Published in final edited form as:

Ir J Med Sci. 2017 November ; 186(4): 981–987. doi:10.1007/s11845-016-1525-4.

Lymphocyte integrin expression differences between SIRS and sepsis patients

Daithi S. Heffernan, MD, AFRCSI, FACS, Sean F. Monaghan, MD, and Alfred Ayala, PhD

Division of Surgical Research, Department of Surgery, Warren Alpert Medical School of Brown University, Rhode Island Hospital, Providence, Rhode Island 02903.

Abstract

Background—Systemic Inflammatory Response Syndrome (SIRS) and sepsis remain leading causes of death. Despite many similarities, the 2 entities are very distinct clinically and immunologically. T-Lymphocytes play a key pivotal role in the pathogenesis and ultimately outcomes following both SIRS and sepsis. Integrins are essential in the trafficking and migration of lymphocytes. They also serve vital roles in efficient wound healing and clearance of infections. Here, we investigate whether integrin expression, specifically $\beta 1$ (CD29) and $\beta 2$ (CD18), are disrupted in SIRS and sepsis, and assess differences in integrin expression between these 2 critically ill clinical categories.

Methods—T-Lymphocytes were isolated from whole blood collected from ICU patients exhibiting SIRS or sepsis. Samples were analyzed for CD18 ($\beta 2$) and CD29 ($\beta 1$) on CD3⁺ T cells through flow cytometry. Septic patients were stratified into either exclusively abdominal or non-abdominal source of sepsis.

Results—CD18 was almost ubiquitously expressed on CD3⁺ T cells irrespective of clinical condition. However, CD29 ($\beta 1$ integrin) was lowest in SIRS patients (20.4% of CD3⁺ T-cells) when compared with either septic patients (35.5%) or healthy volunteers (54.1%). Further, there was evidence of compartmentalization in septic patients, where abdominal sources had a greater

Address for Correspondence Daithi S. Heffernan MD, DHeffernan@Brown.edu, Division of Surgical Research, Department of Surgery, 211 Aldrich Building, 593 Eddy Street, Rhode Island Hospital, Providence, Rhode Island 02903, Phone: 401-444-5617, Fax: 401-444-6681.

Author contributions;

- (1) conceiving, designing the study; data collection, analysis and interpretation – DSH, SFM, AA
- (2) writing the manuscript or providing critical revisions that are important for the intellectual content; - DSH, SFM, AA
- (3) approving the final version of the manuscript – DSH, SFM, AA

Compliance with Ethical Standards Statement

There was no external funding for this study

Conflict of Interest – no author has any conflict of interest with respect to this manuscript. No author has received any research grant from any company for this project. None of the authors has received any speaker honorarium, or owns any stock in any company that has any relationship to this study.

No animal was used in this study.

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Full Rhode Island Hospital Institutional Review Board approval was obtained and active during the period of the study.

Informed assent was obtained from all individual participants included in the study

percentage of CD3⁺CD29⁺ T-cells (41.7%) when compared with those with non-abdominal sources (29.5%).

Conclusion—Distinct differences in T-cell integrin expression exists between patients in SIRS versus sepsis, as well as relative to the source of sepsis. Further work is needed to understand cause and effect relative to the progression from SIRS into sepsis.

Introduction

The Systemic Inflammatory Response Syndrome (SIRS) is the inflammatory and immune response to injury and traumatic insult, whereas Sepsis is defined as the systemic inflammatory response to severe microbial infection. The physiological derangement associated with both SIRS and sepsis continue to be leading causes of death among intensive care unit patients with mortality rates continuing to be reported as high as 40 to 50%(1, 2). In both SIRS and sepsis, the inflammatory and immune systems appear dysfunctional(3-6). This can ultimately cause tissue damage leading to multiple organ failure and death. However, despite many clinical similarities between these two entities, they are immunologically very different conditions(4).

