J Gastroenterol 1996; 31:199–206

Journal of Gastroenterology

Extraintestinal manifestations of granulomatous enterocolitis induced in rabbits by long-term submucosal administration of muramyl dipeptide emulsified with Freund's incomplete adjuvant

Kiyoo Kuroe, Yoichi Haga, Osamu Funakoshi, Ichiro Mizuki, Kosuke Kanazawa, and Yutaka Yoshida

First Department of Internal Medicine, Hirosaki University School of Medicine, 5 Zaifucho, Hirosaki, Aomori 036, Japan

Abstract: We examined whether extraintestinal manifestations of granulomatous enterocolitis in rabbits might be produced by the long-term administration of muramyl dipeptide which represents the basic fragment of the bacterial cell wall, emulsified with Freund's incomplete adjuvant. Muramyl dipeptide emulsion was injected submucosally at six sites in the rectum and colon, 10 cm proximal to the anus, each time with a flexible endoscope. Seven rabbits were injected nine times or more every month, and all were sacrificed 1 month after the last injection. The histological changes in the colon in the seven rabbits were mononuclear cell infiltration, epithelioid granulomas, granulomatous lesion, and denuded and regenerative epithelia, although the changes differed in degree. In five of the seven rabbits, histological examination of the liver showed pericholangitis and periductal fibrosis, findings analogous to sclerosing cholangitis in patients with inflammatory bowel disease. In four of the seven rabbits, fibrosis bridging mainly between portal and portal veins, and, in places, between portal and central veins, was seen. Two of the seven rabbits developed polyarthritis. The histological changes in our model suggest that continuous stimulation with bacterial cell wall fragments may be involved in the extraintestinal manifestations of chronic intestinal inflammation such as that seen in inflammatory bowel disease.

Key words: muramyl dipeptide, granulomatous enterocolitis in rabbits, extraintestinal manifestations, pericholangitis, arthritis

Offprint requests to: K. Kuroe

(Received for publication on May 8, 1995; accepted on July 28, 1995)

Introduction

The commensal intestinal flora produce or contain a number of potent inflammatory products such as lipopolysaccharide, peptidoglycan-polysaccharide (PG-PS) complexes, muramyl peptides, and N-formylmethionyl-oligopeptides, to which the intestinal mucosa is exposed continuously. The healthy gut mucosa is known to have a defensive barrier against the potentially proinflammatory products of luminal commensal bacteria. This barrier may be destroyed as a consequence of mucosal damage in intestinal inflammation. In intestinal inflammation, bacterial products may be absorbed through the defective barrier, and initiate and perpetuate local and systemic inflammation. We have previously reported granulomatous enterocolitis induced in rabbits by the injection of N-acetylmuramyl-L-alanyl-D-isoglutamine (muramyl dipeptide, MDP), emulsified with Freund's incomplete adjuvant (FIA). MDP, which represents the basic fragment of the bacterial cell wall and has adjuvant activity, is normally present within the gut lumen. The histological changes of the colon in our animal models showed mononuclear cell infiltration, epithelioid granulomas with occasional giant cells, poorly-formed granulomas, lymphoid aggregations, and transmural infiltration.[1] There results suggested that bacterial products could be involved in the pathogenesis of human chronic intestinal inflammation such as inflammatory bowel disease (IBD).

Here we report the extraintestinal manifestations of granulomatous enterocolitis in rabbits induced by the long-term administration of MDP mixed with FIA.

Materials and methods

Male Japanese white rabbits, weighing 2 kg, were purchased from a local breeder and used for all experiments.

