E-selectin combined with soluble CD44 as predictors of acute graft-versus-host disease after allogeneic hematopoietic stem cell transplantation

Li YANG
Wuhan Children's Hospital, Huazhong University of Science and Technology
Wei WANG
Wuhan Children's Hospital, Huazhong University of Science and Technology
Zhi CHENG
Wuhan Children's Hospital, Huazhong University of Science and Technology
Fei LONG
Wuhan Children's Hospital, Huazhong University of Science and Technology
Ming SUN
Wuhan Children's Hospital, Huazhong University of Science and Technology
Wenjie LU
Wuhan Children's Hospital, Huazhong University of Science and Technology
Shanshan Qi
Wuhan Children's Hospital, Huazhong University of Science and Technology
Hao XIONG
xionghao@zgwhfe.com

Wuhan Children's Hospital, Huazhong University of Science and Technology

Research Article

Keywords: Allogeneic hematopoietic stem cell transplantation (allo-HSCT), Acute graft-versus-host disease (aGVHD), E-selectin (CD62E), soluble CD44, Pediatric patients

Posted Date: February 26th, 2024

DOI: https://doi.org/10.21203/rs.3.rs-3974402/v1

Additional Declarations: No competing interests reported.

Abstract

Background

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is the mainly curable treatment options in children with high-risk malignancies, bone marrow failure diseases and inherited metabolic diseases. Acute graft-versus-host disease (aGVHD) accompanied with series of serious complications are the most severe obstacle of allo-HSCT because the early and accurate diagnostic markers and effective treatment are still lacked. Non-organ-specific injury induced activated endothelial cells and tissue integrity biomarkers may have higher specificity for the occurrence and development of aGVHD.

Methods

The blood from 52 pediatric patients who underwent allo-HSCT including 16 recipients with aGVHD and 36 recipients without aGVHD were collected to check the level of adhesion molecules. The vitro experiments, transwell experiments, and aGVHD mouse model are used to verify the effects of E-selectin in the occurrence and development of aGVHD.

Results

We found that E-selectin secreted by endothelial cells was remarkably increased while the level of soluble CD44, a widely distributed tissue structure molecule, was significantly decreased in aGVHD patients. The level of E-selectin was negatively correlated with the soluble CD44 and associated with the severity of the aGVHD. After that, the vitro experiments suggested the elevated E-selectin could recruit immune cells that result in a series of inflammatory response and tissue injury. The aGVHD mouse model revealed that the level of E-selectin in the intestine occurred aGVHD was obviously increased than that without aGVHD. The expression level of CD44 in organs was related to the incidence of organ aGVHD. More importantly, the area under the ROC curve (AUC) of E-selectin and CD44 can reach 0.85 indicating that these two parameters have strong prediction ability of aGVHD.

Conclusions

E-selectin and CD44 could play an important role in the occurrence and development of aGVHD. E-selectin combined with soluble CD44 could act as efficient biomarkers for the diagnosis of aGVHD.

Background

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is the mainly curative treatment option for children with high-risk blood malignancies, bone marrow failure diseases, and inherited metabolic diseases[1]. However, acute graft-versus-host disease (aGVHD) might be still a major cause of transplant

failure and the most serious complications that affects the clinical application and long-term survive of patients after allo-HSCT[1]. There are 40–50% of patients who undergo transplant procedures occurring various degree of aGVHD[2]. aGVHD is the result of a series of pathological processes that range from local inflammation to systematic organ failure resulting from injury and activation of endothelial cells, tissue damage, pathogenic effector cells and inflammatory mediators, among other effects[3, 4]. Although most cases of mild aGVHD (I-) could be treated with classic steroids and calcineurin inhibitor, cases that progress to severe aGVHD (-) have lower treatment effectiveness and poor prognosis[5]. Specifically, 15% of patients with severe acute aGVHD (to) would undergo poor survival[2, 6].

Rash, hyperbilirubinemia, and diarrhea are the typical clinical features of aGVHD that are widely used as standard signs for the diagnosis of aGVHD[7, 8]. However, these clinical signs and symptoms are not specific to aGVHD. For example, skin rashes resulting from drug eruption or engraftment syndrome[9], diarrhea, and elevated bilirubin are common clinical symptoms of infection after HSCT[10]. In addition, inflammation, tissue damage and the activation of immune cells, widely-spreading in microbial infections after HSCT, would be the major pathophysiology of aGVHD. Further, the levels of diagnostic markers, such as IL-6, soluble suppression of tumorigenicity-2 (sST2) and regenerating islet-derived protein 3α, which are secreted by damaged end organs, are also increased in transplant recipients who develop severe infection and pathological damage but do not develop to aGVHD[11–15]. Thus, it is still difficult to diagnose aGVHD in the early phase as the lack of specific markers and clinical signs [10]. Pathological examination could be used to detect damage to immune cells in target organs[16], but it is quite difficult to obtain specimens without the risk of invasive procedures[17]. In addition, the therapeutic agents and approaches based on current theories, such as glucocorticoids, calcineurin inhibition, and antimetabolites, have a low success ratio, especially in cases of severe aGVHD[18, 19]. Therefore, it is necessary to develop novel markers that could predict the occurrence of aGVHD in its early phases and also serve as potential therapeutic targets to alleviate the terrible situation of aGVHD. More importantly, accurate diagnosis of aGVHD could reduce unnecessary immunosuppressant therapy and the incidence of serious post-transplantation infection, and greatly improve the outcomes of allo-HSCT.

Pan-organ immune activation and tissue damage in aGVHD would be the key difference to the common inflammatory damage after HSCT. Thus, the activation of endothelial cells and impairment of parenchymal cells in multi-organ tissue might be specific key pathological mechanisms for distinguishing aGVHD from common inflammatory conditions. The molecules from plasma secreted by activated endothelial cells or tissue structural damage might be more precise indicators of the risk of aGVHD. The family of adhesion molecules mediated the interaction between cells and cells and constituted the extracellular matrix[20, 21], are widely involved in the immune recognition-activation process and play an important role in tissue structure and integrity[22]. Accordingly, our study focused on the level of adhesion molecules in allo-HSCT recipients. The findings firstly revealed that E-selectin and CD44 play an important role in the occurrence and development of aGVHD and could serve as valuable biomarkers for the diagnosis of aGVHD in early phase.

Materials and methods

Patients and samples

This study included 52 pediatric patients who underwent allogeneic hematopoietic stem cell transplantation (allo-HSCT) at Wuhan Children's Hospital (Wuhan, China) between 2021.03 and 2022.12. A comprehensive summary of the characteristics of all enrolled patients is presented in Table 1 and detailed information for the patients in this study are listed in Supplemental Table 1. Blood samples were collected from 52 child patients including 16 recipients with aGVHD and 36 recipients without GVHD. The grading and organ grading criteria for aGVHD are described in the International Alliance for Acute Graft-versus-Host Disease (MAGIC) grading criteria, excluding similar symptoms caused by other factors[7]. All patients gave written, informed consent in accordance with the Declaration of Helsinki. No patient compensation was provided. This study was approved by the Ethics Committee of Wuhan Children's Hospital and registered in the Chinese Clinical Trial Registry (**Project Number ChiCTR2100050665**).

