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IL-17 producing CD4⁺ T cells mediate accelerated ischemia/reperfusion-induced injury in autoimmunity-prone mice^{1,2}

Colin Edgerton^{*,3}, José C. Crispín^{†,3}, Chantal M. Moratz^{*,§}, Estelle Bettelli[‡], Mohamed Oukka[‡], Milomir Simovic^{*,§}, Athina Zacharia^{*}, Ryan Egan[§], Jie Chen[†], Jurandir J. Dalle Lucca[§], Yuang-Taung Juang[†], and George C. Tsokos[†]

^{*}Department of Medicine, Uniformed Services University for the Health Sciences, Bethesda, MD 20814

[†]Department of Medicine, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA 02115

[‡]Center for Neurologic Diseases, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts 02115

[§]Department of Cellular Injury, Walter Reed Army Institute of Research, Silver Spring, MD 20910

Abstract

Elements of the innate and adaptive immune response have been implicated in the development of tissue damage after ischemic reperfusion (I/R). Here we demonstrate that T cells infiltrate the intestine of C57BL/6 mice subjected to intestinal I/R during the first hour of reperfusion. The intensity of the T cell infiltration was higher in B6.MRL/*lpr* mice subjected to intestinal I/R and reflected more severe tissue damage than that observed in control mice. Depletion of T cells limited I/R damage in B6.MRL/*lpr* mice, whereas repletion of B6.MRL/*lpr* lymph node-derived T cells into the I/R-resistant *Rag-1*^{-/-} mouse reconstituted tissue injury. The tissue-infiltrating T cells were found to produce IL-17. Finally, IL-23 deficient mice, which are known not to produce IL-17, displayed significantly less intestinal damage when subjected to I/R. Our data assign T cells a major role in intestinal I/R damage by virtue of producing the pro-inflammatory cytokine IL-17.

Keywords

autoimmunity; IL-17; IL-23; inflammation; ischemia reperfusion; MRL/*lpr* mice; p19^{-/-} mice; systemic lupus erythematosus; T cell

Ischemia/reperfusion (I/R) represents a model of tissue injury in which circulation is reinstalled in an organ transiently deprived of blood flow. The ischemic insult alters the affected tissue making it susceptible to inflammatory damage during reperfusion. Furthermore, mediators produced in the ischemic areas diffuse when circulation is restored and cause inflammation in

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Corresponding author: George C. Tsokos, MD, Beth Israel Deaconess Medical Center, Harvard Medical School, 330 Brookline Avenue, CLS-937, Boston, MA 02115, USA. Tel. 617 735-4161; Fax 617-435 4170, gtsokos@bidmc.harvard.edu.

³C. E. and J. C. C. contributed equally to this work

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remote organs not exposed to ischemia [1]. Several molecules and cells have been implicated in I/R. These include mainly elements of the innate immune response such as reactive oxygen species, cytokines and chemokines, complement, natural antibodies, and neutrophils [1]. The adaptive immune system has not yet been assigned a definitive role in the production of tissue injury in classical models of I/R. Nevertheless, recent evidence suggests that T cells participate actively in I/R injury as they have been found to enter the damaged tissue early after circulation is restored [2]. T cell depletion has been shown to diminish organ damage in intestinal [3], hepatic [4], and kidney models of I/R [5]. Although the mechanism by which T cells contribute to tissue injury is not completely understood, secretion of pro-inflammatory cytokines is probably involved. Hence, IFN- γ and TNF- α have been linked to tissue damage in the setting of I/R [5,6].

I/R-mediated organ damage has relevance to a number of clinical settings that include acute organ infarction, shock, and organ transplantation [1]. The model is also relevant in the study of systemic lupus erythematosus (SLE), a chronic inflammatory disease of autoimmune etiology strongly associated with vascular injury [7,8]. In patients with SLE, a number of pathogenic pathways converge to cause vascular damage. These include thrombotic diathesis [9], accelerated atherosclerosis [8], and vasculitis [10]. In this setting, the aforementioned factors are concomitant to an abnormal pro-inflammatory skewed immune response [11]. The coincidence of an inflammatory milieu with the proclivity to vascular damage makes patients with SLE prone to develop organ damage due to I/R phenomena. The importance of this fact is evidenced by the finding that mice with lupus-like disease are more susceptible to organ damage due to I/R than non-autoimmune mice [12]. Several facts could account for the increased susceptibility of lupus-prone mice to I/R. In a previous communication, we demonstrated that the presence of autoantibodies significantly increased the tissue injury score after I/R [12].

