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Case Report

Concurrent Epstein-Barr virus associated NK/T cell lymphoma after immunosuppressive therapy for aplastic anemia: report of a case and review of literature

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Abstract: Aplastic anemia (AA) patients with prolonged immunosuppression have a risk of development of lymphoproliferative disorders (LPDs), especially combined with Epstein-Barr virus (EBV) infection. However, development of nature killer/T (NK/T) cell lymphoma, in a nontransplantation setting, has not been documented for AA patients with immunosuppressive therapy (IST). Herein, we described a middle-aged man, Han ethnic, who presented with swelled parotid gland after a long history of IST for AA. Fever, night sweating, weight loss had not been found. Increased heterotypic lymphocytes had been detected in the left side of parotid gland demonstrated as $cCD3^+$, $CD56^+$, $GranB^+$, $TIA\text{-}1^+$, $MUM\text{-}1^+$, KI-67 (50%-75%)$^{++}$, $Bcl\text{-}6^-$, MPO^- by immunohistochemistry, and in-situ hybridization (ISH) indicated EBER positive. Chromosome analysis by R banding method revealed 46, XY [20]. NK/T cell lymphoma concurrent with aplastic anemia was diagnosed and a mild chemotherapy regimen including vincristine, prednisone, L-asparaginase was administered. The parotid mass was gradually regressed after the first cycle of chemotherapy. The patient discharged from the hospital voluntarily and lost the follow-up.

Keywords: Aplastic anemia, lymphoma, immunosuppressive therapy

Introduction

Aplastic anemia (AA) is a hematological disorder presenting with pancytopenia in peripheral blood and hypocellularity in bone marrow [1], which is deemed as the disease of T-cell mediated autoimmune disorder targeted against hematopoietic stem cell descendants. HLA matched sibling donor transplantation or immunosuppressive therapy (IST), including antithymocyte globulin (ATG) and cyclosporine A (CsA), is considered to be effective treatment for severe aplastic anemia (SAA). Currently, IST is widely used to treat AA owing to its underlying immune pathophysiology. It's well known that patients with IST have a higher risk of infecting EBV, which is associated with the development of lymphoproliferative disorders (LPDs). Extranodal natural killer/T (NK/T) cell lymphoma, a rare type of non-Hodgkin's lymphoma, is an aggressive lymphoma originated from NK or T cells [2]. Relatively speaking, it is more common in American and Asian population and closely associated with EBV infection, irrespective of the ethnic origin [3]. A few of B cell neoplasms have been reported among immunodeficiency associated LPDs. However, as far as we know, NK/T cell lymphoma was rarely reported in immunodeficiency patients, especially in AA patients with a history of IST.

We reported a patient suffering from NK/T cell lymphoma concurrent with AA after a long-term treatment of IST, as well as the clinicopathologic characteristics. It may contribute to better understanding the potential correlations between AA and lymphoma for both clinicians and research fellows.

Case report

A 34-year-old male, Han ethnic, complained of epistaxis and scattered petechiae in his extremities in local hospital in 1995. He had no other

