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Protection from Radiation-Induced Colitis Requires MHC Class II Antigen Expression by Cells of Hemopoietic Origin

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Ulcerative colitis, an inflammatory bowel disease, is believed to result from a breakdown of dominant tolerance mechanisms that normally control intestinal immunity. Although CD4+ T lymphocyte subpopulations and expression of MHC class II molecules have been shown to play a role in the pathogenesis of the disease, the nature of the responsible mechanisms remains unclear. In this paper we describe a novel mouse model for inflammatory bowel disease, radiation-induced colitis, that occurs with complete penetration 6–8 wk postinduction. A combination of high dose gamma-irradiation and lack of MHC class II expression on cells of hemopoietic origin results in development of colitis in C57BL/6 mice. Because of its versatility (due to susceptibility of mice of the widely genetically manipulated C57BL/6 background), high reproducibility, and 100% penetration, radiation-induced colitis will be a useful mouse model for colitis and a significant tool to study dominant immunological tolerance mechanisms. Moreover, our data imply that tolerization to enteric Ags requires MHC class II mediated presentation by APC of hemopoietic origin. The Journal of Immunology, 1999, 163: 4033–4040.

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* Abbreviations used in this paper: TNBS, 2,4,6-trinitrobenzene sulfonic acid; RIC, radiation-induced colitis; PFA, paraformaldehyde; MHC II, MHC class II deficient; NOD, nonobese diabetic; CIA, collagen-induced arthritis; AEC, 3-amino-9-ethylcarbazole; KO, knockout; wt, wild type; DC, dendritic cells.
coli, recombinase-activating gene-1 (RAG-1)-deficient mice do not (1), suggesting that B lymphocytes play a role in its etiology. Therefore, a cognate interaction between B or T lymphocytes and (foreign) antigenic determinants seems to elicit inflammation, which under normal conditions is controlled by regulatory T cells. However, colitis can be induced in SCID mice by dextran sulfate sodium feeding, suggesting that, at least in this model, B and T cells are not required (35–37).

As mentioned above, mice deficient in MHC class II expression develop colitis at 4–6 mo of age (1). Therefore, expression of MHC class II molecules somehow protects mice from the development of this disease. Because these molecules are known to play a major role in development (38), peripheral survival (39, 40), and activation of CD4+ T lymphocytes (41), we wished to investigate the mechanism(s) by which MHC class II molecules protect mice from colitis. In this paper we report that irradiation hematopoietic chimeras that lack MHC class II expression on bone marrow-derived cells but express these molecules on radiosensitive cells invariably develop colitis ~8 wk postreconstitution. The model of radiation-induced colitis (RIC) will be a useful tool to study the development of colitis and to investigate the mechanism(s) of MHC class II mediated protection from this disease and from immunopathology in general.

Materials and Methods

Mice

Wild-type C57BL/6 mice were obtained from Harlan Netherlands (Zeist, The Netherlands). Mice deficient for MHC class I expression (MHC I⁻) because of targeted disruption of the β₂-microglobulin gene (42) were obtained from Dr. B.-J. Fowlkes (National Institutes of Health, Bethesda, MD). These mutants had been crossed at least seven generations to C57BL/6 mice, after which intercrossing yielded mice homozygous for the disrupted allele. Mice of H-2b haplotype deficient in MHC class II expression (MHC II⁻) due to disruption of the I-Aα gene in C57BL/6 stem cells (I-Eα⁻) (43) were obtained from Dr. H. Blumentha (Roche, Basel, Switzerland). MHC I⁻ and MHC II⁻ animals were interbred in our conventional animal facilities to obtain MHC I⁺II⁺ mice. Bone marrow chimeras

Hematopoietic chimeras were prepared essentially as described previously (44). In brief, age and sex-matched anti-NK1.1-treated (100 µg of PK136 i.p. (45)) hosts were lethally irradiated (1000 rad, 160 rad/min) using a Cs⁺⁺ source and injected next day i.v. with 10⁻⁶ bone marrow cells depleted of T cells by complement killing using anti-Thy1 Ab AT83 (46). As a standard procedure in the generation of irradiation bone marrow chimeras, mice were kept on antibiotic (0.2% Bactrim; Roche, Basel, Switzerland) containing water for the duration of the experiment.