In this communication, we confirm that tissue injury after I/R is increased in lupus-prone mice (B6.MRL/*lpr*) when compared to normal non-autoimmune mice. Depletion of T cells suppresses the development of I/R injury, whereas transfer of MRL/*lpr* T cells to the I/R injury-resistant *Rag-1*^{-/-} mouse, reconstitutes tissue damage. We show that tissue-infiltrating T cells produce IL-17, and demonstrate the pathogenic importance of this cytokine using a mouse deficient in IL-23. Although T cell infiltration is not modified in IL-23 deficient mice, T cells fail to produce IL-17 and tissue injury is significantly reduced. Thus, we propose a model in which T cells infiltrate ischemic tissues early during reperfusion and secrete IL-17 that acts as a major pathogenic element in intestinal I/R tissue damage.

Materials and Methods

Mice

Adult male B6.MRL*Tnfrsf6*^{lpr} (B6.MRL/*lpr*), B6.*Rag-1* (*Rag-1*^{-/-}), and control C57BL/6 mice were obtained from The Jackson Laboratory (Bar Harbor, ME), and housed in the animal facility of the Uniformed Services University for the Health Sciences. IL-23p19^{-/-} (IL-23-deficient mice) and their WT controls were generated on a mixed B6 X 129 background.

Reagents

The following antibodies were used for immunofluorescence studies: FITC-labeled Armenian hamster anti-mouse CD3 ϵ (Clone 145-2C11); FITC-labeled rat anti-mouse CD4 (Clone RM4-5); FITC-labeled rat anti-mouse CD8 (Clone 53-6.7); FITC-labeled rat-anti mouse Ly-6G and Ly-6C (Gr-1; Clone RB6-8C5); FITC-labeled rat anti-mouse CD11b (Integrin α_M chain; Clone M1/70); rat anti-mouse IL-17A (Clone TC11-18H10); FITC-labeled rat IgG,

all from BD Biosciences (San Jose, CA). As secondary antibody, affinity purified Texas Red-labeled goat-anti rat IgG was used (Jackson Immunoresearch Laboratories, West Grove, PA).

Ischemia reperfusion protocol

After an acclimation period of at least 7 days, mice were prepared for surgery. Anesthesia was induced with ketamine (16 mg/kg) and xylazine (8 mg/kg) administered by i.p. injection. All procedures were performed with the animals breathing spontaneously and body temperature maintained at 37°C using a water-circulating heating pad. Research was conducted in compliance with the Animal Welfare Act and other Federal statutes and regulations relating to animals and experiments involving animals, and adheres to principles stated in the Guide for the Care and Use of Laboratory Animals, NRC Publication, 1996 edition. All procedures were reviewed and approved by the Institute's Animal Care and Use Committee, and performed in a facility accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, International.

Animals were subjected to I/R as previously described [13]. Briefly, a midline laparotomy was performed before a 30-min equilibration period. The superior mesenteric artery was identified and isolated, and a small non-traumatic vascular clamp (Roboz Surgical Instruments, Rockville, MD) was applied for 30 min. After this ischemic phase, the clamp was removed and the intestine was allowed to reperfuse for 1 or 3 hours. In some experiments, one day prior to surgery, 250 µg of rat anti-mouse CD4 IgG monoclonal Ab (BD Biosciences, Clone RM4-5) was administered by i.p. injection. As sham procedure, animals were subjected to the same surgical intervention without artery occlusion. The laparotomy incisions were sutured and the animals were monitored during the reperfusion period. Additional ketamine and xylazine was administered by i.m. injection immediately before euthanasia. There was no significant difference in survival between treatment and control groups. After euthanasia, the small intestine 10–20 cm distal to the gastroduodenal junction was removed for histological and immunofluorescence analysis as described below.

