Check for updates

To the editor:

Targeting host B-cell immune responses by persistent donor NK-cell alloreactivity following nonmyeloablative allogeneic stem cell transplantation

Allogeneic bone marrow transplantation (BMT) is an established treatment for a variety of hematological and immunological disorders. It is postulated that the beneficial effects after myeloablation and haploidentical BMT are mediated by alloreactive donor natural killer (NK) cells, and host-reactive NK-cell clones have been isolated from human transplant recipients up to 3 months after transplantation.[1,2] However, little is known about the ability of graft-derived NK cells to target host immune cells after nonmyeloablation and haploidentical BMT. Likewise, the persistence of donor NK-cell alloreactivity in chimeric hosts is poorly defined in this setting.[3] Here, we report the ability of alloreactive donor NK cells to eradicate host immune cells after haploidentical BMT in mice, without inducing graft-versus-host disease (GVHD).

The Experimental Animal Commission of Leiden University Medical Center (Leiden, The Netherlands) approved the experiments described in this letter.

Recently, we showed the effective treatment of B-cell–mediated autoimmune disease (AID) in mice by allogeneic BMT.[4] We postulated the rapid eradication of host B cells produced pathogenic autoantibodies by donor NK cells. To further analyze donor NK-cell alloreactivity toward antibody-secreting B cells, we used a model of haploidentical (F1→P) BMT involving host B cells recognizing a model antigen, ovalbumin (OVA). In this model, donor T cells are tolerant for host cells,[5,6] but donor NK cells are activated as they lack inhibitory signals provided by self–major histocompatibility complex (MHC) class I.[7,8] After immunization, high levels of anti-OVA antibodies were detected. Subsequently, the mice were treated with nonmyeloablative conditioning (ie, low-dose total-body irradiation (6 Gy) and anti-CD40L antibodies (0.5 mg), and BMT from syngeneic, haploidentical or fully MHC-mismatched donor mice. Both fully MHC-mismatched and haploidentical BMT resulted in the early elimination of OVA-specific B cells (Figure 1A) and anti-OVA antibodies (Figure 1B), in contrast to syngeneic BMT. Recipient mice had no signs of acute GVHD (ie, no weight loss and/or detectable histologic abnormalities of liver, gut, and skin), supporting the notion that donor NK cells can mediate beneficial anti–host B-cell alloresponses, without causing GVHD.

Next, we examined whether host-reactive NK-cell alloresponses after haploidentical BMT are still present 3 months after BMT using an in vivo cytotoxicity assay.[9,10] In this experiment, differentially carboxyfluoroscein succinimidylester (CFSE)-labeled splenocytes from syngeneic (host) and haploidentical (control) mice were injected into chimeric hosts (4% to 5% host Gr-1$^+$ cells). Prior to the challenge with CFSE-labeled splenocytes, NK cells in recipient mice were (or were not) depleted by anti-NK1.1 antibodies. At day +7, peripheral-blood cells were analyzed for the presence of CFSE$^+$ cells, and the extent of host-cell elimination was determined. Syngeneic cells were eliminated in control mice, while this elimination was inhibited after depletion of NK1.1$^+$ cells (Figure 1C). Although the effect of depletion of NK1.1$^+$ cells is probably underestimated, as not all F1-derived NK-cells express NK1.1, these data indicate that host-reactive NK-cells contribute to the eradication of host immune cells, and that this alloreactivity persists up to 3 months after BMT.

In conclusion, our data suggest that donor NK-cell alloreactivity toward host immune cells may be exploited in the setting of allogeneic BMT to treat AID in which B cells play a pathogenic role.

