10.1148/radiol.2016152387

Appendix E1

Immunohistochemistry

Fixed colon tissue segments from mice with inflammatory bowel disease (IBD) or from healthy mice were frozen at −80°C and were embedded in Tissue-Tek optimal cutting temperature compound (Sakura Finetek, Torrance, Calif). The 7-μm-thick slices were cut by using a cryotome at −20°C and were mounted on glass slides. Tissue slices were washed with phosphate-buffered saline and were made permeable with 0.2% Triton X-100 (Sigma-Aldrich, St Louis, Mo). Slices were immunostained overnight at 4°C with the rat antimouse macrophage-specific antibody F4/80 (MCA497GA; AbD Serotec, Raleigh, NC) at a 1:100 dilution. Then, slices were stained with rabbit antirat immunoglobulin G secondary antibody conjugated to Alexa 488 (21210; Invitrogen, Carlsbad, Calif) at a 1:500 dilution for 2 hours at room temperature and were counterstained with 4',6-diamidino-2-phenylindole, dihydrochloride (or DAPI). Slices were examined by using a microscope (Apotome; Zeiss, Oberkochen, Germany) to examine the colocalization of fluorescence from the VS-1000H DM Red perfluorocarbon (PFC) emulsion with that of green fluorescent Alexa 488.

Histologic Grading of Inflammation and Dysplasia

Fixed colon tissues were washed with phosphate-buffered saline and were cut longitudinally to investigate possible tumor outgrowth. On the basis of hydrogen 1 (^{1}H) and fluorine 19 (^{19}F) merged magnetic resonance (MR) images and anatomic structures as a spatial reference, colon tissues were divided into multiple segments of equal length with axial cuts and were classified as segments with ^{19}F signal or segments without signal. A total of 28 colon segments (ascending colon, n = 14; descending colon, n = 14) were analyzed in the experimental group, and six colon segments were analyzed in the control group. Segments were embedded in paraffin blocks and were cut transversely into 5-μm-thick slices. This was followed by hematoxylin-eosin staining. Slices were independently analyzed by two pathologists who were blinded to the identity of samples. The degree of inflammation was scored based on the method proposed by Hogan et al, with some modification (42). Briefly, the degree of inflammation was assessed by using six categories classified as *(a)* area involved, *(b)* crypt loss, *(c)* erosion or ulceration, *(d)* edema, *(e)* polymorphonuclear infiltration, and *(f)* mononuclear infiltration. For the area involved, the scale was 0%, normal; 1, 1%–10%; 2, 11%–25%; 3, 26%–50%; and 4, 51% or more. For crypt loss, the scale was as follows: 1, one to two per focal missing crypts; 2, contiguous crypt loss; and 3 or 4, total crypt loss. Erosions and ulcerations were defined as follows: 0, intact epithelium; 1, involvement of the lamina propria; 2, ulcerations involving the submucosa; and 3, transmural ulcerations. For edema, the severity was defined as follows: 0, none; 1, multifocal or mild separation of crypts; 2, diffusely separating crypts; 3, diffusely separating crypts and submucosal-transmural edema. For leukocyte (polymorphonuclear or mononuclear) infiltration, the severity was scaled as follows: 0, normal and scattered cells in the lamina propria; 1, multiple aggregates of more than 20 cells; 2, larger or coalescing aggregates expanding in the lamina propria and separating glands; and 3, transmural presence. The total colitis score was expressed

as the sum of all scores from the individual parameters. Any appearance of adenomas and dysplastic lesions was also graded based on their size and invasiveness, as follows: 0, none; 1, less than 1 mm; 2, 1–3 mm; and 3, more than 3 mm or clear invasion.

Reference

43. Hogan SP, Seidu L, Blanchard C, et al. Resistin-like molecule beta regulates innate colonic function: barrier integrity and inflammation susceptibility. J Allergy Clin Immunol 2006;118(1):257–268.