T cell adoptive transfer

Mesenteric lymph nodes from B6.MRL/*lpr* mice were isolated, rinsed, and disrupted to form a cell suspension. The suspension was then filtered through a sterile nylon mesh screen (0.45 µm, BD) to obtain a single cell suspension. T cells were isolated by negative selection using a combination of biotinylated antibodies (anti-B220, anti-CD11b, anti-Ter119, and anti-CD49b) and streptavidin coated magnetic beads (Dyna, M-280). T cells (5×10^6 per mouse) were transferred into *Rag-1*^{-/-} mice by tail vein injection. One week later, mice were subjected to I/R.

Histology and immunofluorescence

To prepare specimens for histological analysis, 2-cm segments of small intestine specimens were fixed in 10% buffered Formalin phosphate immediately after euthanasia. Next, tissues were embedded in paraffin, sectioned transversely in 5- to 7-µm sections, and stained with Giemsa. In each section, 50–100 villi were graded on a six-tiered scale, as previously described [13]. Briefly, a score of 0 was assigned to a normal villus; villi with tip distortion were scored as 1; villi lacking goblet cells and containing Guggenheims' spaces were scored as 2; villi with patchy disruption of the epithelial cells were scored as 3; villi with exposed but intact lamina propria and epithelial cell sloughing were assigned a score of 4; villi in which the lamina propria was exuding were scored as 5; and finally, villi displaying hemorrhage or denudation were scored as 6. All histological analysis was performed in a blinded manner.

For immunofluorescence, small intestine sections were snap-frozen to -70°C, and sections were cut with a cryostat and fixed in acetone. Samples were blocked in PBS + 10% FCS. Sections

were incubated overnight with the primary antibodies (1:100). After thorough washing, secondary antibodies were incubated for one hour. Sections were counterstained with DAPI (0.5 µg/mL) and mounted using anti-fade solution (Slowfade Gold, Invitrogen). Finally, slides were scanned in a Nikon Eclipse Ti confocal microscope. Images were analyzed with EZ-C1 v.3.6 software. To quantify cellular infiltrates, digital photomicrographs of stained sections were processed with the ImageJ software (ImageJ 1.35, NIH) which quantifies the pixel density (pixel density per area unit) of stained cells present in each field. Five random fields (at a power of 200 ×) were examined per tissue per animal in a blinded fashion.

Statistical analysis

Data are presented as mean ± SEM. Data were compared by one-way ANOVA with post hoc analysis using Newman-Keuls test (GraphPad/Instat Software). For nonparametric analysis of mucosal injury, data was compared using Kruskal-Wallis test with Dunn post analysis. A $p < 0.05$ was considered significant.

Results

CD4⁺ T cells infiltrate damaged intestine early during reperfusion

To determine which cell types are involved in I/R injury, C57BL/6 mice were subjected to intestinal ischemia by applying a clamp to the superior mesenteric artery for 30 minutes. After either 1 or 3 hours of reperfusion, mice were sacrificed and the intestine was stained for immunofluorescence analysis. As shown in Figures 1A and B, a significant increase in the number of CD4⁺ T cells present in the intestinal tissue was observed in mice that had been subjected to I/R, as compared to mice in which sham surgery had been performed ($p < 0.001$). Interestingly, the CD4⁺ T cell infiltrate appeared rapidly, within the first hour of reperfusion, and was short lived. It could no longer be observed 3 hours after reperfusion (Figure 1C). CD4⁺ T cells were mainly observed in central areas of the villi, where they formed small aggregates adjacent to blood vessels and lamina propria (Figure 1 A and B). The early tissue infiltration response was highly specific for CD4⁺ T cells and analogous phenomena were not observed when other cell types were studied. The numbers of CD8⁺ T cells, granulocytes (Gr-1⁺ cells), and CD11b⁺ cells (monocytes and granulocytes), did not increase significantly when we compared intestines subjected to I/R injury with intestines in which sham procedures had been performed (Figure 1D, E, F).