Induction of colitis and study protocol

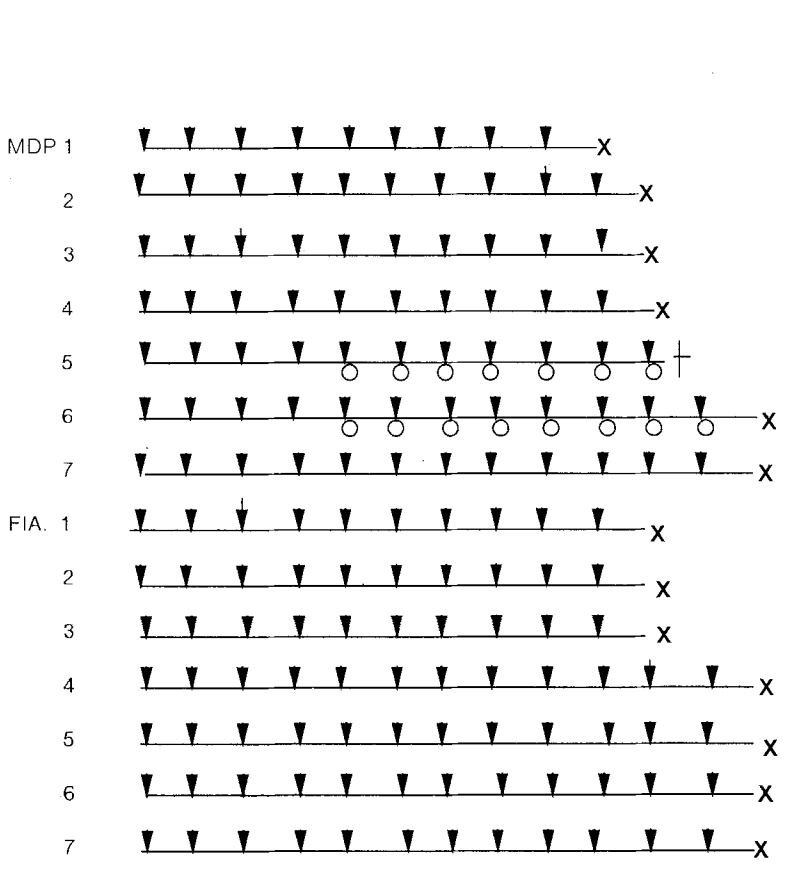
This animal model was induced by the method described in our previous study.[1] Briefly, 100 μg of MDP (Chemicon, Temecula, Calif.) was dissolved in 0.05 ml of phosphate-buffered saline (PBS, pH7.2) and emulsified with an equal volume of Freund's incomplete adjuvant (FIA; Difco, Detroit, Mich.). The emulsion 0.1 ml was injected submucosally at six sites in the rectum and colon, 10 cm proximal to the anus. Injection to the rectum and colon was performed by using a flexible endoscope (Olympus BF type p10; Tokyo, Japan) and a polyethylene catheter with a 23-gauge, 5-mm retractable needle (Sumitomo Bakelite, Tokyo, Japan) for variceal sclerotherapy of the esophagus. MDP emulsion was injected every month nine or more times into seven rabbits. The animals were subsequently sacrificed under ether anesthesia 1 month after the last injection. As controls, FIA alone, emulsified with an equal volume of PBS, was injected into seven rabbits (Fig. 1).

Assessment of the colon, liver, and arthropathy

The small and large intestines, liver, spleen, and lung were examined grossly, and prepared for pathological examinations. In addition, the right swollen ankle joint in one of two rabbits with polyarthritis was decalcified with formic acid. These tissues were fixed in buffered-formalin and embedded in paraffin wax. Sections (5-μm-thick) were stained with hematoxylin and eosin, or with azan.

Measurement of anti-MDP antibody and immune complex

MDP was conjugated to methylated bovine serum albumin (MeBSA; Sigma, St. Louis, Mo.) according to the method of Reichert et al.[2] slightly modified by Bahr et al.[3] Two normal rabbits were immunized subcutaneously with 10 mg of conjugated MDP-MeBSA, equivalent to 1.05 mg of MDP, each time. They were immunized every week, five times, and were



	pericholangitis and periductal fibrosis	arthritis	optical density anti-MDP	optical density I C
MDP 1	+	-	0.083	0.115
2	+	-	0.105	0.092
3	-	-	0.094	0.103
4	+	-	0.143	0.242
5	+	+	0.149	0.925
6	-	+	0.087	0.540
7	+	-	0.107	0.262
FIA. 1	-	-	0.088	0.060
2	-	-	0.089	0.158
3	-	-	0.087	0.123
4	-	-	0.091	0.172
5	-	-	0.107	0.132
6	-	-	0.090	0.172
7	-	-	0.080	0.159

Fig. 1. Regimens for the submucosal injections of muramyl dipeptide (*MDP*) emulsion or Freund's incomplete adjuvant (*FIA*) only in the colon. Time points of injection, sacrifice, occurrence of arthritis and death or sickness, are shown as arrows, ×, circles, and †, respectively. The presence or absence of pericholangitis and periductal fibrosis, and arthritis, are shown as + and −, respectively, and the optical densities of sera from the rabbits injected with MDP emulsion or FIA alone are shown

