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Herpes Virus Entry Mediator(HVEM): A novel potential mediator of trauma induced immunosuppression

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Abstract

Background—HVEM is a co-inhibitory molecule which can both stimulate and inhibit host immune responses. Altered expression of HVEM and its ligands is associated with increased nosocomial infections in septic patients. We hypothesize critically ill trauma patients will display increased lymphocyte HVEM expression and that such alteration is predictive of infectious events.

Materials and Methods—Trauma patients prospectively enrolled from the ICU were compared with healthy controls. Leukocytes were isolated from whole blood, stained for CD3 (lymphocytes) and HVEM, and evaluated by flow cytometry. Charts were reviewed for injuries sustained, APACHE II score, hospital course, and secondary infections.

Results—Trauma patients (N=31) were older (46.7 +/−2.4 vs 36.8+/− 2.1 years; p=0.03) than healthy controls (N=10), but matched for male sex (74% vs 60%; p=0.4). Trauma patients had higher presenting WBC (13.9 +/− 1.3 vs 5.6 +/−0.5 $\times 10^6$/ml; p=0.002), lower percentage of $CD3^+$ lymphocytes (7.5% +/−0.8 vs 22.5% +/−0.9; p<0.001), but significantly greater expression of $HVEM^+/CD3^+$ lymphocytes (89.6% +/−1.46 vs 67.3% +/−1.7; p<0.001). Among trauma patients, secondary infection during the hospitalization was associated with higher APACHE II scores (20.6 +/− 1.6 vs 13.6 +/−1.4; p=0.03) and markedly lower $CD3^+$ lymphocyte HVEM expression (75% +/−2.6 vs 93% +/−0.7; p<0.01).

Conclusion—HVEM expression on $CD3^+$cells increases after trauma. Patients developing secondary infections have less circulating $HVEM^+CD3^+$. This implies HVEM signaling in lymphocytes plays a role in maintaining host defense to infection in after trauma. HVEM expression may represent a marker of infectious risk as well as a potential therapeutic target, modulating immune responses to trauma.

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Keywords

HVEM; Trauma; Immunosuppression; Lymphocytes

Background

Despite significant advances in trauma systems and care, trauma remains a leading cause of death and long-term functional disability. Trauma related deaths continue to demonstrate a trimodal distribution with the delayed third phase driven by secondary infections and end-organ dysfunction. Traumatic injuries induce significant disruptions in both the immune and inflammatory systems[1]. The $CD3^+$ lymphocyte component of the immune system does not merely contribute to delayed adaptive functions, but rather lymphocytes play key central regulatory roles in the early initial immune response to surgical critical illness and traumatic injuries. It is now recognized that trauma induced immune-paralysis, specifically lymphocyte dysfunction, has been associated with secondary infection, long term organ dysfunction and an increased risk of death.[2] Severe critical illness induced immune dysfunction can persist for considerable duration of time. Pelligrini *et al* noted trauma induced lymphocyte dysfunction over a month from the initial traumatic injury, and this persisted well after clinical resolution of the initial event.[3] The early immune and inflammatory events can set in motion a perpetual cycle of inflammatory dysfunction leading to long term clinical effects.[4] Despite the recognition of this pattern of trauma induced immune dysfunction, many of the mechanisms are poorly understood, with few studies addressing whether these findings are translatable to patients. Some of the mechanisms that lead to this trauma/injury and critical illness induced immune-paralysis in patients involve the role of check point proteins, specifically showing a central role for lymphocytes and co-inhibitory molecules upon lymphocytes.[2, 5]

HVEM (Herpes Virus Entry Mediator) is a TNF family transmembrane receptor expressed across a spectrum of immune cells including lymphocytes[6–8] and dendritic cells, as well as epithelial cells.[9] Much of the early work delineating HVEM's signaling cascade was completed using T-cells, demonstrating this regulator's interesting and powerful role as a bidirectional switch on these essential adaptive immune cells.[10–13] HVEM interacts with a variety of ligands from both the TNF-related cytokine family and the immunoglobulin superfamily and its ultimate signaling effect is dependent on which ligand it binds, as well as its own confirmation within the membrane.[14] Ligation with immunoglobulin superfamily member ligands CD160 and B sand T Lymphocyte Attenuator (BTLA) induces immune inhibition via Shp-1/2 phosphorylation while TNF-related cytokine family member ligands LIGHT and Lymphotoxin alpha (LTα) binding resulting in activation through an NFκB mediated mechanism.[11, 15] HVEM has multiple binding domains, allowing it to bind more than one ligand at once, even forming trimeric complexes with stimulatory and inhibitory ligands all at once. This allows a level of environmental specificity to HVEM signaling not possible with other immune regulators.