Table 1
Comprehensive clinical characteristics of the patients enrolled in this Study

Parameter	Acute GVHD	Non-GVHD	P value
Age(year)	5.5(0.72-16)	5.5(0.25-15)	
Male/Female	9/7	22/14	
CD44*	4948(3779–6117)	6803(5296–8310)	< 0.01
CD62E(E-selectin)*	717(195–1239)	349(177–521)	0.014
ICAM-1*	47(20–74)	121(0-257)	0.03
ICAM-2*	459(234–684)	494(109–879)	0.738
ICAM-3*	2156(1363–2949)	2447(1893–3001)	0.134
PSGL-1*	89(61–117)	76(57–95)	0.108
NCAM*	6317(4098–8536)	7178(4810–9546)	0.223
ALCAM*	1873(1412–2334)	1571(1189–1953)	0.017
VCAM*	33942(27767–40117)	32953(22440–43466)	0.728
PECAM*	914(755–1073)	857(678–1036)	0.285
CD62P*	205(109–301)	149(98–200)	0.041
EPCAM*	14(10–18)	15(11–19)	0.160
CD62L*	66687(46518–86856)	79156(46316–111996)	0.167
Neutrophils(10^9/L)	3.17(1.64–4.7)	3.29(1.09–5.49)	0.841
Red blood cells(10^{12}/L)	3.25(2.69–3.81)	3.36(2.99–3.73)	0.432
Lymphocytes(10^9/L)	0.59(0.03–1.15)	0.71(0.09–1.33)	0.506
Monocytes(10^9/L)	0.59(0.22–0.96)	0.61(0.3–0.92)	0.837
Platelets (PLT)(10^9/L)	80.5(25.54-135.46)	137.9(67.73-208.07)	0.029
White blood cells(10^9/L)	4.69(2.75–6.63)	4.71(2.53–6.89)	0.972
C reaction protein (CRP)(mg/L)	11.18(0.81–21.55	4.628(0-9.562)	0.742

Data are shown as mean (range); *:pg/ml.

Parameter	Acute GVHD	Non-GVHD	P value
IL-6*	60.32(0-133.33)	16.01(0-32.71)	0.0301
Data are shown as mean (range); *:pg/ml.			

The preparation of platelet-free serum and detection of the adhesion molecules

Anticoagulant peripheral blood by sodium citrate was collected from allo-HSCT recipients involved in this study at 28 days after transplantation. Prostaglandin E1 (1 μM) and RGDS (1 mM) were immediately added into the blood samples with gently resuspending to inhibit the activation of platelets[23]. After that, the samples were centrifuged at 5000g and 4℃ for 20 minutes. The platelet-free serum was gained and stored at -80℃ refrigerator. The adhesion molecules were detected by multi-analyte flow assay kit (Human Adhesion Molecule Panel (13-plex), Cat. No. 740945, Biolegend). Data were collected using FACSCanto (BD Bioscience) and analyzed by FlowJo software (Tree Star). The concentration of indicated adhesion molecules was calculated through the standard curve.

E-selectin vitro experiments

Anticoagulant peripheral blood by sodium citrate was collected from healthy volunteers to verify the effect of E-selectin. E-selectin (MCE, HY-P72661) was added into peripheral blood in terminal concentrate 0μg/ml, 5μg/ml, 10μg/ml, 20μg/ml and 50μg/ml for 5 minutes and gently resuspended. Adenosine 5'-diphosphate (ADP,10μM) was used for positive control. For the transwell experiments, E-selectin (2.5μg/ml,5μg/ml,10μg/ml) were preincubated at the bottom chamber for 2 hours. After that PBMCs isolated from the healthy donors were added into the top chamber for co-culturing 12 hours. The migrated cells in the bottom chamber were digested to collect together for detecting by the following antibodies from BD Bioscience including BV510-conjugated anti-human CD45(2D1), APC-cy7-conjugated anti-human CD14(MφP9), Fitch-conjugated anti-human CD3(SK7), BV421-conjugated anti-human CD16(3G8), APC-conjugated anti-human CD19(SJ25C1), PE-conjugated anti-human CD8 (SK1), Percp-cy5.5-conjugated anti-human CD4(SK3).

Co-culture experiments

Human umbilical vein endothelial cells (HUVEC, provided by Transfusion Research Department, Wuhan Blood Center, Wuhan, Hubei, China) were co-culture with the follows for 24 hours: 1. supernatant of THP1 cells purchased from Procell (Cat.NO.CL-0233) with or without the stimulation of lipopolysaccharides (LPS,100ng/ml, Cat.NO.L8880, Solarbio); 2. soluble CD14 (10μg/ml, Cat.NO. 383-CD/CF, R&D system) with or without LPS (100ng/ml); 3. TNF-α (100ng/ml, Cat.NO. 570104, Biolegend) was served as positive control. The level of E-selectin in HUVEC was detected by flow cytometry (PE-conjugated anti-human CD62E (E-selectin, clone HAE-1f, Biolegend).

Immunofluorescence and histochemistry

The intestine, brain, kidney, liver and skin tissues from mouse were fixed in 4% paraformaldehyde, embedded in paraffin, and sectioned. Immunofluorescence and histochemistry were performed and whole section were scanned by Pannoramic MIDI (3D HISTECH). Fluorescent double staining of CD31 (Servicebio, Cat.NO. GB113151) and CD44 (Servicebio, Cat.NO. GB112054) in sections was performed with red and green fluorescence, respectively. Immunohistochemical detection of E-selectin (Thermo Fisher, Cat.NO. 14-0627-82) was carried out on paraffin-embedded mice intestine mice tissues with/without aGVHD.

aGVHD mouse model

6–8 weeks-old male C57BL/6 mice and female Balb/c mice were purchased from Hubei Biont Biological Technology Co., Ltd. All husbandry and experimental contact with the mice were maintained under specific pathogen free condition. The aGVHD mouse model was induced as previously described methods [24]. The female Balb/c recipients were irradiated using 8Gy of X-ray irradiation (2.19 Gy/min) at intervals of 4–6h. Donor bone marrow cells (3×10^6) and splenic mononuclear cells (3×10^7) obtained from male C57BL/6J mice were resuspended in 50 μl PBS and transplanted into irradiated female Balb/c mice via tail vein injection. 10 days after transplantation, the mice were sacrificed and target organs were harvested from each group for performance of immunofluorescence and histochemistry. The mice in the aGVHD group had obviously decreased body weight, wrinkled hair, bowed back, decreased activity, diarrhea and bloody stool.

Statistical analysis

Data with normal distribution and equal variance were analyzed by the student's *t* test or ANOVA for comparisons of groups by GraphPad Prism software (version 8.0). For non-normally distributed variables, the Kruskal-Wallis tests was performed. Correlation analysis was assessed by Spearman's rank correlation. P values < 0.05 were considered statistically significant (*P < 0.05; **P < 0.01; ***P < 0.001).

