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Reticular dysgenesis

Reticular dysgenesis: HLA non-identical bone marrow transplants in a series of 10 patients

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Summary:

Reticular dysgenesis is a very rare congenital immunodeficiency classified within the severe combined immunodeficiencies (SCID) and characterized by impairment of both lymphoid and myeloid cell development. We report our experience in 10 patients with RD, treated between 1979 and 1999 with HLA-haploidentical hematopoietic stem cell transplantation (HSCT). All children but one were symptomatic within the first days of their lives. Five patients required two HSCT. Five patients received conditioning therapy with busulfan (16 mg/kg) and cyclophosphamide. Three of them are alive and well with myeloid and T and B cell lymphoid reconstitution, whereas two patients died (one chronic graft-versus-host disease, one pneumonitis). Transplantation without or with other conditioning regimens in the other five cases led to absent or incomplete engraftment and none of these cases survived. These results demonstrate the mandatory need for intensive conditioning before haploidentical HSCT in RD to achieve full lymphoid and myeloid engraftment.
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Reticular dysgenesis (RD) is a rare inherited disorder characterized by congenital agranulocytosis and severe combined immunodeficiency.[1,2] There is a maturation arrest in the myeloid lineage and also a global impairment of lymphoid maturation leading to severe reduction in both B and T cell numbers. The underlying inherited defect affecting the differentiation of both lymphoid and myeloid cell precursors is unknown. Hematopoietic stem cell transplantation (HSCT) is thus by far the only curative treatment as shown in a small number of cases.[3–6] All patients not receiving HSCT have died from infections within the first days or weeks of life.[1,2,7–10] There is no larger series of RD described to date.

We analyzed retrospectively the outcome of HSCT from HLA non-identical donors performed in 10 patients with RD from three European centers (Ulm, Paris, London) during a 20-year period. Three of these patients are long-term survivors and are considered as cured from all manifestations of RD.

Patients and methods

Between 1979 and 1999, 10 patients suffering from RD who received HLA non-identical HSCT in three European centers were reported to the European registry (EBMT/ESID). The diagnosis of RD was based on established clinical and biological data: agranulocytosis, lymphocytopenia, early arrest of myeloid differentiation in bone marrow, failure of *in vitro* assays to produce mature granulocytes from precursors, lack of lymphocyte functions, and absence of thymus. The diagnosis was made after exclusion of other variants of SCID presenting with neutropenia, associated with infection or graft-versus-host disease.[11] One patient has been previously reported.[4] Patient characteristics at HSCT are given in Table 1. Age at first HSCT ranged from 0.5 months to 4 months (median 2.5 months). All the patients were nursed within a protective environment and were given non-absorbable antibiotics to suppress their intestinal microflora; they all received weekly intravenous immune globulin therapy.

In the 10 patients, a total of 15 HSCT were performed. Five transplants were performed without the use of conditioning, the others, including three repeat transplants, were preceded by conditioning. Donors were the mothers in 10 transplants, the fathers in three transplants and in one transplant both parents.

In one case, the second HSCT was from a matched unrelated donor (MUD) (patient 8).

Conditioning regimens (Table 2): Conditioning consisted of either cyclophosphamide (four doses at 50 mg/kg) plus busulfan (four doses at 4 mg/kg in five, four doses at 2

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Table 1 Ten patients with reticular dysgenesis: clinical characteristics

Patients	*Sex*	*Age at BMT (m)*	*Clinical status*	*Lymphocytes/μl*
1	M	1 7	Bacterial sepsis, maternal GVHD	0
2	M	3.5	Enteritis, pneumonia, maternal GVHD	400
3	M	3	Enteritis	2800
4	F	2	Enteritis, pneumonia, maternal GVHD	300
5	M	2.5	—	—
6	F	2.5 10	Enteritis, pneumonia	950
7	M	2 10	Enteritis, pneumonia	—
8	M	0.5 11	Enteritis, maternal GVHD	1100
9	M	4	Enteritis, fungal sepsis, hepatitis	200
10	M	2.5 6	Bacterial sepsis	300

mg/kg in three) in eight HSCT, or of cyclophosphamide plus antithymocyte globulin (ATG) in one HSCT. One patient received busulfan alone (four doses of 4 mg/kg) (Table 2). Three recipients also received anti-LFA1 antibody to prevent graft rejection. The mean number of nucleated cells per kilogram infused was 1.4×10^8 (range 0.4 to 29×10^8).