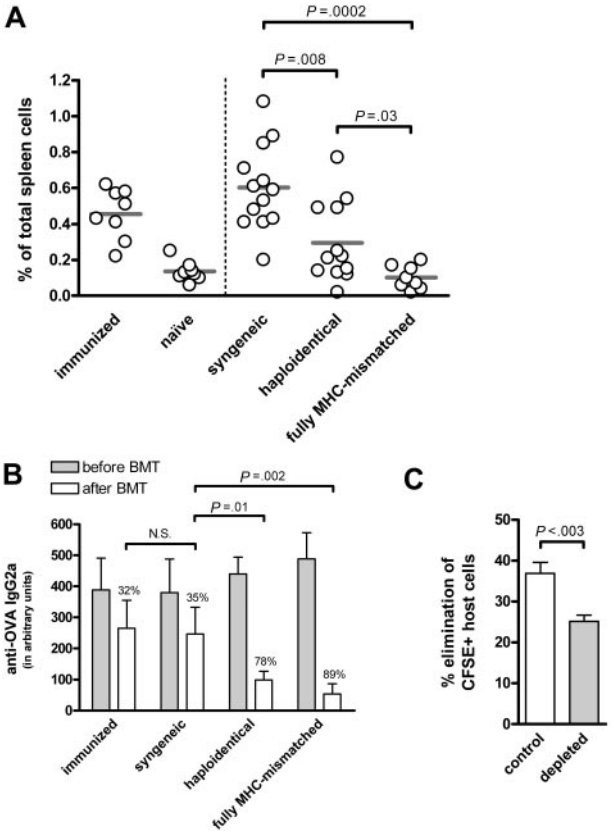

Figure 1. Persistent donor NK-cell alloreactivity toward host B cells after haploidentical BMT. (A,B) Haploidentical BMT results in a significant decrease in OVA-specific B cells and anti-OVA antibodies. DBA/1 mice were immunized with OVA, and were either left untreated or were subjected to nonmyeloablative conditioning consisting of low-dose total body irradiation (6 Gy) and a single injection of 0.5 mg of anti-CD40L antibodies (MR1), followed by transplantation with 10^7 syngeneic, haploidentical (F1→P) or fully MHC-mismatched BM cells from DBA/1, (DBA/1 x C57BL/6) F1 or C57BL/6 donor mice, respectively. The percentage of OVA-specific B cells in spleen was analyzed 2 weeks after BMT. Compared to syngeneic controls, both haploidentical (F1→P) and fully MHC-mismatched BMT recipients showed a significant decrease in the percentage of OVA-specific B cells ($P = .008$ and $P = .0002$, respectively). Sera were taken before and after BMT, and tested by ELISA for the presence of anti-OVA IgG2a antibodies. At the start of treatment, no differences in anti-OVA antibody levels were observed between the groups of mice. After treatment, all groups displayed a decrease in anti-OVA antibodies; syngeneic BMT-treated mice showed a decrease in OVA-specific antibody levels of 35%, similar to that of controls (32%), while both haploidentical (F1→P) and fully MHC-mismatched BMT resulted in a strong decrease in antibodies against OVA of 78% ($P = .01$) and 89% ($P = .002$), respectively. As negative control, naive mice were analyzed, while immunized mice served as positive control. (N.S. = statistically not significant.) (C) Donor NK cells persistently eradicate host immune cells after haploidentical BMT. At 3 months after BMT, chimeric C57BL/6 mice treated with haploidentical (F1→P) BMT from (BALB/c x C57BL/6) F1 were injected with 10^7 CFSE-labeled splenocytes (containing 50% to 60% B cells) from syngeneic (CFSE^{dim}) and haploidentical (CFSE^{high}) mice mixed at a 1:1 ratio. Before adoptive transfer, NK cells in recipient mice were depleted with 0.25 mg anti-NK1.1 antibodies (PK136), while PBS was used as control. At day +7 after adoptive transfer, the presence of CFSE$^+$ cells in peripheral blood was analyzed by FACS. Error bars indicate mean ± SEM.

Downloaded from http://ashpublications.net/blood/article-pdf/109/12/5524/1480584/zh801207005524.pdf by guest on 18 May 2024

Roelof Flierman, Geert Westerhuis, Marjolijn Hameetman, Leonie M. van Duivenvoorde, Thorbald van Hall, Jacob M. van Laar, Willem E. Fibbe, René E. M. Toes

R.F. and G.W. contributed equally to this letter.

Correspondence: Roelof Flierman, Department of Nephrology (D3-P), Leiden University Medical Center, Albinusdreef 2, 2333 ZA, Leiden, The Netherlands, email: r.flierman@lumc.nl

Conflict-of-interest disclosure: The authors declare no competing financial interests.

References

1. Ruggeri L, Capanni M, Urbani E et al. Effectiveness of donor natural killer cell alloreactivity in mismatched hematopoietic transplants. Science. 2002;295: 2097-2100.
2. Ruggeri L, Capanni M, Casucci M et al. Role of natural killer cell alloreactivity in HLA-mismatched hematopoietic stem cell transplantation. Blood. 1999;94:333-339.
3. Johansson MH, Hoglund P. Low number of H-2Dd-negative haematopoietic cells in mixed bone marrow chimeras convey in vivo tolerance to H-2Dd-negative cells but fail to prevent resistance to H-2Dd-negative leukaemia. Scand J Immunol. 2004;59:71-78.
4. Flierman R, Witteveen HJ, van der Voort EI et al. Control of systemic B cell-mediated autoimmune disease by nonmyeloablative conditioning and major histocompatibility complex-mismatched allogeneic bone marrow transplantation. Blood. 2005;105:2991-2994.
5. Von Boehmer H, Sprent J, Nabholz M. Tolerance to histocompatibility determinants in tetraparental bone marrow chimeras. J Exp Med. 1975;141:322-334.
6. Katz DH, Skidmore BJ, Katz LR, Bogowitz CA. Adaptive differentiation of murine lymphocytes. I. Both T and B lymphocytes differentiating in F1 transplanted to parental chimeras manifest preferential cooperative activity for partner lymphocytes derived from the same parental type corresponding to the chimeric host. J Exp Med. 1978;148:727-745.
7. Karre K, Ljunggren HG, Piontek G, Kiessling R. Selective rejection of H-2-deficient lymphoma variants suggests alternative immune defence strategy. Nature. 1986;319:675-678.
8. Karlhofer FM, Ribaudo RK, Yokoyama WM. MHC class I alloantigen specificity of Ly-49+ IL-2-activated natural killer cells. Nature. 1992;358:66-70.
9. Westerhuis G, Maas WG, Willemze R, Toes RE, Fibbe WE. Long-term mixed chimerism after immunologic conditioning and MHC-mismatched stem-cell transplantation is dependent on NK-cell tolerance. Blood. 2005;106:2215-2220.
10. Johansson S, Johansson M, Rosmaraki E et al. Natural killer cell education in mice with single or multiple major histocompatibility complex class I molecules. J Exp Med. 2005;201:1145-1155.

