TNFR1 mediates the radioprotective effects of lipopolysaccharide in the mouse intestine

Terrence E. Riehl, Rodney D. Newberry, Robin G. Lorenz, and William F. Stenson

Division of Gastroenterology, Washington University School of Medicine, St. Louis, Missouri 63110; and Department of Pathology, University of Alabama, Birmingham, Alabama 35294

Submitted 20 December 2002; accepted in final form 18 September 2003

Riehl, Terrence E., Rodney D. Newberry, Robin G. Lorenz, and William F. Stenson. TNFR1 mediates the radioprotective effects of lipopolysaccharide in the mouse intestine. Am J Physiol Gastrointest Liver Physiol 286: G166–G173, 2004. First published October 2, 2003; 10.1152/ajpgi.00537.2002.—LPS is radioprotective in the mouse small intestine through a mechanism that involves the synthesis of cyclooxygenase-2 (COX-2) and PGE2. The goal of this study was to identify the intermediate steps in this process. We used wild-type (WT) C57BL/6 mice and knockouts for tumor necrosis factor receptors 1 and 2 (TNFR1WT, TNFR2WT) and recombination-activating gene 1/TNFFR2−/− mice. Mice were given parenteral LPS and then subjected to 12 Gy total body gamma irradiation. The number of surviving intestinal crypts was assessed 3.5 days after irradiation using a clonogenic assay. Crypt cell apoptosis was assessed by histology. Parenteral administration of LPS induced COX-2 expression, PGE2 production, and radioprotection in WT and TNFR2−/− mice but not in TNFR1−/− mice. TNFR1−/− mice were radioprotected by administration of exogenous 16,16-dimethyl PGE2. Immunohistochemical studies localized TNFR1 and COX-2 expression to subepithelial fibroblasts and villus epithelial cells. Radiation-induced apoptosis was reduced by pretreatment with LPS in WT and TNFR2−/− mice but not in TNFR1−/− mice. In the absence of LPS, crypt survival was elevated in TNFR1−/− when compared with WT mice. These findings demonstrate that TNFR1 function is required for LPS-induced radioprotection in C57BL/6 mice and define an essential role for TNFR1 function in the induction of COX-2 expression and PGE2 production in this process. The immunolocalization of TNFR1 and COX-2 expression to subepithelial fibroblasts following LPS administration suggests that this cell type plays an intermediate role in LPS-induced radioprotection in the intestine.

GAMMA RADIATION, AN EXTENSIVELY characterized model of intestinal injury, affects rapidly proliferating cell populations (21, 25, 26). After total body irradiation, the major sites of injury are the bone marrow and the intestinal epithelium. Exposure of mice to 7–15 Gy total body irradiation results in the death of most proliferating cells in the bone marrow, but some stem cells survive and repopulate the marrow (10). With higher doses of radiation, the number of surviving bone marrow stem cells is insufficient to repopulate the marrow, and the animal dies. Similarly, after 8–14 Gy of total body irradiation, proliferating transit cells in the intestinal crypt are killed; but some stem cells survive (21, 25, 26). Surviving stem cells proliferate and give rise to transit cells that form regenerative crypts and eventually repopulate the mucosa. Higher doses of radiation kill more of the stem cells and, consequently, reduce the number of regenerative crypts.

The cellular response to radiation injury in the bone marrow and intestine can be modulated by agents given before irradiation. Agents that decrease the amount of radiation-induced injury are said to be radioprotective. IL-1, IL-11, IL-12, TNF-α, granulocyte-macrophage colony-stimulating factor, stem cell factor, PGE2, and LPS are radioprotective in the bone marrow (16, 17, 27, 35). In the intestine, IL-1, IL-11, PGE2, and transforming growth factor-β (TGF-β) are radioprotective; given before radiation, they increase the number of surviving crypts after radiation (22, 23, 34).