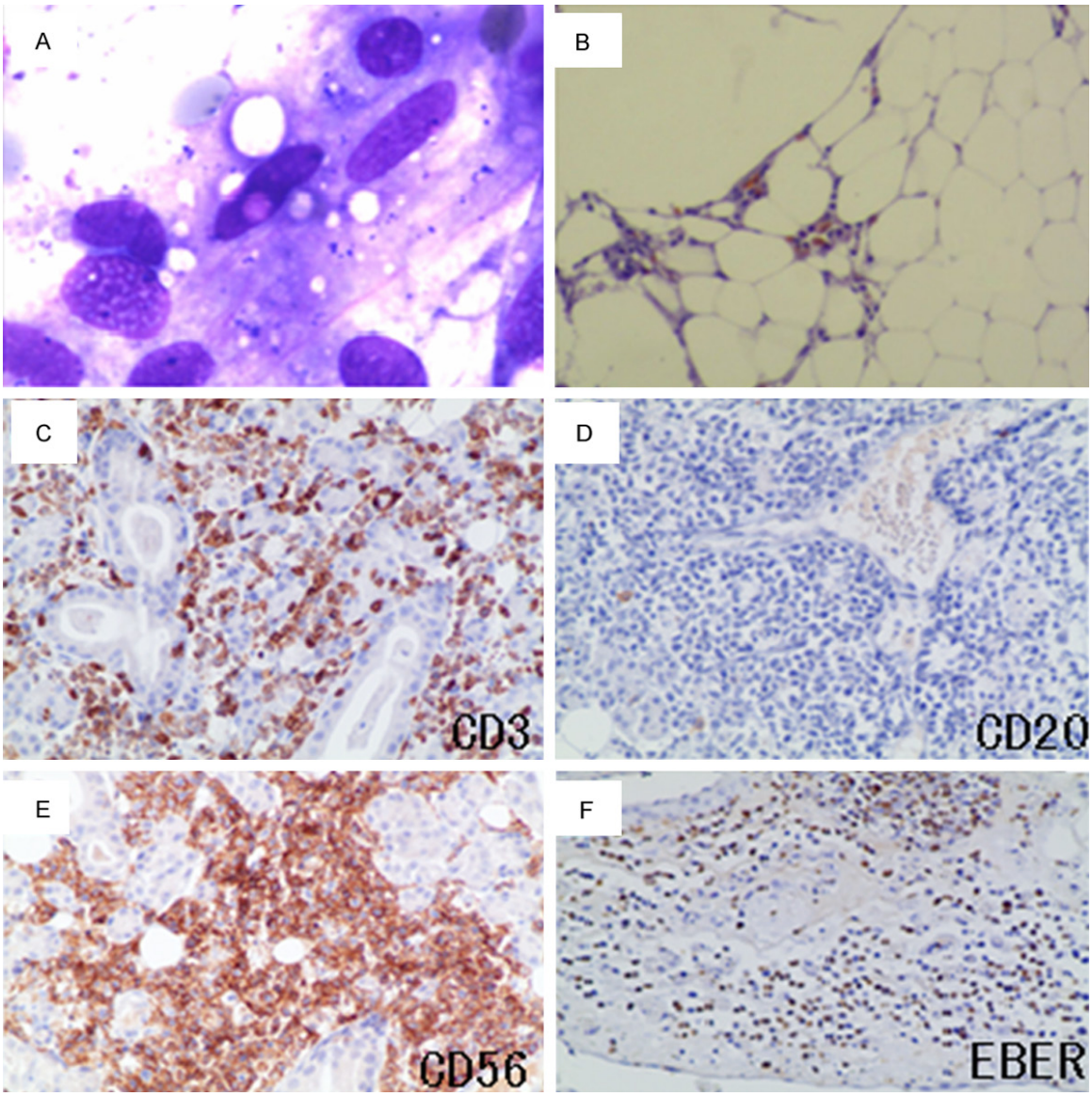

Figure 1. The composite pictures showed the histopathologic and cytomorphologic features of this patient. A. Bone marrow aspiration showed hypocellular marrow with failure of trilineage hematopoietic cells, Giemsa, × 1000. B. Bone marrow biopsy analysis also confirmed marked decrease of the proliferation of hematopoietic cells, HE, × 100. C-E. Biopsy of swelled parotid gland found heterotypic lymphocytes, which clearly expressed CD3, CD56, while they were negative for CD20 by immunohistochemistry at low magnification. F. EBV-encoded RNA (EBER) was detected in the heterotypic lymphocytes of the swelled parotid gland by in situ hybridization using a 100 × magnification.

subjective symptoms, such as fever, coughing, night sweating, and weight loss. No hepatosplenomegaly or lymphadenopathy was noted. His complete blood cell (CBC) count was hemoglobin (Hb) of 6.3 g/dL (normal range 13-17.5 g/dl), white blood cell (WBC) of 2,100/ul (normal range 3,500-9,500/ul), neutrophil of 1,000/ul (normal range 2,000-4,000/ul) and platelet of 12,000/uL (normal range 125,000-350,000/uL). Bone marrow aspirate and biopsy showed a markedly hypocellular marrow. According to his symptoms and signs, CBC count, as well as hypoplasia of bone marrow, the diagnosis of AA was confirmed definitely. Since the patient took neither consideration about ATG nor sibling donor transplantation for economic reasons, CsA and androgens were initiated as the main treatment for him and he got hematologic improvement by these attempts. Eighteen years later, he was transferred