bled 1 week after the last injection. The sera from the 2 rabbits served as positive controls for anti-MDP antibody. In addition, the sera of 7 rabbits, taken just before the eighth injection of MDP emulsion or FIA alone, and the sera of 16 normal rabbits were stored at −20°C until use to examine anti-MDP antibody titers and immune complex (IC) levels. Anti-MDP antibody titers were measured in triplicate by enzyme-linked immunosorbent assay (ELISA), performed by the method of Bahr et al.[3] Briefly, A--L-MDP (Sigma; 10 μg/ml), conjugated according to the method of Chedid et al.,[4] was coated onto the wells of micro-ELISA plates (Corning, Corning, N.Y.) in a carbonate/bicarbonate buffer, pH9.6, for 16 h at 4°C. After the coating, the wells washed in PBS, pH7.4, containing 0.05% v/v Tween 20, and rabbit sera, diluted 100-fold with PBS containing 2% bovine serum albumin, were added to the wells. Horseradish peroxidase (HP)-labeled goat anti-rabbit Ig antibody (Dako, Glostrup, Denmark) was diluted to 1:2000 in PBS and added to the coated wells. Incubation was performed for 16 h at 4°C, and the unbound enzyme was washed out with PBS. The amount of enzyme bound to the wells was assayed using *o*-phenylenediamine and hydrogen peroxide. The reaction was stopped by the addition of 5N H_2SO_4, and absorbance at 490 nm was immediately read with a model 3550 Microplate Reader (Bio-Rad, Tokyo, Japan). IC levels were measured in triplicate with a Clq solid-phase enzyme immunoassay. In this assay, Clq-coated plates (SRL, Tokyo, Japan) were used as solidphase and HP-labeled swine anti-rabbit IgG (Dako) as an indicator system. Aggregated rabbit IgG (ARG), prepared by a modification of the method of Nydegger and Svehag,[5] was used as a model of IC, and the sera of nine rabbits immunized with various human antigens were used as positive controls for IC: the sera of two rabbits immunized with α1-antitrypsin, the sera of three rabbits immunized with IgM, the sera of three rabbits immunized with haptoglobin, and the sera of one rabbit immunized with myoglobin. ARG and sera were diluted to 1:200 with PBS and added to the Clq-coated wells. Incubation was performed for 90 min at room temperature. The wells were washed, and HP-labeled anti-rabbit IgG, at 1:5000 dilution, was then added and the plates were incubated for 90 min at room temperature. The amount of enzyme bound to the wells was assayed in the same way as the ELISA of the anti-MDP antibody. Variations in absorbance values among triplicate experiments were negligible; hence, only mean values are presented (Fig. 1).

Since anti-MDP antibody titers were 0.088 ± 0.011 (M ± SD) in 16 normal rabbits by an ELISA method, values exceeding 0.110 (M + 2SD) were assessed as positive. IC levels measured by the Clq assay were 0.118 ± 0.039 (M ± SD) in 16 normal rabbits. Values exceeding 0.194 (M + 2SD) were assessed as positive.

Statistical analysis

Statistical significance was evaluated by Wilcoxon's test or by Fisher's exact probability test; $P < 0.05$ was regarded as significant. Since sample numbers were small, values greater than mean + 2 SD were defined as positive, similar to values determined by drawing a receiver operator characteristic (ROC) curve.[6]

Results

Two of the seven rabbits injected with MDP emulsion developed clinical redness and swelling of both wrist and ankle joints after the 5th injection of MDP emulsion. One of the two rabbits developed diarrhea and weight loss, and died of illness 4 days after the 11th injection. In the seven rabbits injected with MDP emulsion, colonic specimens examined at necropsy showed intestinal thickening. The mucosal surfaces of the fixed colons were slightly granular in places. No gross abnormal findings were seen in the livers, spleens, kidneys, or lungs.

Colon damage

The histological changes of the colon in the 7 rabbits injected with MDP emulsion were similar to those seen in our previous study.[1] Additional changes were degeneration, extensive denudation, and regeneration of the epithelia of the colon, observed 10 cm proximal to the anus in 3 of the 7 rabbits, and distant from the injection sites, approximately 40 cm from the anus, in 2 rabbits. Colonic specimens of the 7 rabbits injected with FIA alone showed sporadic granulomatous lesions composed of foamy histiocytes and scattered eosinophils similar to those seen in our previous study. The sera of 2 of 7 rabbits injected with MDP emulsion were positive for anti-MDP antibody. However, the sera from the 7 rabbits injected with FIA alone were all negative. The ODs of two positive controls were 0.621 and 0.437, respectively. The sera of 4 of 7 rabbits injected with MDP emulsion revealed positivity for ICs. ARG and positive controls showed a striking increase of ICs (0.517 and M ± SD; 1.235 ± 0.689, respectively. Fig. 2). The levels of anti-MDP antibody in all 7 rabbits with colitis injected with MDP emulsion were significantly higher than those in the 16 normal control rabbits ($P < 0.05$), while the levels of IC were not higher than those in controls. There was no significant correlation between the positive rate for anti-MDP antibody and that for IC by Fisher's exact probability test.