We recently demonstrated that LPS is radioprotective in the intestine through a mechanism that involves the induction of cyclooxygenase-2 (COX-2) and the production of PGE2 (28). LPS induces COX-2 expression in villus epithelial cells and subepithelial fibroblasts but not in stem cells or other crypt epithelial cells. LPS is radioprotective in the intestine through a COX-2-mediated mechanism, but COX-2 was not induced in stem cells. Radioprotective agents act by promoting stem cell survival. The absence of LPS-induced COX-2 expression in stem cells suggests that the radioprotective effects of LPS are mediated through other cell types in which COX-2 is induced and PGE2 synthesized. In this study, we sought to identify the intermediate steps in LPS-induced radioprotection in the intestine.

MATERIALS AND METHODS

Animals. Mice were maintained on a 12:12-h light-dark schedule and fed standard laboratory mouse chow. Animal procedures and protocols were conducted in accordance with the institutional review board at Washington University School of Medicine (St. Louis, MO). C57BL/6, TNF receptor 1 (TNFR1)-deficient (13, 19), TNF receptor 2 (TNFR2)-deficient, and recombination-activating gene (RAG)1-deficient on the C57BL/6 background were purchased from The Jackson Laboratory (Bar Harbor, ME). Whole body irradiation of mice was carried out in a gamma cell 4137Cs irradiator at a dose rate of 80.7 cGy/min. Animals were killed at various times and rapidly dissected as described previously (1). The proximal jejunum was fixed in Bouin’s solution and divided into 5-mm segments before paraffin embedding and immunohistochemical analysis. The distal jejunum was snap-frozen in liquid nitrogen for Western blotting and analysis of TNF-α levels. The proximal ileum was snap-frozen for measurement of PGE2 levels.

Crypt survival. Crypt survival was measured in animals killed 3.5 days after irradiation, as described previously (28), using a modification of the microcolony assay (21, 33), with signal detection using 3,3′-diaminobenzidine tetrahydrochloride (Sigma, St. Louis, MO).

Address for reprint requests and other correspondence: W. F. Stenson, Box 8124 Washington Univ. School of Medicine, 660 South Euclid Ave., St. Louis, MO 63110.

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
Immunohistochemistry. For immunohistochemical localization of mouse COX-2 or TNFR1, deparaffinized sections of Bouin’s fixed tissue were incubated with either a 1:2,000 dilution of rabbit anti-mouse COX-2 (Cayman Chemical, Ann Arbor, MI) (28) or a 1:50 dilution of Armenian hamster anti-mouse TNFR1 monoclonal antibody (BD Pharmingen, San Diego, CA). Endogenous peroxidase activity was quenched with 3% hydrogen peroxide. Nonspecific staining was blocked by incubating sections sequentially in blocking buffer (New England Nuclear, Boston, MA), avidin-D solution (Vector Laboratories, Burlingame, CA), and biotin-D (Vector Laboratories), and either normal donkey serum for COX-2 (Sigma) or normal goat serum for TNFR1 (Sigma) before incubating slides overnight at 4°C with the primary antibody. Sections incubated with preimmune rabbit serum (for COX-2), preimmune goat serum (for TNFR1), or without primary antibody served as negative controls. A 1:2,000 dilution of biotinylated donkey anti-rabbit immunoglobulin G (Jackson Immuno Research Laboratory, West Grove, PA) was used as the secondary antibody for COX-2, and a 1:2,000 dilution of biotinylated goat anti-Armenian hamster immunoglobulin G (Jackson Immuno Research) was used as the secondary antibody for TNFR1. Signal amplification was carried out with the indirect biotinyl tyramide system (New England Nuclear). The amplified antibody signal was detected using 3,3’-diaminobenzidinetetrahydrochloride.

Measurement of PGE2 levels. PGE2 concentrations in extracts from distal jejunum were determined by a PGE2-specific enzyme-linked immunosorbent assay (Cayman Chemical) according to the manufacturer’s directions.

Measurement of TNF-α levels. Extracts from distal jejunum were obtained from supernatants of tissue lysates prepared in proteinase inhibitor cocktail (30). Samples were assayed by a TNF-α-specific enzyme-linked immunosorbent assay (B-D Pharmingen, San Diego, CA) according to the manufacturer’s directions.

