Langenbecks Arch Surg (2007) 392:339–343
DOI 10.1007/s00423-007-0164-7

ORIGINAL ARTICLE

Depressed T cell-derived IFN-γ following trauma-hemorrhage: a potential mechanism for diminished APC responses

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Received: 19 January 2007 / Accepted: 30 January 2007 / Published online: 22 March 2007
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Abstract
Introduction Prolonged immunosuppression has been demonstrated after trauma-hemorrhage resulting in an increased susceptibility to sepsis. The contribution of antigen-presenting cells (APC) vs T cells to this diminished immune response, however, remains unknown.
Materials and methods To study this, male mice were trauma-hemorrhaged (35±5 mmHg for 90 min and resuscitation) or sham operated. At 24 h thereafter, spleens were harvested and T cells (via Microbeads) and APC (via adherence) were isolated. Cocultures of combined T cells and APC were established for 48 h, stimulated with ConA and LPS. The T cell-derived cytokine IFN-γ and IL-12 for APC responses were measured in the supernatants by the multiplex assay.
Results The release of IFN-γ was suppressed by T cells after trauma-hemorrhage irrespective of whether sham or trauma-hemorrhage APC were added. Trauma-hemorrhaged APC did not affect T cells-derived IFN-γ release by sham T cells. In contrast, trauma-hemorrhaged T cells depressed the release of IL-12 by APC. The release of IL-12 by trauma-hemorrhaged APC was not altered when sham T cells were cocultured.
Conclusion Prolonged immunosuppression after trauma-hemorrhage appears to be predominantly due to diminished T cell function. Thus, attempts to prevent immunodysfunction should be directed towards T cells.

Keywords T cells · Antigen-presenting cells · Trauma-hemorrhage · Immunosuppression

Introduction

Cell-mediated immunity is depressed after trauma and severe blood loss resulting in an increased susceptibility to infection [1]. In particular, the interaction between antigen-presenting cells (APC) and T cells has been shown to play a central role in cell-mediated immunity [2].

In this respect, LPS-induced cytokine release capacities of APC are diminished after trauma and blood loss [3]. APC-derived cytokines, i.e., IL-12, however, have been shown to be responsible for the activation of T cells [4, 5].

With respect to T cell function, a depressed Th1 cytokine release, i.e., IL-2, IFN-γ, and diminished lymphocyte proliferation, have been reported after trauma and hemorrhagic shock [6]. Studies indicate that, in particular, IFN-γ affects APC responses [7–9]. Moreover, the depression of T cells and APC persists for up to 7 days after trauma-hemorrhage and is associated with an increased susceptibility to infections [10]. Although APC appear to be the culprit for initiating immunosuppression after trauma and blood loss [11], it remains unknown whether alterations in T cells or APC function are responsible for persisting immunodysfunction under those conditions. The data of the present study might help to further understand the pathophysiological mechanisms of immunodysfunction, thereby allowing the developing of new therapeutic targets for immuno-maintaining therapies.

Best abstracts – Surgical Forum 2007
Presented at the 29th Annual Conference on Shock, Broomfield, CO, 3–6 June 2006.
Awarded with the travel award of the Shock Society 2006.

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Materials and methods

Animals C3H/HeN mice (Charles River, Sulzfeld, Germany), aged between 6 and 8 weeks, were used in this study. All procedures were carried out in accordance with the guidelines set forth in the Animal Welfare Act and the Guide for the Care and Use of Laboratory Animals by the National Institutes of Health. The Institutional Animal Care and Use Committee of the *Regierung von Oberbayern* and the Ludwig Maximillian University, Munich, Germany, approved this project.

Experimental groups Male mice were randomly assigned to the sham operation or trauma-hemorrhage (n=8–10 per group).

Trauma-hemorrhage procedure Mice were lightly anesthetized with a mixture of Isoflurane (Forene®, ABBOTT Laboratories, Abbott Park, IL), N_2O, and O_2 and turned to a supine position. Then, a 2.5-cm middle-line laparotomy was performed (i.e., trauma was induced), which was then closed aseptically in two layers using Ethilon 6-0 sutures (Ethicon, Somerville, NJ). After this, femoral arteries were aseptically cannulated with a polyethylene 10 tubing (Clay-Adams, Parsippany, NJ) using a minimal-dissection technique. Blood pressure was constantly monitored by attaching one of the catheters to a blood pressure analyzer (Digi-Med™, Louisville, KY). Upon awakening, the animals were bled rapidly through the other catheter to a mean arterial blood pressure (BP) of 35±5 mmHg (BP prehemorrhage was 95±5 mmHg), which was maintained for 90 min. At the end of that period, the animals were resuscitated intra-arterially with lactated Ringer's solution (four times the shed blood volume over 30 min) to provide adequate fluid resuscitation. Lidocaine was applied to the groin incision sites, the catheters were removed, the vessels were ligated, and the groin incisions were closed.