Graft-versus-host disease (GVHD) prophylaxis: All grafts were T cell-depleted using E-rosetting in five cases, soybean agglutination and E-rosetting in three cases, anti-T monoclonal antibodies plus complement in four cases and positive selection of $CD34^+$ cells in two cases. Post-HSCT GVHD prophylaxis in patients receiving E-rosette depleted bone marrow consisted of cyclosporine for 60 days.

Chimerism was studied by karyotyping, HLA typing, blood group typing and more recently by restriction fragment length polymorphism or polymorphic microsatellite genotyping. Lymphocyte populations were identified by immunofluorescence staining with T and B cell-specific monoclonal antibodies.

Results

Clinical features

Clinical findings at diagnosis in the 10 children are summarized in Table 1. As previously reported in RD, the disorder was characterized in our series by the early onset of the symptoms, with severe infections in the first days of life. Among the 10 patients, seven presented with bacterial sepsis and/or pneumonia. Besides agranulocytosis, profound lymphopenia was observed in almost all the cases. When T cells were present and analyzed, they were often maternal T cells that had crossed the placenta,[4,12] but some patients had residual T cells.

Treatment and outcome

In the 10 patients, a total of 15 HSCT was performed.

HSCT without conditioning: Of the four patients who did not receive conditioning prior to initial haploidentical HSCT, two showed evidence of engraftment, which, however, remained incomplete and restricted to T cells, while in two cases engraftment failed completely. Three of these four patients received second transplants. In two of them a

Table 2 Outcome of HLA-haploidentical bone marrow transplantation in 10 patients with reticular dysgenesis

Patients	*Condt*	*Sex donor*	*HLA Comp*	*T cell depletion*	*Engraft T*	*Engraft Myel*	*Outcome*
1	—	F	3/6	Soybean + E-rosett	+	−	Agranulocytosis
	Bu(8)	F	3/6	Soybean + E-rosett	NE	NE	Death. Aspergillosis
2	—	F	3/6	Soybean + E-rosett	+	−	Death. Infection
3	Bu(16),Cy	F	3/6	mAB	+	+	Alive and well
4	Bu(16),Cy	M	3/6	E-rosett	+	+	Alive and well
5	Bu(16),Cy	F	3/6	E-rosett	+	+	Death. cGVHD
6	Bu(8),Cy	M	4/6	mAB	−	−	Graft failure
	Bu(16),Cy	F	5/6	mAB	+	+	Death. Pneumonitis
7	—	F	3/6	E-rosett	−	−	Graft failure
	Cy, ATG	F+M	3/6	E-rosett	+	−	Death. Agranulocytosis
8	—	F	3/6	E-rosett	−	−	Graft failure
	—	M	6/6 MUD		+	−	Death. B-cell LPD, GVHD, Pneumonitis
9	Bu(8),Cy	F	3/6	mAB	NE	NE	Death. Pneumonitis
10	Bu(8),Cy	M	3/6	CD34+selection	−	−	Graft failure
	Bu(16),Cy	F	4/6	CD34+selection	+	+	Alive and well

Bu = busulfan (total dose, mg/kg); Cy = cyclophosphamide; ATG = antithymocyte globulin; mAB = monoclonal antibodies; Condt = conditioning regimen; Comp = compatibility; Engraft = engraftment; Myel = myeloid; GVHD = graft-versus-host disease; LPD = lymphoproliferative disease; E-rosett = E-rosetting; NE = non evaluable.

preparative regimen was used prior to repeat transplants. In the third patient a MUD HSCT without conditioning regimen was performed.

HSCT with conditioning therapy: Ten transplants were performed after conditioning. Two patients died of infectious complications before engraftment could be evaluated.