Apoptosis. The proximal jejunum was obtained from mice 6 h after 12 Gy whole body irradiation, fixed overnight in Bouin’s solution, and embedded in paraffin. Sections were stained with hematoxylin and eosin, and the number of apoptotic cells per crypt section was assessed by morphological criteria as described by Potten and Grant (24).

LPS. LPS from Escherichia coli K-235 was purchased from Sigma (St. Louis, MO). Animals treated with LPS received a single dose (0.5 mg/kg) injected intraperitoneally.

RESULTS

We (28) had previously demonstrated that LPS is radioprotective in the small intestine in FVB/N mice. Treatment with LPS (0.5 mg/kg given 14 h before irradiation) increases the number of surviving crypts per cross section from 10 to 20. In FVB/N mice, the radioprotective effects of LPS in the intestine are mediated through COX-2 and PGE2. In the current study, we used a series of knockout mice on the C57BL/6 background to define the intermediate steps in the induction of radioprotection by LPS in the mouse small intestine. In untreated C57BL/6 mice, there were 12 surviving crypts per cross section 3.5 days after gamma irradiation (12 Gy; Fig. 1). In C57BL/6 mice given 0.5 mg/kg of LPS 14 h before radiation, there were 26 surviving crypts per cross section. Thus the fold increase in crypt survival with LPS was similar in FVB/N and C57BL/6 mice, although the baseline crypt survival was somewhat higher in the C57BL/6 strain. We used RAG1−/− mice to determine whether the radioprotective effects of LPS are mediated through T lymphocytes or B lymphocytes. Baseline crypt survival in the RAG1−/− mice was somewhat lower than in C57BL/6 controls; however, LPS induced a threefold increase in crypt survival in the RAG1−/− mice, demonstrating that the radioprotective effects of LPS are not mediated through T lymphocytes or B lymphocytes.

TNF-α is a candidate intermediate molecule for LPS-induced radioprotection, because LPS is known to induce TNF-α production, which, in turn, may induce COX-2 expression and PGE2 production (9). In addition to these observations, previous studies (17) have demonstrated that TNF-α is radioprotective in the bone marrow. The biological effects of TNF-α are mediated through binding to two distinct receptors. It is not known which TNFR(s) are required for COX-2 induction in the intestine in response to LPS. Signaling through TNFR1 is associated with apoptosis and cytokine production; whereas signaling through TNFR2 is associated with proliferation and enhanced cell survival. To define the role of TNF-α in LPS-induced radioprotection in the intestine and to identify the relevant TNFR(s) for this process, we measured crypt survival in TNFR1−/− and TNFR2−/− mice in the presence and absence of LPS. Baseline crypt survival in TNFR1−/− mice was more than double that seen in the C57BL/6 wild-type (WT) mice; however, treatment with LPS did not induce a further
increase in crypt survival in the TNFR1−/− mice. In contrast, baseline crypt survival in TNFR2−/− mice was similar to that seen in WT C57BL/6 mice, and administration of LPS-induced a 2.7-fold increase in crypt survival. These data demonstrate that the absence of TNFR1 results in a significant increase in baseline crypt survival and that TNFR1 is essential for LPS-induced radioprotection in the intestine. Conversely, the absence of TNFR2 has no effect on either the baseline response to radiation injury or LPS-induced radioprotection.

These data suggest that TNF-α production after LPS administration and subsequent ligation of the TNFR1 are essential for LPS-mediated radioprotection. To support this observation, we measured TNF-α levels in the intestines of control mice and mice treated with parenteral LPS. TNF-α protein levels increase five- to sixfold in response to LPS in both WT and TNFR1−/− mice (Fig. 2).

