Effect of interleukin-15 on depressed splenic dendritic cell functions following trauma-hemorrhage

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Kawasaki T, Choudhry MA, Schwacha MG, Bland KI, Chaudry IH. Effect of interleukin-15 on depressed splenic dendritic cell functions following trauma-hemorrhage. Am J Physiol Cell Physiol 296: C124–C130, 2009. First published November 5, 2008; doi:10.1152/ajpcell.00447.2008.—Although trauma-hemorrhage (T-H) induces suppressed splenic dendritic cell (DC) maturation and antigen presentation capacity, it remains unclear whether IL-15 modulates splenic DC functions. The aim of this study therefore was to investigate the effect of IL-15 on splenic DC functions after T-H. Male C3H/HeN mice (6–8 wk old) were randomly assigned to T-H or sham operation. T-H was induced by midline laparotomy and ~90 min of hemorrhagic shock (blood pressure 35 mmHg), followed by fluid resuscitation (4× the shed blood volume in the form of Ringer lactate). Two hours later, mice were killed, splenic DCs were isolated, and the effects of exogenous IL-15 on their costimulatory factors, major histocompatibility class II expression, ability to produce cytokines, and antigen presentation were measured. The results indicate that IL-15 production capacity of splenic DCs was reduced following T-H. Ex vivo exposure to IL-15 attenuated the suppressed production of TNF-α, IL-6, and IFN-γ from splenic DCs following T-H. In addition, expression of surface antigen studies demonstrate that exogenous IL-15 attenuated T-H-induced downregulation of the activation of DC. The suppressed splenic DC antigen presentation function following T-H was also attenuated by IL-15 treatment. Moreover, IL-15 enhanced IL-12-induced IFN-γ production and antigen presentation by splenic DCs. These data suggest that ex vivo treatment with IL-15 following T-H provides beneficial effects on splenic DCs. The depression in IL-15 production by splenic DCs could contribute to the host’s enhanced susceptibility to infections following T-H.

antigen presentation; tumor necrosis factor-α; major histocompatibility class II

SEVERE INJURY LEADS TO A MARKED immunsuppression that is associated with an increased susceptibility to sepsis, organ failure, and mortality (4, 5, 36). Previous studies demonstrated that immune cell dysfunctions are pivotal in the development and function of trauma patients who develop multiple organ failure and infectious complications (24–26, 31). Simultaneous with the often-exaggerated inflammatory response, these patients’ macrophages show depressed human leukocyte antigen HLA-DR expression and a failure of antigen-presenting functions. This in turn may contribute to a suppression of T cell effector responses. A recent report demonstrated that trauma is a major health problem in the United States, causing 4.5% of total deaths in the year 2003 (13). Furthermore, the depression of cell-mediated and humoral immunity has been postulated to be a critical factor for predisposing these injured patients to opportunistic as well as nosocomial infections (10).

Dendritic cells (DCs) are recognized as the key antigen-presenting cells for the initiation of T cell-dependent immune responses (33). In mediating this role, DCs pass through different functional states of activation. Resting DCs reside in peripheral tissues in an immature state and are highly efficient in the capture and uptake of antigen. Upon receipt of various activating stimuli, they are induced to migrate to secondary lymphoid organs, with concomitant reduction in their capacity for antigen uptake. However, their ability to present antigen and activate naïve T cells is markedly increased. Activated DCs are also capable of directing the type of T cell response, a process that may be affected by cytokines secreted by the DCs.