HVEM has emerged as an important coregulatory molecule in critical illness.[5, 16] Altered expression of HVEM and one of its ligands, BTLA, are associated with poor outcomes and

increased nosocomial infections in critically ill septic patients. The HVEM/BTLA axis has been studied in the murine and human septic populations revealing BTLA and HVEM expression is increased in the setting of these extreme stresses, and that increased BTLA expression is associated with poor outcomes and increased risk of nosocomial infection.[17, 18] We have previously demonstrated a role for the BTLA axis in trauma induced immunosuppression.[17] Furthermore, alterations in HVEM signaling have been shown to play a significant role in the immune response to sterile inflammation.[19–22] To date, the role of HVEM in sterile trauma induced immunosuppression has not been translated to critically ill trauma patients.

Given that infections are a driving force in poor long-term trauma outcomes, we hypothesize critically ill trauma patients will induce an early alteration in HVEM expression on $CD3^+$ lymphocytes and that such an alteration would be predictive of secondary infectious events.

Materials and Methods

Patients

Critically ill trauma patients, ages 18 years and older, who were admitted to the Trauma ICU at a single level I trauma center were prospectively consented and enrolled within 24 hours of presentation. Patients were included in the study if they had a trauma related critical illness requiring ICU admission. Exclusion criteria included death within 48 hours of arrival, pregnancy, or any patient with a known history of lymphoma or leukemia. Age and sex matched healthy controls were enrolled as the control group. This study was approved by the institutional review board of Rhode Island Hospital. For clinical information, charts were reviewed for demographics and patient characteristics, as well as comorbidities and all injuries sustained. Hematologic profiles from the day of lab draw, including White Cell Count, as well as all clinical features needed to calculate Acute Physiology of Chronic Health Evaluation II (APACHE II score). ICU and hospital courses were reviewed for all complications, including development of a secondary trauma related infections. Determination of infection as well as all clinical care was at the discretion of the treating team.

Specimen preparation and flow cytometry

Whole blood was obtained in heparin containing tubes. A portion of each sample was separated to collect serum which was then stored at −80°C for later cytokine analysis. The remaining whole blood was layered with Ficoll Histopaque −1077 and centrifuged. The layer containing leukocytes was aspirated and washed with phosphate buffered saline then centrifuged. Cells were stained using monoclonal antibodies for CD3 (BD Pharmingen, FITC Mouse Anti-Human CD3, Cat#555332; Beckman Coulter, APC Anti-Human CD3, PN#IM2467U), CD4 (Biolegend, PE-anti-human CD4, Cat# 318806) or CD8 (Biolegend, PE-anti-human CD8, Cat#344706) to identify lymphocytes, as well as for HVEM (Biolegend, PE-anti-human CD270, Clone 122, Cat#318806). Cellular populations were analyzed using flow cytometry. Initially, distinct forward and side scatter patterns were used to identify lymphocytes. Gating based on isotype controls was then used to identify HVEM $^+CD3^+$ lymphocytes. Flow cytometry data analysis was completed using FlowJo software.

For cytokine analysis we chose to examine patient plasma IL-6, IL-10, IL-2 and TNF-α levels using a Cytometric Bead Array (CBA) (BD Biosciences) according to the manufacture's specifications and as we have previously described[23].

Statistical Analysis

When data was normally distributed a t-test was used, however, if data was not normally distributed a Mann-Whitney U test was applied. Results are presented as means +/− standard error of the mean. Linear regression was used to correlate HVEM expression and APACHE II Score, and R^2 was calculated. Statistical analysis was undertaken using SigmaPlot version 12 (Systat Software, Inc, Chicago, IL). Alpha was set to 0.05.

Results

Thirty-one trauma patients and 10 healthy controls were enrolled for this study. Trauma patients were slightly older (46.7 +/−2.4 versus 36.8 +/−2.1 years; p=0.03) but matched for male sex (74% versus 60%; p=0.4). Among the trauma group as a whole, the average APACHE II score was 14.7+/−1.3). The overall mortality of the trauma group was 19% (6 patients) (Table 1). Trauma patients had higher presenting White Cell Count (13.9 +/− 1.3 versus 5.6 +/−0.5 $\times 10^6$/ml; p=0.002). Blood samples were processed, and samples were gated and stained for $CD3^+$, $CD4^+$, and $HVEM^+$ expression. A representative image is depicted in Figure 1. Although trauma patients, compared to controls displayed a lower percentage of $CD3^+$ lymphocytes (7.5% +/−0.8 versus 22.5% +/−0.9; p<0.001), there was no difference in the expression of either $CD4^+CD3^+$ lymphocytes (approximately 65%), or the $CD8^+CD3^+$ lymphocytes (approximately 30%) (Table 1 & Figure 2). Among the $CD3^+$ population, trauma patients did display a significantly greater frequency of $HVEM^+CD3^+$ lymphocytes (89.6% +/−1.5 versus 67.3% +/−1.7; p<0.001) (Figure 3). With respect to cytokine levels, it was noted that all measured cytokine levels (IL-6, IL-10, IL-2 and TNF-α) were significantly elevated among trauma patients when compared with healthy controls.

