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Chimerism-directed adoptive immunotherapy in the prevention and treatment of post-transplant relapse of leukemia in childhood

We present the role of frequent monitoring of hematopoietic chimerism in the prediction of post-transplant relapse and our initial experience with adoptive immunotherapy in the prevention and treatment of hematologic relapse in children after allogeneic hematopoietic stem cell transplantation.

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Relapse of leukemia remains the major cause of treatment failure in allogeneic hematopoietic stem cell transplantation (HSCT) in children. In a prospective study we used frequent monitoring of hematopoietic chimerism (HC)[1,2] to identify patients with a high risk of post-transplant relapse and thus indicated for adoptive immunotherapy (AI).[3-6] Between January 1997 and June 2001 we performed a total of 54 unmanipulated allogeneic HSCT from HLA-identical siblings (28) or matched unrelated donors (26) in 50 consecutive children with hematologic malignancies in the University Hospital Motol, Prague. Fifty-two evaluable follow-ups from forty-eight patients at a median age of 10 years (2-18 years) with acute lymphoblastic leukemia (ALL; 18/17), acute myelogenous leukemia (AML; 17/14), chronic myelogenous leukemia (CML; 8), myelodysplastic syndrome (MDS; 6) and juvenile myelomonocytic leukemia (JMML; 3) were included in this prospective chimerism study. Written informed consent was obtained from the parents. We analyzed HC in peripheral blood samples using polymerase chain reaction of variable number of tandem repeats (ApoBII, CoI2A1, YNZ22, D1S80, HVR-Ig, TPO) with a maximum sensitivity of 0.5%.[7] Peripheral blood samples were taken weekly from day +8 until day +100, then once a month during the first year following HSCT and thereafter according to clinical and laboratory outcome.[1] Complete donor chimerism (CC), characterized by the disappearance of recipient cells until day +28 and sustained emergence of donor cells was documented in 29/52 follow-ups. Mixed chimerism (MC), characterized by the re-emergence or persistence of recipient cells after day +28, was found in 23/52 follow-ups. Transient MC (trMC) was seen in 9 follow-ups and increasing MC (inMC) in 14 follow-ups. Considering the transplant-related mortality until day +100, 44 follow-ups were evaluable for relapse-free survival (RFS). At a median follow-up of 16.5 months RFS for the CC group was 20/22, while that for the MC group was 11/22. RFS for the trMC group was 7/9 as compared to 4/13 for the inMC group (Figure 1). Two patients with CC (1 ALL, 1 AML) relapsed after transplantation without prior detection of MC; in both extramedullary relapse occurred.

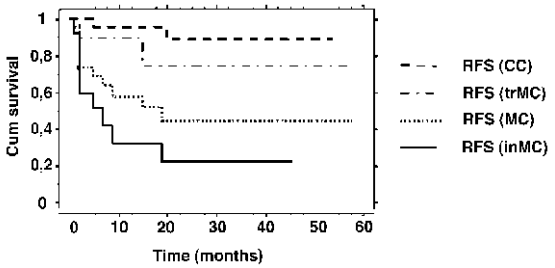

Figure 1. Kaplan-Meier estimates of relapse-free survival for the CC and the MC groups. Forty-four patients were evaluable for RFS. RFS for the CC group was 20/22, whilst that for the MC group was 11/22. RFS for the MC group was 7/9 as compared to 4/13 for that in the MC group.

AI was used in the prevention and treatment of post-transplant relapse in 13 patients/14 follow-ups (ALL 4, AML 5, CML 3, JMML 1/2). Treatment was started on the basis of inMC (9), in molecular relapse[8] (1) or in hematologic relapse (3/4). Withdrawal of post-transplant immunosuppression (IS) was performed in 11 patients, 5 patients with no or only transient response to withdrawal of IS received second-line therapy by donor lymphocyte infusion (DLI). In 3 follow-ups without IS, DLI was applied as a front-line therapy.[4] Doses of $CD3^+$ cells varied between 1×10^5 and 2.4×10^8/kg body weight according to type of donor and indication for DLI. Complete response to AI, defined as sustained recurrence of CC and continuous complete remission (CCR), was documented in 6/14 follow-ups (second post-transplant relapse in a patient with JMML, 3/3 patients with CML, and in only 2/9 patients with acute leukemia) at a median follow-up of 28 months (range 6 to 46 months). One patient with ALL achieved CC but died soon after of severe graft-versus-host disease (GVHD). Only temporary responses (transient decrease or disappearance of MC) were seen in 3 follow-ups (2 AML, 1 JMML) with subsequent hematologic relapse 8, 9 and 20 months after the initiation of AI. No response to AI was seen in 4 follow-ups (2 ALL, 2 AML). Overall survival of the patients treated with AI was 8/13 (ALL 1/4, AML 3/5, CML 3/3, JMML 1/1). Out of 3 patients/4 follow-ups treated in hematologic relapse only 1 (JMML) is alive in CCR. Pre-emptive AI was performed in 10 patients. Initial response was documented in