Sham-operated animals underwent the same groin dissection, which included ligation of both femoral arteries; however, neither hemorrhage nor fluid resuscitation was carried out. Mortality was not observed in this model of trauma-hemorrhage. The trauma-hemorrhage procedure was performed at the same time of the day to avoid fluctuations of plasma hormone levels because of circadian rhythm.

Cell-harvesting procedure The animals were killed by an overdose of Isoflurane (Forene®) 24 h after the trauma-hemorrhage or sham operation. Thereafter, spleens were removed aseptically and placed in cold and sterile phosphate-buffered solution (PBS; Merck, Darmstadt, Germany). The spleens were dissociated by grinding between two cold and sterile microscope slides, suspended in PBS, and centrifuged at 300 *g* for 10 min. After resuscitation, the erythrocytes were lysed hypotonically.

T cells separation T cells were separated by using a "Pan T cell Isolation Kit" (Milthenyi Biotecs, Bergisch Gladbach, Germany) as described by the manufacturer. This system allows negatively selecting T cells via magnetically labeling all other immune cells (negative selection). Nonlabeled T cells remained undetected and were allowed to rinse through the magnetic column. In brief, after a two-step labeling of all non-T cells—respectively, macrophages (MØ), dendritic cells (DC), natural killer cells, and B cells—the suspension was allowed to run through a depletion column (composed of ferromagnetic fibers) inserted within a strong magnetic field. The unlabeled T cells were eluted by repeated washing of the column within the magnetic field. After this isolation procedure, T cell purity was found to be 95%. Moreover, T cell and APC functions were not significantly affected using cell sorting via Microbeads.

APC separation Using the remaining cell suspension after T cell isolation, APC were separated using their ability to adhere on culture plates. In brief, using 1×10^6 cells/well of this cell suspension, the cells were incubated for 2 h on 24 well plastic tissue culture plates. The APC were allowed to adhere on the plastic surface, and the nonadherent cells were removed carefully by washing with a warm cell medium. Approximately 100,000 of APC remained in each well.

Cell culture In an attempt to establish physiologically relevant coculture conditions, 0.5×10^6 trauma-hemorrhaged (hem) or sham T cells were added to the adherent APC from trauma-hemorrhaged or sham animals to achieve a ratio of 5:1. This coculture system resulted into four study groups: group I, sham T cells+sham APC; group II, sham T cells+hem APC; group III, hem T cells+sham APC; and group IV, hem T cells+hem APC (Fig. 1). As controls, single cultures of T cells and APC without coculturing were established. Cocultures were incubated in 1 ml of Dulbecco's modified Eagle medium (GIBCO™, Invitrogen, Carlsbad, CA) with 10% inactivated fetal calf serum (Cambrek, Belgium) and 0.1% Gentamycin (Bio Whittaker, Belgium) for 48 h at 37°C and 5% CO_2 stimulated with 2.5 μg/ml concanavalin A (ConA; Sigma-Aldrich, Saint Louis, MO) and 1 μg/ml lipopolysaccharide (LPS) from *Escherichia coli* (Sigma, Saint Louis, MO). After 48 h of incubation, the supernatants were harvested, aliquoted, and stored at −70°C. In addition, cultivated cells were harvested and investigated via flow cytometry.