Interleukin (IL)-15 is a 14–15 kDa member of the four α-helix bundle family of cytokines (3, 6, 12, 37). IL-15 and IL-2 share many functional activities on natural killer (NK) and T cells; this is probably due to receptor sharing, which results from structural and functional homology. IL-15 is a member of the IL-2 family. Both IL-2 and IL-15 have high affinity binding to the IL-2Rα subunit, whereas IL-2Rβγ c subunits are required for high affinity binding to IL-15Rα (11). In contrast, distinct types of cells produce IL-2 and IL-15. Whereas IL-2 is predominantly produced by T cells, IL-15 mRNA is barely detectable in T cells and is mainly expressed in placentas, skeletal muscle, kidney, lung, heart, fibroblasts, epithelial cells, monocytes, and DCs. IL-15 is a pivotal cytokine that influences the development and function of innate immune cells, including NK cells, NK T cells, intestinal γδ T cells, and DCs (9, 17, 20).

Although our previous studies have shown that trauma-hemorrhage induces suppressed splenic DC maturation and antigen presentation capacity (15, 16), it remains unclear whether IL-15 modulates splenic DC functions under such condition. The aim of this study therefore was to investigate the effect of IL-15 on splenic DC functions following trauma-hemorrhage.

MATERIALS AND METHODS

Mice. Male C3H/HeN mice (Charles River, Wilmington, MA), 6–8 wk old and weighing 20–25 g, were used in the experiments. These mice were allowed to acclimate to the animal facility for 1 wk before the experiments. Animal experiments were conducted in accordance with guidelines set forth in the Animal Welfare Act and the Guide for the Care and Use of Laboratory Animals, published by the National Institutes of Health (NIH Publication No. 85-23, Revised 1996) and were approved by the Institutional Animal Care and Use Committee at the University of Alabama at Birmingham.

Trauma-hemorrhage. Animals were anesthetized with isoflurane (Attane; Minrad, Buffalo, NY) and restrained in supine position. A
2.0-cm midline laparotomy (i.e., induction of soft tissue trauma) was performed and then was closed aseptically in two layers using 6-0 Ethilon sutures (Ethicon, Somerville, NJ). Subsequently, both femoral arteries were aseptically catheterized with polyethylene-10 tubing (Clay-Adams, Parsippany, NJ), and the animals were allowed to awaken. Blood pressure was monitored continuously through one of the femoral catheters using a blood pressure analyzer (Digi-Med BPA-190; Micro-Med, Louisville, KY). Upon awakening, the animals were bled through the other catheter to mean arterial pressure of 35 ± 5 mmHg that was maintained for 90 min. At the end of that period, animals were resuscitated with four times the shed blood volume in the form of lactated Ringer solution over 30 min. Lidocaine was applied to the groin incision sites, the catheters were removed, the vessels were ligated, and the incisions were closed. Sham-treated animals underwent the same anesthetic and surgical procedures, but neither hemorrhage nor fluid resuscitation was performed. The animals were anesthetized by isoflurane administration 2 h after trauma-hemorrhage, at which time blood and spleen were collected for analysis.

Isolation of splenic DCs and flow cytometric analysis. Spleens were digested by Liberase Cl (Roche, Indianapolis, IN) and teased apart by repeated pipetting in PBS containing 5% FCS and 5 mM EDTA. The red blood cells were osmotically lysed, and splenocytes were blocked with 1 μg/ml Fc block (clone: 93) antibody for 15 min on ice. Cell suspensions were enriched with anti-CD11c magnetic beads and positive selection columns MS+ according to the manufacturer’s instructions (Miltenyi Biotec, Auburn, CA). Flow cytometric analysis demonstrated that cells contained >90% CD11c-positive cells. After incubation with or without murine recombinant (r)IL-15 (1 ng/ml) (PeproTech, Rocky Hill, NJ) (34) for 24 h at 37°C, 5% CO2, cell suspensions were stained with antibody against CD11c and antibody against surface markers major histocompatibility class (MHC)-II (clone: MS5/114,15.2), CD40 (clone: 1C10), CD80 (clone: 16–10A1), CD83 (clone: Michel-17), or CD86 (clone: GL1) for DCs (eBioscience, San Diego, CA). Appropriate fluorescein-conjugated IgGs were used as isotype controls. Cells were gated for CD11c-positive cells and were analyzed using a BD LSRII flow cytometer (Becton Dickinson, San Diego, CA). A total of 10,000 events were collected for analysis.