Table 1. Cases about LPDs after IST for AA

	Age/gender	Immunosuppression drugs	Virus	Histopathology	Treatment	Outcome	reference
1	17/female	ATG+CSA+Pre	NR	B-EBV-LPD	RT	CR	10
2	38/male	rATG+CSA+Met-Pre	EBV	IM	Cessation of CSA+Rit	CR	11
3	42/female	rATG+CSA	EBV	DLBCL	Rit+CTX	CR	12
4	54/male	rATG+CSA+Met-Pre	EBV	DLBCL	NT	death	13
5	61/male	ATG+CSA	NR	DLBCL	R-CHOP+R-VP	PR	14
6	55/male	rATG+Met-Pre	EBV	EBV-LPD	Cessation of CSA,regression	CR	15
7	46/female	rATG+CSA+Pre	EBV/VZV/CMV	EBV-plasmacytic LPD	Cessation of CSA,regression	CR	16
8	53/male	CSA+Pre	EBV	NK/T	VPL	death	Our case

ATG: antithymocyte, CSA: cyclosporine A (CSA), Pre: Prednisone, Met-Pre: methylpredonisolone, NR: not reported, EBV: Epstein-Barr-virus, VZV: Varicella-zoster-virus, CMV: Cytomegalovinus, B-EBV-LPD: B cell origin from Epstein-Barr virus associated lymphoproliferative disorder, IM: infectious monocytosis, DLBCL: diffuse large B cell lymphoma, NK/T: natural killer and natural killer like T cell lymphoma, RT: radiation treatment, Rit: rituximab, CTX: cyclophosphamide, R-CHOP: rituximab, cyclophosphamide, adriacin, vincristine, prednisolone, R-VP: rituximab, vincristine, predn-isolone, VPL: vincristine, prednisone, L-asparaginase, CR: complete remission.

to stomatology department in our hospital due to the swelled parotid gland and the biopsy suspected EBV-associated lymphoproliferative disorder (EBV-LPD). The further immunohistochemistry analysis showed heterotypic lymphocytes were cCD3$^+$, CD30$^+$, CD56$^+$, GranB$^+$, TIA$^+$, MUM1$^+$, Ki-67 (50%-75%) $^{++}$ and in-situ hybridization (ISH) indicated EBER was also strongly positive (**Figure 1**). And the NK/T cell lymphoma involving the parotid gland was diagnosed due to the ISH and immunohistochemistry results.

He was then transferred to hematology department for the treatment of lymphoma. Physical examination found several scattered petechiae on his extremities without any superficial lymphadenopathy or hepatosplenomegaly. The left side of facial was swelled. CBC count showed leukocyte count was 1,340/uL with 81.4% neutrophils, hemoglobin of 9.9 g/dL, and a platelet count of 8,000/uL, and reticulocyte percentage of 0.5%. The serum ferritin leverl (more than 1,500 ng/ml) was markedly elevated (normal range 23.9-336.2 ng/ml). Bone marrow aspirate examination demonstrated hypocellular marrow with failure of trilineage hematopoietic cells. The percentage of lymphocytes was increased and granular lymphocytes were easy to be found (**Figure 1**). A bone marrow biopsy showed bone marrow failure with less than 20% normal cellularity (**Figure 1**). Conventional cytogenetic analysis by R banding method revealed 46, XY [20]. Flow cytometry for bone marrow cells identified the mild inversion of CD4/CD8 cell ratio and detected an increased proportion of NK/T cells (22%) with the phenotype of CD3$^+$ CD16$^+$ CD56$^+$. T cell receptor (TCR) gene rearrangement was negative by polymerase chain reaction (PCR) method. The quantities of EBV-DNA viral load in peripheral blood were 1.03×10^4 copy/ml. Besides the parotid gland, the whole body CT scan failed to reveal any other sites of tumor invasion. CsA was discontinued, and the chemotherapy regimen including vincristine, prednisone, L-asparaginase was administered. The parotid mass was gradually regressed after the first cycle of chemotherapy. The patient and his family demanded to discharge from our hospital for economic reasons and lost follow-up.