Within the immune response it has been shown that T-Lymphocytes, including their sub-populations, play key roles in the modulation and survival from both SIRS and sepsis in both murine models and humans(3, 7-10). Sepsis has been noted to induce significant lymphocyte dysfunctionality, often noted to be an immunoparalysis and CD3⁺-T-Lymphocytes are essential to recovery from sepsis(4). The immune dysfunction induced in both SIRS and sepsis is thought to lead to patients being at risk for the development of secondary infections, prolonged end organ dysfunction and ultimately death. We have previously demonstrated that an inappropriate lymphocyte response to traumatic injury (SIRS) in humans is associated with increased mortality(11). As part of their role in coordinating the inflammatory and/or immune response foreign pathogenic challenge, lymphocytes need to be able to competently migrate and traffic to and from the sites of injury and infection(12). We have demonstrated that in abdominal sepsis, a failure of lymphocyte sub-populations to migrate led to significant alterations in the innate immune response the infection(13).

Integrins are known to play a key role in regulating the immune system(14-16). They are responsible for efficient migration of, among other cells, lymphocytes from the lymph nodes to the site of wound or infection(17, 18). Among the integrins expressed upon T-Lymphocytes, Integrin β 1 and β 2 are involved in lymphocyte functions, such as mediating T-cell interactions with other cells as well as modulating intra-cellular signaling. Integrins β 1 and β 2 also play prominent roles in T-cell migration to peripheral nodes and inflammatory sites, antigen presentation and cytotoxic killing(16, 19-21). The β 2 integrin plays a critical role in the leukocyte response to inflammatory states, an importance underscored by the roles in diverse states such from embryogenesis to wound healing. β 1 integrins are involved in lymphocyte adhesion, proliferation, migration, and often serves a co-stimulatory role in inflammatory conditions.

Much of the work on T-cell integrins relates to their function in murine models, healthy humans or patients with chronic medical conditions(14, 22-24). There is a paucity of data

addressing the effect of critical illness upon human peripheral blood T-Lymphocyte integrin expression and no data regarding differences in expression between the sterile inflammatory state of SIRS compared with the microbiologically driven condition of sepsis in humans. Given the key role of lymphocyte dysfunction in outcomes from SIRS and sepsis,

We postulate that the generalization of the inflammatory response in tissue destruction (SIRS) would require less active directed migration of lymphocytes when compared to infectious processes (Sepsis) which would mandate a more directed migration of lymphocytes to the site of infection, thereby inducing an alteration of integrin expression between these two patient populations. We undertook this analysis of integrin expression on T-lymphocytes derived from SIRS versus Septic critically ill surgical patients.

Methods

Patients

Patients admitted to the surgical or trauma intensive care unit of tertiary care facility were prospectively enrolled. The diagnosis of SIRS was assigned following the parameters defined by the American College of Chest Physicians/ Society of Critical Care Medicine. Sepsis was defined as patients with SIRS and a source of infection, requiring Intensive Care Unit (ICU) care. Data was collected to ascertain presence and degree of SIRS at the time of the blood draw and the source of sepsis. Blood draws were undertaken within 24 hours of identification of either SIRS or sepsis. In addition, clinical data was collected to calculate an APACHE II score. In patients with sepsis, charts were reviewed to assess the source of the sepsis, and we operationally divided patients into either abdominal or non-abdominal sepsis. To serve as a control, we collected blood from healthy volunteers. In-hospital mortality was also assessed for each patient. This study was executed under the approval of the Institutional review board of Rhode Island Hospital.

Antibody Staining

Peripheral blood lymphocytes were collected, erythrocytes lysed, and isolated from whole blood samples as previously described by us and others(3). In brief, CD18 and CD29 expression were assessed on CD3+ T cells through flow cytometry. The monoclonal antibodies used for staining were allophycocyanin (APC) anti-CD3 (clone UCHT1, Beckman Coulter) and phycoerythrin (PE) conjugated anti-CD18, (clone 6.7, BD Pharmingen) or PE conjugated anti-CD29 (clone HUTS21, BD Pharmingen). Antibody optimization for staining was established according to manufacturers' recommendations. 100ul of whole blood samples were stained in a 5ml disposal conical with APC-conjugated anti-CD3 (clone UCHT1) and PE-conjugated antiCD18 (clone 6.7) or PE-conjugated antiCD29 (clone HUTS21) for 45minutes at room temperature. Red blood cells were then lysed with ammonium chloride lysis buffer and washed. Samples were then analyzed FACS Array (BD Bioscience). Appropriate isotype controls of the antibodies were also run.