Assessment of cytokines IL-12 and IFN-γ were determined in the supernatants by the Bio-Plex array system (BioRad

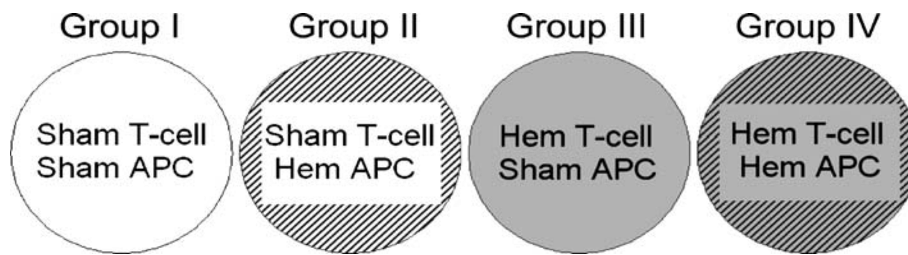

Fig. 1 Schematic illustration of the established Cocultures of T cells and antigen-presenting cells (*APC*) harvested 24 h after sham operation or trauma-hemorrhage (*Hem*). This coculture system resulted into four study groups: *group I*, sham T cells+sham APC; *group II*, sham T cells+hem APC; *group III*, hem T cells+sham APC; and *group IV*, hem T cells+hem APC

Laboratories, Hercules, CA) as proposed by the manufacturer. In brief, for the multiplex assay, 50 µl of each sample was allowed to react with the cytokine-specific antibody-conjugated beads in the microplate wells. The flow-based Bio-Plex protein array system identifies and quantifies each specific reaction based on bead color according to the manufacture's information. The magnitude of the reaction was measured using fluorescently labeled reporter molecules also specific of each target protein.

Statistics The results are presented as mean±SEM. One-way analysis of variance (ANOVA) method followed by the Student–Newman–Keuls or Tukey's test as a post hoc test for multiple comparisons was used to determine the significance of the differences between experimental means. A p value of less than 0.05 was considered to be significant.

Results

T cells-derived cytokines The release of T cells-derived IFN-γ (Fig. 2) by sham T cells were similar, irrespective whether cocultured APC were harvested from the sham (group I) or trauma-hemorrhaged animals (group II). Hemorrhaged T cells cocultured with sham or hemorrhaged APC displayed significantly depressed IFN-γ release capacities.

In summary, the release of T cells-derived cytokines was not affected by APC, irrespective whether APC were subjected to the sham operation or trauma-hemorrhage.

APC-derived cytokines Similar to T cells-derived cytokines, the release of IL-12 (Fig. 3) characterizing APC function was significantly suppressed in the presence of hemorrhaged T cells (groups III/IV vs groups I/II). Trauma-hemorrhaged APC per se were not altered in their capacity to release IL-12 when cocultured with sham T cells. Hemorrhaged T cells, however, resulted in significantly suppressed IL-12 release capacity by sham and hemorrhaged APC.

In summary, the release of APC-derived cytokine was depressed by hemorrhaged T cells, irrespective whether APC were subjected to the sham operation or trauma-hemorrhage.

Discussion

After trauma and severe blood loss, immunodysfunction has been found, which results in an increased susceptibility to infectious complications contributing to considerable morbidity [12, 13]. Zellweger et al. [14] demonstrated persistently depressed immune responses for up to 7 days following the experimental trauma-hemorrhage model used in the present study. Moreover, this immunosuppression was associated with an increased mortality rate after subsequent polymicrobial sepsis [15–17]. On the cellular level, trauma-hemorrhage results in case of unseparated splenic cells in reduced T cell proliferation and lower quantity of released cytokines, i.e., IL-2 and IFN-γ [18].

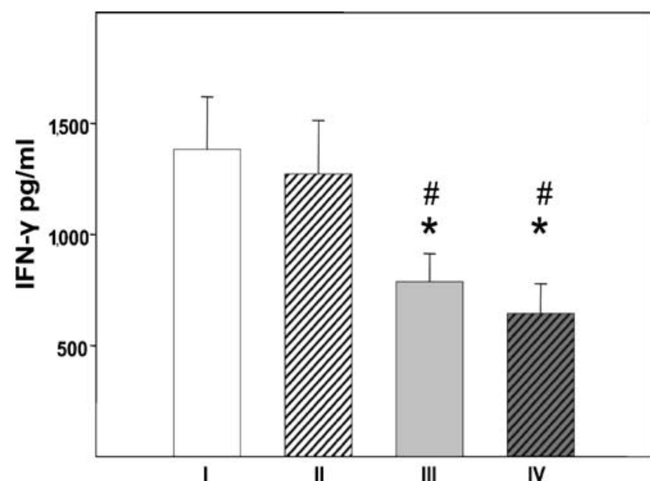

Fig. 2 Cocultures of combined T cells and antigen-presenting cells (*APC*) harvested 24 h after sham operation (*Sham*) or trauma-hemorrhage (*Hem*). *Group I*: sham T cells+sham APC, *group II*: sham T cells+hem APC, *group III*: hem T cells+sham APC, and *group IV*: hem T cells+hem APC. Cocultures were stimulated for 48 h with ConA and LPS. The T-cell-derived cytokine IFN-γ was measured in the supernatants by multiplex assay. N=8–10 per group, mean±SEM, ANOVA, *p<0.05 vs group I. $^{\#}p$<0.05 vs group II