Determination of cytokine levels in CD11c-positive cell culture supernatants. Purified CD11c-positive cells (1 × 10^6 cells/well) from spleen were cultured in 96-well tissue culture plates in RPMI-1640 medium containing 10% FCS and 50 ng/ml gentamicin with or without LPS (10 μg/ml). In some experiments, CD11c-positive cells were incubated with murine rIL-12 (10 ng/ml) (PeproTech) and/or murine rIL-15 (1 ng/ml) (34). After 24 h incubation at 37°C, 5% CO2, the plate was centrifuged at 400 g for 10 min. Supernatants were collected and frozen at −80°C until use. The levels of TNF-α, IL-6, and IFN-γ in the DC supernatant were measured using Cytometric Bead Array (CBA) mouse inflammation kit (BD Biosciences, San Diego, CA) according to the manufacturer’s instructions.

Antigen presentation. The capacity of purified splenic CD11c-positive cells to present antigen to D10.G4.1 cloned helper T cells (American Type Culture Collection, Manassas, VA) was carried out according to the method of Ayala et al. (2). After incubation with murine rIL-12 (10 ng/ml) and/or murine rIL-15 (1 ng/ml) for 24 h at 37°C, 5% CO2, splenic DCs were incubated for 30 min (37°C, 5% CO2 in the dark) with 50 μg/ml mitomycin C (Sigma-Aldrich, St. Louis, MO), following which the cells were washed four times in PBS, and a series of dilutions was made. The DCs were then cocultured with 2 × 10^5 cells of D10.G4.1 in the presence or absence of 300 μg/ml conalbumin (Sigma Chemical, St. Louis, MO) for 48 h (37°C, 5% CO2). 5-Bromo-2′-deoxyuridine was added to the cells, and cells were reincubated for 24 h. After incubation, T-cell proliferation was determined using the Cell Proliferation Biotrak ELISA System (Amersham Biosciences, Piscataway, NJ).

Fig. 1. IL-15 production by CD11c-positive cells from sham-operated (sham) and trauma-hemorrhage animals. At 2 h following resuscitation, splenic dendritic cells (DCs) were purified by magnetic-activated cell separation (MACS). Purified CD11c-positive cells were cultured in 96-well tissue culture plates with LPS (10 μg/ml) for 24 h. The levels of IL-15 in DC supernatant were measured by ELISA. Data are shown as means ± se of 6 animals in each group. *P < 0.05, **P < 0.001 compared with no LPS stimulation. #P < 0.001 compared with equivalent sham.
MHC-II expression on splenic DCs. Exogenous IL-15 improved but did not restore MHC-II expression to sham levels following trauma-hemorrhage.

Effects of exogenous IL-15 on cytokine production by splenic DCs. There were no significant differences between sham and trauma-hemorrhage DC production of TNF-α and IL-6 without stimulation. After stimulation, these cytokine concentrations increased significantly in both sham and trauma-hemorrhage mice. However, LPS-induced production of TNF-α and IL-6 was significantly suppressed in splenic DCs following trauma-hemorrhage (Fig. 4). The suppression in TNF-α and IL-6 production was significantly attenuated by exogenous IL-15 in trauma-hemorrhage mice; however, the levels were not restored to those observed in sham animals. Previous study demonstrated that IL-12 synergizes with IL-15 to stimulate production of TNF-α in human NK cells (7); therefore, we also
investigated the effect of IL-12 on LPS-induced TNF-α and IL-6 production. Ex vivo treatment with IL-15 and IL-12 increased production of these cytokines in trauma-hemorrhage mice. Furthermore, the result of incubation with both IL-12 and IL-15 demonstrated that they had an additive effect on TNF-α and IL-6 production of splenic DCs. DCs have been shown to produce IFN-γ in response to certain stimuli, such as IL-12 (27). We investigated whether IFN-γ production was also altered by IL-15-mediated DC activation. There were no significant differences between sham and trauma-hemorrhage DC production of IFN-γ without stimulation. IL-12-induced IFN-γ production by splenic DCs also decreased following trauma-hemorrhage (Fig. 5). Ex vivo treatment with IL-15 increased these cytokines production in both sham and trauma-hemorrhage groups. Furthermore, the result of incubation with both IL-12 and IL-15 demonstrated that they had an additive effect on IFN-γ production of splenic DCs.

