Proinflammatory human 6-sulfo LacNAc-positive dendritic cells accumulate in intestinal acute graft-*versus*-host disease

Allogeneic hematopoietic stem cell transplantation (HSCT) represents a potentially curative therapy for various malignant and non-malignant hematologic diseases. However, a frequent and severe complication of this therapeutic approach is acute graft-versus-host disease (aGVHD) characterized by immune-mediated inflammation and tissue destruction.[1] In this context, donor-derived CD4$^+$ T-helper (Th) cells and CD8$^+$ cytotoxic T lymphocytes (CTLs) play an essential role in the pathogenesis of aGVHD, which is based on their profound capability to produce various proinflammatory cytokines and to lyse target cells of the recipient.[1]

Dendritic cells (DCs) are professional antigen-presenting cells (APCs), which display a unique capacity to induce and expand proinflammatory CD8$^+$ CTLs and CD4$^+$ T cells.[2] Recent murine models revealed that DCs essentially contribute to T-cell-mediated inflammation and tissue destruction in aGVHD.[3] However, in contrast to mouse DCs, little is known about the potential role of native human DCs in the pathogenesis of aGVHD. In this context, it has been demonstrated that patients who develop aGVHD after HSCT show lower numbers of blood circulating DCs.[4] Furthermore, it has been reported that human DCs are able to induce GVHD in a SCID mouse model and that the administration of anti-CD83 antibodies can prevent the development of GVHD.[5] More recently, Bossard *et al.* demonstrated that native human plasmacytoid DCs, which may play an essential role in the pathogenesis of various autoimmune diseases, accumulate in intestinal tissues of aGVHD patients.[6] The same group also found a significant increase of plasmacytoid DCs in affected skin of aGVHD patients, further substantiating their previous results.[7]

To gain novel insights into a potential participation of human myeloid DCs in the inflammatory processes underlying aGVHD, we explored the presence and cytokine expression of 6-sulfo LacNAc$^+$ (slan) DCs (formerly termed M-DC8$^+$ DCs) in affected tissues. SlanDCs represent a particular proinflammatory subset of human myeloid blood DCs. In previous studies, we demonstrated that activated slanDCs produce large amounts of tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-6, IL-12 and IL-23, efficiently stimulate CD4$^+$ and CD8$^+$ T cells, and promote the polarization of naïve CD4$^+$ T lymphocytes into Th1 or Th17/Th1 cells.[8-11] In addition, we found that the high proinflammatory capacity of slanDCs is retained after granulocyte-colony stimulating factor (G-CSF) treatment of peripheral blood stem cell donors.[12] Thus, G-CSF-mobilized slanDCs were able to secrete large amounts of proinflammatory cytokines and to promote the polarization of naïve CD4$^+$ T lymphocytes into Th1 cells. We also observed a significantly reduced frequency of slanDCs in the blood of patients with severe aGVHD[13] that could be explained by an increased DC migration into affected tissues.