Results

Elevation of serum E-selectin levels in patients with aGVHD and its correlation with the degree of aGVHD

In this study, blood samples were collected from 52 pediatric patients underwent allo-HSCT, including 36 patients without GVHD and 16 patients with aGVHD (Fig. 1A). Platelet-free plasma obtained from the blood samples was used to determine the level of adhesion proteins. We assayed a series of adhesion proteins, including immunoglobulin-like adhesion molecules, integrins, cadherins, and selectins (Figure S1), and detected significant differences in the levels of E-selectin (aGVHD vs non- GVHD, 717 ± 522pg/ml vs 349 ± 172pg/ml), soluble CD44 (aGVHD vs non-GVHD, 4948 ± 1169pg/ml vs 6803 ± 1507pg/ml in patients with aGVHD compared to non-GVHD. As described in the previous study, the level of P-selectin is affected by several factors, such as the activation of platelets and the elevation of pro-inflammatory factors[25, 26]. Therefore, we focused on the obvious change of E-selectin and soluble CD44 levels (Fig. 1B). Patients were divided into mild (,) and severe (,) aGVHD groups based on the presence of

rash, presentation of gastrointestinal symptoms, and total bilirubin levels[7]. The level of E-selectin in patients with severe aGVHD was obviously elevated compared to that in patients with mild aGVHD (Fig. 1C, 946.6 ± 606.2pg/ml vs 421.9 ± 101.3pg/ml). In contrast, the level of soluble CD44 in patients with severe aGVHD was definitely downregulated compared to that in patients with mild aGVHD (Fig. 1D, 4297.9 ± 709.8pg/ml vs 5783.8 ± 1139.2pg/ml). Moreover, the level of E-selectin was negatively correlated with the level of soluble CD44 in patients with aGVHD (Fig. 1E). These findings imply that E-selectin may be associated with the occurrence and development of aGVHD, and we conducted further in vivo and in vitro experiments to confirm this role of E-selectin.

Increase in the production of E-selectin by endothelial cells stimulated by increased recruitment of activated monocytes by E-selectin

Infections are the most common trigger of aGVHD[27, 28]. Accordingly, in this study, the level of C-reactive protein in aGVHD patients, which is considered an indicator of infection or inflammation[29], was significantly higher than non-GVHD (Fig. 3A), which implies that the patients with aGVHD had a higher incidence of infections than those without aGVHD. As the infectious state is associated with high numbers of monocytes, we performed further in vitro experiments with a monocyte cell line (THP1) to examine its association with the E-selectin. As endothelial cells are known to produce E-selectin[30], human umbilical vein endothelial cells (HUVEC) were cultured with the supernatant of THP1 cells stimulated with LPS. The results showed that the production of E-selectin by HUVEC was significantly promoted. However, the supernatant of THP1 cells without the stimulation of LPS had no such effect on HUVEC (Fig. 3B). With regard to the underlying mechanism, the THP1 cells were found to produce a large amount of TNF-α (Fig. 3C), which is known to promote the production of E-selectin by HUVEC [30, 31]. In addition, HUVEC, co-cultured with the supernatant of THP1 cells stimulated with LPS, produced more IL-6 than HUVEC co-cultured with THP1 cell supernatant without the stimulation of LPS (Fig. 3E). This is consistent with the elevated IL-6 levels observed in patients with aGVHD compared to non-GVHD (Fig. 3F).

The transmigration and activation of leukocytes are important for the occurrence and development of aGVHD[32]. Therefore, we examined the effects of E-selectin on the chemotaxis of leukocytes. To this end, peripheral blood mononuclear cells were collected and co-cultured with E-selectin in a transwell system (Fig. 2A). In the presence of E-selectin, a higher number of monocytes were transferred to the bottom of the plate (Fig. 2B and C). This implies that E-selectin promoted the activation and migration of leukocytes, which may be one of the mechanisms via which involved in the development of aGVHD.

Altogether, the above findings imply that infection in the pre-aGVHD stage or the early aGVHD stage may lead to the increased production of pro-inflammatory factors by monocytes, and these factors may lead to the increased production of E-selectin from endothelial cells. E-selectin might then lead to the increased activation and migration of monocytes, which would, in turn, induce the pathogenesis of aGVHD and further promote the production of E-selectin. In this way, increase in the severity of aGVHD may be associated with increase in the levels of E-selectin.

Activation of platelets by E-selectin and its association with thrombocytopenia after allo-HSCT

Thrombocytopenia is a common clinical symptom of aGVHD that is associated with a series of serious outcomes[33]. The activation of platelets is the main reason for the decrease in the level of platelets[34]. Accordingly, we found that the number of platelets was significantly lower in aGVHD cases than in cases non-GVHD (Fig. 4A). To determine the effect of E-selectin on platelets, we collected blood samples from healthy donors for co-culture with E-selectin. The results revealed that E-selectin could efficiently promote the activation of platelets in a dose-dependent manner (Fig. 4B and C) which suggests the elevated E-selectin may act as a strong risk factor for the occurrence and development of thrombocytopenia.

Association of the distribution of endothelial cells or CD44 with aGVHD-affected target organs

The intestine and the skin are the most common targets of aGVHD[35, 36]. As E-selectin is mainly produced by activated endothelial cells[37], we detected the distribution of endothelial cells based on CD31 expression in different mouse organs, including the skin, the intestine, the brain, and the lung through immunofluorescence staining. A higher number of endothelial cells and a higher level of expression of CD44 were detected in intestine and skin than in brain and kidney (Fig. 5A & B and Figure S2). Accordingly, it has been reported that the brain and kidney are rarely affected by aGVHD[1, 38]. Although the number of endothelial cells in the kidney was much higher than that in the intestine and skin, renal CD44 expression was low (Fig. 5C)[39, 40]. This may explain why thrombotic microangiopathies were mostly observed in the kidney[41]. More importantly, we detected higher levels of E-selectin in the intestines of the aGVHD mouse group than in the group without aGVHD (Fig. 5D).

Furthermore, the area under the ROC curve (AUC) of E-selectin and CD44 reached 0.85, which indicates that these two parameters were strong predictors of aGVHD (Fig. 6 and Figure S3). The best cut-off value in the ROC curve were 5344pg/ml (CD44) and 433pg/ml(E-selectin). The sensitivity of E-selectin and CD44 were 0.875 with specificity of 0.972(CD44) and 0.861(E-selectin). Altogether, these results indicate that E-selectin combined with CD44 could act as potential biomarkers for the early diagnosis of aGVHD.

Discussion

In this study, we have examined the potential of E-selectin, an adhesion protein secreted by endothelial cells, as a predictor of aGVHD. E-selectin levels were found to be significantly elevated in patients with aGVHD as compared to the patients without aGVHD, and the soluble CD44 levels showed the opposite trend. These findings indicate the E-selectin and CD44, which are essential for tissue architecture[42], regulate tight-junction assembly and barrier function[43], could be served as combined biomarkers for predicting aGVHD.

The current results indicated that endothelial cells could efficiently express E-selectin on the stimulation with LPS and soluble CD14, as previously reported[44]. In addition, TNF-α was also found to promote the secretion of large amounts of E-selectin by HUVEC[45]. The secretion of E-selectin reflects two important pathological conditions in aGVHD, namely, the activation of endothelial cells and the triggering of inflammatory response associated with microbial infection. Our study revealed that the increased levels of E-selectin have two important physiological effects: (1) chemotactic effect on immune effector cells such as monocytes and lymphocytes and (2) activation of platelets. In particular, there was a significant increase in the platelet activation rate in the presence of E-selectin. However, as the concentration of E-selectin in platelet-free peripheral plasma was lower than that observed in in vitro experiments, we proposed that the local concentration of E-selectin secreted by activated endothelial cells may be enough to activate platelets, which needs to be verified in future experiments.

Endothelial injury induces the activation of endothelial cells and producing of E-selectin, which promotes the migration of mononuclear cells to the damaged sites. On stimulation with LPS, infiltrated mononuclear cells secreted a large amount of TNF-α, which could efficiently induce endothelial cells to produce E-selectin. In addition, endothelial cells showed obvious upregulation of the expression of E-selectin on stimulation with soluble CD14 and LPS. More importantly, accumulated E-selectin could significantly activate platelets and lead to the wear out of platelets. This is consistent with the significant thrombocytopenia observed in patients with aGVHD. Therefore, the infiltration and activation of monocytes induced by E-selectin play a key role in the progression of aGVHD which partly explains the hyperacute aGVHD could occurred after monocyte-granulocyte reconstruction but before the complete reconstruction of T lymphocytes [46, 47].