Statistical Analysis

SigmaPlot 10.0 (Systat Software, San Jose, CA) was used for all analysis. Chi-squared analysis was used for categorical analysis One way ANOVA and one way ANOVA on ranks

analysis was used to compare findings among the three groups: SIRS, sepsis, or normal controls. Statistical significance was set at $p < 0.05$.

Results

Over the course of the study 88 patients were enrolled. 10 healthy volunteer controls were also enrolled (Group 1). 35 patients exhibited SIRS with no evidence of infection (Group 2) whereas 53 of the patients had sepsis upon enrollment (Group 3). There was no significant difference between the three groups with respect to gender. Although both patient groups were slightly older than controls, there was no difference in age between patients with SIRS versus Sepsis (54.5 versus 56.3 years; $p = 0.9$). Further, there was no difference in APACHE II scores among SIRS and Sepsis patients (16.8 versus 19.5; $p = 0.07$). Both patient groups had significantly higher WCC compared with controls, but there was no difference in WCC between SIRS and Septic patients (12.5 ± 0.9 versus $14.4 \pm 0.7 \times 10^9/L$; $p = 0.09$) (Table 1). All patients with sepsis were receiving antimicrobial therapy, and 11 (21%) were receiving vasoactive pressors at the time of the blood draw.

Both patient groups had significantly lower percentage as well as absolute number of circulating $CD3^+$ lymphocytes when compared with controls. However, there was no significant difference between SIRS and Sepsis patients with respect to percent or number of circulating $CD3^+$ Lymphocyte populations (table 2).

Samples were analyzed for CD18 expression on $CD3^+$ T cells. CD18 was noted to be almost ubiquitously expressed on all $CD3^+$ Lymphocytes among normal controls (98.5%), SIRS (98.2%) or septic (97.9%) patients ($p = 0.91$) (Figure 1). This led to no significant difference in absolute number of circulating $CD18^+CD3^+$ Lymphocytes between SIRS and Sepsis patients (0.821 versus $0.819 \times 10^9/L$; $p = 0.95$) (table 2)

Next, samples were analyzed for the expression of CD29 on $CD3^+$ Lymphocytes. CD29 expression on $CD3^+$ Lymphocytes was significantly different across all three groups. Normal controls had the greatest percent of $CD3^+$ Lymphocytes expressing CD29 (54.1%). Notably Septic patients had significantly greater percentage of $CD29^+CD3^+$ Lymphocytes (35.5%) when compared to patients with SIRS (20.4%) (Figure 2). Assessing for absolute cell counts, both patient groups were noted to have lower numbers of $CD29^+CD3^+$ Lymphocytes compared to normal controls. Furthermore, patients with Sepsis compared with SIRS had greater numbers of circulating $CD29^+CD3^+$ Lymphocytes (0.294 versus $0.162 \times 10^9/L$; $p < 0.05$) (Table 2)

Of the septic patients, 21 had an abdominal source of sepsis compared with 32 patients who had a non-abdominal source of sepsis. We analyzed the CD29 expression on $CD3^+$ Lymphocytes with respect to abdominal versus non-abdominal sources of sepsis. Patients with abdominal sepsis had significantly elevated percentage of blood $CD3^+$ lymphocytes expressing CD29 ($41.7\% \pm 2.9$ versus $29.5\% \pm 3.9$; $p = 0.014$) (Graph 3). Accounting for absolute number of cells, patients with an abdominal source of sepsis had significantly higher numbers of circulating $CD3^+CD29^+$ Lymphocytes (0.36 ± 0.038 versus $0.24 \pm 0.043 \times 10^9/L$; $p = 0.035$).