Discussion

AA is rare and potentially a fatal disease which is characterized by hypocellular bone marrow and pancytopenia; it is thought to be an aberrant immune response involving the T-cell mediated destruction of hematopoietic stem cells, which may precede, co-occur, or follow a lymphoproliferative neoplasm. The disease of NK/T lymphoma coexisted with AA is a unique neoplasm, which needs us to master the clinical feature and explore the biological characteristics deeply.

Since the survival of AA improved, more and more late complications of clonal evolutions have been observed, such as myelodysplastic syndrome (MDS), acute myeloid leukemia (AML) and paroxysmal noctumal hemoglobinuria (PNH). European group for blood and marrow transplantation (EBMT) [4] reported the 10-year incidence of MDS/AML was 5.8% after IST for AA. It was also reported [5] the cumulative incidence of clonal evolutions was 3.7%, and 5-year incidences of PNH and MDS/AML were 2.1% and 1.7%, respectively. It was still unclear that whether the clonal evolutions were a part of the extended natural history or were related

to the applied therapies. It was well documented that EBV infection was an important complication of prolonged immune deficiency, which was also strongly associated with EBV-LPDs. EBV-LPDs have also been reported in AA patients after the treatment of allo-HSCT [6, 7] or IST [8, 9]. Most cases of LPD after IST were originated from B cells, while this case presented with NK/T cell lymphoma, which was an aggressive lymphoma originated from NK/T cells. The best molecularly clarified scenario was that of concurrent AA and T-cell large granular lymphocyte leukemia. It was still unknown whether the therapeutic use of IST, especially the use of CsA alone, was the main contributor to developing EBV-LPDs. The reported clinicopathological features of LPD cases after the treatment of IST in AA were summarized in **Table 1** [10-16]. It could be easily seen that ATG combined with CsA could induce a lymphoproliferative change, but it was not clear CsA used alone could accelerate developing LPDs. To our best knowledge, the late clonal evolution involving NK/T cell lymphoma for AA had never been reported with the treatment of IST.

Hematologic improvement after IST implicated the immune system, especially T cell mediated immune reaction, played an important role in destruction of bone marrow stem and progenitor cells. ATG made a significant contribution to curative treatment of AA and had been shown to obtain satisfactory results [17]. Due to ATG had a higher degree of T-cell depletion than CsA, clonal evolutions including EBV-LPDs were increased in patients with AA who received ATG than CsA therapy. Ryota et al [11] reported a case of plasmacytic lymphoproliferative disorder after ATG therapy for AA patient, who presented with progressive pancytopenia with increase of atypical plasma cells in his bone marrow. At the same time, EBV, Varicella-zoster virus (VZV) and cytomegalovinus (CMV) reactivation were all detected by the biopsy of lesions. It reported the concurrent infection of the three viruses attacked the patient's immune system just like the case we presented after IST for AA.

EBV is known to be an extremely strong association with development of NK/T cell lymphoma [18], especially in immuno-compromised patients, who had a high-risk factor obtained primary EBV or other viral infection and further EBV-associated LPDs. In addition, EBV load was significantly related to the tumor load and treatment response [19, 20]. In current studies, EBV associated lymphoma had a poorer prognosis and more extensive necrosis. Considered from prognosis based on the NK/T characteristics, Ko et al [21] has reported $CD56^+$ EBV^+ TCR^+ lymphoma at extra-nasal sites was a clinically more aggressive malignancy, more extensive necrosis and shorter overall survival (OS) than $CD56^+$ EBV^- TCR^+. The high level of serum ferritin (>300 ng/ml) at the time of diagnosis for extranodal NK/T cell lymphoma patients was associated with a remarkably low remission rate (23%) and a short OS time (median 4 months) [22]. Our patient, a mid-aged man, who had $CD56^+$ EBV^+ TCR^- and high level of serum ferritin, which could be the risk factors for his clinical outcome. So it's essential to monitor the EBV-DNA load and the level of serum ferritin after IST for AA. Several studies have already reported the benefit of combining with a closely and early monitoring of EBV reactivation and EBV-specific T cell reconstitution, and provided preemptive treatment with CD20 antibodies for EBV infected B lymphocytes [23-25].

