GENETICS

Resistance of Mice Exposed to Whole-Body Irradiation to Transplanted Hemopoietic Cells Modified with RNA Preparations

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The resistance of irradiated mice to bone marrow grafts treated with RNA from the liver of syngenic, allogenic, or xenogenic (rat) species was studied. Syngenic recipients developed resistance to grafts treated with allogenic RNA. The resistance to allografts increased after treatment with xenogenic RNA, whereas treatment of allo- and xenografts with syngenic RNA facilitated their take.

Key words: *bone marrow grafts; RNA; modulation of resistance to grafts*

Our previous studies showed that total or poly-A$^+$ RNA extracted from mouse or rat liver can induce in mouse tumor cells the expression of antigens characteristic of RNA donor cells and capable of increasing the resistance of the mice to transplantation of these cells [2-5]. Expression of the corresponding antigens can be induced by exogenous RNA in normal cells [1,6-8], including murine hemopoietic stem cells [1]. Experiments with transplantation of bone marrow (BM) cells from homozygous parents to lethally irradiated F_1 hybrid mice showed that preincubation hemopoietic cells with recipient's RNA abolished hybrid resistance verified by the spleen colony formation test [1]. In light of this, the possibility of regulation of the host-graft relationship by targeted phenotypic (antigenic) modification of grafted cells with RNA preparations is of special interest. This work was designed to study the resistance of BM recipient to grafts treated with RNA from various sources.

MATERIALS AND METHODS

BM recipients were male A/He, CC57BR, and BALB/c mice; the donors were syngenic and allogenic mice and Wistar rats. The recipients were subjected to whole-body irradiation in a RUP-150/300-10-1 x-ray apparatus in a dose of 0.47 Gy/min 24 h before grafting. BM cells isolated from the femur were washed with cold Eagle's medium and resuspended in the same medium to a concentration of 0.5×10^6 cells/ml for syngenic and 3×10^7 cells/ml for allogenic and xerogenic grafting. The cells were incubated in the absence or presence of RNA (2 mg/ml) for 1.5 h at 4°C, and then for 30 min at 37°C. After incubation, the cells were washed with Eagle's medium, and 0.5 ml aliquots containing 2×10^5 syngenic or 1.5×10^7 nonsyngenic nucleated cells were injected into the caudal vein of irradiated mice. RNA was isolated from the liver as described elsewhere [4]. Alternatively, yeast RNA (Vector) and synthetic poly-A ribonucleotide (polyadenylic acid, Reanal) were used. The ability of liver RNA preparations to induce during incubation with tumor cells the expression of surface antigens of the RNA donor was assayed by cytotoxicity test with the corresponding antiserum [2]. In some experiments, control tests were performed with RNA samples that cause no such effect; our previous studies [4] showed that partial degradation of RNA during isolation abolished this effect. The 90-day survival rates were recorded, and the