Discussion

The morbidity and mortality following either severe tissue injury, as seen in the Systemic Inflammatory Response System (SIRS) or with severe infection (Sepsis) remains dismally high(2). This is often thought to be due to a dysregulation of the inflammatory and immune system(4). SIRS and sepsis share many similar clinical characteristics, such as tachycardia, leukocytosis and capillary leak. Indeed, many components of therapy are similar between both clinical entities, including large volume fluid resuscitation and the need for vasoactive agents. Furthermore, both SIRS and sepsis have been associated with immune-paralysis and immune dysfunction(4-6). Specifically, an appropriate lymphocyte response is critical to resolving inflammation, clearing bacteria, and ultimate survival in both cases. However, patients with SIRS are at high risk of developing infections and, thus, progressing into sepsis; thereby, increasing their mortality. The understanding of how T-cells change and transition from SIRS to sepsis in humans remains elusive. Understanding differences in lymphocyte dysfunction between SIRS and sepsis could potentially offer critical insights into advancing therapeutic options.

Integrins are essential to normal T-cell functioning. T-lymphocytes use integrins to migrate in and out of lymph nodes and, following infection, to migrate into other tissues(14, 17, 18, 25). Integrins are key to the immunological cross-talk between T cells and antigen-presenting cells. Much of the work on lymphocyte integrins relates to their function in animal models, T-cells from normal humans or from patients with chronic non-acute diseases. There is a paucity of data pertaining to the effects of SIRS or sepsis on human lymphocyte T-cells integrin expression and no data regarding a differential expression between SIRS and sepsis. We have demonstrated here marked differences in lymphocyte integrin expression in two distinct critically ill conditions, SIRS and Sepsis.

A robust leukocyte tissue influx has been associated with increased mortality in SIRS and sepsis, potentially from cell mediated tissue destruction(26). Increased integrin expression, specifically ICAM and $\beta 2$, mediates an excessive neutrophil influx into tissues(27, 28). $\beta 1$ integrins mediate their effects on lymphocyte migration via, among others CXCR4 - an important chemokine involved in lymphocyte migration(29, 30). CXCR4 expression has been shown to be up-regulated upon lymphocytes from patients with sepsis(31). Transmigration is the final and least well understood step in lymphocyte homing cascade. It has recently been shown that lymphocytes can migrate directly through endothelial cells (transcellular). Both $\beta 1$ and $\beta 2$ integrins have been shown to be involved in this process via interactions with endothelial junction integrin ligands such as JAM-B and JAM-A, respectively(32, 33). Modulating the chemokine/integrin axis may offer a potential therapeutic target to modulate sufficient leukocyte infiltration to allow bacterial clearance, while limiting tissue destruction(34).

Integrin $\beta 2$, CD18, is typically expressed on all lymphocytes(16, 25, 35) with the exception of certain rare immuno-compromised diseases, such as leukocyte adhesion deficiency (LAD)-1 syndrome. From this disease, it has shown that the lack of CD18 causes adhesion and mobility deficiencies that result in multiple skin infections and slow wound healing(36). Consistent with established data, we have shown that CD18 is ubiquitously expressed on all

lymphocytes in the peripheral blood(15, 25). The levels of lymphocyte CD18 expression were unchanged by the clinical circumstances with all groups displaying approximately 100% expression. Between healthy controls, SIRS or sepsis, or considering source of sepsis, CD18 expression does not display any significant difference.

Integrin $\beta 1$, CD29, is a protein encoded by the ITGB1 gene. It interacts with ligands VCAM-1 and MAdCAM-1 during adhesions and also interacts with collagen, fibronectin, and laminin(14, 17, 37). Deficiency of CD29 is known to affect cellular migration in a variety of conditions(38-40). Specifically, the Huts21 CD29 antibody represents activated integrin(41). We herein demonstrate that patients with either critical condition, SIRS or sepsis, displayed significantly lowered CD29 expression on peripheral lymphocytes, potentially a reflection of the immune dysfunction induced by critical illness. Compared to those with SIRS, a state of sterile inflammation, patients with sepsis have a greater need for a directed cellular response to a specific site of infection, requiring leukocyte transmigration from the vascular space to a focus of infection. The increased CD29 expression on lymphocytes of patients with sepsis would provide better endothelial adherence or trans-endothelial migration of T-cells. Our finding of the lowered CD29 expression in patients with SIRS rather than sepsis would potentially fit this observation. Patients with sepsis have a greater need for cellular localization (transmigration) to the source of the infection(42), rather than patients exhibiting SIRS where there is no focal point of infection.

