Stem Cell Transplantation

B-cell repertoire reconstitution after hematopoietic stem cell transplantation In children evaluated by immunoglobulin heavy chain third complementarity determining region fingerprinting

Immunodeficiency after hematopoietic stem cell transplantation leads to a high risk of opportunistic infections. We evaluated B-lymphocyte reconstitution in 36 children by heavy chain third complementarity determining region (CDR3)-fingerprinting and immunophenotypic analysis. The time necessary to return to the normal immunoglobulin heavy chain-CDR3 polyclonal situation was basically related to the type of transplant and this process did not recapitulate fetal ontogenesis.

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A delay in immune reconstitution after hematopoietic stem cell transplantation (HSCT), influenced by the intensity of the conditioning regimen, source of stem cells and the type of transplant, is the cause of several opportunistic infections and profoundly affects patients' return to regular social activities.[1,2] Moreover, in the allogeneic setting, acute and chronic graft-versus host disease (GvHD) and the immunosuppressive therapies to control them, interfere with immune reconstitution.

We used heavy chain third complementarity determining region (CDR3) fingerprinting to analyze reappearance and diversification of functional B cells post-HSCT in 36 children. Molecular synthesis of third CDR3, the hypervariable segment of immunoglobulin heavy chain (IgH), is a multistep process that generates the great diversity and specificity of antibodies[3] and involves rearrangements of variable (V), diversity (D) and joining (J) gene segments. We analyzed two VH families (V_H3 and V_H6). V_H3 is one of the largest family, whereas V_H6 is one of the smallest with only one gene used in the rearrangement process. V_H6 is reported to be particulary expressed in the fetal repertoire.[4] Molecular data were compared with information from fluorescent activated cell sorter (FACS) analysis.

Table 1. HSCT patients.

Pts	*Age*	*Sex*	*Disease*	*Stem Cell Source*	*Pre-transplant Regimen*	*GvHD A/C*	*Infections*	*Clinical outcome*
Allogeneic HSCT								
1, Fl	11	F	ALL	MUD	TBI/TT/Cy	A1	cmv	alive
2, SSt	1	M	ALL	MUD	BU/TT/Cy	A2	–	dead+180,relapse
3, CD	2	M	ALL	MUD	BU/TT/Cy	A3	cmv	alive
4, FL	17	M	ALL	MUD	TBI/TT/Cy	A4	–	dead+100,GvHD
5, RA	16	F	ALL	MUD	TBI/TT/Cy	A1	cmv	alive
6, LM	8	F	ALL	MUD	TBI/TT/Cy	A1	–	alive
7, Ajo	10	M	ALL	MUD	TBI/TT/Cy	A2	cmv	dead+200,relapse
8, PrA	14	M	ALL	MUD	TBI/TT/Cy	A1	EBV	alive
9, VS	12	F	AML	MUD	TT/Cy	abs	cmv	dead+180,relapse
10, Mgi	1	M	AML	MUD	BU/TT/VP16	abs	–	relapse+180
11, BI	16	F	CML	MUD	TBI/Cy	A3/Cext/sev	cmv	dead+180,GvHD
12, MAl	6	M	JMML	MUD	BU/Cy/L PAM	A3	cmv	alive
13, DSM	15	F	MDS	MUD	BU/Cy/L PAM	A4	–	dead+90,GvHD
14, CC	10	M	FEL/NHL	MUD	FLU/Cy	abs	EBV	alive
15, DPL	1	M	osteopetrosis	MUD	BU/TT/Cy	A3	–	alive
16, GG	7	F	ALL	MRD	TBI/TT/Cy	abs	cmv	alive
17, CGG	5	M	ALL	MRD	TBI/TT/Cy	A3	–	alive
18, SC	9	M	ALL	MRD	TBI/TT/Cy	A2/Clim/mild	–	alive
19, SN	8	F	ALL	MRD	TBI/TT/Cy	A4	–	dead+80,GvHD
20, MD	9	F	ALL	MRD	TBI/TT/Cy	A2/3	–	alive
21, AJ	8	M	AML	MRD	BU/Cy/L PAM	A2/Cext/mild	–	alive
22, Ban	9	M	AML	MRD	BU/Cy/L PAM	A4	cmv	alive
23, CFr	1	F	JMML	MRD	BU/Cy/L PAM	A2	–	alive
Autologous HSCT								
1, CFi	14	F	AML	BM	TBI/L PAM	–	–	alive
2, PA	11	M	AML	BM	L PAM	–	–	dead+365,relapse
3, AC	5	F	AML	PBSC	ARA-C/VP16/TT	–	–	alive
4, CS	6	M	NB	PBSC	BU/L PAM	–	–	dead+180,relapse
5, BIG	5	F	NB	PBSC	BU/L PAM	–	–	alive
6, MomG	2	F	NB	PBSC	BU/L PAM	–	–	dead+90,relapse
7, DiMa	4	M	NB	PBSC	BU/L PAM	–	–	relapse
8, LDR	5	F	NB	PBSC	BU/L PAM	–	–	alive
9, BO	9	M	EWS	PBSC	BU/L PAM	–	–	alive
10, DCF	11	M	EWS	PBSC	TT/L PAM	–	–	dead+160,relapse
11, CA	5	F	MB	PBSC	BU/L PAM	–	–	alive
12, RoA	2	F	MB	PBSC	TT/L PAM	–	–	dead+250,relapse
13, GL	10	F	OSTEO	PBSC	CARBO-VP	–	–	relapse