CD4⁺ T cell infiltration is amplified in MRL mice

Mice deficient in Fas molecule (MRL/*lpr*) develop a T cell driven autoimmune disease that resembles SLE [14]. T cells from MRL/*lpr* mice exhibit an activated phenotype and have been shown to induce auto-antibody production from auto-reactive B cells [15], secrete pro-inflammatory cytokines [16], and infiltrate organ tissues [17]. Likewise, in humans with SLE, peripheral blood T cells have been shown to share an activated phenotype with the T cells that infiltrate kidneys affected by lupus nephritis [18]. These facts suggest that T cells from MRL/*lpr* mice and from humans with SLE have a propensity to infiltrate tissues and cause inflammation. To test this hypothesis we subjected B6.MRL/*lpr* mice to intestinal ischemia and compared the CD4⁺ T cell infiltration after 1 hour of reperfusion to that observed in congenic B6 mice. As shown in Fig. 2, intestinal infiltration by CD4⁺ T cells was significantly increased in B6.MRL/*lpr* mice when compared to non-autoimmune B6 mice ($p < 0.001$). B6.MRL/*lpr* mice showed an increased amount of CD4⁺ T cells even in the absence of ischemic insult. After I/R, the number of CD4⁺ T cells was dramatically amplified ($p < 0.001$). The distribution of the infiltrating CD4⁺ T cells was not different in MRL/*lpr* mice than in wild type mice. CD4⁺ T cells were mostly found close to the vasculature at the base of the villi and within the lamina propria.

Infiltrating CD4⁺ T cells play a role in I/R-mediated tissue damage

The amplified intestinal CD4⁺ T cell infiltration observed in B6.MRL/*lpr* mice could be a non-pathogenic phenomenon. However, tissue injury scores were significantly higher in B6.MRL/*lpr* mice than in non-autoimmune B6 mice indicating that the autoimmune proclivity of B6.MRL/*lpr* mice leads to enhanced susceptibility to ischemic injury. The degree of tissue damage (Fig. 3A) was mirror-imaged in the overall intensity of the T cell infiltration (Fig. 3B), suggesting a role for CD4⁺ T cells in the enhanced tissue damage. To test this hypothesis, CD4⁺ T cells were depleted from B6.MRL/*lpr* mice 24 hours prior to the induction of I/R. As expected, cell depletion with anti-CD4 Ab resulted in a decrease in the amount of infiltrating CD4⁺ T cells (data not shown). As shown in Fig. 3C, CD4⁺ T cell depletion decreased basal tissue injury levels, indicating that the low level intestinal damage observed in sham-operated B6.MRL/*lpr* mice is caused by the spontaneously infiltrating CD4⁺ T cells observed in lupus-prone mice. Importantly, CD4⁺ T cell depletion reduced intestinal injury scores in B6.MRL/*lpr* mice subjected to I/R to levels similar to those found in sham-operated mice ($p < 0.001$), providing further evidence of the pathogenic role of CD4⁺ T cells in I/R-mediated intestinal injury.

To further establish the contribution of CD4⁺ T cells in the I/R-mediated bowel injury, we transferred lymph node T cells from B6.MRL/*lpr* mice into mice deficient in lymphocytes (*Rag-1*^{-/-}). *Rag-1*^{-/-} are known to be resistant to I/R damage [12]. Thus, this approach would allow the evaluation of the degree of damage caused by T cells in the absence of other possible pathogenic factors such as antibodies and B cells. As shown in Fig. 3D, T cell repletion increased significantly the degree of tissue damage induced by I/R in *Rag-1*^{-/-} mice ($p = 0.02$) confirming the claim that the mere presence of B6.MRL/*lpr* T cells is capable of promoting tissue injury upon an I/R insult.