Acute GVHD is associated with parenchymal damage, as indicated by various indexes measured in different organs, such as elevated soluble ST2 levels (associated with intestinal crypt injury) and abnormal liver function and bilirubin levels (related to hepatocellular injury). However, the levels of these markers are also elevated after non-aGVHD-related injury, and often, these markers are not changed in the early stage of aGVHD. In contrast, changes in the levels of adhesion molecules, structural proteins, and matrix components, rather than markers associated with organ parenchymal cell damage, may be more accurate indicators of the occurrence of aGVHD.

In line with this notion, we found that aGVHD was associated with significantly decreased levels of soluble CD44, an important structural protein that is widely distributed and can mediate the cell polarity and barrier function[43]. This implies that tissue density was severely damaged in patients with severely aGVHD. Remarkably, we also found that the level of soluble CD44 is negatively correlated with the level of E-selectin in plasma from patients with different grade aGVHD. Moreover, T cells also play an important role in the damage caused to endothelial cells[48]. As described in a previous study, activated T cells upregulate the expression of CD44[49], which is a ligand of E-selectin[50]. The increased level of E-selectin may not only attract monocytes, but also T cells, to accelerate the progression of aGVHD in target organ. Therefore, it is essential to control the secretion of E-selectin which accelerated the process of aGVHD through promoting the infiltration of monocytes and activation of platelet.

Conclusions

Our study revealed that E-selectin and CD44 plays an important role in the occurrence of aGVHD. Based on the findings, monitoring during the early period of post-allo-HSCT for the level of E-selectin and soluble CD44 may contribute to the diagnosis of aGVHD. Further, the findings also showed that endothelial cells can secrete large amounts of E-selectin on TNF-α stimulation and stimulation with soluble CD14 combined with LPS. Therefore, blocking E-selectin and TNF-α may help control infection and have beneficial therapeutic effects in preventing the progression of aGVHD.

Abbreviations

allo-HSCT: allogeneic hematopoietic stem cell transplantation;

aGVHD: acute graft-versus-host disease;

sST2: soluble suppression of tumorigenicity-2

CRP: C-reactive protein;

PLT: platelet;

PBMCs: peripheral blood mononuclear cells;

HUVEC: human umbilical vein endothelial cells;

LPS: lipopolysaccharides;

TNF-α: tumor necrosis factor-α;

ROC curve: receiver Operating Characteristic curve;

AUC: area under the ROC curve;

Declarations

Ethics approval and consent to participate

All patients gave written, informed consent in accordance with the Declaration of Helsinki. No patient compensation was provided. This study was approved by the Ethics Committee of Wuhan Children's Hospital and registered in the Chinese Clinical Trial Registry (**Project Number ChiCTR2100050665**).

Consent for publication

All patients provided written informed consent for their data to be used for clinical research in accordance with the modified Declaration of Helsinki and Good Clinical Practice guidelines.

Availability of data and materials

The detail information of the samples enrolled in this research are available in the main text or in the supplementary materials. All other data that support the findings of this study are available from the corresponding author (XIONG Hao, xionghao @zgwhfe.com) upon reasonable request.

Competing Interests

All authors declare no conflicts of interest.

Funding

This research project has been funded by Construction Project of Research Division of pediatric hematology of Wuhan Children's Hospital (2022FEYJS006).

Authors contributions

YANG Li and WANG Wei designed and executed the experiments. LONG Fei performed the experiments.LU Wenjie, SUN Ming and QI Shanshan collected the clinical samples and provided the detailed information of patients enrolled in this study.

YANG Li, WANG Wei and XIONG Hao analyzed and wrote the manuscript.

Acknowledgments

We are grateful for experimental support from Transfusion Research Department, Wuhan Blood Center, Wuhan, Hubei, China for the Human umbilical vein endothelial cells (HUVEC) in this study.

Author details

[1] Department of Hematology, Wuhan Children's Hospital (Wuhan Medical Care Center for Women and Children), Tongji College, Huazhong University of Science and Technology, Wuhan, Hubei, China

References

1. Zeiser R, Blazar BR. Acute Graft-versus-Host Disease - Biologic Process, Prevention, and Therapy. N Engl J Med. 2017;377(22):2167–79.
2. Jamy O, Zeiser R, Chen YB. Novel developments in the prophylaxis and treatment of acute GVHD. Blood. 2023;142(12):1037–46.
3. Ferrara JLM, Chaudhry MS. GVHD: biology matters. (2473–9537 (Electronic)).
4. Ara T, Hashimoto D. Novel Insights Into the Mechanism of GVHD-Induced Tissue Damage. (1664–3224 (Electronic)).

5. Choe H, Ferrara JLM. New therapeutic targets and biomarkers for acute graft-versus-host disease (GVHD). Expert Opin Ther Targets. 2021;25(9):761–71.
6. Malard F, Holler E, Sandmaier BM, Huang H, Mohty M. Acute graft-versus-host disease. Nat reviews Disease primers. 2023;9(1):27.
7. Harris AC, Young R, Devine S, Hogan WJ, Ayuk F, Bunworasate U, Chanswangphuwana C, Efebera YA, Holler E, Litzow M, et al. International, Multicenter Standardization of Acute Graft-versus-Host Disease Clinical Data Collection: A Report from the Mount Sinai Acute GVHD International Consortium. Biol Blood Marrow Transpl. 2016;22(1):4–10.
8. Przepiorka D, Weisdorf D, Martin P, Klingemann HG, Beatty P, Hows J, Thomas ED. 1994 Consensus Conference on Acute GVHD Grading. *Bone Marrow Transplant* 1995, 15(6):825–828.
9. Jang KT, Song KY, Kim BK. Histological features and immune cell changes in skin lesions of engraftment syndrome of children undergoing hematopoietic stem cell transplantation. Histol Histopathol. 2012;27(2):235–40.
10. Cox GJ, Matsui SM, Lo RS, Hinds M, Bowden RA, Hackman RC, Meyer WG, Mori M, Tarr PI, Oshiro LS, et al. Etiology and outcome of diarrhea after marrow transplantation: a prospective study. Gastroenterology. 1994;107(5):1398–407.
11. Calò Carducci FI, Aufiero LR, Folgori L, Vittucci AC, Amodio D, De Luca M, Li Pira G, Bergamini A, Pontrelli G, Finocchi A. D'Argenio P: Serum soluble ST2 as diagnostic marker of systemic inflammatory reactive syndrome of bacterial etiology in children. Pediatr Infect Dis J. 2014;33(2):199–203.
12. Wendt R, Lingitz MT, Laggner M, Mildner M, Traxler D, Graf A, Krotka P, Moser B, Hoetzenecker K, Kalbitz S et al. Clinical Relevance of Elevated Soluble ST2, HSP27 and 20S Proteasome at Hospital Admission in Patients with COVID-19. Biology 2021, 10(11).
13. Zhu Q, Li H, Zheng S, Wang B, Li M, Zeng W, Zhou L, Guan Z, Wang H, Liu Y, et al. IL-6 and IL-10 Are Associated With Gram-Negative and Gram-Positive Bacteria Infection in Lymphoma. Front Immunol. 2022;13:856039.
14. Ding X, Liu S, Zhu S, Zhou B, Ma H. A Pilot Study on Human Circulating System Indicated That Regenerating Islet-Derived Protein 3 Gamma (REG3A) is a Potential Prognostic Biomarker for Sepsis. Am J Cardiol. 2023;190:90–5.
15. Dudek M, Kałużna-Oleksy M, Migaj J, Sawczak F, Krysztofiak H, Lesiak M, Straburzyńska-Migaj E. sST2 and Heart Failure-Clinical Utility and Prognosis. J Clin Med 2023, 12(9).
16. Yip RHL, Naso JR, Yang HM. Terminal ileum is the most sensitive site for the histologic diagnosis of grade 4 graft-versus-host disease (GvHD) in the lower GI tract and is a harbinger of poor outcome. Virchows Archiv: Int J Pathol. 2021;479(5):919–25.
17. Iqbal N, Salzman D, Lazenby AJ, Wilcox CM. Diagnosis of gastrointestinal graft-versus-host disease. Am J Gastroenterol. 2000;95(11):3034–8.
18. Westin JR, Saliba RM, De Lima M, Alousi A, Hosing C, Qazilbash MH, Khouri IF, Shpall EJ, Anderlini P, Rondon G, et al. Steroid-Refractory Acute GVHD: Predictors and Outcomes. Adv Hematol.

