Changes in Antigenic Characteristics of Erythrocytes and Number Metaphase Chromosome in Bone Marrow Cells after Experimental and Clinical Bone Marrow Transplantation

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Transplantation of allogenic bone marrow from HLA-identical sibs to patients with acute and chronic leukemia receiving immunosuppressive therapy is associated with the appearance of erythrocytes simultaneously carrying donor and recipient antigenic markers: AB0 system, rhesus factor and its subtypes, M and N antigens. Integration of genes responsible for each antigen is realized independently presumably at the level of stem cell, which ensures long-term (>3 years) repopulation of these erythrocytes. Experiments on inbred mice showed that transplantation of allogenic bone marrow is associated with an increase of chromosome number in 39% bone marrow cells 4 days after transplantation, which indicate the possibility of integration of whole chromosomes.

Key Words: *bone marrow transplantation; "hybrid" erythrocytes; chromosomes; hyperploidy*

Transplantation of allogenic bone marrow (BM) to patients with suppressed immune reactivity from HLA-identical sibs results in BM take and formation of virtually 100% blood chimerism [3,4,7,8,10,11]. This manifests in replacement of the recipient blood group and rhesus factor with the donor blood group and rhesus factor. Apart from this known fact, erythrocytes simultaneously carrying both donor and recipient antigenic markers were for the first time detected in recipients after transplantation of BM of a different group [2,4]. Long-term circulation of these erythrocytes with double antigenic markers (hybrid erythrocytes) attests to gene exchange between the donor and recipient cells at the early stages of erythropoiesis (hemopoietic stem cells). If not, repopulation of the hybrid clone would not be maintained for a period exceeding the mean erythrocyte life span.

The mechanism of gene exchange remains unclear and deserve further investigation under clinical (transplantation of allogenic bone marrow) and experimental conditions.

Here we studied manifestation of erythrocytes hybridization after BM transplantation under different conditions. Another purpose of this study was to create an experimental model as an approach to explaining the mechanism of hybrid erythrocyte appearance.

MATERIALS AND METHODS

The study was carried out in 26 patients mainly with acute and chronic myeloleukemia after HLA-identical BM transplantation from sibs with the same and different blood groups, rhesus factor and its subtypes,

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and M and N antigens. Blood groups and rhesus factor of the donor, recipient, and sometimes their parents were determined by standard immunological methods. Before BM transplantation the patients with acute and chronic leukemia received cytostatic (immunosuppressive) therapy. Since the appearance of hybrid cells indicates changes in the chromosome system of these cells, we investigated changes in the recipient BM cell chromosome system in animals subjected to immunosuppression and BM transplantation.

In animals experiments female CBA mice served as recipients. The immunoreactivity of animals was suppressed by exposure to whole-body γ-irradiation (^{137}Cs) in a dose of 350 R (22.5 R/min power). Intact males of the same strain served as donors. Donor BM cells were isolated from tibial diaphyses and injected to irradiated recipients into the retrobulbar sinus in a dose of 5×10^5 cells/mouse in 0.24 ml medium 199 (Sigma).

In group 1 recipients were sacrificed by cervical dislocation 4 days after transplantation (start of BM recovery after irradiation). It is considered that radiation-induced changes in chromosome number appear starting from mitosis II [1].

Group 2 mice were sacrificed 14 days after BM transplantation and 4 days after blood loss (1% body weight) preceding sacrifice. Blood loss was used for stimulation of BM proliferation which, as we suggested, will promote changes in their chromosome system. Chromosome preparations from recipient BM cells were prepared by routine methods [6,7]. Colchicine (0.025% solution) in a dose of 0.1 ml/10 g was injected intraperitoneally 2 h before sacrifice for accumulation of metaphase cells. BM was washed out with warm (37°C) 1% sodium citrate. The resultant cell suspension was incubated at the same temperature for 35-40 min, centrifuged at 1000-15,000 rpm for 5 min, and fixed in a methanol-glacial acetic acid mixture (3:1), after which the suspension was pipetted onto cold slides and stained with Giemza dye. Metaphases were analyzed under a Zeiss microscope at ×1000. Chromosomes were counted on photographs in cells with round or oval nuclei. The percentage of metaphase plates with abnormal chromosome number was estimated for each animal from the total number of counted metaphases and the mean percentage for the whole group was estimated. The data were statistically processed using Fisher—Student's test.