An intriguing finding in our results was the difference in peripheral blood T-cell CD29 expression between abdominal and non-abdominal sources of sepsis. Patients with abdominal sepsis displayed significantly lower CD29 expression compared to patients with non-abdominal sources of sepsis. There is a growing body of literature suggestive of a compartmentalization effect with abdominal pathologies and inflammatory responses(9, 43, 44). Akin to this work, we speculate that the abdominal cavity offers a containment and protective environment, attempting to limit the systemic nature of the insult. Stromberg et al demonstrated that T-cell infiltration following gut injury and sepsis improves survival following sepsis(45). It has been shown that lymphocytes play a vital role in mediating gut epithelial apoptosis in sepsis but not under normal conditions. However, the exact cause/ effect of lymphocyte migration to the peritoneal cavity remains unclear. Clearly the ability of lymphocytes to travel between tissues in patients with systemic evidence of infection and injury remains critical.

That said, this current work cannot address a possible cause and effect to our observations. It is often uncertain whether the illness leads to alteration of cellular functions, or whether the altered cellular function leads to the predisposition to critical illness and/or infection. This data supports our hypothesis that a differential lymphocyte integrin expression exists between patients with generalized tissue destruction (SIRS) compared with patients needing a more active directed lymphocyte migration to a focus of infection (Sepsis).

CONCLUSION

Sepsis and SIRS remain leading causes of death in ICU patients. Lymphocytes play a key role in the pathogenesis and progression of both entities. We have demonstrated clear

differences in Integrin expression on T-cells from two distinctively different critical conditions which often have overlapping clinical presentation. Understanding changes in lymphocyte integrin expression in each will allow for potential therapeutic targets, leading to better control over cellular migration and ultimately end-organ dysfunction seen in both condition. Further it may begin to explain why certain patients with SIRS progress to sepsis and ultimately septic shock and death.