2011;2011:601953.

19. Rashidi A, DeFor TE, Holtan SG, Blazar BR, Weisdorf DJ, MacMillan ML. Outcomes and Predictors of Response in Steroid-Refractory Acute Graft-versus-Host Disease. Biol Blood Marrow Transpl. 2019;25(11):2297–302.
20. Bazzoni G. Pathobiology of junctional adhesion molecules. Antioxid Redox Signal. 2011;15(5):1221–34.
21. Mousa SA. Cell adhesion molecules: potential therapeutic & diagnostic implications. Mol Biotechnol. 2008;38(1):33–40.
22. Buckley CD. Rainger Ge Fau - Bradfield PF, Bradfield Pf Fau - Nash GB, Nash Gb Fau - Simmons DL, Simmons DL: Cell adhesion: more than just glue (review). (0968–7688 (Print)).
23. Pietrocola G, Schubert A, Fau - Visai L, Visai L, Fau - Torti M, Torti M, Fau - Fitzgerald JR, Fitzgerald Jr. Fau - Foster TJ, Foster Tj Fau - Reinscheid DJ, Reinscheid Dj Fau - Speziale P, Speziale P: FbsA, a fibrinogen-binding protein from Streptococcus agalactiae, mediates platelet aggregation. (0006-4971 (Print)).
24. Zhuoya W, Nannan Z, Aiping Z, Guoyan W, Menghua D, Jiashen Z, Yanlian X, Xiying L. Human placenta derived mesenchymal stromal cells alleviate GVHD by promoting the generation of GSH and GST in PD-1(+)T cells. (1090–2163 (Electronic)).
25. Ferroni P, Martini F, Riondino S, La Farina F, Magnapera A, Ciatti F, Guadagni F. Soluble P-selectin as a marker of in vivo platelet activation. Clin Chim Acta. 2009;399(1–2):88–91.
26. Schrijver IT, Kemperman H, Roest M, Kesecioglu J, de Lange DW. Soluble P-selectin as a Biomarker for Infection and Survival in Patients With a Systemic Inflammatory Response Syndrome on the Intensive Care Unit. Biomark insights. 2017;12:1177271916684823.
27. Chagué C, Gautier T, Dal Zuffo L, Pais de Barros JP, Wetzel A, Tarris G, Pallot G, Martin L, Valmary-Degano S, Deckert V, et al. High-density lipoprotein infusion protects from acute graft-versus-host disease in experimental allogeneic hematopoietic cell transplantation. Am J transplantation: official J Am Soc Transplantation Am Soc Transpl Surg. 2022;22(5):1350–61.
28. Hülsdünker J, Thomas OS, Haring E, Unger S, Gonzalo Núñez N, Tugues S, Gao Z, Duquesne S, Cywes-Bentley C, Oyardi O, et al. Immunization against poly-N-acetylglucosamine reduces neutrophil activation and GVHD while sparing microbial diversity. Proc Natl Acad Sci USA. 2019;116(41):20700–6.
29. Yuan ZA-O, Han M, Li D, Hao R, Guo X, Sang S, Zhang H, Ma XA-O, Jin H, Xing Z, Zhao C. A cost-effective smartphone-based device for rapid C-reaction protein (CRP) detection using magnetoelastic immunosensor. (1473 – 0189 (Electronic)).
30. Rahman A, Kefer J, Bando M, Niles WD, Malik AB. E-selectin expression in human endothelial cells by TNF-alpha-induced oxidant generation and NF-kappaB activation. Am J Physiol. 1998;275(3):L533–544.
31. Del Bo C, Marino M, Riso P, Møller P, Porrini M. Anthocyanins and metabolites resolve TNF-α-mediated production of E-selectin and adhesion of monocytes to endothelial cells. Chemico-Biol

Interact. 2019;300:49–55.

32. Aladağ E, Kelkitli E, Göker H. Acute Graft-Versus-Host Disease: A Brief Review. Turkish J haematology: official J Turkish Soc Haematol. 2020;37(1):1–4.
33. Adams JA, Fau - Jiang GA, Jiang Yz Fau YZ, Macdonald D, Macdonald D. Fau - McCarthy DM, McCarthy Dm Fau - Zuiable A, Zuiable A Fau - Treleaven J, Treleaven J Fau - Powles RL, Powles Rl Fau - Barrett AJ, Barrett AJ: Thrombocytopenia after bone marrow transplantation for leukaemia: changes in megakaryocyte growth and growth-promoting activity. (0007-1048 (Print)).
34. Schoettler ML, Carreras E, Cho B, Dandoy CE, Ho VT, Jodele S, Moissev I, Sanchez-Ortega I, Srivastava A, Atsuta Y, et al. Harmonizing Definitions for Diagnostic Criteria and Prognostic Assessment of Transplantation-Associated Thrombotic Microangiopathy: A Report on Behalf of the European Society for Blood and Marrow Transplantation, American Society for Transplantation and Cellular Therapy, Asia-Pacific Blood and Marrow Transplantation Group, and Center for International Blood and Marrow Transplant Research. Transplantation Cell therapy. 2023;29(3):151–63.
35. Rafei H, Jenq RR. Microbiome-intestine cross talk during acute graft-versus-host disease. Blood. 2020;136(4):401–9.
36. Strong Rodrigues K, Oliveira-Ribeiro C, de Abreu Fiuza Gomes S, Knobler R. Cutaneous Graft-Versus-Host Disease: Diagnosis and Treatment. Am J Clin Dermatol. 2018;19(1):33–50.
37. Roldán V, Marín F, Lip GY, Blann AD. Soluble E-selectin in cardiovascular disease and its risk factors. A review of the literature. Thromb Haemost. 2003;90(6):1007–20.
38. Miyata M, Ichikawa K, Matsuki E, Watanabe M, Peltier D, Toubai T. Recent Advances of Acute Kidney Injury in Hematopoietic Cell Transplantation. Front Immunol. 2021;12:779881.
39. Genest DS, Patriquin CJ, Licht C, John R, Reich HN. Renal Thrombotic Microangiopathy: A Review. Am J kidney diseases: official J Natl Kidney Foundation. 2023;81(5):591–605.
40. Epperla N, Li A, Logan B, Fretham C, Chhabra S, Aljurf M, Chee L, Copelan E, Freytes CO, Hematti P et al. Incidence, Risk Factors for and Outcomes of Transplant-Associated Thrombotic Microangiopathy. *Br J Haematol* 2020, 189(6):1171–1181.
41. Dvorak CC. TA-TMA: state of the art for diagnosis and treatment. Blood Adv. 2020;4(1):217.
42. Goodison S, Urquidi V, Tarin D. CD44 cell adhesion molecules. Mol pathology: MP. 1999;52(4):189–96.
43. Kirschner N, Haftek M, Niessen CM, Behne MJ, Furuse M, Moll I, Brandner JM. CD44 regulates tight-junction assembly and barrier function. J Invest Dermatol. 2011;131(4):932–43.
44. Dayang EZ, Plantinga J, Ter Ellen B, van Meurs M, Molema G, Moser J. Identification of LPS-Activated Endothelial Subpopulations With Distinct Inflammatory Phenotypes and Regulatory Signaling Mechanisms. (1664–3224 (Electronic)).
45. Van Kampen C, Mallard BA. Regulation of bovine E-selectin expression by recombinant tumor necrosis factor alpha and lipopolysaccharide. (0165–2427 (Print)).