Infiltrating T cells produce IL-17

T cells can induce inflammation and tissue damage using different mechanisms. However, the kinetics with which they appeared and disappeared from ischemic tissue during reperfusion suggested that their mechanism of action was the production of cytokines that could induce tissue damage by acting on resident cells and attracting inflammatory cells to the injured site. IL-17 is a potent pro-inflammatory cytokine produced by the effector CD4⁺ T cell subset known as Th17 [19]. IL-17 has been linked to several inflammatory disease models such as experimental autoimmune encephalomyelitis [20] and collagen-induced arthritis [21]. The receptor(s) for IL-17 is expressed by a large variety of cells and thus it has a wide array of effects including the recruitment of macrophages and neutrophils to inflamed tissues [22]. Therefore, we stained intestinal sections from B6 and B6.MRL/*lpr* mice subjected to ischemia followed by 1 h reperfusion with FITC-labeled anti-CD3 and rat anti-mouse anti-IL-17 antibodies (followed by Texas Red-labeled goat anti-rat) and analyzed them using confocal microscopy. As shown in Fig. 4A, we found neither T cells nor IL-17 in the intestinal tissue of normal mice subjected to sham surgery. I/R led to an increase in the number of T cells infiltrating the tissues as well as to the deposition of IL-17. A majority of the T cells co-expressed IL-17 and CD3 (Fig. 4B, yellow color). We could also detect IL-17 in other cells that lacked CD3 expression. Congruent with our earlier findings, we could observe both T cells and IL-17 in intestinal villi from B6.MRL/*lpr* mice even in the absence of ischemic injury (Fig. 4). I/R was associated with a dramatic increase in the number of infiltrating IL-17⁺ CD3⁺ T cells in MRL mice, confirming that T cells produce IL-17 during reperfusion of intestinal tissue subjected to an ischemic assault.

Absence of IL-17 diminishes I/R intestinal injury

To test the hypothesis that IL-17 plays a pathogenic role in tissue damage induced by I/R, we used IL-23p19^{-/-} mice. These mice lack the 19 kD subunit that constitutes IL-23 when coupled

to a 40 kD subunit shared with IL-12. In consequence, they exhibit a markedly reduced IL-17 response [19] and are resistant to IL-17-mediated diseases such as EAE [20]. We subjected p19 KO mice to I/R and analyzed the intensity of tissue damage, T cell infiltration, and IL-17 expression after 1 h reperfusion. As shown in Fig. 5A, the tissue injury score decreased significantly ($p=0.002$) in p19 KO mice when compared to congenic controls. The results were highly reproducible within groups and the experiment was only performed once ($n=3$ per group). As expected, IL-17 was virtually absent in intestinal tissue from p19 KO mice. However, T cell infiltration was not modified (Fig 5B). These results indicate that IL-17 production, rather than the mere presence of infiltrating T cells, is necessary for the development of full-blown I/R-mediated intestinal tissue injury.

Discussion

In this communication, we confirm that T cells play a fundamental role in the early phase of the intestinal I/R-mediated tissue injury and demonstrate that they do so by means of producing the pro-inflammatory cytokine IL-17. Lupus prone mice which are known to suffer more intestinal I/R damage [12], display more intense T cell infiltration, as well as a more pronounced IL-17 tissue expression. The claim that T cells are important in I/R damage is supported by two experiments. First, depletion of CD4⁺ T cells in the B6.MRL/*lpr* mouse limits significantly T cell infiltration and tissue damage, and second, replenishment of the I/R-resistant Rag-1^{-/-} mouse with B6.MRL/*lpr* lymph node T cells reconstitutes the intestinal I/R damage. Finally, we provide first evidence that the infiltrating T cells produce IL-17, and demonstrate the pathogenic importance of this cytokine which apparently enables the subsequent steps of tissue damage.