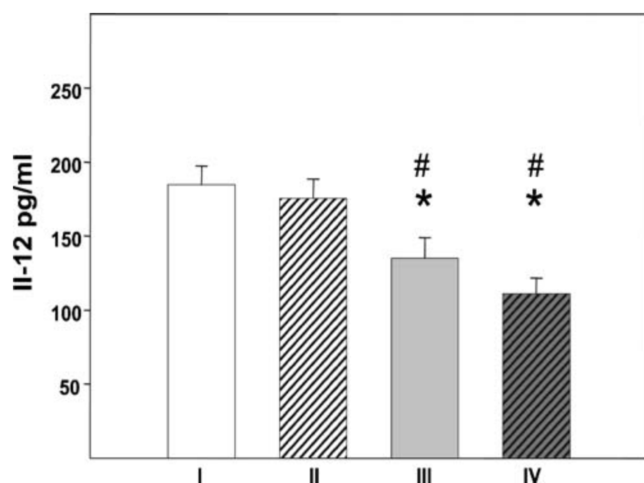

Fig. 3 Cocultures of combined T cells and antigen-presenting cells (*APC*) harvested 24 h after sham operation (*Sham*) or trauma-hemorrhage (*Hem*). *Group I*: sham T cells+sham APC, *group II*: sham T cells+hem APC, *group III*: hem T cells+sham APC, and *group IV*: hem T cells+hem APC. Cocultures were stimulated for 48 h with ConA and LPS. The APC-derived cytokine IL-12 was measured in the supernatants by multiplex assay. N=8–10 per group, mean±SEM, ANOVA, *$p<0.05$ vs group I. $^{\#}p<0.05$ vs group II

For the development of potentially clinically relevant immunomodulatory strategies to prevent this prolonged immunosuppression, it appears important to further elucidate the underlying pathophysiological mechanisms. In this respect, an intact interaction between APC and T cells has been shown to be crucial for an efficient immune response [19, 20]. Recent studies by Kawasaki et al. [11] indicate that DC harvested 2 h after trauma-hemorrhage display depressed cytokine release capacities. Despite this information, it remains unknown whether APC are also responsible for persisting immunosuppression after trauma-hemorrhage. Thus, it was the aim of the present study to investigate the interaction of T cells and APC in a system of cocultures after trauma-hemorrhage or sham operation.

In an attempt to stimulate APC and T cells, cocultures were incubated in the presence of ConA and LPS as previously published [21, 22]. ConA is a polyvalent tetrameric protein with carbohydrate-binding specificity for several mono- and oligosaccharides on T cells resulting in a direct activation [23]. LPS binds to the cell surface receptor CD14 present on APC, thereby activating those immune cells [24].

For characterization of T cell and APC function, IFN-γ and IL-12 were determined. In this respect, the Th1 cytokine IFN-γ has been demonstrated to be predominately released by T cells [25, 26]. In contrast, IL-12 is primarily produced by MØ and DC [27].

The results of the present study indicate that the release capacity of IFN-γ by T cells is markedly depressed by trauma-hemorrhage. This effect of trauma-hemorrhage on T cells is evident irrespective whether T cells are cocultured with sham or trauma-hemorrhaged APC. In this respect, several studies demonstrate a diminished IFN-γ release by nonseparated T cells [1]. The present findings further extend those results that T cells display depressed IFN-γ release capacities also in the presence of sham-operated APC. In contrast, APC harvested after trauma-hemorrhage exhibit diminished IL-12 release capacities only in the presence of hemorrhaged T cells. Moreover, cocultures of hemorrhaged T cells resulted in depressed capacities to release IL-12 also by sham APC. These findings suggest that trauma and hemorrhagic shock by itself does not result in suppressed APC function. Moreover, the presence of suppressed T cells appears to be required for depressed APC cytokine responses seen in nonseparated APC 24 h after trauma-hemorrhage [3].