Effects of exogenous IL-15 on antigen-presenting capacity of DCs after trauma-hemorrhage. In an effort to determine whether IL-15 has a crucial role in the antigen-presenting function of splenic DCs, antigen presentation was also carried

Fig. 3. Changes in surface major histocompatibility class (MHC) II expression of CD11c-positive splenic DCs. At 2 h following resuscitation, splenic DCs were purified by MACS sorting. After incubation with or without murine rIL-15 (1 ng/ml) for 24 h, cells were stained with antibody against CD11c and antibody against MHC-II. Cells were gated for CD11c-positive cells and analyzed by flow cytometry. Data are shown as means ± SE of 6 animals in each group. *P < 0.05 compared with sham with equivalent sham.

Fig. 4. TNF-α and IL-6 production by CD11c-positive cells. At 2 h following resuscitation, splenic DCs were purified. Purified CD11c-positive cells were cultured in 96-well tissue culture plates with LPS (10 μg/ml) for 24 h. In some experiments, CD11c-positive cells were incubated with murine rIL-12 (10 ng/ml) and/or murine rIL-15 (1 ng/ml). The levels of TNF-α and IL-6 in DC supernatant were measured using Cytometric Bead Array (CBA). Data are shown as means ± SE of 6 animals in each group. *P < 0.001 compared with no LPS stimulation. #P < 0.05, ##P < 0.001 compared with sham with LPS stimulation.

Fig. 5. IFN-γ production by CD11c-positive cells. At 2 h following resuscitation, splenic DCs were purified. Purified CD11c-positive cells were cultured in 96-well tissue culture plates with murine rIL-12 (10 ng/ml) and/or murine rIL-15 (1 ng/ml) for 24 h. The levels of IFN-γ in DC supernatant were measured using CBA. Data are shown as means ± SE of 6 animals in each group. *P < 0.05, **P < 0.001 compared with no IL-12 treatment. #P < 0.05, ##P < 0.001 compared with sham with IL-12 stimulation.
out in the presence of murine rIL-12 and/or rIL-15. The CD11c-positive cells isolated from trauma-hemorrhage mice showed a lower capacity to stimulate D10.G4.1 T-cell line proliferation than those from sham controls in the presence of conalbumin (the antigen to which the D10.G4.1 cell responds following appropriate antigen processing and presentation by the DCs) (Fig. 6). Ex vivo treatment with IL-12 and IL-15 increased suppressed antigen-presenting capacity of DCs following trauma-hemorrhage; moreover, they had an additive effect on antigen presentation function.

**Cell viability.** In our experiments, the viability of the cells was >98% at isolation and >95% after incubation. There were no significant differences between the trauma-hemorrhage mice and the sham mice in the viability of the cells at any time point during the incubation period.

**DISCUSSION**

The present study demonstrates that IL-15 production capacity of splenic DCs is reduced following trauma-hemorrhage. Ex vivo exposure to IL-15 increases DC ability to produce TNF-α, IL-6, and IFN-γ following trauma-hemorrhage. In addition, expression of surface antigen studies demonstrate that exogenous IL-15 attenuates trauma-hemorrhage-induced downregulation of the activation of DCs. Furthermore, suppressed splenic DC antigen presentation function following trauma-hemorrhage is also attenuated by IL-15 treatment. IL-15 also has the additive effects of IL-12 on IFN-γ production ability and antigen-presenting function of splenic DCs. These data suggest that IL-15 might influence the immune function of splenic DCs following trauma-hemorrhage.

