tissue destruction in these diseases. This hypothesis is based on the reproducible finding of these cytopathic inducers in patients with these illnesses and the unique ability of these cytotoxins to destroy only a narrow range of host cells. This range includes colon, ileum or chick embryo cells but excludes most other human and animal tissue systems. The finding of this narrow range of cytotoxicity suggests the possibility that these inducers of cytotoxicity may represent one step in the pathway which leads to tissue destruction in these illnesses. One hypothesis for the development of UC or CD is outlined in Figure 1. It is conceivable that the large and small intestine may react in only a limited number of ways to a variety of insults. A number of inciting agents or combinations of inciting agents may initiate the processes leading to these diseases. These inciting agents may include viruses or viral products, bacteria or bacterial products such as endotoxins, environmental toxins and/or food antigens. In a susceptible host, these may stimulate immune mediation which may lead to the process which eventually produces a cytotoxin that in turn produces cell destruction. Alternatively these cytotoxins may have no pathogenic role in spite of their limited range of cytotoxicity.

FIGURE 1

PATHOGENESIS OF ULCERATIVE COLITIS OR CROHN'S DISEASE

HYPOTHESIS

Inciting agent
Virus or viral product
Bacteria or bacterial product
Environmental Toxin
and/or
Food antigen
↓
Susceptible host
↓
Immune mediation
↓
Cytotoxin Effector
↓
Cell Destruction

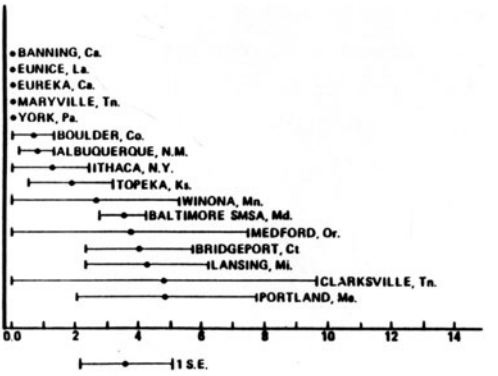

FIGURE 1. ANNUAL AGE ADJUSTED INCIDENCE RATES OF DEFINITE AND PROBABLE CROHN'S DISEASE, PER 100,000 POPULATION, WITH STANDARD ERRORS, BY GEOGRAPHIC AREA, WHITE, PAS AREAS, 1973

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