8/10 children with recurrence of CC in 6 of them. In 2 children, a significant long-term decrease of MC was documented and AI probably postponed hematologic relapse. This allowed us to perform a second transplantation in both patients.[9] Secondary GVHD grade I-III was seen in 5/14 follow-ups and was fatal in one patient.

We confirmed that patients with increasing MC have a significantly higher risk of hematologic relapse.[1] Continuous CC together with trMC usually proved to be a good prognostic factor, but in our experience had limited value in predicting extramedullary relapse. Detection of HC is a simple, reliable and rapid method and when performed frequently, allows us to identify patients indicated for AI. A graft-versus-leukemia effect of AI in our small cohort was evident in patients with CML and JMML, was less effective in patients with AML, and was questionable in patients with ALL. We speculate that in patients with acute leukemia AI methods are more effective when initiated early before full leukemia recurrence.

Renata Formánková, Petr Sedlácek, Lenka Krsková, Hana Říhová, Lucie Srámková, Jan Stary for the Czech Pediatric Hematology Working Group

Correspondence: Renata Formánková, MD, 2nd Department of Pediatrics, University Hospital Motol, V Úvalu 84, CZ-150 06, Prague 5, Czech Republic. Phone & Fax: international +42.02.24432220. E-mail: renata.formankova@lfmotol.cuni.cz

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Inversion of intron 1 of the factor VIII gene for direct molecular diagnosis of hemophilia A

An intron 1 inversion of the factor VIII gene has been recently described as a consequence of an intrachromosomal recombination involving a 1041bp specific duplicon inside and outside the gene. We investigated the intron 1 inversion in a cohort of 201 Spanish hemophilia A (HA) families. The inversion was detected in 4 families with severely affected cases of HA and no inhibitor history. The frequency of the inversion among cases of severe HA cases was 5% (4/79), confirming that this inversion is a recurrent mutational event.

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The most frequent mutation in severe hemophilia A patients is an inversion of intron 22 of the factor VIII gene, described 8 years after the cloning of the gene.[1,2] In 1996 an inversion breaking intron 1 was detected in two hemophilic monozygotic twins.[3] This was originally regarded as a rare event, but 6 years later, the same group in the United Kingdom reported that this inversion was a recurrent event in patients with hemophilia A (HA).[4] A 1041-base pair sequence (int1h-1) of the intron 1 was found to be duplicated (int1h-2) and oriented in the opposite direction 140 kb outside the gene between the C6.1A and VBP1 genes. This inversion arises from a recombination event between the two homologous sequences int1h-1 and int1h-2 (Figure 1).

One hundred and eighty-five unrelated HA patients and 16 mothers of deceased hemophiliacs, in whom inversion of intron 22 had been excluded, were investigated for the presence of inversion of intron 1. Out of 201 cases, 79 had severe disease, 53 had moderate disease and the remaining 69 had a mild phenotype. For inversion analysis, two polymerase chain reactions (PCR) were performed as previously described[4] with slight modifications. In the first reaction, primers specific for int1h-1 (9F, 9cR) plus the primer int1h–2F were used in an amplification reaction that yielded a 1908 bp product from normal DNA and a 1323 bp product if the inversion was present (Figures 1 and 2). In the second reaction, primers specific for int1h-2 (int1h –2F, int1h –2R) plus the primer 9F yielded a 1191 bp product from normal DNA and a 1776 bp product in the presence of an inversion, assuming that the interchange is reciprocal. The pattern of the carriers had both bands (Figure 2). For haplotype analysis, four intragenic (Intron 13 CA repeat, *Bcl*I intron 18 and Intron 22 CA repeat by PCR and *Kpn*I/*Xba*I intron 22 by Southern blot) and two extragenic (DXS52 by PCR and DX13 by Southern blot) markers were used as previously described.[5,6]

The test was positive in 3 out of 185 HA patients and in one out of the 16 HA mothers. The overall frequency of intron 1 inversion in all hemophiliacs without intron 22 inversion was 4/201 (2%). The calculated frequency was 5% (4/79) when considering only severe cases. Three were familial cases and in