The fast kinetics with which T cell infiltration occurs, indicates that the observed CD4⁺ cells are memory T cells since they migrate to inflamed tissues and produce inflammatory cytokines. This behavior is characteristic of the inflammatory response in the setting of I/R and it has been documented in previous work [4]. In agreement with our findings, T cell infiltration has shown to be negligible after 3 hours of reperfusion [4]. A different communication, however reported peak T cell adhesion to intestinal endothelial cells 6 hours after reperfusion [3]. We assume that methodological differences may account for the discrepant kinetics. We have found that the timing of the transfer is particularly relevant for the behavior of T cells, because their homing ability may be diminished during the first hour after reperfusion (Moratz CM, unpublished observation). Interestingly, the same report [3] demonstrated that early I/R-induced phenomena (i.e. albumin leakage and neutrophil accumulation) depended on the presence of T cells, since they were abolished in SCID mice and restored after T cell reconstitution [3]. Thus even though they detected late T cell-endothelial cell adhesion, their data supports an early role for T cells in the I/R injury process.

Tissue injury produced by I/R is increased in mice from autoimmune strains [11]. In this paper we confirm this fact and add T cells as central effector players in I/R injury. T cells from patients with SLE and mice with lupus-like diseases exhibit a number of biochemical abnormalities that alter their activation and effector functions. In patients with SLE, T cells have been found to infiltrate the kidney [23]. Because peripheral blood T cells and kidney infiltrating cells express the adhesion molecule CD44 and its signaling partner pERM, it has been claimed that CD44 guides the kidney infiltrating T cells [18]. T cells from lupus-prone mice express more CD44 (Ref. [24], and unpublished data from the Tsokos lab). This may account for the increased density of infiltrating T cells into the I/R-damaged intestine observed in this report (Fig. 2). It is possible that tissues affected by I/R express more hyaluronic acid –the ligand for CD44. Alternatively, chemokine receptor expression may be altered in SLE T cells [25,26] and the I/R intestine may produce more of the corresponding chemokines.

Th17 cells represent a CD4⁺ T cell subset which produces increased amounts of IL-17 [19]. IL-17 participates in the immune response against various infections, particularly extracellular bacteria and fungi, and has been linked to inflammatory and autoimmune diseases in humans and mice [27]. In particular, IL-17 has proved to play a key role in experimental autoimmune encephalomyelitis [28,29] and collagen-induced arthritis [21,30]. The action of IL-17 is particularly broad, since its receptor is expressed by a large array of immune and non-immune cells, especially epithelial tissues [27]. The production of IL-17 leads to the induction of multiple pro-inflammatory effects including the production of cytokines and chemokines (IL-6, IL-8, GM-CSF, G-CSF, CXCL1, CXCL10) [19]. In addition, it plays a role in the local recruitment and activation of neutrophils [31]. These functional characteristics, along with the demonstration of its early presence in I/R-damaged tissues, suggested that IL-17 plays a pivotal role in I/R injury. Functionally, IL-17A and IL-17F are very similar, and the presence of one can compensate for the absence of the other. Thus, deletion of either gene does not lead to any phenotypic difference. Moreover, the distance that separates both genes has precluded the production of double KO mice. For this reason, IL-23 deficient (p19^{-/-}) mice that fail to produce IL-17 have been extensively used as a surrogate IL-17 deficient model [20,32-34]. IL-23 p19 KO mice have a decreased capacity to develop T cell-dependent humoral immune responses and their ability to mount delayed type hypersensitivity responses is impaired [35]. The presence of IL-23 is particularly relevant for the production of IL-17 in the intestine [19,36]. Thus, we believe that the p19 KO mouse is an ideal model for the study of the IL-23-IL17 axis in intestinal I/R. Our results indicate that the powerful pro-inflammatory effect of IL-17 produced by T cells is responsible for a large proportion of the immune-mediated tissue damage triggered by ischemia. However, we cannot rule out the importance of other IL-23-associated factors.

IL-17 production in response to I/R is present in both normal and lupus-prone mice; however, it is exaggerated in the autoimmune mice. Although we do not have sufficient information to explain why this happens, it is possible that the intestinal local cytokine and chemokine milieu that results following I/R injury may account for the increased rate of Th17 cell infiltration. IL-2, which is able to restrain Th17 cell development [37], is produced in abnormally low levels in lupus-prone mice [38]. Conversely, the secretion of IL-6, which has Th17-inducing capacity [32,39], is increased [40].