We previously demonstrated that the radioprotective effects of LPS in the small intestine are mediated through the induction of COX-2 and increased production of PGE2 (28). To better define the roles TNF-α and TNFR1 play in LPS-induced radioprotection in the intestine, we measured PGE2 levels in the intestine of WT, RAG1−/−, TNFR1−/−, and TNFR2−/− mice given LPS (Fig. 3). Administration of LPS resulted in an increase in intestinal PGE2 levels in WT, RAG1−/−, and TNFR2−/− mice. In TNFR1−/− mice, baseline intestinal PGE2 levels were similar to those in WT mice; however, administration of LPS did not increase PGE2 levels. In other mouse strains, LPS administration induced the expression of COX-2 in the intestine (13). We observed that LPS administration also induces COX-2 protein expression in the intestines of C57BL/6, RAG1−/−, and TNFR2−/− mice (Fig. 4). However, in TNFR1−/− mice, there is little expression of COX-2 in the intestine at baseline and no increase with LPS. These data suggest that the failure of LPS to induce radioprotection in TNFR1−/− mice is due to the inability of LPS to induce increased PGE2 production in these mice and demonstrate an essential role for TNFR1 in the induction of COX-2 expression and PGE2 production in the intestine in response to LPS.

To identify the cell types involved in the intermediate steps of LPS-induced radioprotection in the intestine, we performed immunohistochemistry for COX-2 and TNFR1. Untreated WT intestine shows little COX-2 staining (Fig. 5A), whereas after treatment with parenteral LPS, COX-2 expression is seen in villus epithelial cells and subepithelial fibroblasts (Fig. 5B). At higher power (Fig. 5C), staining of fibroblasts immediately adjacent to crypt epithelial cells is seen. In TNFR1−/− mice, there is little COX-2 expression either at baseline (Fig. 5D) or after treatment with LPS (Fig. 5E). We observed TNFR1 expression in crypt and villus epithelial cells and in subepithelial fibroblasts (Fig. 6). Villus epithelial cells and subepithelial fibroblasts both express TNFR1 at baseline and express COX-2 after treatment of with LPS; however, given the proximity of subepithelial fibroblasts to the crypt epithelial cells, we favor this cell type for playing a role in the intermediate steps of LPS-induced radioprotection in the intestine.

To further support the position of TNFR1 as upstream of PGE2-mediated events in LPS-induced radioprotection in the intestine, we examined the ability of TNFR1−/− mice to be protected from radiation injury by the administration of exogenous dmPGE2. In both WT and TNFR1−/− mice, the admin-

Fig. 2. LPS treatment increases TNF-α production in the small intestines of WT and TNFR1−/− mice. Mice were treated with vehicle (control) or LPS (0.5 mg/kg). After 1 h, mice were killed and the TNF-α content of the small intestine was determined by enzyme immunoassay. Data are the means ± SE of 4 animals. *P < 0.001 compared with WT control; **P < 0.001 compared with TNFR1−/− control.

Fig. 3. LPS treatment increases PGE2 production in WT, RAG1−/−, and TNFR2−/− mice but not in TNFR1−/− mice. Mice were treated with vehicle (control) or LPS (0.5 mg/kg). After 1 h, mice were killed and the PGE2 content of the small intestine was determined by enzyme immunoassay. Data are the means ± SE of 4–8 animals. *P < 0.001 compared with WT control; **P < 0.001 compared with RAG1−/− control; ***P < 0.001 compared with TNFR2−/− control.

Fig. 4. Detection of cyclooxygenase (COX)-2 in the small intestines of control and LPS-treated WT, RAG1−/−, TNFR1−/−, and TNFR2−/− mice by Western blotting. LPS (0.5 mg/kg) was given to mice 1 h before death, intestinal tissue was harvested, and the expression of COX-2 protein in tissue lysates was determined by Western blotting using rabbit anti-mouse antibody. A mouse macrophage cell line treated with LPS was used as positive control.
administration of dmPGE 2 resulted in a two- to threefold increase in the number of surviving crypts per cross section (Fig. 7), demonstrating that PGE 2 is radioprotective in TNFR1−/− mice, and the failure of LPS to induce a radioprotective response in TNFR1−/− mice is not due to the inability to respond to PGE 2. These observations place TNFR1-mediated events upstream of COX-2 induction and PGE 2 production in the cascade of events leading to LPS-induced radioprotection in the intestine.