46. Saliba RM, de Lima M, Giralt S, Andersson B, Khouri IF, Hosing C, Ghosh S, Neumann J, Hsu Y, De Jesus J, et al. Hyperacute GVHD: risk factors, outcomes, and clinical implications. Blood. 2007;109(7):2751–8.
47. Mahabal GD, George L, Peter D, Thomas M, George B, Mathews V, Abraham A, Srivastava A, Pulimood S. Hyperacute Graft-Versus-Host Disease of the Skin following Allogeneic Stem Cell Transplantation: A Paediatric Case Series. Indian J dermatology. 2023;68(1):73–7.
48. Vythoulkas D, Tsirigotis P, Griniezaki M, Konstantellos I, Lazana I. Endothelial Dysfunction Syndromes after Allogeneic Stem Cell Transplantation. Cancers (Basel) 2023, 15(3).
49. DeGrendele HC, Kosfiszer M, Estess P, Siegelman MH. CD44 activation and associated primary adhesion is inducible via T cell receptor stimulation. *Journal of immunology (Baltimore, Md: 1950)* 1997, 159(6):2549–2553.
50. Li L, Ding Q, Zhou J, Wu Y, Zhang M, Guo X, Long M, Lü S. Distinct binding kinetics of E-, P- and L-selectins to CD44. FEBS J. 2022;289(10):2877–94.

Figures

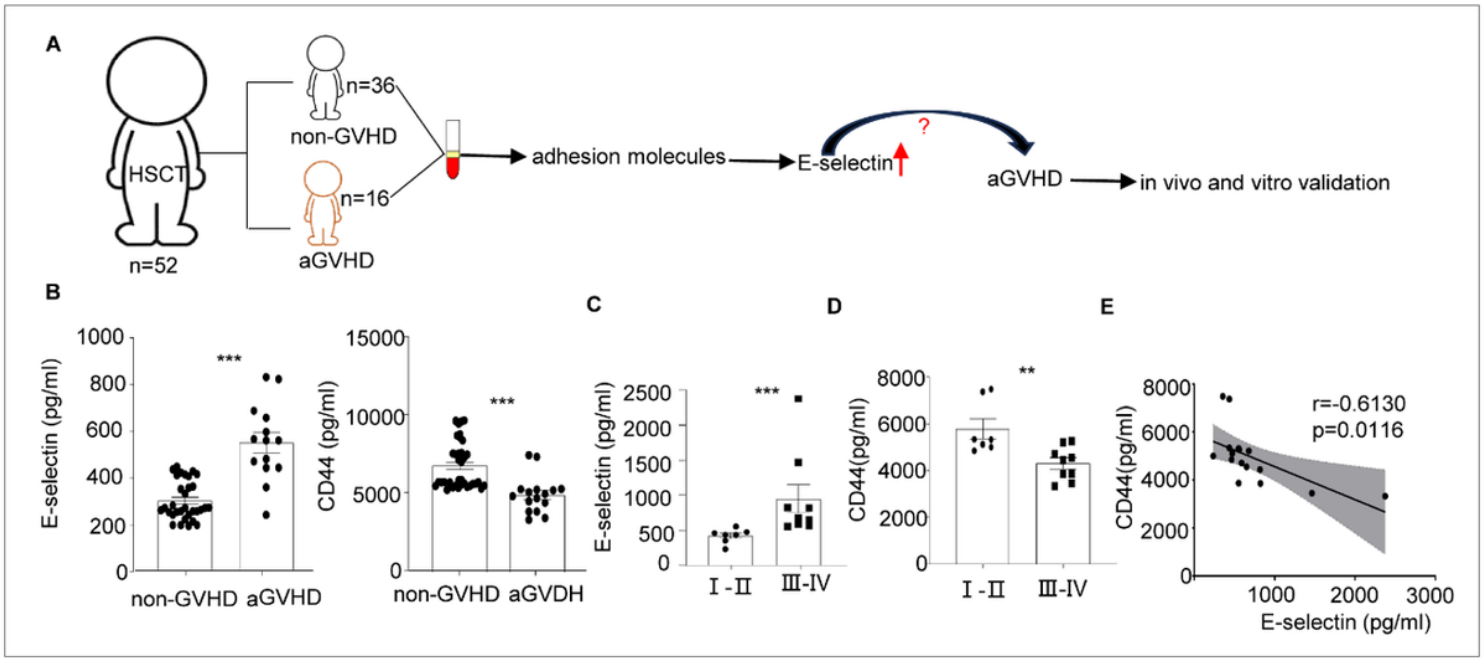

Figure 1

The serum E-selectin are increased in aGVHD and positively correlated with the serious of aGVHD.

(A) Overview of the study design. (B-C) The concentrate of the soluble CD44 and E-selectin in the serum from the allo-HSCT recipients with (n=16)/without (n=36) aGVHD. (C-D) The concentrate of the soluble CD44 and E-selectin in the serum from the mild GVHD (I-II, n=7) and severe (III-IV, n=9) aGVHD recipients. (E) The Spearman correlation between the concentrate of soluble CD44 and E-selectin in the recipients with aGVHD (n=16).

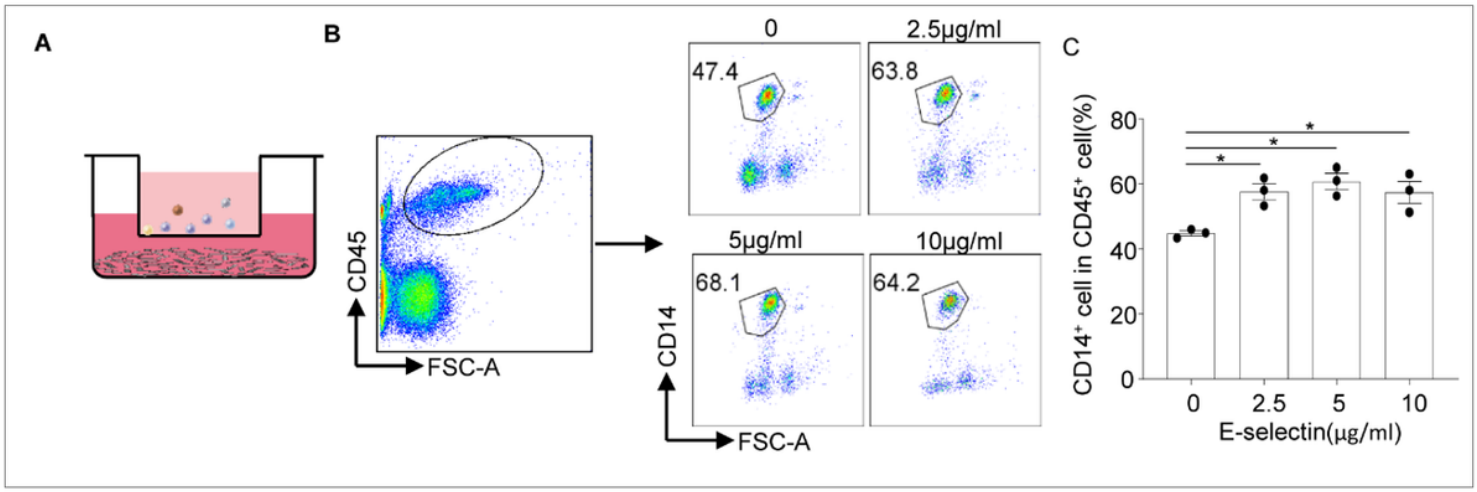

Figure 2

E-selectin can effectively attract monocytes to the injury sites.