In conclusion, we have presented evidence that IL-17 producing T cells infiltrate intestinal tissue after mesenteric I/R and that this response is amplified in lupus-prone mice. Our data suggest that control of IL-17 production may be of benefit to the I/R-instigated tissue damage which represents a component of various clinical conditions including organ transplant, coronary blood flow reinstatement following myocardial infarction and autoimmune diseases where the vascular bed is frequently affected.

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FIGURE 1.

CD4⁺ T cells infiltrate ischemic intestine early during reperfusion. Representative processed photomicrographs of a B6 mouse subjected to sham (A) or I/R (B) procedure (CD4⁺ cells are highlighted in magenta). Increased numbers of CD4⁺ T cells were observed in intestines from mice subjected to I/R (B), as compared to non-ischemic intestines from control mice (A). C. Cumulative data expressed as square pixel density from sham and I/R mice sacrificed at 1 and 3 hours after reperfusion. D. Infiltration of Gr-1⁺ cells in intestinal tissue of mice subjected to sham surgery (white bars) or superior mesenteric artery occlusion (black bars). Intestines were stained 1 hour after reperfusion and cell infiltration was quantified as square pixel density. Although Gr-1⁺ cells showed a trend to increase in I/R mice (D), the difference did not reach

statistical significance ($p=0.26$). Likewise, there was no significant difference in the number of CD11b⁺ ($p=0.12$) and CD8⁺ cells ($p=0.75$) in mice subjected to I/R injury when compared to mice that underwent the sham procedure (*E* and *F*, respectively). $*p<0.001$.

FIGURE 2.

I/R-induced CD4⁺ T cell infiltration is increased in lupus-prone mice. B6 (black bars) and B6.MRL/*lpr* (white bars) mice were subjected to intestinal ischemia followed by 1 hour reperfusion. The magnitude of the induced CD4⁺ T cell infiltration was significantly larger in B6.MRL/*lpr* mice (B) than in congenic control B6 mice (A). C. Cumulative data expressed as square pixel density. * $p < 0.001$.

FIGURE 3.

CD4⁺ T cells mediate I/R-induced injury in B6.MRL/*lpr* mice. Tissue injury (A) closely paralleled CD4⁺ T cell infiltration (B). C. CD4⁺ cell depletion caused a decrease in tissue injury scores in B6.MRL/*lpr* mice and abrogated the effect of I/R diminishing the intestinal damage to levels similar to those of sham operated mice. D. Transfer of T cells obtained from B6.MRL/*lpr* lymph nodes restored tissue damage in I/R-resistant Rag-1^{-/-} mice. * $p < 0.001$; ** $p < 0.05$.

FIGURE 4.

Intestine-infiltrating T cells produce IL-17. Tissues from B6.MRL/*lpr* and control mice subjected to sham-surgery and I/R were stained with FITC-labeled anti-CD3 Ab and rat anti-mouse IL-17 Ab (followed by Texas Red-labeled goat anti-rat Ab). Sections were scanned in a confocal microscope. **A.** A scarce number of T cells can be observed in normal intestinal villi from a sham-operated B6 mouse. In contrast, I/R leads to the accumulation of T cells and IL-17 (upper panels). In intestinal sections from B6.MRL/*lpr* mice, T cells are present in sham-operated animals. Ischemic injury leads to a massive infiltration of IL-17⁺ T cells (lower panels). **B.** Higher magnification demonstrates that a majority of infiltrating T cells produce IL-17.

FIGURE 5.

IL-23 deficient mice are protected from I/R-mediated intestinal injury. p19 deficient and congenic control mice ($n=3$ per group) were subjected to 30 min mesenteric occlusion followed by 1 h reperfusion. **A.** Tissue injury was significantly inhibited in p19 KO mice when compared to control mice ($*p=0.002$). **B.** Staining with Armenian hamster anti-mouse CD3-FITC and rat anti-mouse IL17A (followed by goat anti-rat Texas red) showed comparable T cell infiltration in p19 KO and control mice, but demonstrated absence of IL-17 expression in p19 KO mice. Representative images are shown. The three left side panels show panoramic views (100 $\times$). The six panels on the right show representative villi.