To address the mechanism of the increased crypt survival in TNFR1−/− mice and determine whether the increased baseline crypt survival in TNFR1−/− mice was COX-2 dependent, TNFR1−/− mice and WT mice were treated with a specific COX-2 inhibitor NS-398 and irradiated. Administration of NS-398 had no effect on the baseline crypt survival in either WT or TNFR1−/− mice, indicating that the increased crypt survival in TNFR1−/− mice is not mediated through events dependent on COX-2 (Fig. 7).

We next sought to define the biological mechanism for LPS-induced radioprotection. The epithelial response to radiation injury includes cell cycle arrest, DNA repair, and apoptosis (21). We had previously demonstrated (5) that prostaglandins influence the apoptotic response to radiation in epithelial cells. We next sought to determine whether the radioprotective effects of LPS are mediated through effects on apoptosis. WT, RAG1−/−, TNFR1−/−, and TNFR2−/− mice were given either LPS or saline; 14 h later, all the mice received 12 Gy gamma irradiation. At 6 and 24 h after irradiation, the animals were killed, and the number of apoptotic epithelial cells per small intestinal crypt was assessed. The number of apoptotic cells in irradiated WT, RAG1−/−, and TNFR2−/− mice was similar, but the number of apoptotic cells in irradiated TNFR1−/− mice

Fig. 5. Immunohistochemical localization of COX-2 in the proximal jejunum of control and LPS-treated WT and TNFR1−/− mice.
A: control WT mice showed COX-2 staining in a few villus epithelial cells. B: 1 h after LPS (0.5 mg/kg ip) treatment, there was COX-2 staining of villous epithelial cells (arrow) and subepithelial fibroblasts (arrowhead). C: higher power view of intestine from LPS-treated mouse showed staining of fibroblasts between crypts. Control (D) and LPS-treated (E) TNFR1−/− mice demonstrated COX-2 staining in scattered villus epithelial cells (original magnification: A, B, D, E: ×400; C: ×1,000).
Fig. 6. A: immunohistochemical localization of TNFR1 in the proximal jejunum of control C57BL/6 WT mice shows TNFR1 staining in villus and crypt epithelial cells and in subepithelial fibroblasts. B: higher-power view of intestine shows TNFR1 staining of a crypt epithelial cell (arrow) and a fibroblast between crypts (arrowhead). C: negative staining with isotype control antibody (original magnification: A, C: ×400; B: ×1,000).

was significantly lower (Fig. 8). The 50% reduction in radiation-induced apoptosis in the TNFR1<sup>−/−</sup> mice compared with WT mice is consistent with the 2.5-fold increase in crypt survival. These data are consistent with the suggestion that the increased baseline crypt survival in TNFR1<sup>−/−</sup> mice is the result of decreased radiation-induced apoptosis, and they provide support for a role of TNFR1 in radiation-induced apoptosis. Administration of LPS dramatically reduced the number of apoptotic cells per crypt in WT, RAG1<sup>−/−</sup>, and TNFR2<sup>−/−</sup> mice but had no effect on the number of apoptotic cells in the TNFR1<sup>−/−</sup> mice. Results at 6 and 24 h were similar. The number of apoptotic cells at 6 h after irradiation is shown in Fig. 8; similar results were seen at 24 h. These data demonstrate that in WT mice, the radioprotective effects of LPS are paralleled by a decrease in radiation-induced apoptosis; moreover, the failure of LPS to induce radioprotection in TNFR1<sup>−/−</sup> mice is paralleled by a failure to decrease radiation-induced apoptosis. One potential interpretation of these data is that TNFR1 is essential for LPS-induced COX-2 expression and PGE2 synthesis reducing radiation-induced apoptosis and, as a result, increasing crypt survival.