Donor myeloid and lymphoid cells were detected in five patients (in two cases after a second transplant). All the patients who had myeloid engraftment received pre-BMT conditioning including busulfan (16 mg/kg) and cyclophosphamide and three of them are alive and well. Among the four patients who were given busulfan at a lower dosage of 8 mg/kg, with or without cyclophosphamide, two died early from aspergillosis or pneumonitis and engraftment could not be evaluated, and in the other two cases there was graft failure. In one patient who received conditioning with a combination of ATG and cyclophosphamide, only T cell engraftment was observed with persistent agranulocytosis. This child died from viral pneumonitis.

Acute GVHD (grade >2) occurred in three patients. One patient developed chronic GVHD and died 6 months post HSCT; another patient who received unmanipulated marrow from an unrelated donor developed acute grade 4 GVHD and he died of EBV-associated B-lymphoproliferative syndrome and viral pneumopathy. The third patient developed limited chronic GVHD and is alive and well.

Altogether three of the 10 patients are alive after HLA non-identical HSCT; two patients died of GVHD; the others died from infectious complications (aspergillosis in one case after a second transplant, viral and/or bacterial infections in the other four patients).

Immune and hematological reconstitution

Three of the 10 patients achieved durable myeloid and T cell engraftment. Hematological and immunologic reconstitution in these cases was complete 1 year post HSCT with normal blood counts and functional T and B cells, as based on normal *in vitro* responses to mitogens (PHA), to antigen (tetanus toxoid), normalization of serum immunoglobulin levels and positive antibody response to antigens in the absence of immunoglobulin substitution.

Discussion

The primary defect in RD is as yet undefined. The disease is likely to be autosomal recessively inherited since both parental consanguinity[8] and familial forms affecting males and females have been reported.[1,9,10,13] Several factors can explain the poor prognosis of RD patients with the lack of neutrophils being responsible for severe infections. Of nine patients reported before 1983, all died, six before day 15 and the others at day 50, 84 and 119.[1,2,7–10] As previously reported, the diagnosis of RD in our series was made at a younger age as compared to other SCID patients. In RD, pluripotent hematopoietic stem cells are present, since differentiation of erythropoietic and megakaryocytic lineages is normal in the majority of the cases.[1,8–10,14] The most common finding in the bone marrow is a maturation arrest of the myeloid lineage with complete absence of cells beyond the promyelocyte stage. In addition, lymphoid development is profoundly deficient, with thymic aplasia and lack of lymphoid organs, lymphocytopenia and lack of lymphoid functions.[7–9] Some patients have been reported to have circulating T cells, but these cells were probably of maternal origin.[4,12] In some cases with SCID, suppression of myelopoiesis may also be induced by maternal T cells, thus representing a secondary phenomenon. Niehues *et al*[11] showed in a recent report on twins with SCID and neutropenia, that the abnormality was responsive to treatment with GM-CSF/G-CSF. In contrast, in RD sustained responses to myeloid growth factors have not been observed.[15] The association of bilateral sensorineural deafness and RD was recently published; it should be also considered in the follow-up of such patients.[6]

Experience of HLA genetically identical HSCT in RD as reported to the European registry is limited to three cases. All three patients are alive. However, even after these transplants the observation of persistent agranulocytosis in one patient favors the use of a myeloablative conditioning regimen prior to HSCT. Bujan *et al*[15] mentioned a patient with RD who received an HLA-identical HSCT without conditioning; there was only T cell engraftment. Myeloid engraftment was observed only after a second HSCT with a conditioning regimen including busulfan and cyclophosphamide.

In our study HLA-non identical HSCT resulted in only three of the 10 patients surviving. The main reason was graft failure. This complication seems to be prevented with the use of a myeloablative regimen prior to HSCT, as already reported by De Santes *et al*.[5] These authors described two cases of RD who failed at one and two transplants attempts, respectively; HSCT was successful only after an intensive conditioning regimen utilizing total body irradiation. Patients with RD thus appear to require intensive conditioning. The busulfan dose of 8 mg/kg does not seem sufficient to allow stable engraftment of donor hematopoietic stem cells, since all the attempts with this dosage were unsuccessful. The three patients who survived with full engraftment all received a total dose of 16 mg/kg of busulfan.