References

1. Singer M, Deutschman C, Seymour C, Shankar-Hari M, Annane D, Bauer M, Bellomo R, Bernard G, Chiche J, Coopersmith C, Hotchkiss R, Levy M, Marshall J, Martin G, Opal S, Rubenfeld G, Van der Poll T, Vincent J, Angus D. The third international consensus definitions for sepsis and septic shock (Sepsis-3). *JAMA*. 2016; 315(8):801–10. [PubMed: 26903338]
2. Rhodes A, Phillips G, Beale R, Cecconi M, Chiche J, De Backer D, Divatia J, Du B, Evans L, Ferrer R, Girardis M, Koulenti D, Machado F, Simpson S, Tan C, Wittebole X, Levy M. The surviving sepsis campaign bundles and outcomes: results from the international multicentre prevalence study on sepsis (the IMPReSS) study. *Intensive Care Medicine*. 2015; 41(9):1620–8. [PubMed: 26109396]
3. Heffernan D, Monaghan S, Chung C, Cioffi W, Gravenstein S, Ayala A. A divergent response of innate regulatory T-cells to sepsis in humans: circulating invariant natural killer T-cells are preserved. *Human Immunology*. 2014; 75(3):277–82. [PubMed: 24269692]
4. Thakkar, R., Huang, X., Lomas-Neira, J., Heffernan, D., Ayala, A. Sepsis and the immune response.. In: Eremin, O., Sewell, H., editors. *Essential immunology for surgeons*. Oxford University Press; 2011. p. 303-42.
5. Binkowska A, Michalak G, Slotwinski R. Current views on the mechanisms of immune responses to trauma and infection. *Central European Journal of Immunology*. 40(2):206–16. 201.
6. Hotchkiss R, Monneret G, Payen D. Sepsis induced immunosuppression: from cellular dysfunctions to immunotherapy. *Nature Reviews Immunology*. 2013; 13(12):862–74.
7. Venet F, Chung C, Monneret G, Huang X, Horner B, Garber M, Ayala A. Regulatory T cell populations in sepsis and trauma. *Journal of Leukocyte Biology*. 2008; 83(3):523–35. [PubMed: 17913974]
8. Shubin N, Monaghan S, Heffernan D, Chung C, Ayala A. B and T lymphocyte attenuator expression on CD4+ T-cells associates with sepsis and subsequent infections. *Critical Care*. 2013; 17(6):R276. [PubMed: 24289156]
9. Young J, Monaghan S, Chung C, Cioffi W, Ayala A, Heffernan D. Divergent invariant natural killer T-cell response to sepsis of abdominal vs non-abdominal origin in human beings. *Surgical Infections*. 2015; 16(1):29–35. [PubMed: 25761077]
10. Drewry A, Samra N, Skrupky L, Fuller B, Compton S, Hotchkiss R. Persistent lymphopenia after diagnosis of sepsis predicts mortality. *Shock*. 2014; 42(5):383–91. [PubMed: 25051284]
11. Heffernan D, Monaghan S, Thakkar R, Machan J, Cioffi W, Ayala A. Failure to normalize lymphopenia following trauma is associated with increased mortality, independent of the leukocytosis pattern. *Critical Care*. 2012; 16(1):R12. [PubMed: 22264310]
12. Benezet A, Menon M, Khanna K. Visualizing T cell migration in situ. *Frontiers in Immunology*. 2014; 5(363)
13. Heffernan D, Monaghan S, Thakkar R, Tran M, Chung C, Gregory S, Cioffi W, Ayala A. Inflammatory mechanisms in sepsis: elevated invariant natural killer T-cell numbers in mouse and their modulatory effect on macrophage function. *Shock*. 2013; 40(2):122–8. [PubMed: 23807244]
14. Mitroulis I, Alexaki V, Kourtzelis I, Ziogas A, Hajishengallis G, Chavakis T. Leukocyte integrins: role in leukocyte recruitment and as therapeutic targets in inflammatory disease. *Pharmacology and Therapeutics*. 2015; 147:123–35. [PubMed: 25448040]
15. Zhang Y, Wang H. Integrin signaling and function in immune cells. *Immunology*. 2013; 135(4): 268–75.
16. Nourshargh S, Alon R. Leukocyte migration into inflamed tissues. *Immunity*. 2014; 41(5):694–707. [PubMed: 25517612]