The balance between proliferation and death of cells is crucial for the normal immune response to invading pathogens. The total number of lymphocytes is tightly controlled by cytokines. IL-15 is crucial for the existence of a number of lymphocytes that include NK, NKT, γδT, and CD8+CD44hi T cells (17, 20). Recently, Dubois et al. (9) reported that IL-15 is also necessary for DCs. It is becoming clear that IL-15 is the key regulator of immune cell growth, differentiation, and function. In our previous study, we showed that percentages of CD11c-positive splenocytes and 33D1-positive splenocytes decrease following trauma-hemorrhage (16). In this study, we found that IL-15 production capacity of splenic DCs from trauma-hemorrhage mice is suppressed significantly; therefore, one potential mechanism for the loss of splenic DCs could be due to decreased levels of IL-15 in spleen. Furthermore, it has also been reported that both splenic IL-15 mRNA and IL-15Rα mRNA were suppressed in thermally injured mice (35). Fms-like tyrosine kinase 3 ligand treatments after burn injury increased the number of DCs in spleen, and this increase was associated with the increase of the splenic levels of IL-15 mRNA and IL-15Rα mRNA (34). These studies also suggest that stressful situations, such as trauma-hemorrhage and thermal injury, induce decreased levels of splenic IL-15 and that there is a close relationship between IL-15 levels and the number of DCs.

Immune function of DCs is dependent on their maturation status (33). Immature DCs are strategically located in tissues that represent pathogen entry routes, where they continuously monitor the environment via the uptake of both particulate and soluble products. Immature DCs have several features that allow them to capture antigens such as ingest particles and microbes by phagocytosis. Mature DCs highly express MHC and costimulatory molecules, including CD40, CD80, CD83, and CD86, on their surface. DC maturation is associated with enhanced production of inflammatory cytokines and chemokines, reduced endocytic and phagocytic capacity, and acquisition of migratory functions. They also have the ability to activate both Th1 and Th2 cell responses. In this study, our results demonstrate the relevance of IL-15 as a DC activator. Ex vivo exposure of DCs to IL-15 attenuates the suppressed production of TNF-α, IL-6, and IFN-γ following trauma-hemorrhage. In addition, IL-15 treatment stimulated increased expression of CD40, CD83, and MHC-II molecules and an augmented ability to proliferate T cells. This finding is in accordance with the previous study of Mattei et al. (21), which showed that IL-15 activates DCs, because in vivo or ex vivo

**Fig. 6. Changes in antigen-presenting capacity of CD11c-positive cells.** Antigen-presenting capacity was determined using antigen conalbumin specifically to stimulate D10.G4.1 cloned helper T-cell proliferation. At 2 h following resuscitation, splenic DCs were purified from splenocytes. After incubation with murine rIL-12 (10 ng/ml) and/or murine rIL-15 (1 ng/ml) and treatment with mitomycin C, DCs were cocultured with 2 × 10⁶ cells of D10.G4.1 in the presence or absence of 300 μg/ml conalbumin for 48 h. 5-Bromo-2’-deoxyuridine was added to the cells, and cells were reincubated for 24 h. After incubation, T-cell proliferation was measured using ELISA. Data are shown as means ± SE of 6 animals in each group. *P < 0.05, **P < 0.001 compared with sham. T-H, trauma-hemorrhage; OD₄₅₀, optical density at 450 nm.
exposure of splenic DCs to IL-15 resulted in an upregulation of costimulatory molecules, markedly increased production of IFN-γ by DCs, and an enhanced ability of DCs to stimulate antigen-specific CD8+ T-cell proliferation. DC-derived IL-15 is also required for the functional maturation of DCs, such as IL-12 production in response to LPS and agonistic anti-CD40 mAb combined with IL-4 stimulation ex vivo (28). Taken together, our results indicate that IL-15 plays an important role in the maintenance of immune cell functions of splenic DCs following trauma-hemorrhage.