Histological analysis

Unfixed colon fragments were embedded in OCT medium (Tissue-Tek, Zoeterwonde, The Netherlands). Cryosections of 10 µm were fixed in 4% paraformaldehyde (PFA) and stained with a 1:1 mixture of May-Grünwald and Giemsa solution. At least 3 mice/group and 15 sections/mouse were counted.

Results

MHC II⁻ mice have been reported to develop colitis-like symptoms at 4–6 mo of age (1). To assess which cell type needs to be MHC class II deficient to allow colitis to develop, and to analyze the role of MHC class I molecules in this disease, we produced irradiation bone marrow chimeras. Lethally irradiated C57BL/6 mice were reconstituted with bone marrow derived from wt (wt → wt chimeras), MHC I⁻ (MHC I⁻ → wt), MHC II⁻ (MCH II⁻ → wt), or MHC I⁺II⁺ (MHC I⁺II⁺ → wt) C57BL/6 animals. After 6 wk, some MHC II⁻ → wt and MHC I⁺II⁺ → wt chimeras showed diarrhea and protrusate posture. While without exception all MHC I⁺II⁺ → wt chimeras showed protrusate posture by 8–12 wk postengraftment, all MHC II⁻ → wt mice systematically died between 8 and 9 wk after reconstitution. None of these signs have ever been observed by us in wt → wt or MHC I⁺ → wt chimeras. For the analysis described below, chimeras were killed and analyzed between week 8 and 15 postreconstitution.

Chimeras lacking MHC class II expression by hematopoietic cells develop colitis

Macroscopic observation revealed swollen intestines in MHC II⁻ → wt and MHC I⁺II⁺ → wt chimeras as compared with wt → wt...
and MHC I° → wt mice (Fig. 1, a and b, and data not shown). Cryosections of the colon of chimeras were stained with May-Grunwald/Giemsa solution (Fig. 1, c–f). Control wt → wt chimeras had a healthy colon morphology. Absence of MHC class I molecules on hemopoietic cells in MHC I° → wt chimeras did not cause any morphological change. However, MHC II° → wt and MHC I°II° → wt chimeras derived colons showed a striking hyperplasia of colon mucosa, accompanied by severe elongation of the crypts. Also, the density of goblet cells in the epithelium of diseased chimeras was significantly decreased. Finally, in MHC II° → wt and MHC I°II° → wt chimeras derived colons an infiltration of the lamina propria by mononuclear and polymorphonuclear cells was evident (Fig. 1, c–f). This result indicates that MHC II° → wt and MHC I°II° → wt chimeras had developed a severe ulcerative colitis like disease.

Colitis developed invariably within 2 mo, and all MHC II° → wt and MHC I°II° → wt chimeras were affected (Table I, and data not shown). Colitis has never been observed by us in wt → wt and MHC I° → wt chimeras.

CD4+ and CD8+ cells infiltrate the lamina propria in MHC II° → wt and MHC I°II° → wt chimeras

The mononuclear cell infiltrates observed in diseased animals were characterized by immunohistochemistry (Fig. 2). Although some CD4+ T lymphocytes were clearly visible in the lamina propria of colon from healthy wt → wt chimeras (Fig. 2a), their number was significantly increased in colitis-affected MHC II° → wt (Fig. 2c) and MHC I°II° → wt (Fig. 2e) chimeras. Lamina propria CD8α+ T cells were relatively rare in wt → wt chimeras (Fig. 2b), but MHC II° → wt (Fig. 2d) and MHC I°II° → wt (Fig. 2f) colons showed important infiltration by these lymphocytes. These data show that the development of colitis in MHC II° → wt and MHC I°II° → wt chimeras was accompanied by lamina propria infiltration by CD4+ and CD8+ T lymphocytes.

Table I. Scoring of RIC in bone marrow chimeras

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4 Hyper., scoring of colon hyperplasia; Meta., scoring of colon metaplasia; Poly., infiltration of polymorphonuclear cells in lamina propria; Mono., infiltration of mononuclear cells in lamina propria.
5 Chimeras were analyzed at the given time points after bone marrow reconstitution.
Lamina propria dendritic cells do not express MHC class II in MHC II° → wt and MHC I°II° → wt chimeras