RESULTS

Signs of erythrocyte hybridization were detected in 16 of 26 patients. These findings confirmed our previous data on the appearance of hybrid AB(IV) group erythrocytes after BM transplantation from B(III) donor to A(II) recipient [2,4]. Circulation of these cells (up to 5.5 months) sooner or later eventuates in their disappearance from the blood, because their antigens are foreign to both the donor and recipient. It was also shown that hybrid erythrocytes appear in cases when the donor and recipient had the same blood group, *e. g.* A0(II) group, if the donor-recipient pair was heterozygotic and both had A0(II), which was confirmed by examinations of their parents. Under these conditions hybrid erythrocytes had 00(I) phenotype. The number of these hybrid cells attained 40% and remained high for a long time. In one patient the maximum number of hybrid cells was 35% and 3 years after BM transplantation it was 10%.

The regularities of changes in rhesus factor and its subtypes after the appearance of hybrid 00(I) erythrocytes are of particular importance. When the donor and recipient differ in rhesus factor, some hybrid erythrocytes were rhesus-positive and the others were rhesus-negative (Table 1). Two waves of appearance of hybrid cell were observed in these cases. These cells first appeared, when the level of donor cells was lower than the level of hybrid cells, because of donor cell death, and when erythroid precursor cells actively proliferate yielding high level of erythrocytes of the recipient phenotype. After the start of active proliferation of donor erythroid precursor cells replacing dying recipient erythrocytes, the second wave of hybrid cell circulation was noted. One more observation indicates that a combination of two processes promoted the appearance of hybrid erythrocytes: intense proliferation, on the one hand, and intense cell death, on the other.

TABLE 1. Antigenic Variants of Hybrid Erythrocytes in BM Recipients

Patient	Erythrocyte antigens		Hybrid cells
	recipient	donor	
C.	A0(II)Rh^-	A0(II)Rh^+	0(I)rh^-+0(I)Rh^+
N.	A(II)Rh^+ (DccEe) N	A(II)rh^- (ddccee) M	A(II)Rh^+ (DccEe) MN
L.	A0(II)Rh^+ (DccEe)	A0(II)Rh^+ (DccEe)	0(I)Rh^+ (DccEE)+0(I)Rh^+ (Dccee)
S.	A0(II) Rh^+ (DccEe)	0(I)Rh^+ (DCCee)	0(I)Rh^+ (Dccee)
V.	0(I)Rh^+ (DccEe)	A0(II)Rh^+ (DCCee)	0(I)Rh^+ (DCCEe)

TABLE 2. Changes in Chromosome Number in BM Cells after Allogenic BM Transplantation in Mice (*M*±*m*)

Group		Number of metaphases	Number of chromosomes in metaphase and number of such metaphases in group (%)				
			<38	38-39	40	41-42	>43
Control	intact	244	14.8±2.6	30.5±2.3	47.0±2.9	8.1±2.5	0.5±0.5
	irradiated	109	25.4±7.5	33.4±3.7	33.2±4.3	6.5±1.3	1.3±1.0
Experiment	1st	165	16.4±7.6	22.5±5.0	23.0±4.3	30.3±4.0	8.4±2.8
	2nd	129	28.6±4.2	19.4±4.5	23.1±3.8	15.9±3.8	13.0±3.8