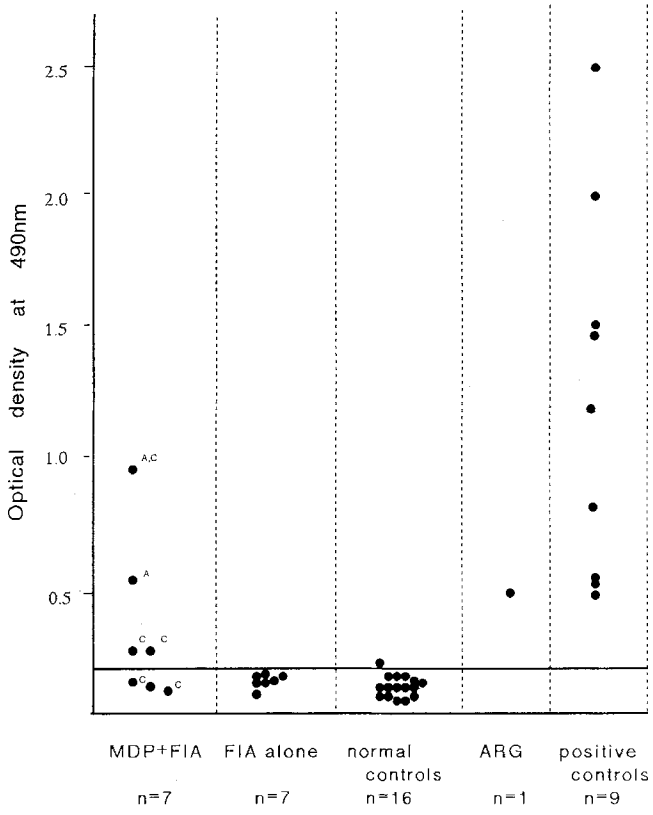


Fig. 2. Immune complex levels in rabbits injected with MDP emulsion. The sera of 4 of 7 rabbits injected with MDP + FIA were positive. The sera of 2 rabbits with polyarthritis in this group showed higher levels of immune complexes than of aggregated rabbit IgG (*ARG*). The sera of rabbits with polyarthritis and pericholangitis are indicated by *A* and *C*, respectively

Liver damage

Histological examination of the liver showed diffuse mononuclear cell infiltration, predominantly lymphocytes (Fig. 3B), and fibrosis around bile ducts in the portal tracts in 5 of the 7 rabbits injected with MDP emulsion, such findings not being detected in normal control rabbits (Fig. 3A). The hepatocytes showed no abnormal changes. Ductal narrowing, obliteration of smaller ducts, or bile duct proliferation were seen in some areas of stellate expansions of the portal tracts (Figs. 3C, 4C). The walls of the damaged ducts were infiltrated with lymphocytes, and the epithelium was degenerative and destroyed. In addition, fibrosis bridging, mainly between portal and portal veins, and, in places, between portal and central veins, was seen in 4 of the 5 rabbits with pericholangitis (Fig. 4B). Colonic specimens in 2 of the 5 rabbits with pericholangitis showed denuded and regenerative epithelia, and those in 3 of the 5 rabbits showed relatively normal surface epithelia with chronic inflammation. Figure 4A shows normal liver tissues stained with azan from a control rabbit. The liver specimens of the 7 rabbits injected with FIA alone showed mild histiocyte infiltration and proliferation of fibroblasts around bile ducts (Figs. 3D,E and 4D,E). The levels of anti-MDP antibody in the 5 rabbits with pericholangitis were significantly higher than those in the 16 normal controls ($P < 0.05$). However, neither anti-MDP antibody nor IC levels were significantly different in the presence and absence of pericholangitis in the 7 rabbits with colitis.

Arthropathy

Histological examination of the red, swollen ankle joint showed profound destruction. The subchondral bony cortex and medulla were replaced by masses of invading connective tissue pannus as well as by inflammatory cells, predominantly plasma cells and lymphocytes. Some residual ragged cartilage remained. Activated inflammatory and fibroblastic pannus had invaded joint spaces as well as the subchondral bone (Fig. 5). The colonic specimens of the 2 rabbits with polyarthritis showed denuded and regenerative epithelia or relatively normal surface epithelia with chronic inflammation. The injection of FIA alone did not induce arthritis in the 7 rabbits. In addition, there were no differences in the degree of colonic inflammation between rabbits with and without arthritis. The IC levels in the 2 rabbits with polyarthritis were significantly higher than those in the 16 normal control rabbits ($P < 0.05$). The sera from the 2 rabbits with polyarthritis had higher levels of ICs than ARG (Fig. 2). However, neither anti-MDP antibody nor IC levels were significantly different in the presence and absence of polyarthritis in the 7 rabbits with colitis. Histological changes of organs other than the liver, i.e., the spleen, kidneys, and lungs, showed no abnormal findings in any of the 14 rabbits in all experiments. In short, of the 7 rabbits injected with MDP emulsion, 7 developed colitis, 5 pericholangitis, and 2 polyarthritis.