To the editor:

Effect of the new HJV-L165X mutation on penetrance of HFE

We present a new homozygous truncating mutation, L165X, of the *hemojuvelin* gene (*HJV*), observed in one male patient with severe juvenile hemochromatosis (JH). Because the C282Y-variant in the *hemochromatosis* gene (*HFE*) was also common in this family, we investigated whether the inactivating mutation HJV-L165X influenced penetrance of HFE-C282Y homozygosity.

The proband, born in 1956 and diagnosed in 1972 by family screening (B III-54),[1] presented with increased serum iron values and heavy iron accumulation in the hepatocytes.[1,2] There was no consanguinity between his parents or grandparents,[1] and linkage to *HLA* was excluded in his familybranch, providing the first clue of genetic heterogeneity in hemochromatosis.[3] In late 2005, he provided contact information on relatives. The institutional review board approved this study and informed consent was obtained from all participants (n = 20). Non-fasting blood and urine samples from the proband and his relatives were collected between 7 and 9 PM on the same day. Information on the number of phlebotomies and the time between the last phlebotomy and sample collection was provided. Urinary hepcidin was measured by mass spectometry.[4] For the proband, we sequenced the *hepcidin* (*HAMP*) and *HJV* genes. Mutations in the *HAMP* gene were absent. The *HJV* gene was sequenced using previously reported primers.[5] For exon 3 we designed new primers: Ex3a1935F 5′-GCAAACTACACTCCGATAGAG-3′ and Ex3a2253R 5′-GCTGGATCATCAGGTCTTCG-3′, resulting in a 319 bp product, and Ex3b2202F 5′-GACCTCGCCTTCCATTCG-3′ and Ex3b2603R 5′-GAATCTCATGAGGTGGATCGG-3′, leading to a 402 bp product (GenBank NT_004 434/gi:88 943 080). We observed a novel homozygous mutation in exon 3 of the *HJV* gene. The 494T→A transversion leads to a premature stop codon at position 165 of the HJV protein: L165X. This probably leads to nonsense-mediated decay of the corresponding messenger RNA. If the aberrant message is translated, however, it would code for a protein that lacks the GPI anchor signal, such that it remains in the endoplasmic reticulum.[6] In both cases, it can be anticipated that upstream regulation of hepcidin is impaired.[7]

Relatives of the proband were investigated for the HFE-C282Y and the HJV-L165X mutation by a restriction fragment length polymorphism analysis using the Ex3b2202F and Ex3b2603R primers and the restriction enzyme HpyCH4V (New England Biolabs, Ipswich, MA).

HJV-L165X homozygosity was only present in the proband, while heterozygosity was common among relatives (allele frequency: 14/40 = 35.0%). Furthermore, the HFE-C282Y mutation was observed frequently (allele frequency: 27/40 = 67.5%) (Table 1). Phlebotomies were only reported in individuals later found to be homozygous for either HJV-L165X (n = 1; proband) or HFE-C282Y (n = 8). Iron indices (current and from the early seventies[1,2]; J. P. G., unpublished data, early 1970s) are copied into Table 1.

Current serum iron parameters are not appropriate as a measure of iron burden, as most relatives have been adequately phlebotomized. We found an alternative in the following parameters: quantity of iron removed by phlebotomies (iron removed/age),[8] a rough estimate hampered by the probability that intestinal iron uptake may increase upon phlebotomy; transferrin saturation (TS) values and desferrioxamine (DFO) test results from the early seventies, before treatment; and urinary hepcidin levels, measured with our improved MS assay.[4] The iron removed/age, urinary hepcidin levels, and iron indices from the early seventies were similar for HJV-heterozygotes (L165X) and the HJV-wildtypes, also when stratified by *HFE* genotype, indicating the absence of a clinically relevant modifying effect. Others reported HJV a modifier,[9,10] although not consistently.[11] Furthermore, our data agree with recent findings that HFE and HJV participate, at least partially, in distinct regulatory pathways.[12] Finally, against a background of multiple small variations, we cannot exclude a minor effect of the heterozygous HJV-L165X mutation on iron homeostasis.

Boukje A. C. van Dijk, Erwin H. J. M. Kemna, Harold Tjalsma, Siem M. Klaver, Erwin T. G. Wiegerinck, Jan-Pieter Goossens, Peter H. Th. J. Slee, Martijn H. Breuning, and Dorine W. Swinkels

Downloaded from http://ashpublications.net/blood/article-pdf/109/12/5524/1480584/zh801207005524.pdf by guest on 18 May 2024