Among the 31 trauma patients, 22.5% developed a secondary infection. The most common source of infection was ventilator associated pneumonia (n=5), one of whom also developed a urinary tract infection. Two patients developed complex wound / soft tissue infections, one of whom also developed a urinary tract infection (Table 2). Comparing those who did versus did not develop a secondary infection, there was no difference in age (48.4 +/− 2.5 versus 39.7 +/− 6.7; p=0.15) or male sex (57% versus 79%; p=0.33) between the groups. Although there was no statistical difference, many of those who developed a secondary infection presented with pre-trauma medical co-morbidities (43% versus 21%; p=0.35). Among all patients, hypertension was the most common medical co-morbidity. With respect to the presenting lymphocyte populations, there was no difference in either presenting $\%CD3^+$ lymphocytes, $\%CD4^+CD3^+$ cells or $\%CD8^+CD3^+$ cells. Patients who developed a secondary infection during the hospitalization had a higher early APACHE II score (20.6 +/− 1.6 vs 13.6 +/− 1.4; p=0.03) (Table 3).

As noted, among the group as a whole, post-traumatically patients demonstrate an elevation of HVEM expression upon $CD3^+$ cells. However, among patients who subsequently developed a secondary infection, the early expression of HVEM upon $CD3^+$ lymphocytes

following the traumatic injury was noted to be markedly reduced compared to patients who did not develop a secondary infection (75% +/−2.6 vs 93% +/−0.7; p<0.01) (Figure 4). Given the association between APAHCE II score and infection as well as the association between HVEM expression and infection, a linear regression was performed to assess the association between the frequency of $HVEM^{+}CD3^{+}$ cells and clinical degree of illness (APACHE II score). An association was noted, showing that increasing APACHE II Score correlated with decreasing HVEM expression upon $CD3^{+}$ lymphocyte (R^2=0.2; p=0.007) (Figure 5)

With respect to cytokine levels, there was no association between initial IL-6 or IL-2 levels with either HVEM expression or development of a subsequent infection. However, it was noted that among trauma patients who later developed a subsequent infection (patients with lowered HVEM expression upon $CD3^{+}$ lymphocytes), both IL-10 (19.7 +/− 0.9 versus 14.6 +/− 0.6 pg/ml; p=0.009) and TNF-α (2.7 +/−0.4 vs. 4.3 +/− 0.3 pg/ml; p=0.04) levels were lower when compared with patients who did not develop a subsequent infection (Figure 6).

Discussion

Trauma remains a leading cause of both death and long-term disability. Traumatic injuries induce a significant and dramatic state of immune suppression and immune dysfunction, persistent inflammation, secondary infections, end organ failure, and ultimately death. Despite significant advances in trauma care, trauma related complications, including infections, continue to show predictable patterns.[24, 25] The trimodal distribution, first described in 1983, relates to immediate, early and late causes of death. The late causes of death occurred weeks to months after the initial traumatic injury, and up to 80% of these late deaths were attributed to infection and organ failure.[26] Despite a flattening of the curve of the later stages of the traditional trimodal distribution[27], later deaths still occur, accounting for 10–30% of trauma-related deaths, and are most commonly infection related.[28–31] Many of these later secondary infectious complications may not lead to mortality but do impose a substantial financial burden and contribute significantly to prolonged hospital length of stay and a greater need for long term rehabilitation.[31, 32]

Trauma and traumatic injuries induce a significant early systemic effect, mediated by immune dysfunction. It is now well recognized that $CD3^{+}$ lymphocytes, despite constituting an element of the adaptive immune system, play a critical role in the response to acute illness and injuries. In keeping with this observation, we have previously demonstrated that the normal lymphocyte response to trauma involves the development of a lymphopenia.[2] We do not believe that our data reflects patients with an underlying history of immune dysfunction, but rather that our data supports the concept that is now well recognized that the traumatic injury itself induces a state of immunosuppression.[33, 34] In one of the earlier studies that identified trauma induced immunosuppression among patients, Pellegrini *et al* demonstrated that trauma induces a systemic effect and is not just a localized effect around the injured tissues; noting systemic lymphocyte dysfunction that may persist for a long period following the injury.[3] They demonstrated a persistence of T-cell anergy, marked by a significant and prolonged suppression of lymphocyte production of IL-2. The cytokine data in our study is in keeping with this observation that trauma patients display a reduced ability

to generate an appropriate immune response. Our data expands upon this observation demonstrating an association with HVEM expression as a potential mechanism for these cytokine observations. Furthermore, Bandyopadhyay *et al* noted that negative signaling receptors among all $CD3^+$ lymphocytes appeared to be capable of contributing to T-cell anergy seen in trauma patients.[35] In a murine model, Chung *et al* demonstrated that survival from acute critical illness can be improved through blockage of lymphocyte anergy and apoptosis pathways including Fas signaling.[36] Furthermore, Murao *et al* demonstrated that one of the immune mediated benefits of hypertonic saline resuscitation for hemorrhagic shock is driven in part through prevention of dysfunction of regulatory lymphocyte populations.[37]