Discussion

An animal model of chronic intestinal inflammation could be useful for promoting understanding of the pathogenesis of IBD and for elucidating the possible causes. PG-PS-induced granulomatous enterocolitis[7] and extraintestinal manifestations have been demonstrated in a rat model, and insulin-like growth factor has recently been suggested to be relevant to the development of fibrosis in this model.[8] This model demonstrated that bacterial cell wall fragments were capable of producing chronic granulomatous inflammation in the intestine and mesenteric lymph nodes. Further, we

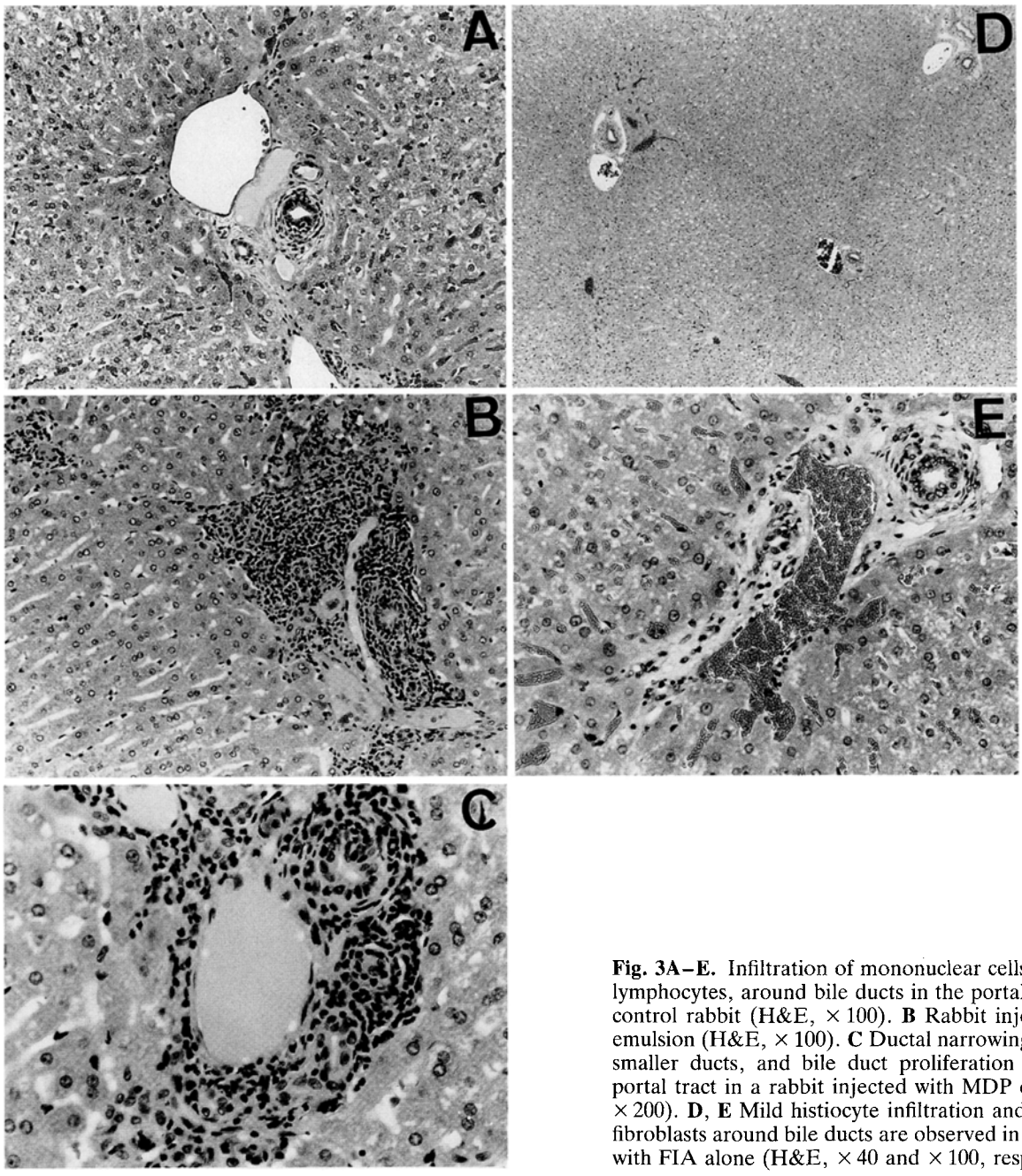


Fig. 3A–E. Infiltration of mononuclear cells, predominantly lymphocytes, around bile ducts in the portal tract. **A** normal control rabbit (H&E, × 100). **B** Rabbit injected with MDP emulsion (H&E, × 100). **C** Ductal narrowing, obliteration of smaller ducts, and bile duct proliferation are seen in the portal tract in a rabbit injected with MDP emulsion (H&E, × 200). **D, E** Mild histiocyte infiltration and proliferation of fibroblasts around bile ducts are observed in a rabbit injected with FIA alone (H&E, × 40 and × 100, respectively)

have reported that granulomatous colitis was induced in rabbits by injections of MDP emulsified with Freund's incomplete adjuvant.[1] MDP, a subunit of peptidoglycan polymers, exists linked to other cell wall components. Many commensal bacteria on mammals possess glycopeptides similar to MDP in structure and bioactivity. MDP is known to have adjuvant activity and antigenicity as a hapten, and to be granulomagenic with mineral oil or branched fatty acids.[9–11]