In conclusion, we described an extremely rare case of NK/T cell lymphoma after IST for AA, presented with swelled parotid gland after a long history of IST for AA. Albeit rare, this malignant disease originated from NK/T cells could still be found. If suspected, careful immunohistochemical and histopathological analyses should be performed to confirm. Additional approach to monitor EBV load was also needed to make the accurate and rapid diagnosis and initiate preemptive treatment. It is also important to explore the potential mechanism in the immunogenetic predisposing factors as well as exposure to the environment, such as viral infection or chemical poison which increases the risk of this disease.

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Disclosure of conflict of interest

None.

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References

[1] Tzankov A and Medinger M. Aplastic anemia: possible associations with lymphoproliferative neoplasms. Int J Lab Hematol 2014; 36: 382-7.

[2] Kidwai SM, Parasher AK and Lin FY. An unusual presentation of NK/T-cell lymphoma, nasal-type in the United States. Am J Otolaryngol 2015; 36: 80-3.

[3] Rezk SA and Huang Q. Extranodal NK/T-cell lymphoma, nasal type extensively involving the bone marrow. Int J Clin Exp Pathol 2011; 4: 713-7.

[4] Socie G, Mary JY, Schrezenmeier H, Marsh J, Bacigalupo A, Locasciulli A, Fuehrer M, Bekassy A, Tichelli A and Passweg J. Granulocyte-stimulating factor and severe aplastic anemia: a survey by the European Group for Blood and Marrow Transplantation (EBMT). Blood 2007; 109: 2794-6.

[5] Li Y, Li X, Ge M, Shi J, Qian L, Zheng Y and Wang J. Long-term follow-up of clonal evolutions in 802 aplastic anemia patients: a single center experience. Ann Hematol 2011; 90: 529-37.

[6] Furuya A, Ishida M, Hodohara K, Yoshii M, Okuno H, Horinouchi A, Nakanishi R, Harada A, Iwai M, Yoshida K, Kagotani A, Yoshida T and Okabe H. Epstein-Barr virus-related post-transplant lymphoproliferative disorder occurring after bone marrow transplantation for aplastic anemia in Down's syndrome. Int J Clin Exp Pathol 2013; 7: 438-42.

[7] Meyer SC, Medinger M, Halter JP, Baldomero H and Hirsch HH. Heterogeneity in clinical course of EBV-associated lymphoproliferative disorder after allogeneic stem cell transplantation. Hematology 2014; 19: 280-5.

[8] Ruiz-Argüelles GJ, Gómez-Rangel JD. Long-term results of the immunosuppressive treatment of patients with severe acquired aplastic anemia: asingle institution study. Acta Haematol 2003; 110: 184-7.

[9] Tzankov A and Medinger M. Aplastic anemia: possible associations with lymphoproliferative neoplasms. Int J Lab Hematol 2014; 36: 382-7.

[10] Dorr V, Doolittle G and Woodroof J. First report of a B cell lymphoproliferative disorder arising in a patient treated with immune suppressants for severe aplastic anemia. Am J Hematol 1996; 52: 108-13.

[11] Calistri E, Tiribelli M, Battista M, Michelutti A, Corbellino M, Viale P, Fanin R and Damiani D. Epstein-Barr virus reactivation in a patient treated with anti-thymocyte globulin for severe aplastic anemia. Am J Hematol 2006; 81: 355-7.

[12] Wondergem MJ, Stevens SJ, Janssen JJ, Oudejans JJ, Ossenkoppele GJ, Middeldorp JM and Zweegman S. Monitoring of EBV reactivation is justified in patients with aplastic anemia treated with rabbit ATG as a second course of immunosuppression. Blood 2008; 111: 1739.

[13] Ohata K, Iwaki N, Kotani T, Kondo Y, Yamazaki H and Nakao S. An Epstein-Barr virus-associated leukemic lymphoma in a patient treated with rabbit antithymocyte globulin and cyclosporine for hepatitis-associated aplastic anemia. Acta Haematol 2012; 127: 96-9.