The mandatory need of a myeloablative conditioning regimen prior to HLA-non identical HSCT is particular to RD. In other SCID variants without myeloid defects, engraftment can be achieved in the absence of myeloablative treatment before HSCT, although usually only of T cells, as reported recently by Buckley *et al*.[16] In this series, 68 of the 72 survivors developed normal T cell function, while B cell function remained abnormal in the majority of patients. We also studied haploidentical bone marrow transplantation in B+ SCID and observed that the rate of T cell engraftment was not statistically significant with or without the use of a conditioning regimen.[17] In summary, our findings in this series of 10 patients with RD indicate that intensive myeloablative preparatory conditioning is required in this disorder to obtain reconstitution of both lymphoid and myeloid functions. This seems to hold true both for HLA haplo-identical, T cell-depleted transplants, as well as for HLA-identical transplants, based on the lim-

ited experience published to date. In future patients, these considerations could improve treatment outcome.

References

1 De Vaal OM, Seynhaeve V. Reticular dysgenesia. *Lancet* 1959; **ii**: 1123–1125.
2 Gitlin D, Vawter G, Craig JM. Thymic alymphoplasia and congenital aleukocytosis. *Pediatrics* 1964; **33**: 184–191.
3 Levinsky RJ, Tiedeman K. Successful bone marrow transplantation for reticular dysgenesis. *Lancet* 1983; **i**: 671–672.
4 Friedrich W, Goldmann SF, Ebell W *et al*. Severe combined immunodeficiency: treatment by bone marrow transplantation in 15 infants using HLA-haploidentical donors. *Eur J Pediatr* 1985; **144**: 125–130.
5 De Santes KB, Lai SS, Cowan MJ. Haploidentical bone marrow transplantation for two patients with reticular dysgenesis. *Bone Marrow Transplant* 1996; **17**: 1171–1173.
6 Small TN, Wall DA, Kurtzberg J*et al*. Association of reticular dysgenesis (thymic alymphoplasia and congenital aleukocytosis) with bilateral sensorineural deafness. *J Pediatr* 1999; **135**: 387–389.
7 Alonso K, Dew JM, Starke WR. Thymic alymphoplasia and congenital aleukocytosis (reticular dysgenesia). *Arch Pathol* 1972; **94**: 179–183.
8 Haas RJ, Niethammer D, Goldmann SF *et al*. Congenital immunodeficiency and agranulocytosis (reticular dysgenesia). *Act Paediatr Scand* 1977; **66**: 279–283.
9 Ownby DR, Pizzo S, Blackmon L *et al*. Severe combined immunodeficiency with leukopenia (reticular dysgenesis) in siblings: immunologic and histopathologic findings. *J Pediatr* 1976; **89**: 382–387.
10 Espanol T, Compte J, Alvarez C *et al*. Reticular dysgenesis: report of two brothers. *Clin Exp Immunol* 1979; **38**: 615–620.
11 Niehues T, Schwarz K, Schneider M *et al*. Severe combined immunodeficiency (SCID) associated neutropenia: a lesson from monozygotic twins. *Arch Dis Child* 1996; **74**: 340–342.
12 Heymer B, Friedrich W, Knobloch C, Goldmann SF. Reticular dysgenesis: primary disorder in differentiation of hematopoietic stem cells? *Verh Dtsch Ges Path* 1990; **74**: 106–110.
13 De La Calle Martin O, Badell I, Garcia A *et al*. B cells and monocytes are not developmentally affected in a case of reticular dysgenesis. *Clin Exp Immunol* 1997; **110**: 392–396.
14 Roper M, Parmley RT, Crist WM *et al*. Severe combined leukopenia (reticular dysgenesis). Immunologic and morphologic characterizations of leukocytes. *Am J Dis Child* 1985; **139**: 832–835.
15 Bujan W, Ferster A, Azzi N *et al*. Use of recombinant human granulocyte colony-stimulating factor in reticular dysgenesis. *Br J Haematol* 1992; **81**: 128–129.
16 Buckley RH, Schiff SE, Schiff RI *et al*. Hematopoietic stem-cell transplantation for the treatment of severe combined immunodeficiency. *New Engl J Med* 1999; **340**: 508–516.
17 Bertrand Y, Landais P, Friedrich W *et al*. Influence of SCID phenotype on the outcome of HLAnon identical, T-cell depleted bone marrow transplantation. A retrospective European survey from the EBMT and the ESID. *J Pediatr* 1999; **134**: 740–748.