We investigated whether extraintestinal manifestations of chronic intestinal inflammation similar to IBD could be produced by continuous, long-term stimulation with bacterial cell wall fragments. On the basis of our previous findings that chronic inflammatory changes of the colon continued for 4 weeks after a single injection of 100 μg MDP, we injected MDP emulsion every month long-term to provide continuous stimulation. In the process of repeated MDP injections, anti-MDP antibodies were induced in some rabbits. However, the pathogenesis of this colitis does not seem to be relevant to anti-MDP antibody, since we were able to induce colitis in rabbits even 1 week after a single shot of MDP emulsion and these animals had no positive anti-MDP antibodies.[1]

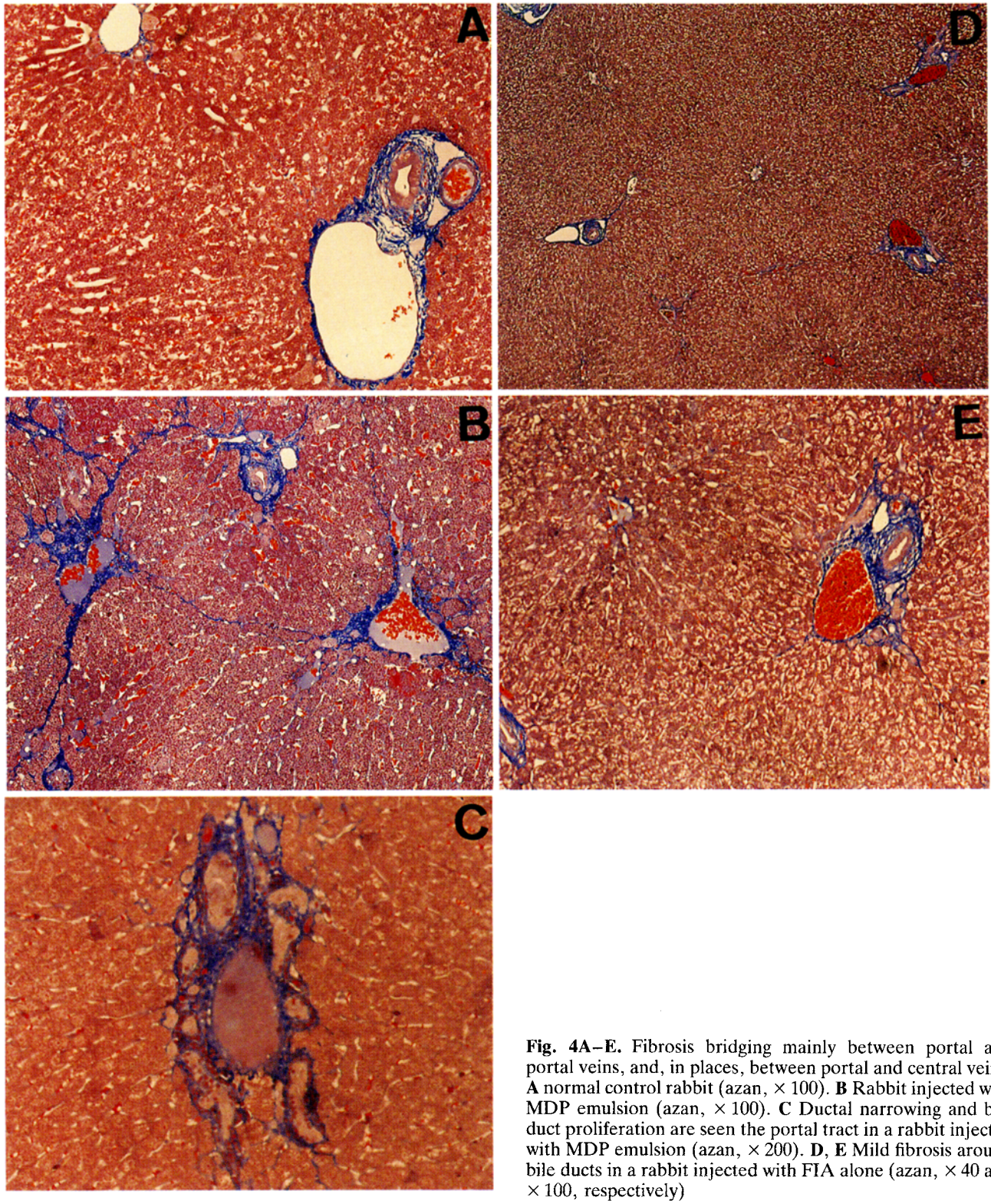


Fig. 4A–E. Fibrosis bridging mainly between portal and portal veins, and, in places, between portal and central veins. **A** normal control rabbit (azan, × 100). **B** Rabbit injected with MDP emulsion (azan, × 100). **C** Ductal narrowing and bile duct proliferation are seen the portal tract in a rabbit injected with MDP emulsion (azan, × 200). **D, E** Mild fibrosis around bile ducts in a rabbit injected with FIA alone (azan, × 40 and × 100, respectively)