The depressed release of IFN-γ by hemorrhaged T cells appears to be at least in part responsible for diminished APC function in the present study. In this respect, Asthana et al. [28] indicated that treatment of nonseparated trauma-hemorrhaged APC with IFN-γ restores the depressed immune competence. In addition, Ertel et al. [29] showed in a clinical trial salutary effect of IFN-γ administration on the release of IL-12 in the blood of septic patients. Moreover, Ayala et al. [30] reported a protective effect of IFN-γ treatment on the depressed expression of MHC II on APC. Nonetheless, further studies are required elucidating the beneficial effect of IFN-γ addition to hemorrhaged T cells on APC cytokine responses.

In the present study, evaluation of T cell and APC responses were restricted to a single time point 24 h after trauma-hemorrhage. In this regard, Kawasaki et al. [31] demonstrated decreased IL-12 release by isolated DC harvested 2 h after trauma-hemorrhage. Furthermore, those depressed DC decreased T cell proliferation of a T-helper cell clone D10.G4.1 [11]. Those studies collectively suggest that APC responses are suppressed early after trauma-hemorrhage thereby initiating immunosuppression. However, 24 h after trauma-hemorrhage, T cells appear to be the immune cells predominately mediating immunosuppression. This might reflect a process of maturation of DC after an inflammatory insult [32].

In summary, our data suggest that T cells are predominantly responsible for persisting immunosuppression after trauma-hemorrhage. In this respect, hemorrhaged T cells depressed APC responses also of sham animals, whereas APC did not influence T cell-derived IFN-γ release capacities. Because IFN-γ is essential for adequate APC function, decreased IFN-γ release by T cells appears to be responsible for APC dysfunction after trauma-hemorrhage. Thus, attempts to prevent prolonged immunodysfunction should be directed towards T cells after trauma and severe blood loss.

Acknowledgment This investigation was supported by a grant from the Deutsche Forschungsgesellschaft DFG AN 357/1-1.