Blocking individual cytokines in response to trauma, sepsis and surgical critical illness has failed across multiple clinical trials[38]. Many of the mechanisms driving the development of trauma induced immunosuppression remain to be properly elucidated and therefore immune modulating therapy lacks direct targeted intervention. However, while the role of checkpoint proteins as regulators of the immune system has begun to be better defined, including among surgical patients, much of the work to date has involved animal models with little correlation to trauma patients. Further a number of the clinical studies aimed at reducing trauma related inflammation have been crude attempts to dampen the entire immune cascade[25] without regard to the underlying mechanistic drivers of inflammation. Many of these studies also include the use of agents such as steroids or free radical scavenging anti-oxidants. It is speculated that it is the non-specific nature of these therapeutic strategies that have failed to produce the desired clinical outcomes, and many have called for a more focused, pathway based approach to modulating immune effects of trauma.[28] However, immune checkpoint directed therapy has shown tremendous potential in a range of medical conditions, most notably in mediating the immune response to malignancies.[39, 40] Checkpoint proteins such as HVEM, BTLA, PD-1 are capable of regulating either an immunosuppressive or immunostimulatory role depending of the specific stressor. As such, these receptors can be neutralized or stimulated by clinically available therapeutic agents, and as such offer potential short-term therapeutic targets. In this study, we have begun to define HVEM as a potential mediator of trauma induced immunosuppression. It has been shown that HVEM, along with one its ligands BTLA, act as key regulators of the immune balance in both murine and human septic critical illness.[17, 18] We describe a novel finding among critically ill trauma patients that traumatic injuries induce an upregulation of HVEM expression and that a failure to upregulate HVEM expression upon $CD3^+$ lymphocytes was associated with an increased risk of secondary infections.

To date much of our understanding of the mechanisms of action of HVEM has been derived from the sepsis or oncology literature, however, there is very limited understanding of the role of the HVEM axis in traumatic injuries among patients[14]. Although HVEM is expressed on many tissue types and immune cell subsets, HVEM was originally described on T-cells and therefore much of our understanding of the downstream signaling generated by HVEM was elucidated using T-cells[10, 11]. As a result, many studies have sought to understand the role of HVEM in the context of T-cell signaling. For example, HVEM mediates effector T-cell survival and function at mucosal barriers[10]. HVEM interacts with a variety of ligands from both the TNF-related cytokine family and the immunoglobulin superfamily and its

ultimate signal effect is dependent on which ligand it binds, as well as its own cis or trans confirmation within the membrane.[14]

HVEM and its ligands are all highly expressed on T-cells, and, as noted above, the ultimate signal generated from ligation is complex. While ligation of HVEM with CD160 or BTLA induces immune inhibition via Shp-1/2 phosphorylation, the ligation of LIGHT and LTα, alternatively, results in activation through NFκB mediated mechanisms.[11, 12, 15] With its multiple cysteine rich binding domains, HVEM can bind more than one ligand simultaneously, often forming 3:3:3 trimeric complexes with LIGHT and BTLA or CD160 simultaneously. In addition to these variations of complexes, it is also noted that HVEM and BTLA can be co-expressed upon the same single cell, forming a stable complex when both proteins are expressed in the *cis* confirmation[41]. Traumatic injuries, ischemia-reperfusion, and tissue injury leads to release of Danger-Associated Molecular Patterns (DAMPs). DAMPs are endogenous mediators capable of signaling and activating the immune system inducing ongoing inflammation.[42] In the compensatory anti-inflammatory response to trauma negative regulators such as HVEM are essential to preventing an overly exuberant immune and inflammatory response. It is the duality of mechanism of HVEM, capable of either immune suppression, or activation, that makes HVEM an intriguing target for immune modulation.

HVEM's ability to target a dampening of the immune and inflammatory responses to a critical illness may also come at a consequence. While negative regulation may prevent excessive tissue damage, it also incurs an increased risk of secondary infections. The benefit of HVEM mediated inhibition has been shown to be prevention of the development or progression of intestinal inflammation. Steinberg *et al* demonstrated that HVEM, through binding with BTLA, triggers inhibitory signaling preventing intestinal inflammation.[22] However, HVEM deficient mice displayed a higher susceptibility to experimental encephalomyelitis, hepatic necrosis and subsequently increased mortality.[43] Other investigators have noted that HVEM mediates effector T-cell mediated immunity across both $CD4^+$ and $CD8^+$ lymphocytes, but demonstrate that this may come at the cost of excessive tissue destruction.[44] Cheung *et al* demonstrated that when BTLA interactions with HVEM in a cis-complex, naïve T-cells are inhibited from activation, thus maintaining T-cells in a naïve state despite activating conditions in the surrounding environment.[45] This inhibition and prevention of long term autoreactivity and autoimmunity has been shown to be beneficial in models of colitis.