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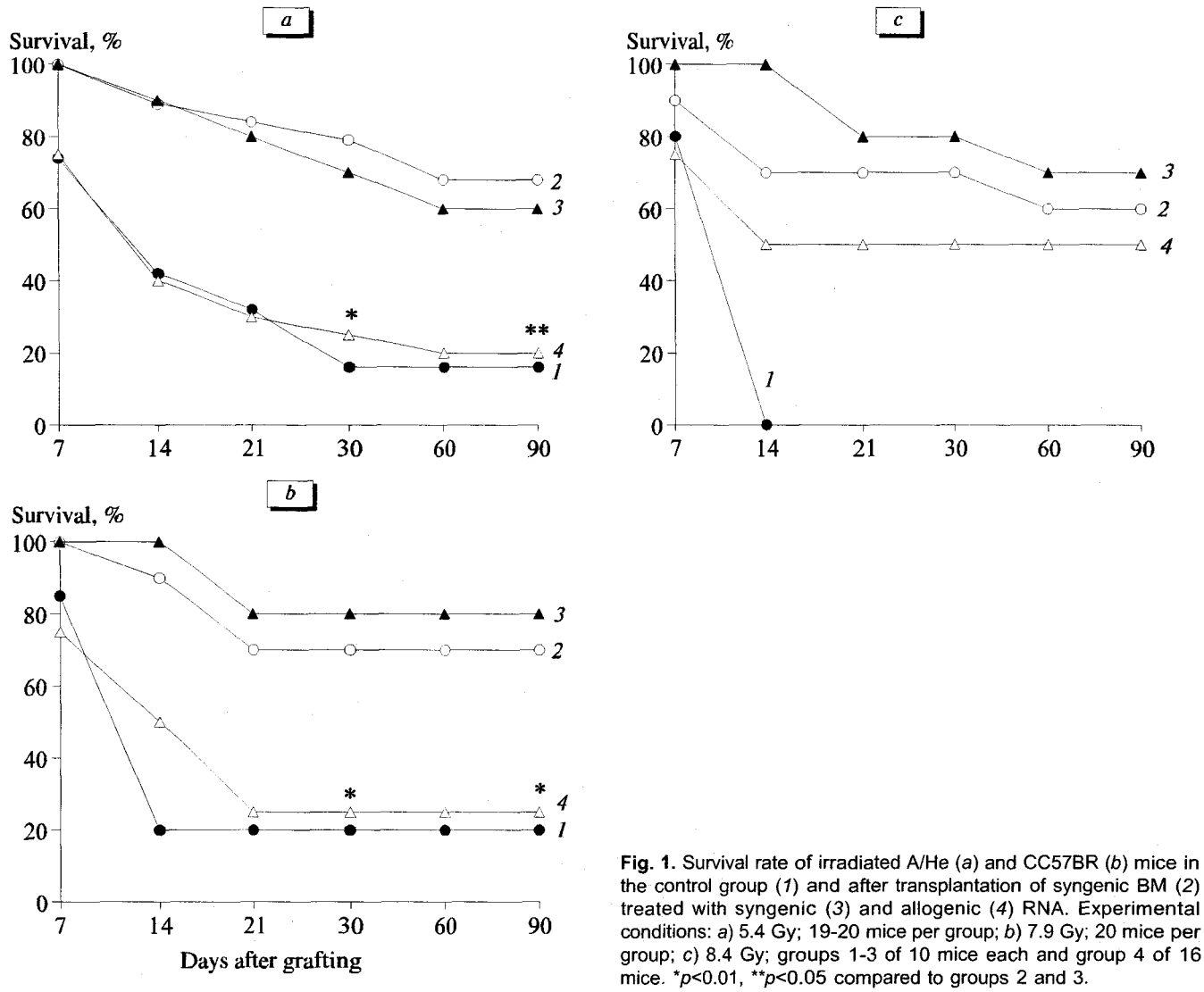

Fig. 1. Survival rate of irradiated A/He (*a*) and CC57BR (*b*) mice in the control group (*1*) and after transplantation of syngenic BM (*2*) treated with syngenic (*3*) and allogenic (*4*) RNA. Experimental conditions: *a*) 5.4 Gy; 19-20 mice per group; *b*) 7.9 Gy; 20 mice per group; *c*) 8.4 Gy; groups 1-3 of 10 mice each and group 4 of 16 mice. *$p<0.01$, **$p<0.05$ compared to groups 2 and 3.

recipient's resistance was determined by the degree of the defense response within the first 21-30 days, *i.e.*, before the "secondary" death of recipients due to the graft-versus-host response (GVHR). The significance of differences determined by Fisher's *f* test (arc sin transformation).

RESULTS

Treatment of BM cells with syngenic RNA had no effect on their ability to prevent radiation-induced deaths irrespective of the dose of irradiation (Fig. 1). Cells treated with allogenic RNA induced no protective effect on recipients receiving relatively low doses (LD_{80}), but protected the mice receiving LD_{100} (*i.e.*, under conditions of deep suppression of recipient's resistance) (Fig. 1). Thus, hemopoietic cells treated with allogenic RNA are inactivated in a syngenic recipient, which is related to the host resistance. It can be assumed that hemopoietic cells treated with allogenic RNA express antigenic structures characteristic of the source strain and induce host responses.

For studying the effects of RNA on inactivation of hemopoietic cells in allogenic recipients, BM cells from CC57BR (H-2^b) were transplanted to A/He (H-2^a) mice irradiated at a mid-lethal dose. Transplantation of intact BM had no protective effect on recipients irradiated at this dose, probably because of preserved capacity of recipient to develop strong reactions to allogenic cells. The treatment of these cells with RNA from CC57BR mice (BM donors), yeast RNA, or poly-A had no effect on their inactivation in allogenic recipients (Table 1). However, treatment of grafted cells with RNA of the recipient strain (A/He) considerably reduced animal postirradiation mortality over 30 days (Table 1), which attested to weakening of host resistance to these cells and successful graft take. In these experiments, recipient's survival rates remained high