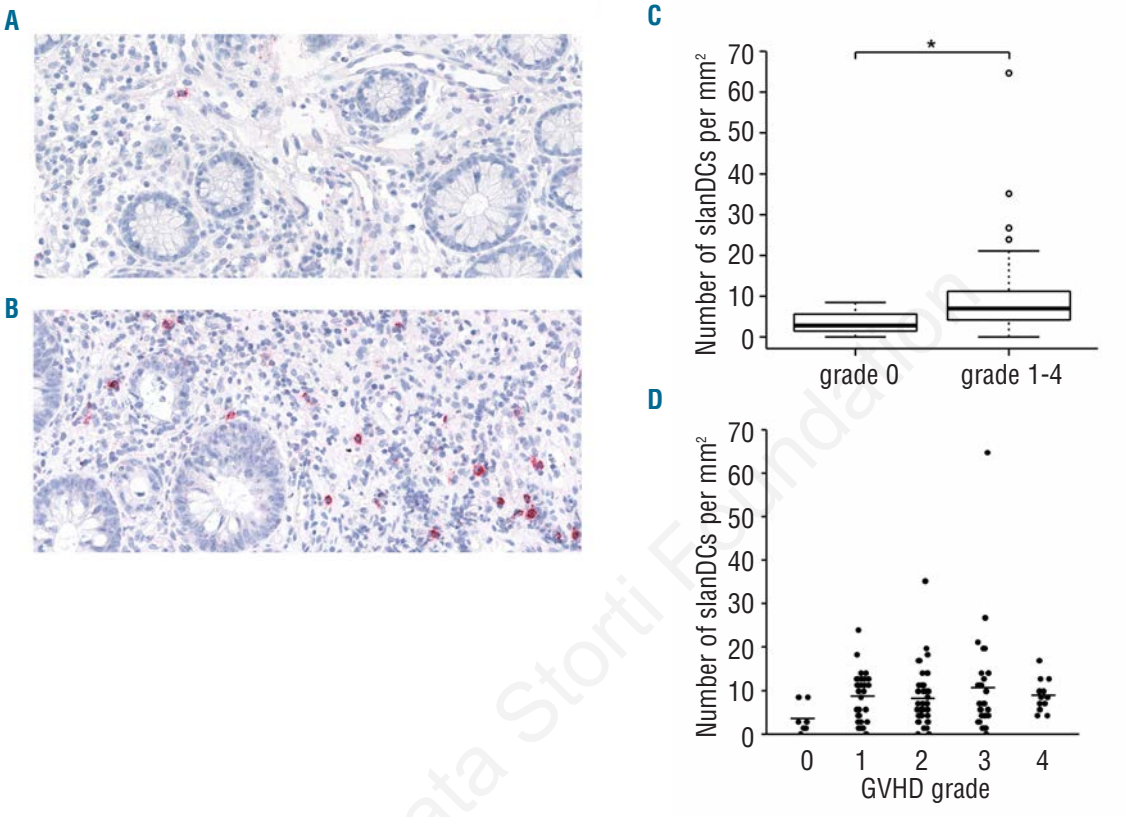
Following these findings, we evaluated the presence of slanDCs in 124 tissue samples derived from 65 patients with aGVHD who underwent HSCT at the University Hospital of Dresden, Germany. Patients, donors and HSCT characteristics are summarized in Table 1. This study was approved by the institutional review board of the University Hospital of Dresden and patients gave their written informed consent. Patients underwent diagnostic

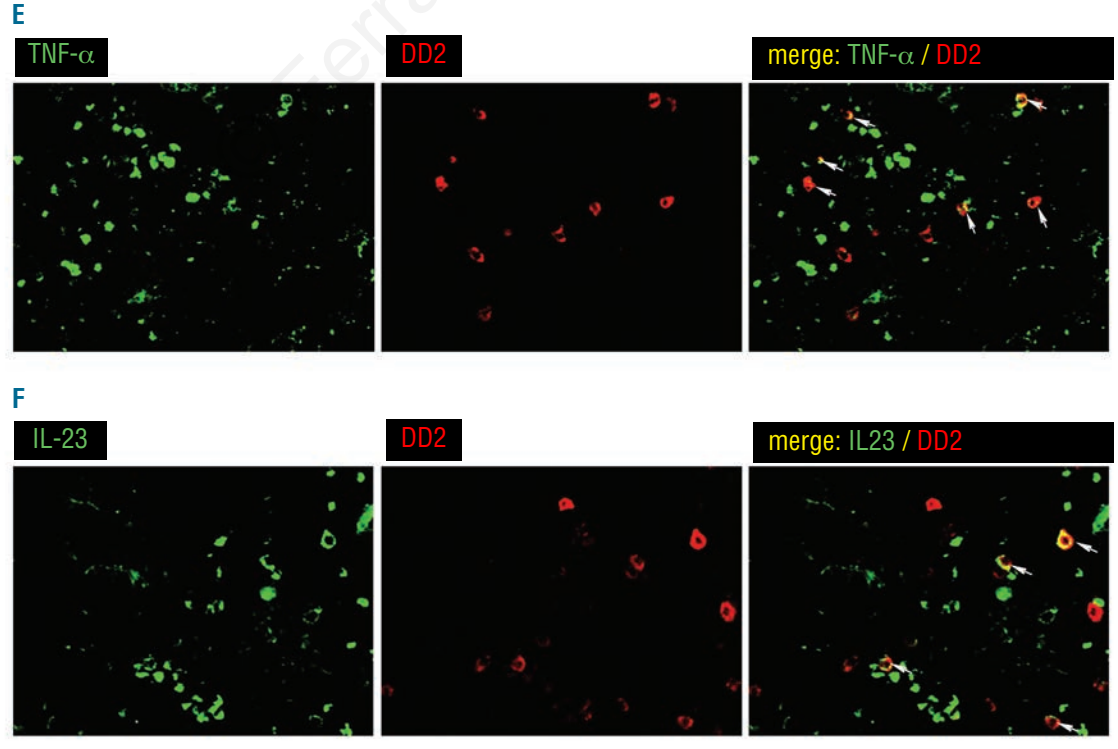
Table 1. Patients, disease and transplant characteristics.