Infiltration of lamina propria by CD4+ and CD8+ T lymphocytes in chimeras lacking MHC class I and/or II expression on hematopoietic cells was rather unexpected. Therefore, we investigated whether lamina propria professional APC are in fact of donor origin. Immunohistological analysis using Abs specific for dendritic cells (DC) (CD11c, (52)) revealed the presence of CD11c+ cells in the lamina propria and epithelium of wt → wt control and colitis-affected MHC I°II° → wt chimeras (Fig. 3, a and c), as previously described in the rat (56). As expected, in MHC I°II° → wt chimeras CD11c+ cells in the colon lacked expression of MHC class II molecules, and MHC class II expression was uniquely observed on intestinal epithelial cells (Fig. 3d). This result indicated that the APC were of donor origin and that the activation event (if any) that lead to infiltration by CD4+ and CD8+ lymphocytes in MHC I°II° → wt chimeras was not mediated by MHC ligands expressed by colon DC.

LPL and IEL in chimeras affected with colitis produce IFN-γ

To investigate whether the lamina propria infiltration by lymphocytes was accompanied by their activation, we analyzed production of the proinflammatory cytokine IFN-γ by these cells. This cytokine is known to be instrumental in the development of colitis in SCID mice induced by injection of CD45RBhigh CD4+ T lymphocytes (57). LPL were isolated from affected and healthy animals and stimulated in vitro in the presence of the Golgi blocker Brefeldin A. Cells were subsequently analyzed for surface expression of CD4 and CD8β and intracellular expression of IFN-γ by flow cytometry (Fig. 4, A and B). Significantly more LPL produced IFN-γ in affected (MHC II° → wt and MHC I°II° → wt) than in healthy (wt → wt and MHC I° → wt) chimeras (p < 0.01) (Fig. 4A). Most (88 ± 7%) of the IFN-γ producers expressed CD4 or CD8β (Fig. 4B and data not shown). Moreover, among CD4+ LPL, significantly more cells produced IFN-γ in affected than in healthy chimeras (p < 0.05). The increased percentage of IFN-γ producing LPL appears to be due to a higher fraction of CD4+ cells producing this cytokine and, in the case of MHC II° → wt chimeras, to the significantly increased percentage of CD8+ T lymphocytes of which a high proportion produces IFN-γ (Fig. 4B).

We also analyzed IFN-γ production by IEL (Fig. 4). Significantly more IEL produced IFN-γ in chimeras affected with colitis (MHC II° → wt and MHC I°II° → wt) than in healthy (wt → wt and MHC I° → wt) animals (p < 0.001, Fig. 4A), and most (76 ±
and E, epithelium.

Cryosections of colon from wt producing cells among CD4 and CD8+ T cells in affected than in healthy chimeras (B4g, and data not shown). Moreover, the percentage of IFN-γ producers expressed either CD4 or CD8 (Fig. 4B, and data not shown). Moreover, the percentage of IFN-γ-producing cells among CD4+ or CD8+ IEL was significantly higher in affected than in healthy chimeras (p < 0.01, Fig. 4B).

Discussion

In this report, we have described a novel murine colitis model, RIC, which is very reproducible and develops with complete penetrance. Irradiation hemopoietic chimeras that had been reconstituted with bone marrow derived from MHC class II expression deficient donors developed symptoms similar to those observed in human Crohn’s disease and ulcerative colitis: hyperplasia of the lamina propria and concomitant elongation of crypts, metaplasia, and infiltration of lamina propria by lymphocytes (of which a large proportion produces IFN-γ), monocytes, and polymorphonuclear leukocytes. RIC develops in C57BL/6 mice, which allows the use of a large selection of induced mutants available on this genetic background.

The etiology of Crohn’s disease and ulcerative colitis remains unknown. Because several types of mutant mice develop colitis much later in life (see Introduction), in RIC the high dose gamma-irradiation is probably instrumental in disease development and in its complete penetrance. Because gamma-irradiation is known to cause damage to intestinal epithelium (58), Ags present in the gut lumen will presumably have more readily access to the lamina propria after irradiation. These Ags are foreign to the immune system, and inflammation followed by massive lymphocyte activation would be expected to occur. An uncontrolled inflammatory reaction may nonspecifically cause mucosal damage (59). The uncontrolled immune response may even develop into autoagression (29, 30) because of molecular mimicry or bystander activation of normally tolerant (anergic) autospecific lymphocytes. While in animals with a normal immune system regulatory T lymphocytes are thought to control intestinal immunity (59, 60), the absence of MHC class II on APC in the MHC IIα→wt and MHC IIβ→wt chimeras may preclude activation of such regulatory cells and thus cause colitis. The previously described development of colitis in allogenic, but not syngenic, bone marrow chimeras (61) may be due to the discrepancy between the haplotypes of MHC-encoded dimers involved in thymic (or peripheral) positive selection and peripheral activation of regulatory T lymphocytes. Similar mechanisms may apply for development of colitis in the absence of irradiation, although the way of entry of Ags is likely to be different and in that case possibly mediated by M cells (59, 62).