Pts: patients; MUD: matched unrelated donor; MRD: matched related donor; F: female; M: male; ALL: acute lymphoblastic leukemia; AML: acute myeloid leukemia; CML: chronic myeloid leukemia; JMML: juvenile myelomonocytic leukemia; MDS: myelodysplastic syndrome; FEL/NHL: familial erythrophagocytic lymphohistiocytosis/non Hodgkin's lymphoma; CGD: chronic granulomatous disease; NB: neuroblastoma; EWS: Ewing sarcoma; MB: medulloblastoma; OSTEO: osteosarcoma; BM: bone marrow; PBSC: peripheral blood stem cells; TBI: total body irradiation; TT: thiotepa; Cy: cyclophosphamide; BU: busulfan; VP16: etoposide; L PAM: melphalam; FLU: fludarabine; ARA-C: cytosine arabinoside; CARBO-VP: carboplatin-etoposide; GvHD: graft-versus-host disease; A: acute GvHD; C: chronic GvHD; 1,2,3,4, acute GvHD grade; abs: absent; ext: extensive; sev: severe; lim: limited; cmv: cytomegalous virus; EBV: Epstein-Barr virus.

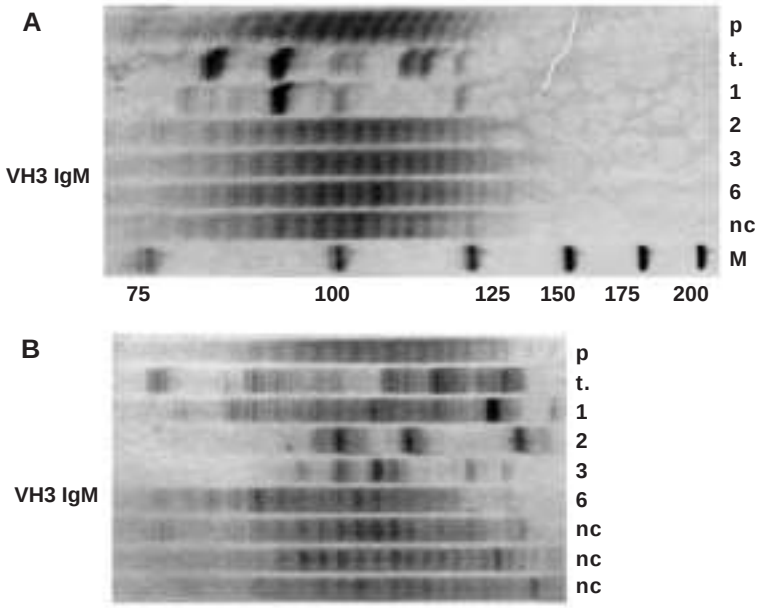

Figure 1. IgM-H-CDR3 fingerprinting: profiles of 2 patients: (A) 5-years old patient affected by neuroblastoma (NB) and recipient of autologous HSCT; (B) 16 year old patient affected by chronic myeloid leukemia (CML) and recipient of an allogeneic HSCT from unrelated donor (MUD); M, 25 ladder molecular weight marker (numbers indicate base pairs); p, pre HSCT; t, take post HSCT (3 days after PMN cells > 500 mm^3); 1, 2, 3, 6, +1, +2, +3, +6 months post HSCT; nc, normal control; V_H3 IgM, clonal repertoire of IgM HCDR3 for V_H3 family.