Characteristics	Study population (n=65)
Median recipient age, years (range)	57 (21-71)
Patient sex, male/female, n. (%)	40/25 (62/38)
Donor sex, male/female, n. (%)	45/20 (69/31)
Median post-transplant time of biopsy, days (range)	32 (10-132)
Diagnosis, n. (%)	
Myeloid malignancies	44 (67.7)
Acute myeloblastic leukemia	29 (44.6)
Myelodysplastic syndrome	10 (15.4)
Osteomyelofibrosis	4 (6.2)
Chronic myeloid leukemia	1 (1.5)
Lymphoid malignancies	20 (30.8)
Chronic lymphocytic leukemia	8 (12.3)
Non-Hodgkin lymphoma	6 (9.2)
Acute lymphoblastic leukemia	2 (3.1)
Multiple myeloma	2 (3.1)
Aplastic anemia	2 (3.1)
Biphenotypic acute leukemia	1 (1.5)
Conditioning regimen, n. (%)	
Myeloablative	14 (21.5)
Bu16/Flu120	9 (13.8)
Bu16/Cy120	4 (6.2)
12 Gy TBI/Cy120	1 (1.5)
Reduced intensity	51 (78.2)
Flu150/Bu8	14 (21.5)
Flu150/Mel140	11 (16.9)
Flu120/2-8 Gy TBI	9 (13.8)
Clo90/Mel140	6 (9.2)
Flu180/Bu10	4 (6.2)
Flu120/Cy	2 (3.1)
Flu120/Cy/2-4 Gy TBI	2 (3.1)
Flu150/Mel140/Thio	1 (1.5)
Flu90/2 Gy TBI/Thio	1 (1.5)
Flu120/Treo	1 (1.5)
GVHD prophylaxis, n. (%)	
CsA alone	11 (16.9)
CsA + mycophenolate mofetil	12 (18.5)
CsA + methotrexate	23 (35.4)
Tacro alone	1 (1.5)
Tacro + mycophenolate mofetil	6 (9.2)
Tacro + methotrexate	3 (4.6)
Tacro + everolimus	3 (4.6)
mycophenolate mofetil alone	3 (4.6)
no prophylaxis	3 (4.6)
Graft source, n. (%)	
Peripheral blood stem cells	64 (98.5)
Bone marrow	1 (1.5)
Donor type, n. (%)	
Matched-related donor	11 (16.9)
Matched-unrelated donor	34 (52.3)
Mismatched-unrelated donor*	19 (29.2)
Haploidentical-related donor	1 (1.5)

*Bu: busulfan, Bu8 (4 x 1 mg/kg p.o., Day -3 and -2, cumulative dose 8 mg/kg), Bu10 (4 x 1 mg/kg p.o., Day -4, -3 and 2 x 1 mg/kg p.o., Day -2, cumulative dose 10 mg/kg), Bu16 (4 x 1 mg/kg p.o., Day -7 to -4, cumulative dose 16 mg/kg p.o. or 4 x 3,2 mg/kg i.v., Day -7 to -4 i.v., cumulative dose 12.8 mg/kg i.v.); Clo 90: Clofarabine (22,5 mg/m^2 on Days -6 to Day -3, 90 mg/m^2 total); CsA: cyclosporine A; Cy: cyclophophamide (500 mg/m^2 on Days -7 to -3); Cy 120: Cyclophophamide (120 mg/kg total); Flu: fludarabine (30 mg/m^2/day for 3-6 days, cumulative dose 90-180 mg/m^2); Mel: melphalan (140 mg/m^2); Tacro: tacrolimus; TBI: hyperfractionated total body irradiation (2 Gy, 4 Gy, 8 Gy or 12 Gy); Thio: thiotepa 10 (10 mg/kg); Treo: treosulfan (12-14 g/m^2/d for 3 Days); *9/10 HLA alleles matched.*

colonoscopy, when symptoms of gastrointestinal aGVHD occurred, at a median time of 32 days (range 10-132 days) after HSCT. Histological grading was performed as described by Lerner *et al.*[14] None had evidence of viral or bacterial infection. Most patients (n=58) received steroid treatment at the time of colonoscopy. The median time of steroid therapy until biopsies was four days (range 1-13 days). The density of slanDCs in formalin-fixed and paraffin-embedded colorectal biopsies from aGVHD patients was determined by using immunohistochemical analysis as described previously.[11] For quantification of slanDCs in tissues, positively stained cells were counted in three different