In septic patients and sepsis models, it has been demonstrated that an absence of the ability to signal via the HVEM pathway increases septic mortality in mucosal gastrointestinal and respiratory infection models.[19, 46, 47] Altered expression of HVEM, as well as its ligand BLTA, have previously been associated with poor outcomes and increased secondary nosocomial infections in critically ill septic patients.[17, 18] In keeping with these prior observations, we demonstrate that a dampened HVEM expression upon $CD3^+$ lymphocytes was associated with an increased risk of secondary infection in this patient cohort, most commonly pneumonia which is a mucosal associated infection. This implies that a robust HVEM signaling in lymphocytes is required to maintain host defenses to infection in critically injured trauma patients.

The single time point draw of this study is a limitation, as it did not allow us to track patient HVEM expression changes over time or relative to the actual infection resolution. Although a single time point may not fully reflect the entire profile of trauma induced immunosuppression, our work is in keeping with several authors who have demonstrated that the initial/early blood draws in patients are highly reflective of later events and/or complications among trauma patients.[48, 49] For example, Wang *et al* demonstrated that initial trauma induced cytokine alterations within 24 hours correlated with trauma related infectious complications[50]. Furthermore, while single time point blood draws may not be ideal at explaining the continuum of activity within a patient, many clinical decisions are made based on single time point blood draws, especially in the care of critically ill trauma patients within the first 24 hours of their care. However, we feel that the strength of this single early time point study is the potential to identify patients at risk of developing a trauma related complication and thereby potentially benefit from checkpoint modulation. The other major limitation of a study of this nature is the inability to assess lymphocyte function or checkpoint protein expression prior to the traumatic injury. APACHE II score and risk of infection following trauma are influenced both by age as well as chronic pre-trauma medical co-morbidities. However, the additive cumulative effect of these factors upon the calculation of APACHE II score cannot be ignored. However, our findings offer insight into the immune mechanisms underlying infection risk in sicker trauma patients.

Conclusion

Lymphocytes play a central role in an appropriate immune and inflammatory response to acute surgical critical illness. Understanding the role of specific regulators of trauma responses will offer the potential for a more focused approach in potential pharmaceutical interventions aimed at regulating the lymphocytic response. Failure to increase frequency of HVEM was associated with an increased risk of secondary infection. Given that known ligands can differentially up or down regulate HVEM, this offers a modifiable mediator of the immune response to traumatic critical illness for potential biomarker/therapeutic consideration.

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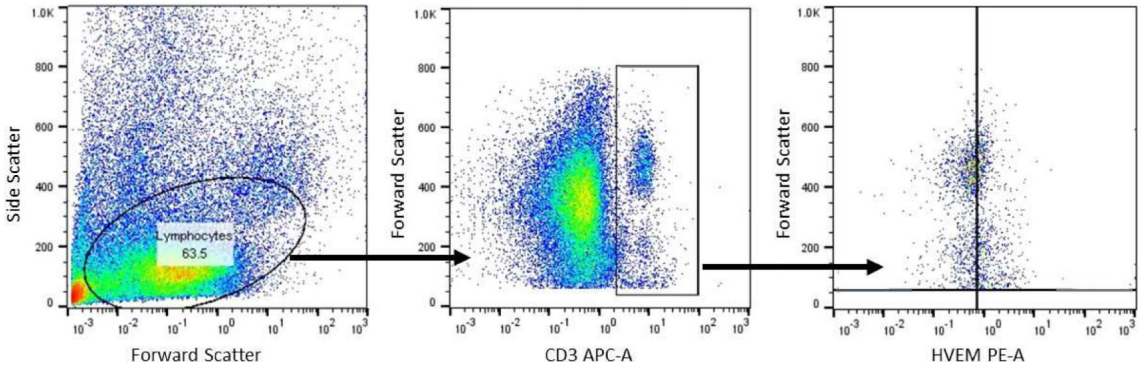

Figure 1:

Representative flow cytometry plots depicting gating strategy as well as significant difference in $CD3^+$ and $HVEM^+$ expression between healthy controls and trauma patients