DISCUSSION

We (28) previously demonstrated that parenteral LPS is radioprotective in the mouse intestine through a mechanism mediated by the induction of COX-2 and the production of PGE2. LPS induced COX-2 expression in villus epithelial cells and subepithelial fibroblasts but not in crypt epithelial cells. Radioprotection reflects the survival and proliferation of epithelial stem cells after radiation injury; therefore, the absence of LPS-induced COX-2 expression in epithelial stem cells suggested that the radioprotective effects of LPS were mediated through other cell types in which COX-2 was induced and PGE2 synthesized.

In this study, WT C57BL/6 mice and knockout mice on the C57BL/6 background were used to explore the intermediates in LPS-induced radioprotection. We found that LPS is not radioprotective in TNFR1<sup>−/−</sup> mice. There are two ligands for
TNFR1, TNF-α and TNF-β, or soluble lymphotixin (LT). In adult mice, LT is expressed exclusively by lymphocytes. We favor TNF-α as the relevant ligand for TNFR1 in LPS-induced radioprotection because both RAG1−/− mice (lacking T lymphocytes and B lymphocytes) and LT-deficient mice are protected from radiation-induced injury following LPS administration (data not shown). Consistent with TNF-α being the relevant TNFR1 ligand for this process, LPS administration increases TNF-α expression in the intestine of both WT and TNFR1−/− mice.

To determine the sequence of events in the cascade leading to protection from radiation injury following LPS administration, we examined COX-2 expression and PGE2 production in the intestine of TNFR1−/− mice following LPS administration. LPS administration does not enhance COX-2 expression or increase PGE2 production in the intestine of TNFR1−/− mice. To further support the role of TNFR1 as being upstream of PGE2, we observed that TNFR1−/− mice, lacking T lymphocytes and B lymphocytes, and LT-deficient mice are protected from radiation-induced injury following LPS administration (data not shown). Consistent with TNF-α being the relevant TNFR1 ligand for this process, LPS administration increases TNF-α expression in the intestine of both WT and TNFR1−/− mice.

The binding of TNF-α to TNFR1 on subepithelial myofibroblast results in the induction of COX-2 and the production of PGE2, which, in turn, binds to receptors on stem cells and thus affects the epithelial response to radiation injury. We have developed data elsewhere demonstrating that the effects of PGE2 on the stem cell response to radiation are mediated through the EP2 receptor (4).

TNF-α is radioprotective in the bone marrow (17); whether or not TNF-α is radioprotective in the intestine has not previously been addressed. The data presented here suggest that exogenous TNF-α is likely to be radioprotective in the intestine through binding to TNFR1 with the induction of COX-2 and PGE2 production. This study also raises the possibility that the radioprotective effects of LPS and TNF-α in the bone marrow are mediated through TNFR1, COX-2, and PGE2.

The epithelial response to radiation injury reflects a complex series of biological events. Gamma radiation damages DNA, resulting in cellular responses including cell cycle arrest, DNA repair, and a choice between apoptosis and survival (21). Radioprotective agents could act through effects on any of these responses. Radioprotection could be induced by agents that protect against DNA damage, shorten cell cycle arrest, promote DNA repair, or diminish apoptosis. TNF-α induces epithelial apoptosis (20), and PGE2 affects radiation-induced apoptosis (5); therefore, we sought to determine whether the radioprotective effects of LPS or TNF-α are the products of effects on apoptosis. Treatment with parenteral LPS-reduced radiation-induced intestinal epithelial apoptosis at 6 and 24 h after 12 Gy gamma radiation. LPS induces COX-2 expression and PGE2 production, and we have previously demonstrated (5) that PGE2 diminishes radiation-induced apoptosis in the intestinal epithelium. Irradiated cells that sustain significant DNA damage accumulate at the G2 phase of the cell cycle. One potential model for the LPS-induced decrease in apoptosis and increase in crypt survival that PGE2, synthesized in response to LPS, allows injured epithelial cells to escape from G2 arrest or to otherwise avoid apoptosis and go on to generate viable crypts.