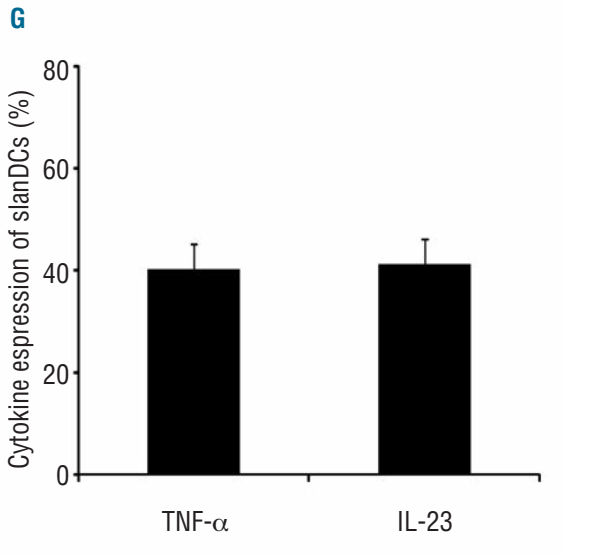


Figure 1. Proinflammatory slanDCs accumulate in colorectal tissues of aGVHD patients. (A-D) Immunohistochemical stainings were performed to detect slanDCs in 124 colorectal tissues from aGVHD patients. As representative examples, the presence of slanDCs in a histologically confirmed aGVHD grade 0 (A) and grade 2 (B) tissue is shown. Original magnification was x200. Positively stained slanDCs were counted and their number per square millimeter was determined. (C) Box plots show the number of slanDCs in histologically confirmed aGVHD grade 1-4 tissues (n=117) in comparison to grade 0 tissues (n=7). Boxes within the plots represent the 25-75th percentiles. Median values are depicted as solid bold lines. The whiskers represent 1.5 times the interquartile range (IQR) between quartile 25 and 75. Circles indicate values more extreme than 1.5 times the IQR. Asterisks indicate a statistically significant difference (*P<0.05). (D) Dot plots demonstrate the number of slanDCs in histologically confirmed aGVHD grade 0 (n=7), grade 1 (n=30), grade 2 (n=44), grade 3 (n=30) or grade 4 (n=13) tissues. The horizontal line shows the mean value. (E, F) Immunofluorescence staining was performed to detect (E) TNF-α- or (F) IL-23-expressing slanDCs (arrows) in aGVHD tissues of 9 patients. As representative examples, images of single TNF-α, IL-23 or slanDC stainings as well as merged images are depicted. Original magnification was x400. (G) Percentage of TNF-α- or IL-23-expressing slanDCs in aGVHD tissues. The results are presented as mean value ± s.e. of slanDCs in nine different aGVHD tissues. Between 17 and 50 slanDCs per tissue were evaluated for cytokine expression dependent on DC frequency. (H) Immunofluorescence staining was performed to detect T cells (anti-CD3 antibody; green) and slanDCs (anti-slan antibody DD2; red) in aGVHD tissues of 5 patients. As representative examples, images of single CD3$^+$ T cell or slanDC stainings as well as merged images are shown. Original magnification was x400.