(A) The overview of the transwell experiments in this study. (B-C) The representative dot plots and summarized frequencies of monocytes (depicted by $CD14^+$ cells).

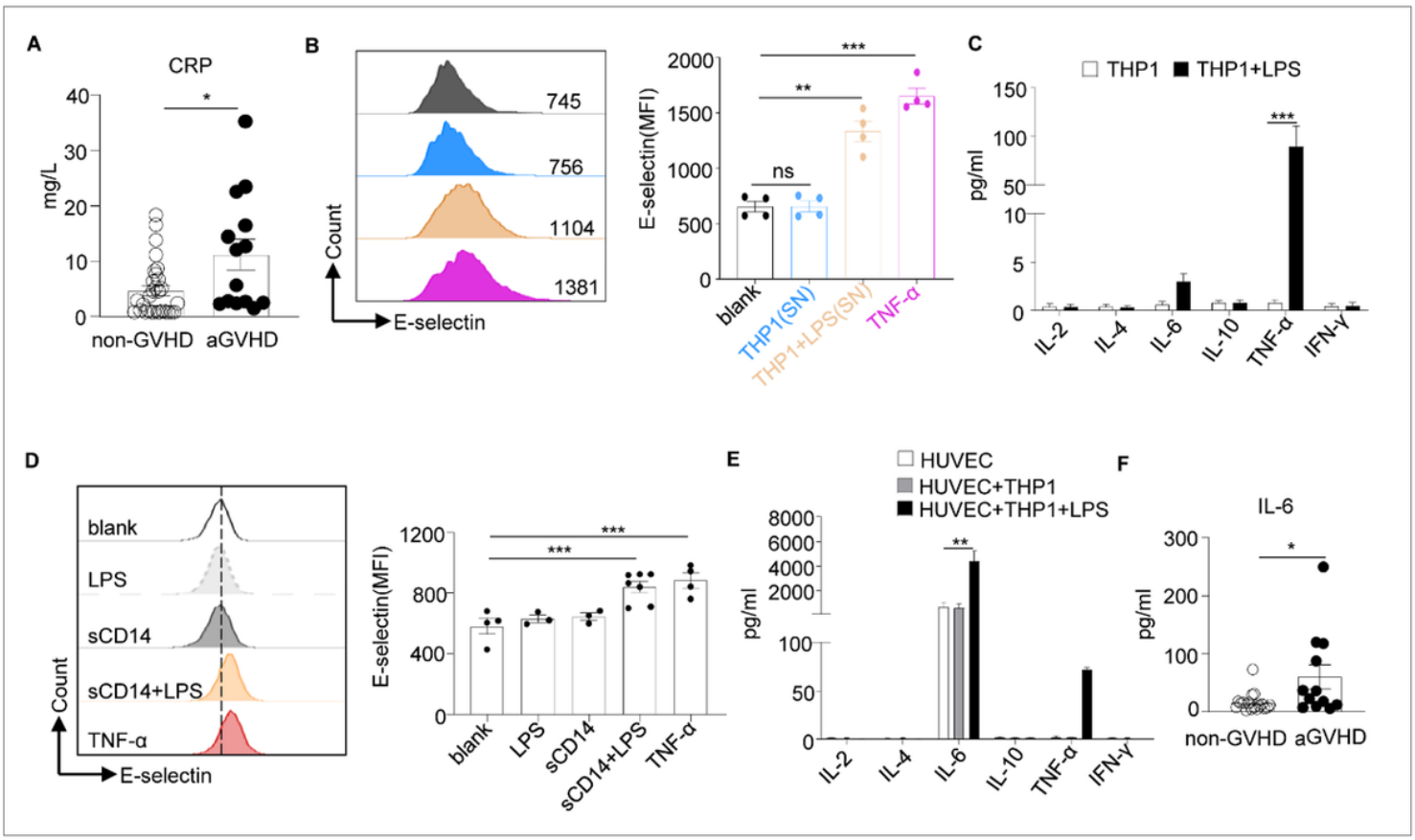

Figure 3

Activated monocytes significantly promote endothelial cells producing more E-selectin and IL-6.

(A) The level of C response protein in the recipients with/without aGVHD. (B) Representative and summarized levels of E-selectin on HUVEC in indicated groups (n=4/group). (C) The concentrate of indicated cytokines from THP1 cells with/without the stimulation of LPS (n=4). (D) Representative and summarized E-selectin levels on HUVEC in indicated groups (n ≥3/group). (E) The concentrate of indicated cytokines from the indicated groups (n=4/group). (F) The level of IL-6 in the serum from the recipients with/without aGVHD.

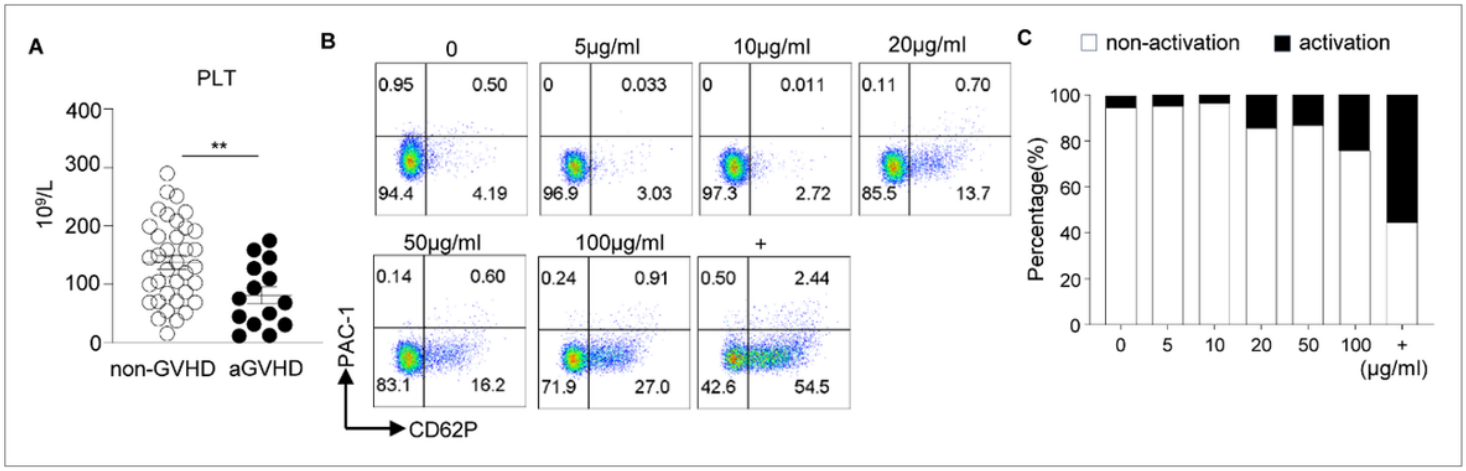

Figure 4

E-selectin could promote the activation of platelets.