References

1. Angele MK, Chaudry IH (2005) Surgical trauma and immunosuppression: pathophysiology and potential immunomodulatory approaches. Langenbeck's Arch Surg 390(4):333–341
2. Lenschow DJ, Walunas TL, Bluestone JA (1996) CD28/B7 system of T cell costimulation. Annu Rev Immunol 14:233–258
3. Angele MK, Knöferl MW, Schwacha MG, Ayala A, Cioffi WG, Bland KI, Chaudry IH (1999) Sex steroids regulate pro- and anti-inflammatory cytokine release by macrophages after trauma-hemorrhage. Am J Physiol 277:C35–C42
4. Robson NC, Beacock-Sharp H, Donachie AM, Mowat AM (2003) The role of antigen-presenting cells and interleukin-12 in the priming of antigen-specific CD4+ T cells by immune stimulating complexes. Immunology 110(1):95–104
5. Heufler C, Koch F, Stanzl U, Topar G, Wysocka M, Trinchieeri G, Enk A, Steinman RM, Romani N, Schuler G (1996) Interleukin-12 is produced by dendritic cells and mediates T helper 1 development as well as interferon-gamma production by T helper 1 cells. Eur J Immunol 26(3):659–668
6. Angele MK, Knoferl MW, Ayala A, Bland KI, Chaudry IH (2001) Testosterone and estrogen differently effect Th1 and Th2 cytokine release following trauma-haemorrhage. Cytokine 16(1):22–30
7. Ayala A, Wang P, Chaudry IH (1993) Insights into the mechanism by which interferon-gamma improves macrophage function following hemorrhage and resuscitation. J Surg Res 54(4):322–327
8. Celada A, Gray PW, Rinderknecht E, Schreiber RD (1984) Evidence for a gamma-interferon receptor that regulates macrophage tumoricidal activity. J Exp Med 160(1):55–74
9. Nambu M, Morita M, Watanabe H, Uenoyama Y, Kim KM, Iwai Y, Kimata H, Mayumi M, Mikawa H (1989) Regulation of Fc gamma receptor expression and phagocytosis of a human monoblast cell line U937. Participation of cAMP and protein kinase C in the effects of IFN-gamma and phorbol ester. J Immunol 143(12)4158–4165
10. Xu YX, Ayala A, Chaudry IH (1998) Prolonged immunodepression after trauma and hemorrhagic shock. J Trauma 44(2):335–341
11. Kawasaki T, Hubbard WJ, Choudry MA, Schwacha MG, Bland KI, Chaudry IH (2006) Depressed splenic dendritic cell antigen presentation function following trauma-hemorrhage. Shock 25 [Suppl]:50
12. Ni Choileain N, Redmond HP (2006) Cell response to surgery. Arch Surg 141(11):1132–1140
13. Angele MK, Schwacha MG, Ayala A, Chaudry IH (2000) Effect of gender and sex hormones on immune responses following shock. Shock 14(2):81–90
14. Zellweger R, Ayala A, DeMaso CM, Chaudry IH (1995) Trauma-hemorrhage causes prolonged depression in cellular immunity. Shock 4(2):149–153
15. Angele MK, Faist E (2002) Clinical review: immunodepression in the surgical patient and increased susceptibility to infection. Crit Care 6(4):298–305
16. Ni Choileain N, Redmond HP (2006) The immunological consequences of injury. Surgeon 4(1):23–31
17. Keel M, Trentz O (2005) Pathophysiology of polytrauma. Injury 36(6):691–709
18. Angele MK, Ayala A, Cioffi WG, Bland KI, Chaudry IH (1998) Testosterone: the culprit for producing splenocyte immune depression after trauma hemorrhage. Am J Physiol 274(6 Pt 1): C1530–C1536
19. Montoya MC, Sancho D, Vicente-Manzanares M, Sanchez-Madrid F (2002) Cell adhesion and polarity during immune interactions. Immunol Rev 186:68–82
20. Delon J, Stoll S, Germain RN (2002) Imaging of T-cell interactions with antigen presenting cells in culture and in intact lymphoid tissue. Immunol Rev 189:51–63
21. Ayala A, Chung Cs, Xy YX, Evans TA, Redmond KM, Chaurdy IH (1999) Increased inducible apoptosis in CD4+ T lymphocytes during polymicrobial sepsis is mediated by Fas ligand and not endotoxin. Immunology 97(1):45–55
22. Shelley O, Murphy T, Paterson H, Mannick JA, Lederer JA (2003) Interaction between the innate and adaptive immune systems is required to survive sepsis and control inflammation after injury. Shock 20(2):123–129
23. Berger SL (1975) Lymphocytes as resting cells. Methods Enzymol 58:486–494
24. Landy M, Baker PJ (1966) Cytodynamics of the distinctive immune response produced in regional lymph nodes by Salmonella somatic polysaccharide. J Immunol 97(5):670–679
25. Smith KA (1988) Interleukin-2: inception, impact, and implications. Science 240(4856):1169–1176
26. Ransom J, Fischer M, Mosmann T, Yokota T, DeLuca D, Schumacher J, Zlotnik A (1987) Interferon-gamma is produced by activated immature mouse thymocytes and inhibits the interleukin 4-induced proliferation of immature thymocytes. J Immunol 139(12):4102–4108
27. Wysocka M, Robertson S, Riemann H, Caamano J, Hunter C, Mackiewicz A, Montaner LJ, Trinchieri G, Karp CL (2001) IL-12 suppression during experimental endotoxin tolerance: dendritic cell loss and macrophage hyporesponsiveness. J Immunol 166(12):7504–7513
28. Asthana D, Yang WC, Fuller L, Zucker K, Lu P, Zheng S, Esquenazi V, Carreno M, Roth D, Burke GW (1993) Differential effects of IFN-gamma on kidney cell expression of MHC class II molecules, kidney cell associated molecules and their stimulatory capacity in mixed lymphocyte kidney cell culture. Transpl Immunol 1(4):282–293
29. Ertel W, Keel M, Neidhardt R, Steckholzer U, Kremer JP, Ungethuem U, Trentz O (1997) Inhibition of the defense system stimulating interleukin-12 interferon-gamma pathway during critical illness. Blood 89(5):1612–1620
30. Ayala A, Ertel W, Chaudry IH (1996) Trauma-induced suppression of antigen presentation and expression of major histocompatibility class II antigen complex in leukocytes. Shock 5(2): 79–90
31. Kawasaki T, Hubbard WJ, Choudry MA, Schwacha MG, Bland KI, Chaudry IH (2006) Trauma-hemorrhage induces depressed splenic dendritic cell functions in mice. J Immunol 177(7):4514–4520
32. Granucci F, Zanoni I, Feau S, Ricciardi-Castagnoli P (2003) Dendritic cell regulation of immune responses: a new role for interleukin 2 at the intersection of innate and adaptive immunity. EMBO J 22(11):2546–2551