highpower fields (HPF) of a section with an Olympus BH-2 microscope and the mean value was determined. The mean number of slanDCs per HPF (area: 0.237 mm^2) was converted into square millimeters. Immunohistochemical staining revealed that slanDCs are present in 119 tissues at varying frequencies and were preferentially located in the stroma (Figure 1A and B). Previously, we had described that most patients displayed an almost complete donor chimerism of blood circulating CD11c$^+$ myeloid DCs comprising slanDCs in blood on Day +28 after HSCT.[15] Furthermore, Langerhans cells and dermal DCs also showed complete donor chimerism in most skin biopsies of patients at early time points after HSCT.[16] These findings suggest that the detected slanDCs in GVHD tissues are most likely of donor origin. Furthermore, we determined the frequency of slanDCs in histologically confirmed aGVHD grade 0-4 tissues. For statistical analysis, a linear mixed model incorporating random effects to take into account dependent observations per patient was applied. The significance of the results was determined by using the statistical software R v.2.15.1 and the 'nlme' package v.3.1-108. P<0.05 was considered as significant. As shown in Figure 1C, higher numbers of slanDCs were detected in aGVHD grade 1-4 tissues compared to grade 0 tissues. However, the relatively low number of analyzed aGVHD grade 0 tissues (n=7) limits the interpretation of this finding. In addition, we found that the density of slanDCs was comparable between aGVHD grade 1-4 (Figure 1D). These results further substantiate our recent findings demonstrating the presence of slanDCs in tissues of patients with inflammatory disorders such as rheumatoid arthritis, psoriasis and lupus erythematosus.[9,11,17] Following our previous results indicating that activated slanDCs secrete large amounts of TNF-α and IL-23 and efficiently stimulate T cells,[8,11] we investigated whether slanDCs locally express these proinflammatory cytokines and co-localize with CD3$^+$ T lymphocytes in affected aGVHD tissues. For immunofluorescence analysis, we applied antibodies and reagents as described previously.[11] Slides were analyzed with a Zeiss Axioimager A1 microscope or a Keyence fluorescence microscope BZ-9000. As depicted in Figure 1E-G, approximately 40% of slanDCs locally express the proinflammatory cytokines TNF-α or IL-23, which are implicated in aGVHD pathogenesis.[1,18] Thus, it has been document-

ed that TNF-α is a key soluble factor of aGVHD by enhancing the ability of APCs to activate T cells and directly destroying target tissues.[1] In addition, it has been shown in mice that IL-23 secreted by donor APCs plays an important role in the induction of proinflammatory cytokine production and pathological damage in the colon during aGVHD.[18] In further experiments, we found that slanDCs co-localize with CD3+ T cells, representing crucial proinflammatory and cytotoxic effector cells, in affected aGVHD tissues (Figure 1H).

In summary, we demonstrated that slanDCs accumulate in colorectal tissues from aGVHD patients, locally express essential proinflammatory cytokines and co-localize with T cells. Together with our previous observation that G-CSF-mobilized, donor-derived slanDCs retain various immunostimulatory properties,[12] these novel findings indicate that slanDCs may contribute to the immunopathogenesis of aGVHD and may represent attractive targets for the prevention and treatment of this important HSCT-associated complication.

Ulrich Sommer,[1] Brit Larsson,[2]* Sebastian Tuve[2]* Rebekka Wehner,[3] Nick Zimmermann,[4] Michael Kramer,[2] Anja Kloß,[3] Claudia Günther,[4] Jana Babatz,[3] Renate Schmelz,[3] Stefan Brückner,[3] Johannes Schetelig,[2] Martin Bornhäuser,[2,5] Knut Schäkel,[6] Michael Philipp Bachmann,[3,5,7] Daniela Aust,[1] Gustavo Baretton,[1] and Marc Schmitz[3,5]*

[1]Institute of Pathology, University Hospital of Dresden; [2]Department of Medicine I, University Hospital of Dresden; [3]Institute of Immunology, Medical Faculty, Dresden University of Technology; [4]Department of Dermatology, University Hospital of Dresden; [5]Center for Regenerative Therapies Dresden; [6]Department of Dermatology, University Hospital of Heidelberg; and [7]Helmholtz-Zentrum Dresden-Rossendorf, Institute of Radiopharmaceutical Cancer Research, Deparment of Radioimmunology, Dresden, Germany

Acknowledgments: the authors thank Bärbel Löbel and Karin Günther from the Institute of Immunology, Medical Faculty, Dresden University of Technology, for technical support.

Funding: this work was supported by grants from the Deutsche Knochenmarkspenderdatei to MB, ST, and MS, the Deutsche Forschungsgemeinschaft to CG and the Medical Faculty, Dresden University of Technology to ST and GC.

Correspondence: marc.schmitz@tu-dresden.de

doi:10.3324/haematol.2013.101071

Key words: dendritic cells, acute graft versus host disease, b-sulfo LacNAc positive, proinflammatory.

Information on authorship, contributions, and financial & other disclosures was provided by the authors and is available with the online version of this article at www.haematologica.org.