(A) The number of platelets in the recipients with/without aGVHD. (B-C) The representative and summarized levels of activation of platelets (n=4/group).

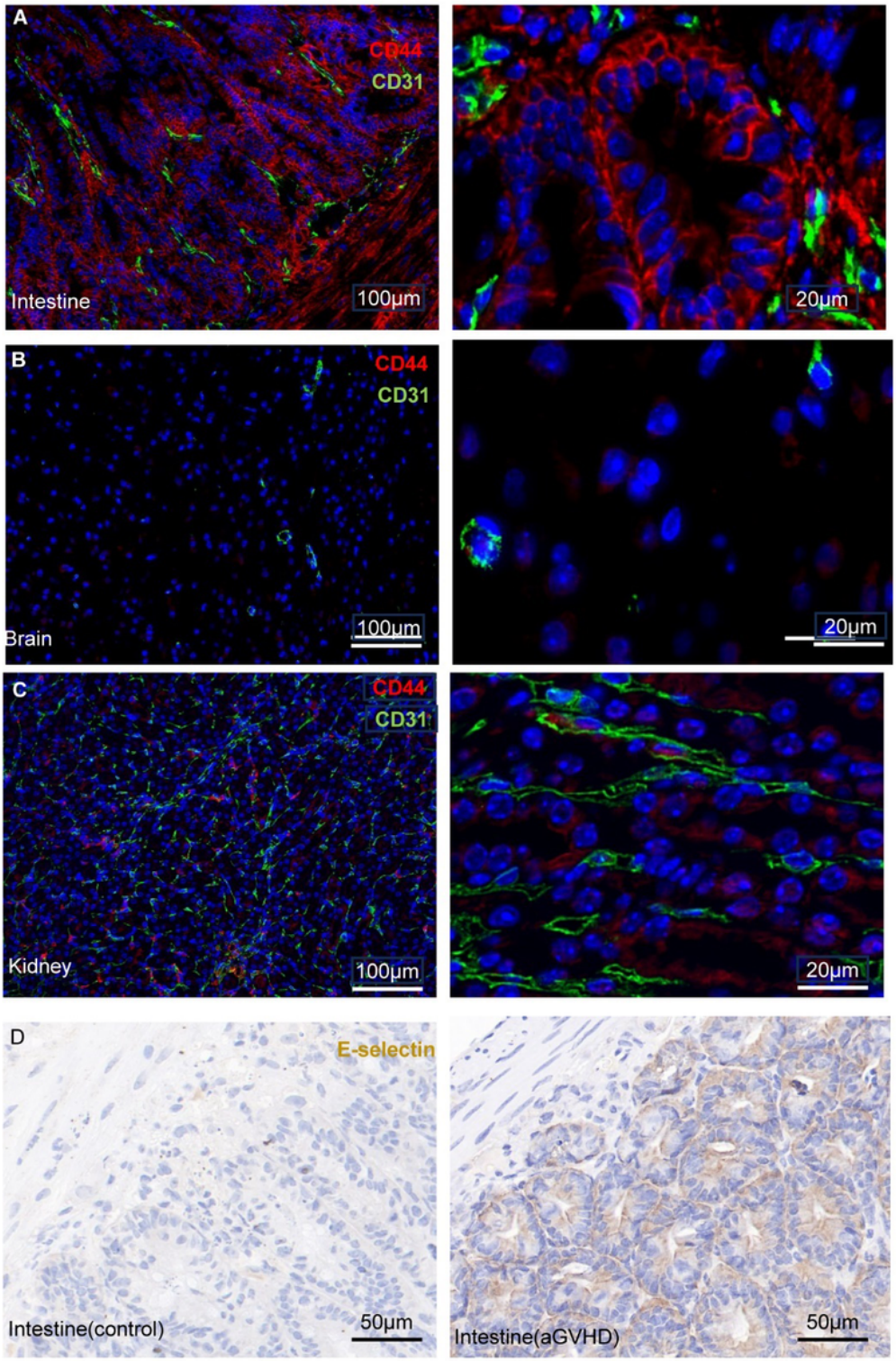

Figure 5

The organ enriched CD44 are more likely occurring aGVHD with elevated E-selectin.

(A-C) The immunofluorescence staining of the CD31(green) and CD44(red) in the indicated organs from mouse. (D) The immunohistochemical staining of E-selectin in the intestine from the mouse with/without aGVHD.

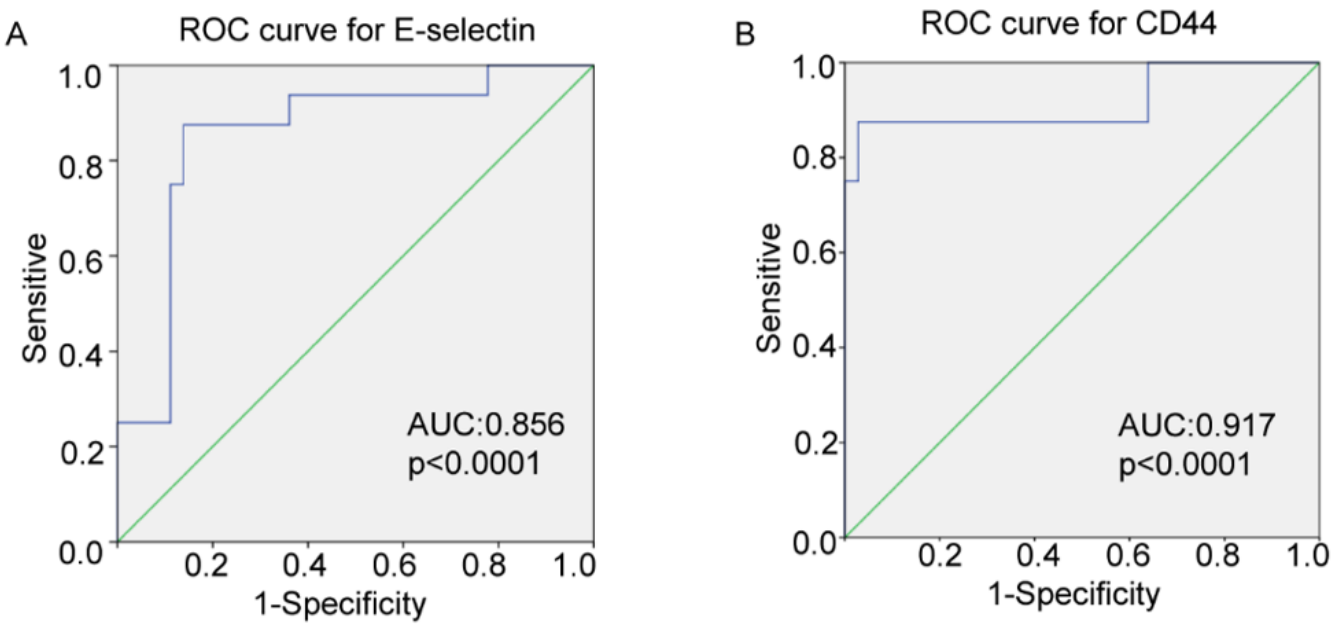

Figure 6

The level of E-selectin and CD44 were analyzed by ROC curve.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- supplementalfigure1.pdf
- supplementaltable11.pdf