In irradiated TNFR1−/− mice, baseline intestinal crypt survival is increased compared with that in WT mice. To address the mechanism of the increased crypt survival in TNFR1−/− mice, we measured radiation-induced apoptosis and found that the number of apoptotic cells per crypt was signiﬁcantly less in TNFR1−/− mice compared with WT mice. This suggests that in WT mice, the binding of TNF-α to TNFR1 mediates radiation-induced apoptosis. An association between the apoptotic effects of TNF-α and signaling through TNFR1 has been demonstrated; parenteral injection of TNF-α in mice induced enterocyte apoptosis through TNFR1 (20). TNFR1−/− mice have also been demonstrated to have diminished intestinal epithelial apoptosis 4 h after exposure to 5 Gy (gamma radiation) (7). Thus, in this system, TNF-α has two conﬂicting effects on radiation-induced apoptosis. In the absence of LPS, TNF-α binding to TNFR1 mediates radiation-induced apoptosis. However, in the presence of LPS, the production of TNF-α is increased and TNF-α binding to TNFR1 results in COX-2 induction, PGE2 synthesis, and a reduction in radiation-induced apoptosis. In LPS-treated mice, the anti-apoptotic effects of PGE2 outweigh the direct proapoptotic effects of TNF-α. The explanation for this distinction may relate to the cells.
producing the TNF-α and their anatomic position in the intestinal mucosa. LPS stimulates macrophages to release TNF-α, which binds to TNFR1 on the adjacent subepithelial myofibroblasts. This results in COX-2 expression and PGE2 production. In contrast, the TNF-α produced in response to radiation appears to bind to TNFR1 on epithelial cells resulting in apoptosis. The cellular source of the TNF-α produced in response to radiation is not clear, but it may be the epithelial cells themselves.

LPS protected against radiation-induced injury in TNFR2−/− mice, and in fact, we observed an augmented crypt survival in TNFR2−/− mice after LPS administration compared with WT mice. Previous studies have demonstrated that LPS-induced TNF-α production in TNFR2−/− mice remains intact (19). The augmented crypt survival in TNFR2−/− mice following LPS administration could be explained by the increased availability of ligand for binding TNFR1 in the absence of TNFR2 or by the loss of TNFR2 effects that normally oppose those of TNFR1, as previously demonstrated in other animal models (19). TNF-α binding to TNFR2 modulates both the level of inflammation and epithelial proliferation in response to injury in mouse models of colitis (15). The importance of signaling through TNFR2 in epithelial proliferation in response to inflammation is consistent with the established role of TNFR2 signaling in cell proliferation and cell survival. Similarly, the importance of TNFR1 signaling in inducing COX-2 expression is consistent with the established role of TNFR1 signaling in gene transcription.

In this study, we have demonstrated that TNF-α and TNFR1 mediate the induction of COX-2 by LPS. Moreover, we have found that the LPS-induced increase in radioprotection is paralleled by a decrease in radiation-induced apoptosis. These findings suggest this sequence of events: LPS stimulates CD14 and toll-like receptor-bearing cells inducing the secretion of TNF-α which binds to TNFR1 on subepithelial fibroblasts, inducing the synthesis of COX-2 and the production of PGE2. PGE2 produced by subepithelial fibroblasts acts locally and binds to EP receptors on adjacent epithelial stem cells reducing radiation-induced apoptosis, resulting in increased stem cell survival and increased crypt survival. We have demonstrated that TNFR1 is essential for the radioprotective effects of LPS in the intestine. The TNFR1-dependent events occurring in the cascade leading to LPS-induced radioprotection occur upstream from COX-2 induction and PGE2 production. Our observations suggest that LPS or other agents that induce TNF-α production may be useful in protecting the intestinal epithelium in patients receiving radiation therapy.

GRANTS

This work was supported by National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) Grants DK-33165 and DK-55753 (to W. F. Stenson) and the Washington University Digestive Disease Research Cores Center NIDDK Grant P30DK-52574.

REFERENCES