Extraintestinal manifestations such as pericholangitis and portal fibrosis occur frequently in IBD patients, but the pathogenetic mechanism has yet to be clarified. Kono et al.[12] described experimental portal fibrosis in rabbits produced by the intraportal injection of killed nonpathogenic *Escherichia coli* after laparotomy. Hobson et al.[13] and Yamada et al.[14] reported bile duct changes in a rat colitis model induced by the infusion of

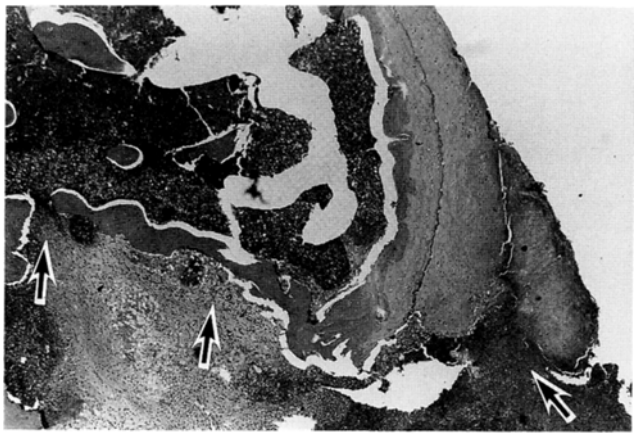


Fig. 5. Subchondral bony cortex and medulla have been replaced by masses of invading connective tissue pannus, as well as by inflammatory cells (*arrows*) in a rabbit injected with MDP emulsion. H&E, × 70

acetic acid and N-formyl methionyl leucyl tyrosine (fMLT). In our experiment, repeated submucosal injections of MDP emulsion induced pericholangitis and portal fibrosis. In addition, the liver specimens of four rabbits showed bridging fibrosis, which has been suggested to progress to cirrhosis. These histologic features mimic the pericholangitis that is frequently seen in IBD patients. However, these models exhibited milder histological findings in regard to the degree of periductal fibrosis compared with those in IBD patients. In three of the five rabbits with pericholangitis, the surface epithelia of the colon remained relatively normal, with an undamaged epithelial barrier but chronic inflammation. Therefore, it would seem that luminal antigens did not play a secondary role in the pericholangitis in our experiment. In our previous study,[1] the submucosal injection of MDP alone induced negligible infiltration of lymphocytes and plasma cells in the lamina propria of the colon. In addition, MDP alone did not induce abnormal findings in the liver (data not shown). It is possible that part of the MDP incorporated in the water-in-oil emulsion may have leaked out and entered the portal vein from the colon, then being slowly released and eliciting an inflammatory response in the liver. However, the reason that only bile ducts were destroyed has yet to be clarified. The histological changes of the liver in rabbits injected with FIA alone were slight histiocyte infiltration and proliferation of fibroblasts around bile ducts, in contrast to the diffuse lymphocyte infiltration and fibrosis in rabbits injected with MDP emulsion. FIA, which does not contain a mycobacterial component, is reported to induce infiltration of histiocytes with no plasma cells and inconspicuous lymphocytes.[15,16] These changes may have been provoked by a small amount of FIA that entered the portal vein.

Arthritis is the most common extraintestinal manifestation in IBD. In our model, arthritis was seen in two of the seven rabbits injected with MDP. MDP is reported to induce polyarthritis, similar to adjuvant-induced arthritis, when injected in the form of an oily emulsion.[17,18] The pathologic changes in our model were basically indistinguishable from those of classic adjuvant-induced arthritis.[19] No differences were detected in the degree of colonic inflammation between the rabbits with arthritis and those without arthritis, suggesting that luminal antigens do not play a secondary role in polyarthritis. Thus, it is possible that MDP emulsion was deposited around joints, particularly in the synovial membrane and subsynovial tissues, and that it induced inflammatory reactions, through the various biologic activities of cytokines, such as the activation of macrophages and lymphocytes.

The extraintestinal manifestations of chronic intestinal inflammation have long been considered to be mediated immunologically. The presence of circulating autoantibodies or ICs in IBD patients with extraintestinal manifestations supports this idea, but it is still controversial.[20–22] In our experiment, in the seven rabbits with colitis, there was no correlation between the presence of extraintestinal manifestations and the levels of anti-MDP antibody or IC. However, since our sample number was small, further study is clearly needed.