The limitation of this study is that splenic DCs were only treated ex vivo with IL-15. Since many different cells express IL-15R on their surface, in vivo administration of IL-15 is likely to affect different types of cells. Therefore, it will be interesting to examine whether in vivo IL-15 treatment has a beneficial role in mice following trauma-hemorrhage. Further studies are needed to investigate this potential benefit of IL-15.

In this regard, however, many studies have demonstrated that IL-15 is a pivotal cytokine for the development and function of innate immune cells (9, 17, 20). Our results also showed ex vivo effect of IL-15 on the maturation of splenic DCs following trauma-hemorrhage. In contrast, previous studies suggest that IL-15 is also involved in the development of immunopathological disorders. The elevated numbers of IL-15-expressing cells and/or elevated levels of IL-15 production correlate with the activities of rheumatoid arthritis (22, 23), inflammatory bowel disease (18), type C chronic liver disease (14), sarcoidosis (1), and multiple sclerosis (19). Furthermore, evidence supporting a role for endogenous IL-15 in the initiation of T-cell responses has been provided by studies in which an IL-15 antagonist, a soluble fragment of IL-15Rα, inhibited the development of collagen-induced arthritis (30) or allograft rejection (32). These observations suggest a harmful effect of IL-15 in various immunopathological and inflammatory diseases.

However, as IL-15 is produced by many cell types, it is unclear whether development of these diseases is attributed to DC-derived IL-15. Recently, Ohteki et al. (29) demonstrated that IL-15 is essential for Propionibacterium acnes- and zymosan-induced granuloma formation and subsequent LPS-induced endotoxin shock through induction of proinflammatory cytokines and chemokines in vivo. They identified an irreplaceable function of DC-derived IL-15 in bacterial product-mediated inflammatory responses. Therefore, DC-derived IL-15 may be critical for endotoxin shock induction in vivo. This raises the possibility that in vivo treatment of mice with IL-15 following trauma-hemorrhage may have harmful effects on mice. In this study, we investigated only ex vivo treatment of splenic DCs with IL-15 and found that it has a beneficial effect on splenic DCs following trauma-hemorrhage. However, it remains unclear whether in vivo modulation of IL-15 by treating animals with IL-15, anti-IL-15 antibodies, or with its agonist following trauma-hemorrhage has any beneficial effect. Further studies are required to identify this possibility.

We also recognize that the present study used measurement at a single time point, i.e., at 2 h following resuscitation, and thus it remains unknown whether splenic DC functions are altered and whether those alterations persist for a period longer than 2 h. Although a time course study of splenic DCs was not carried out, our previous studies on Kupffer cells, splenic or peritoneal macrophages indicate that the depression in their function that was evident at 2 h was evident for a prolonged period of time and that it took ~7 days for their functions to return to normal (8, 38). Thus, although a time point other than 2 h was not examined in the present study, on the basis of our previous studies (8, 38), it would appear that the deleterious effects of trauma-hemorrhage on splenic DCs would also persist for ~7 days following resuscitation. However, more studies are needed to confirm whether splenic DCs follow the pattern similar to Kupffer cells and other macrophages following trauma-hemorrhage.

In summary, our results suggest that the IL-15-producing capacity of splenic DCs decreases following trauma-hemorrhage. Trauma-hemorrhage induces depressed splenic DC maturation; however, administration of exogenous IL-15 after trauma-hemorrhage is effective in normalizing the cytokine production capacity and antigen-presenting capacity of splenic DCs. It therefore appears that the salutary effects of IL-15 on splenic DC functions are likely mediated via an attenuation of suppressed splenocyte maturation under those conditions.

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GRANTS

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