Patient N. with A(II)Rh^+ (DccEe)N group received BM from donor with A(II)Rh^- (ddccee) MN group. After successful transplantation erythrocytes of the recipient phenotype were completely replaced with cells with the donor phenotype. Five months after transplantation a relapse of the disease developed with complete restoration of erythrocytes with the recipient phenotype and rhesus factor. However 24% recipient Rh^+ erythrocytes carried not only N, but also M antigen, which was absent in the recipient before transplantation. In case of complete identity of the rhesus factor and its subtypes, for example, rhesus-positive donor A0 Rh^+ (DcE/Dce) and rhesus-positive recipient A0 Rh^+ (DcE/Dce), after BM transplantation hybrid 00 erythrocytes remained Rh^+, but DccEE and Dccee, which did not correspond to the phenotype of rhesus variants of the transplantation partners but corresponded to their parents' phenotype.

Circulation of erythrocytes with rhesus factor subtypes different from both the donor and recipient factors indicates hybridization of erythroid cells. For example, patient S. A(II)Rh^+ (DccEe) received BM from her sister 0(I)Rh^+ (DCCee); after 3 months apart from erythrocytes of the donor phenotype, there were erythrocytes with 0(I)Rh^+ (DCcee). Patient V. with erythrocyte phenotype 0(I)Rh^+ (DCcEe) received BM from his sister with A(II)Rh^+ (DCCee) phenotype; after 2 months there were no A(II) erythrocytes, but there were 0(I)Rh^+ (DCCEe) erythrocytes, differing by the rhesus factor variant from both the donor and recipient. In many patients identification of hybrid erythrocytes was difficult because in some combinations of donor and recipient antigens hybrid erythrocyte antigens could coincide with donor antigens. The actual number of hybrid erythrocytes can be much higher than we detected in this study, and integration of the donor and recipient genetic material can involve genes located on other chromosomes. These data indicate that at least chromosomes 1 and 9 are independently involved in hybridization (Table 1).

Experimental part of our study was aimed at elucidation whether donor or recipient cells could integrate whole chromosomes, which can be seen from their increased number (hyperploidy) after BM transplantation. Analysis of the number of chromosomes in recipient mouse BM cells 4 days after transplantation showed that this intervention was associated with a significant increase ($p<0.001$) in the number of chromosomes in 39% cells.

The number of cells with 41-42 chromosomes and particularly with 43 and more chromosomes several times surpassed the values in intact and irradiated controls (Table 2).

Up to 52 chromosomes were found in some cells. Fourteen days after BM transplantation the number of cells with 41-42 chromosomes decreased significantly in comparison with group 1, while the number of cells with 43 chromosomes increased significantly. A significant decrease in the number of cells with normal chromosome number can result from their loss during preparation [1] and, presumably, integration of their chromosomes by other cells. Increase of chromosome number virtually cannot be an artifact [1]. We can only hypothesize that an increase of chromosome number can result from either incomplete or complete fusion of some donor and recipient cells with subsequent release of the greater part of unnecessary chromosomes, as is observed in creation of hybridomas. Monocytes and macrophages are liable to fuse. The former form osteoclasts with sometimes up to 100 nuclei as a result of fusion. The latter fuse to form giant cells round a foreign body. Chorionic cells also can fuse. Fusion of cells of different types, for example B lymphocytes and dendritic cells, is observed in Hodgkin's disease; it is the so-called Reed—Sternberg cells. Macrophages absorb apoptotic bodies of tumor cells and carry them to other tissues, which is believed to be responsible for tumor metastasizing [9]. Apoptotic bodies are larger than chromosomes, and through macrophages they can get into a cell. But the cell has an important barrier: nuclear membrane. It is considered to reliably protect the cell genome from foreign information. However the nuclear membrane disappears at the moment of cell division and hence, the chromosome in the cytoplasm can mix with chromosomes freely lying in the cytoplasm during

mitosis, when the nuclear membrane disappears. Increasing capacity of regenerating BM to phagocytosis can promote this process. Such an interpretation of experimental data explains the above clinical and experimental findings and offers an answer to the problems of the mechanism of therapeutic effect and sources of regeneration material in the so-called cell therapy, widely discussed today.

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