17. Koivisto L, Heino J, Hakkinen L, Larjava H. Integrins in wound healing. *Advances in wound care*. 2014; 3(12):762–83. [PubMed: 25493210]
18. Baaten B, Cooper A, Swain S, Bradley L. Location, location, location: the impact of migratory heterogeneity on T cell function. *Frontiers in Immunology*. 2013; 4:311. [PubMed: 24115949]
19. Valignat M, Theodoly O, Gucciardi A, Hogg N, Lellouch A. T lymphocytes orient against the direction of fluid flow during LFA-1 mediated migration. *Biophysical Journal*. 2013; 104(2):322–31. [PubMed: 23442854]
20. Reichardt P, Patzak I, Jones K, Etemire E, Gunzer M, Hogg N. A role for LFA-1 in delaying T-lymphocyte egress from lymph nodes. *EMBO Journal*. 2013; 32(6):829–43. [PubMed: 23443048]
21. Tan S. The Leukocyte beta2 (CD18) integrins: the structure, functional regulation and signalling. *Bioscience Reports*. 2012; 32(3):241–69. [PubMed: 22458844]
22. Cantor J, Rose D, Slepak M, Ginsberg M. Fine tuning tumor immunity with integrin trans-regulation. *Cancer Immunology Research*. 2015; 3(6):661–7. [PubMed: 25600437]
23. Uotila L, Jahan F, Soto Hinojosa L, Melandri E, Gronholm M, Gahmberg C. Specific phosphorylations transmit signals from leukocyte beta2 to beta1 integrins and regulate adhesion. *Journal of Biological Chemistry*. 2014; 289(46):32230–42. [PubMed: 25278023]
24. Verma N, Kelleher D. Adaptor regulation of LFA-1 signaling in T lymphocyte migration: Potential druggable targets for immunotherapies? *European Journal of Immunology*. 2014; 44(12):3484–99. [PubMed: 25251823]
25. Dupre L, Houmadi R, Tang C, Rey-Barroso J. T Lymphocyte migration: An action movie starring the actin and associated actors. *Frontiers in Immunology*. 2015; 6:586. [PubMed: 26635800]
26. Gerner R, Moschen A, Tilg H. Targeting T and B lymphocytes in inflammatory bowel diseases: Lessons from clinical trials. *Digestive Diseases*. 2013; 31(3-4):328–35. [PubMed: 24246983]
27. Weaver L, Bao F, Dekaban G, Hryciw T, Schultz S, Cain D, Brown A. CD11b integrin blockade reduces the systemic inflammatory response syndrome after traumatic brain injury in rats. *Experimental Neurology*. 2015; 271:409–22. [PubMed: 26169930]
28. Gorlino C, Ranocchia R, Harman M, Garcia M, Crespo M, Moron G, Maletto B, Pistoresi-Palencia M. Neutrophils exhibit differential requirements for homing molecules in their lymphatic and blood trafficking into draining lymph nodes. *Journal of Immunology*. 2014; 193(4):1966–74.
29. Wostradowski T, Gudi V, Pul R, Gingele S, Lindquist J, Stangel M, Lindquist S. Effect of interferon-beta1b on CXCR4-dependent chemotaxis in T cells from multiple sclerosis patients. *Clinical and Experimental Immunology*. 2015; 182(2):162–72. [PubMed: 26212126]
30. Moser B, Loetscher P. Lymphocyte traffic control by chemokines. *Nature Immunology*. 2001; 2(2):123–8. [PubMed: 11175804]
31. Ding Z, Jia S, Marshall J, Downey G, Waddell T. Up-regulation of functional CXCR4 expression on human lymphocytes in sepsis. *Critical Care Medicine*. 2006; 34(12):3011–7. [PubMed: 17075377]
32. Manetti M, Guiducci S, Romano E, Rosa I, Ceccarelli C, Mello T, Milia A, Conforti M, Ibbamanneschi L, Matucci-Cerinic M. Differential expression of junctional adhesion molecules in different stages of systemic sclerosis. *Arthritis and Rheumatism*. 2013; 65(1):247–57. [PubMed: 23001478]
33. Sladojevic N, Stamatovic S, Keep R, Grailer J, Sarma J, Ward P, Andjelkovic A. Inhibition of junctional adhesion molecule-A/LFA interaction attenuates leukocyte trafficking and inflammation in brain ischemia/reperfusion injury. *Neurobiology of disease*. 2014; 67:57–70. [PubMed: 24657919]
34. Huang Y, Clarke F, Karimi M, Roy N, Williamson E, Okumura M, Mochizuki K, Chen E, Park T, Debes G, Zhang Y, Curran T, Kambayashi T, Burkhardt J. CRK proteins selectively regulate T cell migration into inflamed tissues. *Journal of Clinical Investigation*. 2015; 125(3):1019–32. [PubMed: 25621495]
35. Morrison V, MacPherson M, Savinko T, Lek H, Prescott A, Fagerholm S. The beta2 integrin-kindlin-3 interaction is essential for T-cell homing but dispensable for T-cell activation in vivo. *Blood*. 2013; 122(8):1428–36. [PubMed: 23823319]
36. Gonzalez-Alvaro I, Munoz C, Garcia-Vicuna R, Sabando P, Cabanas C, Sanchez-Madrid F, Diaz-Gonzalez F. Interference of nonsteroidal antiinflammatory drugs with very late activation antigen 4/