The histological changes shown in our model in this study suggest that continuous stimulation with bacterial cell wall fragments may be involved in the extraintestinal manifestations of chronic intestinal inflammation. Further studies of lipid-MDP complexes, such as fatty acid-MDP complex, are necessary to elucidate these mechanisms.

References

1. Kuroe K, Haga Y, Funakoshi O, et al. Rabbit granulomatous enterocolitis induced by injection of muramyl dipeptide emulsified with Freund's incomplete adjuvant. J Gastroenterol 1995;30: 472–477.
2. Reichert CM, Carelli C, Jolivet M, et al. Synthesis of conjugates containing N-acetylmuramyl-L-alanyl-D-isoglutaminyl (MDP). Their use as hapten-carrier systems. Mol Immunol 1980;17: 357–363.
3. Bahr GM, Carelli C, Audibert F, et al. Analysis of the antigenic relationship of various derivatives of N-acetyl-muramyl-L-ala-D-isoglutamine (MDP), using anti-MDP antibodies. Mol Immunol 1982;19:737–745.
4. Chedid L, Parant M, Parant F, et al. Enhancement of certain biological activities of muramyl dipeptide derivatives after conjugation to a multi-poly(DL-alanine)–poly(L-lysine) carrier. Proc Natl Acad Sci USA 1979:76:6557–6561.
5. Nydegger UE, Svehag S-E. Improved standarization in the quantitative estimation of soluble immune complexes making use of an international reference preparation. Results of a collaborative multicentre study. Clin Exp Immunol 1984;58:502–509.

6. Sackett DL, Haynes RB, Tugwell P. Clinical epidemiology. 1st Ed. Boston: Little, Brown, 1985;106–107.
7. Sartor RB, Cromartie WJ, Powell DW, et al. Granulomatous enterocolitis induced in rats by purified bacterial cell wall fragments. Gastroenterology 1985;89:587–595.
8. Zimmermann EM, Sartor RB, McCall RD, et al. Insulin-like growth factor I and interleukin 1β messenger RNA in a rat model of granulomatous enterocolitis and hepatitis. Gastroenterology 1993;105:399–409.
9. Emori K, Tanaka A. Granuloma formation by synthetic bacteria cell wall fragment: Muramyl dipeptide. Infect immun 1978; 19:613–620.
10. Tanaka A, Emori K. Epithelioid granuloma formation by a synthetic bacterial cell wall component, muramyl dipeptide (MDP). Am J Pathol 1980;98:733–748.
11. Emori K, Nagao S, Shigematsu N, et al. Granuloma formation by muramyl dipeptide associated with branched fatty acids, a structure probably essential for tubercle formation by *Mycobacterium tuberculosis*. Infect Immun 1985;49:244–249.
12. Kono K, Ohnishi K, Omata M, et al. Experimental portal fibrosis produced by intraportal injection of killed nonpathogenic *Escherichia coli* in rabbits. Gastroenterology 1988;94:787–796.
13. Hobson CH, Butt TJ, Ferry DM, et al. Enterohepatic circulation of bacterial chemotactic peptide in rats with experimental colitis. Gastroenterology 1988;94:1006–1013.
14. Yamada S, Ishii M, Liang LS, et al. Small duct cholangitis induced by N-formyl L-methionine L-leucine L-tyrosine in rats. J Gastroenterol 1994;29:631–636.
15. Carter RL, Jamison DG, Vollum RL. Histological changes evoked in mice by Freund's incomplete adjuvant. J Pathol Bacteriol 1968;95:471–476.
16. Yamanaka M, Hiromatsu K, Hirahara T, et al. Pathological studies on local tissue reactions in guinea pigs and rats caused by four different adjuvants. J Vet Med Sci 1992;54:685–692.
17. Kohashi O, Aihara K, Ozawa A, et al. New model of a synthetic adjuvant, N-acetylmuramyl-L-alanyl-D-isoglutamine-induced arthritis. Lab Invest 1982;47:27–36.
18. Chang Y, Pearson CM, Chedid L. Adjuvant polyarthritis. V. Induction by N-acetylmuramyl-L-alanyl-D-isoglutamine, the smallest peptide subunit of bacterial peptidoglycan. J Exp Med 1981;153:1021–1026.
19. Pearson CM. Development of arthritis, periarthritis and periostitis in rats given adjuvants. Proc Soc Exp Biol Med 1956;91:95–101.
20. Chapman RWG, Marborgh BÅ, Rhodes JM, et al. Primary sclerosing cholangitis: A review of its clinical features, cholangiography, and hepatic histology. Gut 1980;21:870–877.
21. Zauli D, Schrumpf E, Crespi C, et al. An autoantibody profile in primary sclerosing cholangitis. J Hepatol 1987;5:14–18.
22. McEwen C, Lingg C, Kirsner JB, et al. Arthritis accompanying ulcerative colitis. Am J Med 1962;33:923–941.