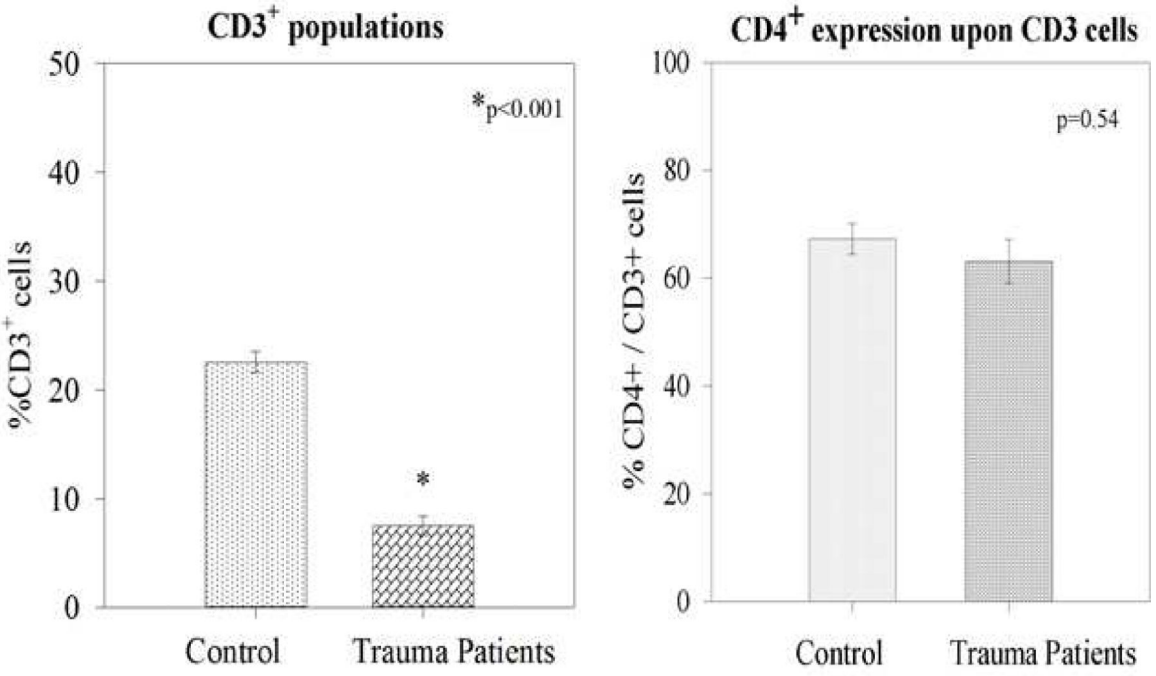

Figure 2–.

Trauma patients displayed a lowered $CD3^+$-lymphocyte population. However, there was no difference in $CD4^+$ expression upon $CD3^+$-lymphocytes.

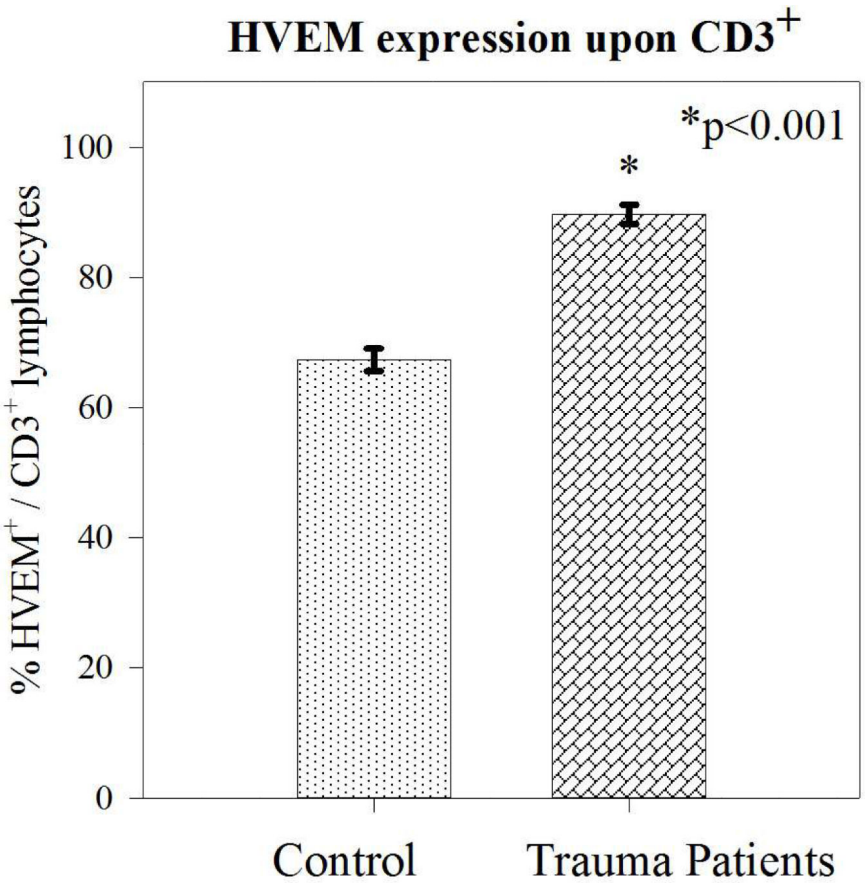

Figure 3–.
Trauma patients displayed a marked increase in HVEM expression upon $CD3^+$-lymphocytes.