Many aspects of the above described model for colitis development in RIC remain to be experimentally addressed. First, because of the requirement for costimulation in activation of naive T lymphocytes, their activation in the absence of expression of MHC molecules on APC (in MHC IIα→wt chimeras) is rather surprising. APC could deliver costimulation in trans (63), and Ags may be presented by MHC expressing intestinal epithelial cells. Because T lymphocytes are efficiently killed by lethal irradiation and the T cells detected in chimeras must therefore have developed after reconstitution, the involvement of Ag-experienced T cells whose activation depends less on costimulation (64) seems rather unlikely. The use of costimulation-deficient bone marrow to reconstitute hosts would be useful to investigate these possibilities.

Lack of expression of MHC class II molecules on cells of hemopoietic origin is sufficient to render mice susceptible to RIC. Thymic positive selection is not expected to be affected in MHC IIα→wt and MHC IIβ→wt chimeras because positively selecting MHC class II molecules seem to be exclusively expressed on radioresistant thymic epithelial cells (65). Lack of thymic clonal deletion because of the absence of MHC class II on thymic APC (66, 67) is an unlikely explanation because TCRαβ expressing lymphocytes are not even required for development of colitis (1). The most likely explanation appears to be that protective T lymphocytes require activation by APC of hemopoietic origin. Whatever the explanation, it appears that tolerization to enteric Ags is not mediated by MHC class II expressing epithelial cells but rather...
by APC of hemopoietic origin. It will be of interest to analyze in a similar chimeric mouse system if oral tolerance also depends on presentation by hemopoietic APC (68, 69).

The situation in the autoimmune models NOD, experimental autoimmune encephalomyelitis, and CIA is substantially different from that in RIC. In the former models, MHC expression is clearly required for disease development and therefore T lymphocytes are involved as effector cells. The protective effect of MHC class II molecules may therefore affect effector and/or protector T lymphocytes. For induction of murine colitis, T lymphocytes are definitively not required (1), and expression of protective MHC class II molecules therefore almost obligatorily affects protective T lymphocytes. RIC therefore seems to be an ideal system to study MHC class II mediated protection from immunopathology.

The mechanism(s) responsible for MHC class II mediated protection from colitis (and murine autoimmune disorders) remains a mystery. Based on our data, we favor the hypothesis that protective T lymphocytes require activation by APC. Whatever the precise mechanism(s), RIC is a very versatile and reproducible model that should prove useful for the elucidation of MHC class II mediated protection from colitis with possible implications for autoimmunity.

**FIGURE 4.** A. IFN-γ production by chimera derived LPL and IEL. LPL and IEL from chimeras were isolated, restimulated in vitro in the presence of Brefeldin A, and assessed for intracellular IFN-γ. The percentage of IFN-γ producing LPL and IEL from chimeras affected with colitis (MHC II’ → wt and MHC I’II’ → wt combined) was significantly higher than that of unaffected (wt → wt and MHC I’ → wt combined) chimeras (p < 0.01 and p < 0.001, respectively (Student’s t test)). B. IFN-γ production by chimera derived CD4+ and CD8+ LPL and IEL. LPL and IEL were isolated, restimulated in vitro in presence of Brefeldin A, and analyzed for surface CD4 and CD8 expression as well as intracellular IFN-γ by flow cytometry. Data from representative experiments are shown. Numbers indicate percentage within indicated gate. The percentage of IFN-γ producing CD4+ LPL and IEL from chimeras affected with colitis (MHC II’ → wt and MHC I’II’ → wt combined, n = 6) was significantly higher than that of unaffected (wt → wt and MHC I’ → wt combined, n = 7 for LPL, n = 5 for IEL) chimeras (p < 0.05 and p < 0.01, respectively). The percentage of IFN-γ producing CD8+ IEL was significantly higher in affected (n = 3) than in healthy (n = 4) chimeras (p < 0.01).
References