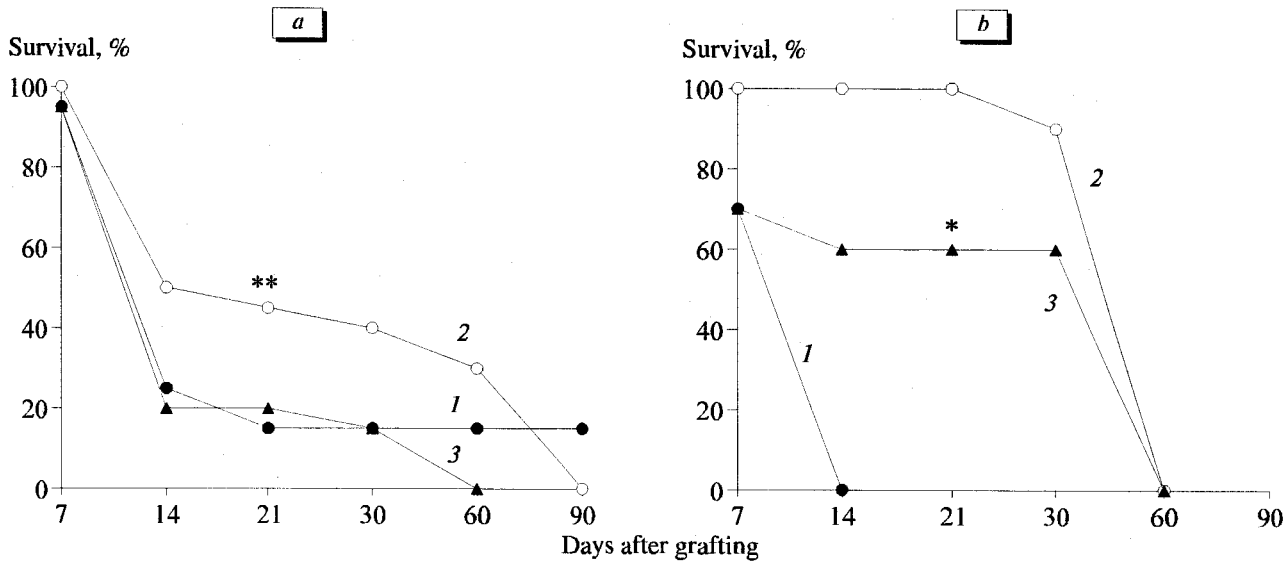

Fig. 2. Survival rate in irradiated A/He mice in the control group (*1*) and after transplantation of BM from CC57BR mice (*2*) treated with rat RNA (*3*). Irradiation doses 6.9 (*a*) and 7.4 (*b*) Gy; each group consisted of 20 (*a*) or 10 (*b*) mice. *$p<0.01$, **$p<0.05$ compared to the control.

over 2-month follow-up. The take of intact BM cells that providing similar 30-day survival rate required absolutely lethal irradiation; however, all animals survived the acute period died of GVHR by day 60. Treatment of BM cells with rat liver DNA decreased their protective effect even after irradiation of recipients in absolutely lethal doses (Fig. 2).

Reduced recipient's resistance to BM cells treated with RNA of the recipient strain was observed in experiments with allogenic transplantation C3H ($H2^k$)→ BALB/c (H-2^d) and grafting of BM from Wistar rats to BALB/c (Fig. 3). RNA species unable to change the antigenic phenotype of tumor cells produced no such effects. The early (21 days) and delayed (60 days) mortalities in lethally irradiated recipients after grafting of allogenic BM treated with RNA of the recipient strain were 7 and 53%, respectively, compared to 33 and 60% after transplantation of intact BM (Fig. 3). This means that changes induced by this RNA in hemopoietic cells promoted their take under conditions of allogenic resistance, but had no effect on the development of GVHR.

Taken together, these results suggest that the treatment of grafted BM cells with high-polymer RNA extracted from mouse or rat liver strongly modulates recipients resistance to these cells and their take, depending on the genetic relationship between the BM recipient and RNA source trains. In the case of allogenic relationships, recipients develop the resistance to syngenic cells, whereas syngenic relationships decrease recipient's resistance to allogenic and xenogenic hemopoietic cells. This effect can be explained by the ability of RNA to induce specific changes in antigen structures and the appearance of antigens characteristic of the RNA donor. These changes in grafted hemopoietic cells considerably modulate the interac-

TABLE 1. Survival Rate (%) of A/He Rats Irradiated in a Dose of 4.7 Gy after Transplantation of BM Cells from CC57BR Mice Treated with Various RNA Preparations

Group	Day after grafting					
	7	14	21	30	60	90
Control (n=63)	100	61	54	54	54	54
BM (n=69)	99	69	51	51	51	47
BM+yeast RNA (n=20)	100	65	60	55	55	55
Bm+polyA (n=29)	96	65	52	52	52	41
BM+CC57BR RNA (n=20)	95	60	60	50	50	50
BM+A/He RNA (n=20)	100	100	100	95*	90	90**

Note. *$p<0.001$, **$p<0.01$ compared to the control.