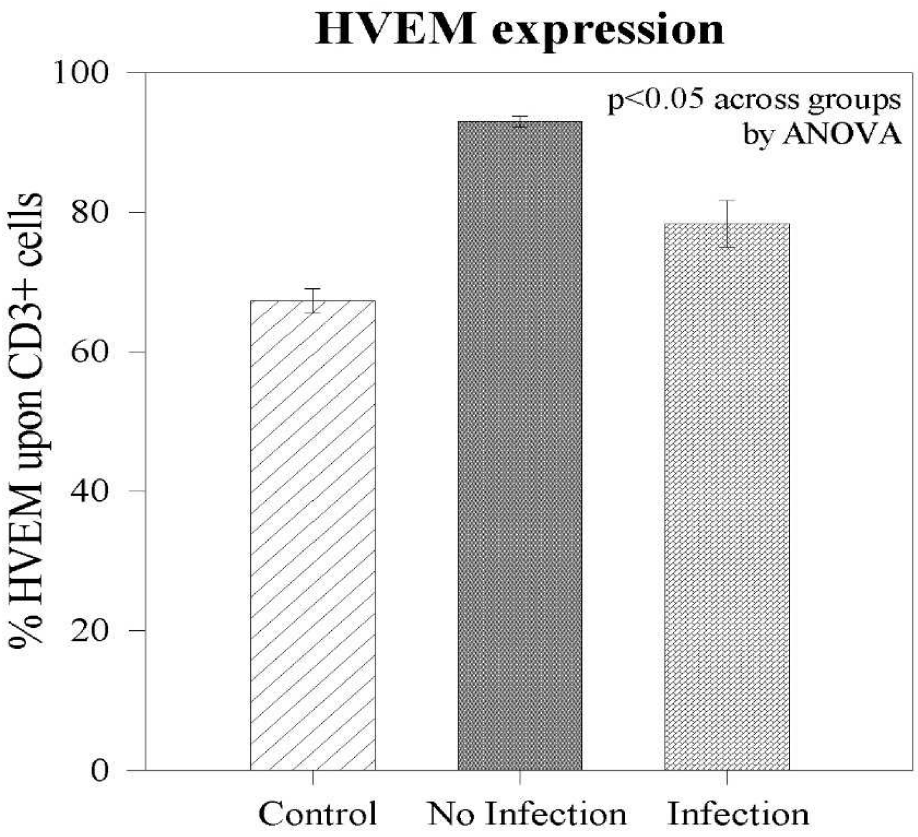

Figure 4 –.

HVEM expression upon $CD3^+$ lymphocytes. HVEM expression increased following trauma. Failure to increase HVEM expression was noted in patients who developed an infection.

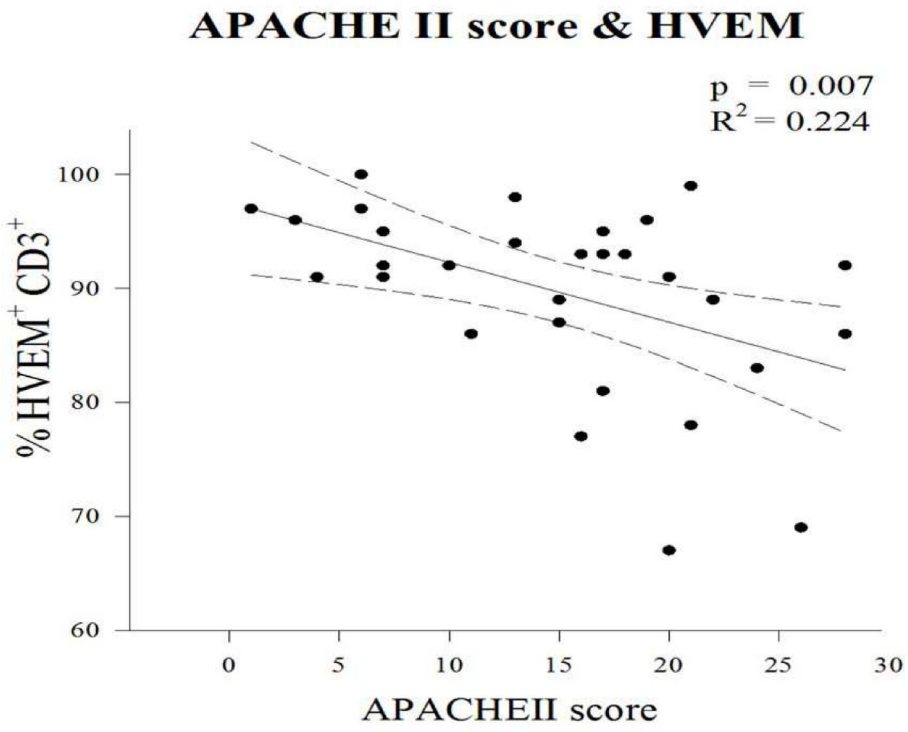

Figure 5 –.

Increasing APACHE II score was correlated with decreasing expression of HVEM upon $CD3^+$ lymphocytes.