- vascular cells adhesion molecule 1-mediated lymphocyte-endothelial cell adhesion. *Arthritis and Rheumatism*. 1998; 41(9):1677–88. [PubMed: 9751102]
37. Rosenthal-Allieri M, Ticchioni M, Breittmayer J, Breittmayer J, Shimizu Y, Bernard A. Influence of beta 1 integrin intracytoplasmic domains in the regulation of VLA-4 mediated adhesion of human T cells to VCAM-1 under flow conditions. *Journal of Immunology*. 17(2):1214–23. 200.
38. Parkash J, Cimino I, Ferraris N, Casoni F, Wray S, Cappy H, Prevot V, Giacobini P. Suppression of b1- integrin in gonadotropin-releasing hormone cells disrupts migration and axonal extension resulting in severe reproductive alterations. *Journal of Neuroscience*. 2012; 32(47):16992–7002. [PubMed: 23175850]
39. Howe G, Addison C. Beta-1 integrin: an emerging player in the modulation of tumorigenesis and response to therapy. *Cell Adhesion and Migration*. 2012; 6(2):71–7. [PubMed: 22568952]
40. Kadioglu A, De Filippo K, Bangert M, Fernandes V, Richards L, Jones K, Andrew P, Hogg N. The integrins Mac-1 and alpha4beta 1 perform crucial roles in neutrophil and T cell recruitment to lungs during *Streptococcus pneumoniae* infection. *Journal of Immunology*. 2011; 186(10):5907–15.
41. Luque A, Gomez M, Puzon W, Takada Y, Sanchez-Madrid F, Cabanas C. Activated conformations of very late activation integrins detected by a group of antibodies (HUTS) specific for a novel regulatory region (355-425) of the common beta-1 chain. *Journal of Biological Chemistry*. 1996; 271(19):11067–75. [PubMed: 8626649]
42. Langer H, Chavakis T. Leukocyte-endothelial interactions in inflammation. *Journal of Cellular and Molecular Medicine*. 2009; 13(7):1211–20. [PubMed: 19538472]
43. Nachtsheim R, Dudley B, McNeil P. The peritoneal cavity is a distinct compartment of angiogenic molecular mediators. *Journal of Surgical Research*. 134(1):28–35. 200.
44. Cavaillon J, Annane D. Compartmentalization of the inflammatory response in sepsis and SIRS. *Journal of Endotoxin Research*. 12(3):151–70. 200.
45. Stromberg P, Woolsey C, Clark A, Clark J, Turnbull I, McConnell K, Chang K, Chung C, Ayala A, Buchman T, Hotchkiss R, Coopersmith C. CD4+ lymphocytes control gut epithelial apoptosis and mediate survival in sepsis. *FASEB J*. 2009; 23(6):1817–25. [PubMed: 19158156]

Figure 1.
%CD18⁺CD3⁺ lymphocytes in normal, SIRS and Sepsis patients.

Figure 2.
%CD29⁺CD3⁺ lymphocytes in normal, SIRS and Sepsis patients.

Figure 3. Compartmentalization of Integrin Expression. %CD29⁺CD3⁺ lymphocytes in abdominal versus non-abdominal sepsis.

Table 1

Demographics of the three groups SIRS, sepsis and resolution of clinical entities.

	Normal Controls (n=10)	SIRS (n=35)	Sepsis (n=53)	p value
Age (mean)	38.2+/-2.5	54.5+/-1/7	56.3+/-2.1	<0.05 vs control
Male (%)	60%	60%	56.6%	0.9
APACHE II (mean)		16.8+/-0.9	19.47+/-1.05	0.07
White Cell Count (WCC – $\times 10^9/L$)	6.3 +/- 0.45	12.5 +/- 0.9	14.4 +/- 0.7	<0.05 vs control

Table 2

Absolute number of CD3⁺ Lymphocytes as well as CD18⁺CD3⁺ and CD29⁺CD3⁺ lymphocytes between the three clinical entities of normal, SIRS and Sepsis.

	Normal (n=10)	SIRS (n=35)	Sepsis (n=53)	p value vs normal
% CD3 ⁺ T-Lymphocytes	26.4%+/-0.8	7.3%+/-0.7	6.5%+/-0.7	<0.05
Absolute Number of CD3 ($\times 10^9/L$)	1.64+/-0.09	0.84+/-0.07	0.84+/-0.08	<0.05
Absolute Number of CD18 ⁺ CD3 ⁺ cells ($\times 10^9/L$)	1.61+/-0.09	0.82+/-0.08	0.82+/-0.07	<0.05
Absolute Number of CD29 ⁺ CD3 ⁺ cells ($\times 10^9/L$)	0.93+/-0.04	0.16+/-0.03	0.29+/-0.03	<0.05