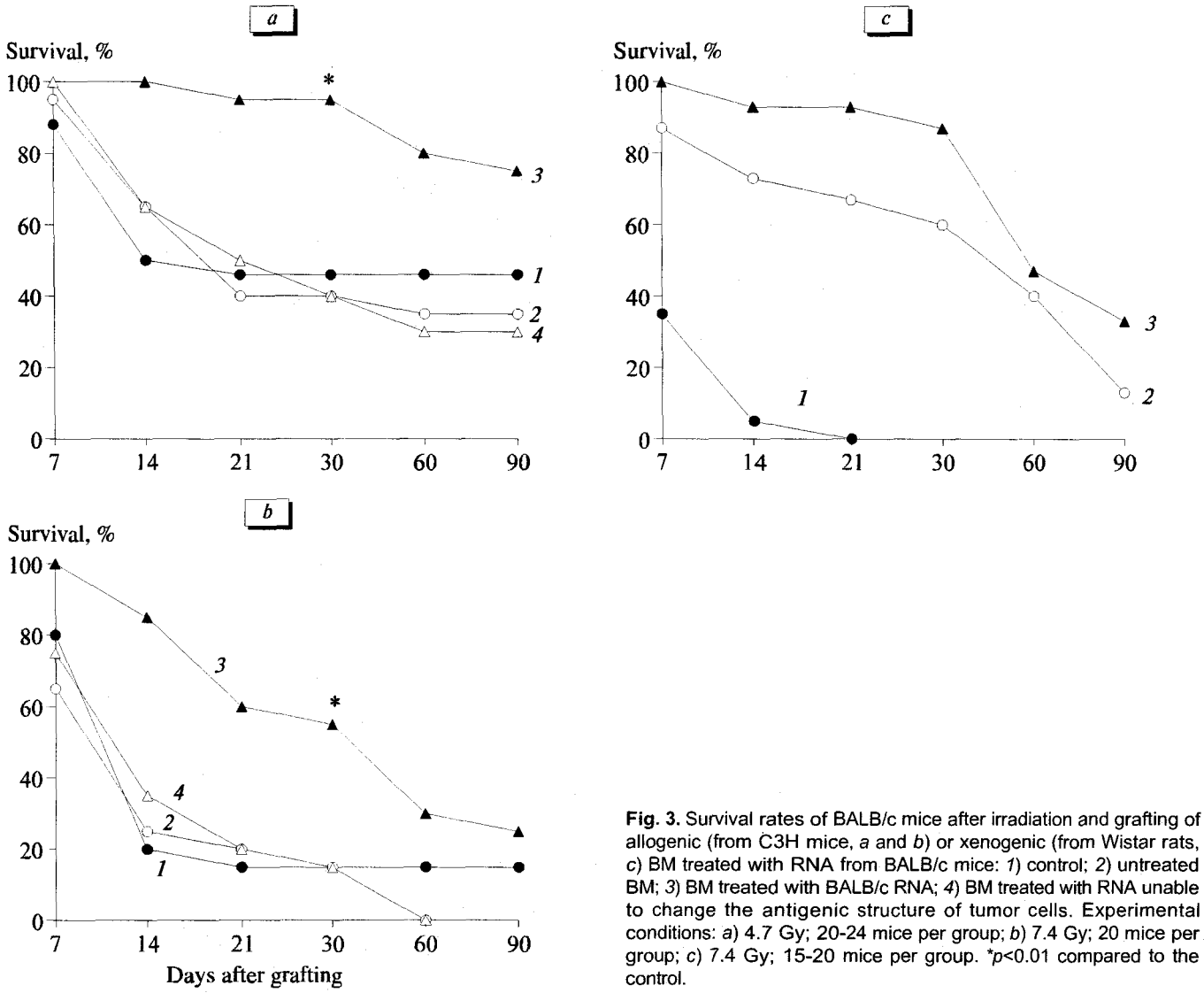

Fig. 3. Survival rates of BALB/c mice after irradiation and grafting of allogenic (from C3H mice, *a* and *b*) or xenogenic (from Wistar rats, *c*) BM treated with RNA from BALB/c mice: *1*) control; *2*) untreated BM; *3*) BM treated with BALB/c RNA; *4*) BM treated with RNA unable to change the antigenic structure of tumor cells. Experimental conditions: *a*) 4.7 Gy; 20-24 mice per group; *b*) 7.4 Gy; 20 mice per group; *c*) 7.4 Gy; 15-20 mice per group. *$p<0.01$ compared to the control.

tion of grafted cells with the host hemopoietic environment [1] and the effector function of cells responsible for resistance.

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