Twenty-three of the 36 children received an allogeneic-bone marrow transplant: 15 from unrelated donors and 8 from HLA-identical siblings (Table 1). Thirteen patients received autologous-HSCT, using bone marrow in 2 cases and peripheral blood (PB) in the other 11 cases (Table 1). In all patients, PB samples were collected before the preparative regimen, at engraftment and +1,+2,+3,+6, and +12 months post-transplant. cDNA was amplified by two consecutive polymerase chain reactions (PCR), the first, using the V_H3 or V_H6 consensus-primer and the IgM1 antisensus-primer and the nested one, using the framework[3] ($FR3C_{3,4,6}$) consensus-primer and the JHC antisensus-primer.[5] PCR products were separated on polyacrylamide gel and visualized by silver staining.[6] Lymphocyte subsets were identified on the same samples by flow cytometry using lineage-specific antigens. IgHCDR3 fingerprinting profiles obtained from HSCT recipients differ strongly from the normal polyclonal repertoire which shows 16-20 bands, each band corresponding to a particular heavy chain CDR3 length.[7]

We compared the speed of immune recovery according to the HSCT type, analyzing the V_H3 - V_H6/IgM CDR3 profiles. Figure 1 shows a comparison of two V_H3 profiles, the first (A) from a recipient of an autologous HSCT and the second (B) from a recipient of an allogeneic HSCT, representative of the immune recovery pattern of all 36 patients. For a short period post-HSCT, both children demonstrated a restricted number of bands; the child who had received an autologous graft (A) achieved a polyclonal pattern earlier (within 1-2 months) than the child who had received an allogeneic HSCT (B), in whom the polyclonal pattern appeared 3-6 months after the HSCT. Accordingly, FACS analysis showed that it took at least 2 and 6 months for B-lymphocyte and NK-cell reconstitution in childhood recipients of autologous and allogeneic HSCT, respectively. Combining data from molecular and phenotypic analysis, it is quite evident that immune recovery after both autologous and allogeneic transplants occurs very gradually and that for up to 6 months post-transplant the absolute counts of B, T and NK cells are lower than those of age-matched healthy subjects. Extensive chronic GvHD and its intensive and prolonged treatment were associated with a more pronounced delay in B-lymphocyte recovery (up to 12 months, *data not shown*). Molecular profiles show that the IgM heavy chain CDR3 repertoire early post-HSCT is clonally restricted in children as it is in adult patients.[6] The profiles differ from those in the normal healthy donors not only in the limited number of bands obtained, but also in the relative intensity of some bands. This suggests the presence of some expanded B clones in the early post-transplant period. The restricted repertoire just after HSCT has been related to recapitulation of fetal immune system.[8,9] However, we found that the size distribution of the CDR3 regions for both the V_H3 and V_H6 families was very similar to that detected in adult PB. These data, assuming the return to a polyclonal repertoire post-HSCT as paradigmatic of normal reconstitution, demonstrate that a preferential expression of the V_H6 family, peculiar of the fetal repertoire, is not present. Data in transplanted children are limited[10] and our study contributes information on the kinetics of the reconstitution of the pediatric B-cell immune repertoire post-HSCT.

We can conclude that post-transplant B-lymphocytes reconstitution in children does not recapitulate fetal ontogeny at a molecular level and that immunological reconstitution post-HSCT is a slow process, especially in cases of allogeneic HSCT. In our experience, the type of transplant, presence of chronic GvHD and prolonge immunosuppressive treatments play major roles in the slow immune recovery.

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References

Errata Corrige

On page 229 of the article by Barbui et al. Practice guidelines for the therapy of essential thrombocythemia. A statement from the Italian Society of Hematology, the Italian Society of Experimental Hematology and the Italian Group for Bone Marrow Transplantation published on Haematologica 2004;89:215-232, the following statement «*...this study was funded by Shire Italia, a pharmaceutical company that sells anagrelide.*» must be substituted with: «*...this study was funded by Shire Pharmaceutical Company, a pharmaceutical company that sells anagrelide.*»