[14] Suzuki Y, Niitsu N, Hayama M, Katayama T, Ishii R, Osaka M, Miyazaki K, Danbara M, Horie R, Yoshida T, Nakamura N and Higashi-hara M. Lymphoproliferative disorders after immunosuppressive therapy for aplastic anemia: a case report and literature review. Acta Haematol 2009; 121: 21-6.

[15] Sugimoto-Sekiguchi H, Tashiro H, Shirasaki R, Arai T, Yamamoto T, Oka Y, Akiyama N, Kawasugi K and Shirafuji N. Colonic EBV-associated lymphoproliferative disorder in a patient treated with rabbit antithymocyte globulin for aplastic anemia. Case Rep Gastrointest Med 2012; 2012: 395801.

[16] Nakanishi R, Ishida M, Hodohara K, Okuno H, Yoshii M, Horinouchi A, Shirakawa A, Harada A, Iwai M, Yoshida K, Kagotani A, Yoshida T and Okabe H. Occurrence of Epstein-Barr virus associated plasmacytic lymphoproliferative disorder after antithymocyteglobulin therapy for aplastic anemia: a case report with review of the literature. Int J Clin Exp Pathol 2014; 7: 1748-54.

[17] Shin SH and Lee JW. The optimal immunosuppressive therapy for aplastic anemia. Int J Hematol 2013; 97: 564-72.

[18] Chan JK, Sin VC, Wong KF, Ng CS, Tsang WY, Chan CH, Cheung MM and Lau WH. Nonnasal lymphoma expressing the natural killer cell marker CD56: a clinicopathologic study of 49 cases of anuncommon aggressive neoplasm. Blood 1997; 89: 4501-13.

[19] Chuang SS. In situ hybridisation for Epstein-Barr virus as a differential diagnostic tool for T-and natural killer/T-cell lymphomas in non-immunocompromised patients. Pathology 2014; 46: 581-91.

[20] Ruf S and Wagner HJ. Determining EBV load: current bestpractice and future requirements. Expert Rev Clin Immunol 2013; 9: 139-51.

[21] Ko YH, Cho EY, Kim JE, Lee SS, Huh JR, Chang HK, Yang WI, Kim CW, Kim SW and Ree HJ. NK

and NK-like T-cell lymphoma in extranasal sites: a comparative clinicopathological study according to site and EBV status. Histopathology 2004; 44: 480-9.

[22] Yamazaki Tomita N, Koyama S, Ogusa E, Ishii Y, Takahashi H, Miyashita K, Matsuura S, Tachibana T, Takasaki H, Takemura S, Fujimaki K, Sakai R, Fujisawa S and Ishigatsubo Y. Serum ferritin level is prognostic of patient outcome in extranodal NK/T cell lymphoma, nasal type. Med Oncol 2014; 31: 149.

[23] Annels NE, Kalpoe JS, Bredius RG, Claas EC, Kroes AC, Hislop AD, van Baarle D, Egeler RM, van Tol MJ and Lankester AC. Management of Epstein-Barr virus (EBV) reactivation after allogeneic stem cell transplantation by simultaneous analysis of EBV DNA load and EBV-specific T cell reconstitution. Clin Infect Dis 2006; 42: 1743-8.

[24] Clave E, Agbalika F, Bajzik V, Peffault de Latour R, Trillard M, Rabian C, Scieux C, Devergie A, Socié G, Ribaud P, Adès L, Ferry C, Gluckman E, Charron D, Esperou H, Toubert A and Moins-Teisserenc H. Epstein-Barr virus (EBV) reactivation inallogeneic stem cell transplantation: relationship between viral load, EBV-specific T-cell reconstitution and rituximab therapy. Transplantation 2004; 77: 76-84.

[25] D'Aveni M, Aïssi-Rothé L, Venard V, Salmon A, Falenga A, Decot V, Virion JM, Wang Y, Clement L, Latger-Cannard V, Tomowiak C and Stoltz JF. The clinical value of concomitant Epstein Barr virus (EBV)-DNA load and specific immune reconstitution monitoring after allogeneic hematopoietic stem cell transplantation. Transpl Immunol 2011; 24: 224-32.