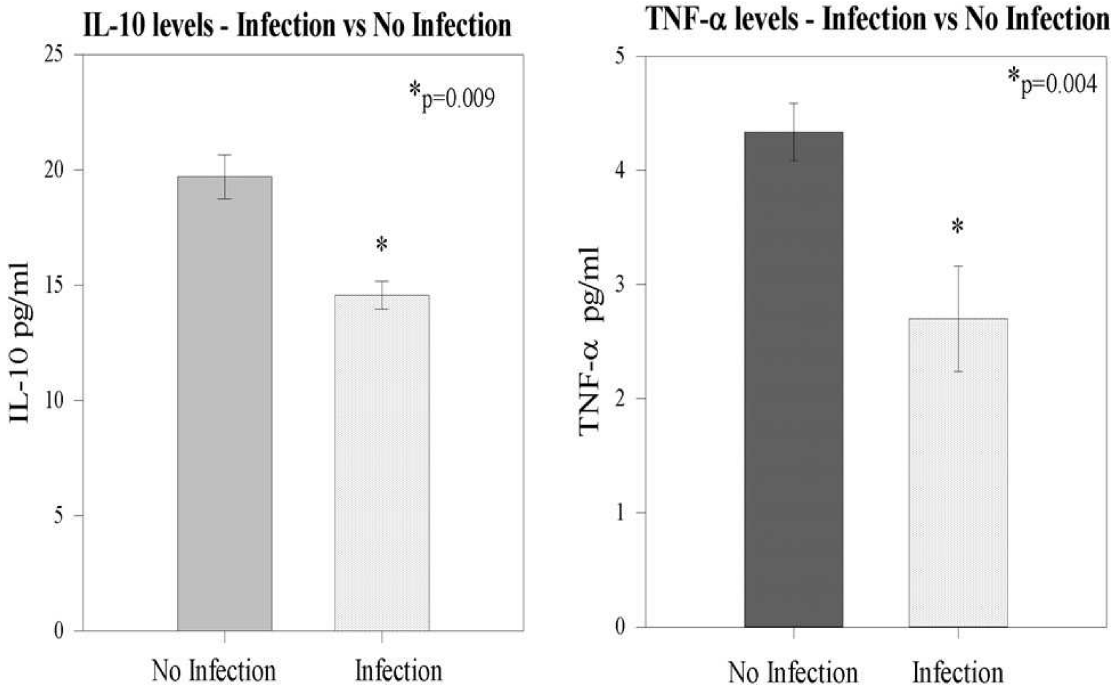

Figure 6 –.

Both initial IL-10 and TNF-α levels were noted to be lower among patients who subsequently developed a trauma related infection.

Table 1:

Patient clinical characteristics: Trauma patients had significantly lowered $CD3^+$ lymphocytes, but no difference in $CD4^+/CD3^+$ cells or $CD8^+/CD3^+$ cells.

Variable	Control N=10 (%)	Trauma Patients N=31 (%)	*p* Value
Age, mean (years) [SEM]	36.8 [2.1]	46.7 [2.4]	0.03
Male sex	60 (6)	74 (23)	0.4
APACHE II Score, mean [SEM]		14.7 [1.3]	
Mortality		19 (6)	
Presenting WCC ($x10^6$/mL)	5.6 [0.5]	13.9 [1.3]	0.002
$CD3^+$ lymphocytes	22.5 [0.9]	7.5 [0.8]	<0.001
$CD4^+CD3^+$ lymphocytes	67.3 [2.7]	63.1 [4.1]	0.54
$CD8^+CD3^+$ lymphocytes	29.1 [1.1]	32.2 [1.2]	0.78
$HVEM^+CD3^+$ lymphocytes	67.3 [1.7]	89.6 [1.5]	<0.001

SEM= Standard error of the mean: WCC=White Cell Count

Table 2:

Types of infection among trauma patients. Pneumonia was the most frequent infection. Two patients had multiple sources of infection. Both UTIs occurred in patients with other infections.

Source of Infection	Number of patients
Pneumonia	5
Complex wound / soft tissue infection	2
Urinary tract infections	2
Multiple sites of infection	2

Table 3:

Patient clinical characteristics of those who did versus did not develop an infection: Trauma patients had significantly lowered $CD3^+$ lymphocytes, but no difference in $CD4^+/CD3^+$ cells

Variable	No Infection N=24 (%)	Infection N=7 (%)	*p* Value
Age, mean (years) [SEM]	39.7 [6.7]	48.4 [2.5]	0.15
Male sex	79 (19)	57 (4)	0.33
APACHE II Score, mean [SEM]	13.6 [1.5]	20.7 [1.6]	0.03
Medical comorbidities (mean)	5 (20.8%)	3 (42%)	0.35
$CD3^+$ lymphocytes	7.2 [0.9]	8.5 [1.9]	0.5
$CD4^+CD3^+$ lymphocytes	65.5 [4.9]	55.4 [6.9]	0.3
$CD8^+CD3^+$ lymphocytes	27.7 [3.5]	35.2 [4.2]	0.28
$HVEM^+CD3^+$ lymphocytes	75 [2.6]	93 [0.7]	<0.001

SEM = Standard error of the mean