References

1. Ferrara JL, Levine JE, Reddy P, Holler E. Graft-versus-host disease. Lancet. 2009;373(9674):1550-61.
2. Steinman RM, Banchereau J. Taking dendritic cells into medicine. Nature. 2007;449:419-26.
3. Stenger EO, Turnquist HR, Mapara MY, Thompson AW. Dendritic cells and regulation of graft-versus-host disease and graft-versus-leukemia activity. Blood. 2012;119(22):5088-103.
4. Mohty M, Blaise D, Faucher C, Bardou VJ, Gastaut JA, Viens P, et al. Impact of plasmacytoid dendritic cells on outcome after reduced-intensity conditioning allogeneic stem cell transplantation. Leukemia. 2005;19(1):1-6.
5. Wilson J, Cullup H, Lourie R, Sheng Y, Palkova A, Radford KJ, et al. Antibody to the dendritic cell surface activation antigen CD83 prevents acute graft-versus-host disease. J Exp Med. 2009;206(2):387-98.
6. Bossard C, Malard F, Arbez J, Chevallier P, Guillaume T, Delaunay J, et al. Plasmacytoid dendritic cells and TH17 immune response contribution in gastrointestinal acute graft-versus-host disease. Leukemia. 2012;26(7):1471-4.
7. Malard F, Bossard C, Brissot E, Chevallier P, Guillaume T, Delaunay J, et al. Increased plasmacytoid dendritic cells and RORγt-expressing immune effectors in cutaneous acute graft-versus-host disease. J Leukoc Biol. 2013;94(6):1337-43.
8. Schäkel K, Kannagi R, Kniep B, Goto Y, Mitsuoka C, Zwirner J, et al. 6-Sulfo LacNAc, a novel carbohydrate modification of PSGL-1, defines an inflammatory type of human dendritic cells. Immunity. 2002;17(3):289-301.
9. Schäkel K, Von Kietzell M, Hänsel A, Ebling A, Schulze L, Haase M, et al. Human 6-sulfo LacNAc-expressing dendritic cells are principal producers of early interleukin-12 and are controlled by erythrocytes. Immunity. 2006;24(6):767-77.
10. Straube C, Wehner R, Wendisch M, Bornhäuser M, Bachmann M, Rieber EP, et al. Bortezumib significantly impairs the immunostimulatory capacity of human myeloid dendritic cells. Leukemia. 2007;21(7):1464-71.
11. Hänsel A, Günther C, Ingwersen J, Starke J, Schmitz M, Bachmann M, et al. Human slan (6-sulfo LacNAc) dendritic cells are inflammatory dermal dendritic cells in psoriasis and drive strong Th17/Th1 T-cell responses. J Allergy Clin Immunol. 2011;127(3):787-94.
12. Baumeister SH, Hölig K, Bornhäuser M, Meurer M, Rieber EP, Schäkel K. G-CSF mobilizes slanDCs (6-sulfo LacNAc+ dendritic cells) with a high proinflammatory capacity. Blood. 2007;110(8):3078-81.
13. Mager K, Wehner R, Bahr F, Oelschlägel U, Platzbecker U, Wermke M, et al. Reconstitution of 6-sulfo LacNAc dendritic cells following allogeneic stem cell transplantation. Transplantation. 2012; 93(12):1270-5.
14. Lerner KG, Kao GF, Storb R, Buckner CD, Clift RA, Thomas ED. Histopathology of graft-vs.-host reaction (GvHR) in human recipients of marrow from HLA-matched sibling donors. Transplant Proc. 1974;6(4):367-71.
15. Bornhäuser M, Eger L, Oelschlaegel U, Auffermann-Gretzinger S, Kiani A, Schetelig J, et al. Rapid reconstitution of dendritic cells after allogeneic transplantation of CD133+ selected hematopoietic stem cells. Leukemia. 2005;19(1):161-5.
16. Auffermann-Gretzinger S, Eger L, Bornhäuser M, Schäkel K, Oelschlaegel U, Schaich M, et al. Fast appearance of donor dendritic cells in human skin: dynamics of skin and blood dendritic cells after alloegeneic hematopoietic cell transplantation. Transplantation. 2006;81(6):866-73.
17. Hänsel A, Günther C, Baran W, Bidier M, Lorenz HM, Schmitz M, et al. Human 6-sulfo LacNAc (slan) dendritic cells have molecular and functional features of an important pro-inflammatory cell type in lupus erythematosus. J Autoimmun. 2013;40:1-8.
18. Das R, Chen X, Komorowski R, Hessner MJ, Drobyski WR. Interleukin-23 secretion by donor antigen-presenting cells is critical for organ-specific pathology in graft-versus-host disease. Blood. 2009;113(10):2352-62.