Effect of γ-Irradiation on the Plasma Content and Glycosylation of Transferrin in Mice

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The content and glycosylation of transferrin are studied in mice 30 days after whole-body γ-irradiation in doses of 775, 825, and 900 rad using polyacrylamide gel electrophoresis with amido black and Schiff reagent staining. Two electrophoretically distinct transferrin fractions are identified. The total content of transferrin decreases more than 2-fold; the decrease in the sialylated fraction being even greater. Irradiation lowers the degree of glycosylation in both fractions. These changes may play an important role in suppression of hemopoiesis in irradiated mice.

Key Words: *transferrin; glycosylation; blood plasma; mice; ionizing irradiation*

Damage to hemopoietic organs is one of the mechanisms underlying the effect of ionizing irradiation on the living organism. Production of blood cells, particularly of the erythroid row cells, is coupled with iron metabolism. These processes may be important for restoration of bone marrow cells and their function after irradiation. Since transferrin (TF), one of the main plasma glycoproteins (GP), is the major protein of iron metabolism [11], the evaluation of its role in postirradiation processes is relevant. There is controversy over the blood TF content after irradiation. It was reported that plasma TF content decreases in rats irradiated with lethal doses [10] as well as in humans exposed to radiation [8]. At the same time, there is evidence that plasma TF markedly increases in mice and dogs exposed to mixed γ-neutron irradiation [12]. Nevertheless, the effects of γ-irradiation on TF glycosylation are unknown, although functional properties of this protein strongly depend on its glycosylation. For example, desialylated GP more actively donates iron than sialylated protein [11].

The aim of this study was to determine the blood content and the degree of glycosylation of TF in mice during the postirradiation period (30 days after γ-irradiation) using zonal electrophoresis in polyacrylamide gel. The primary objective was to identify TF after electrophoretic separation of mouse plasma proteins under our experimental conditions.

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MATERIALS AND METHODS

Male (CBA×C57Bl/6) F_1 mice weighing 24-28 g were used. The animals were maintained on standard vivarium diet. They were irradiated in an IGUR installation at a dose power of 1.74 Gy/min (whole-body γ-irradiation, ^{137}Cs). On day 30 after irradiation, the survived mice were decapitated under light ether anesthesia. Plasma was obtained by centrifugation of heparinized blood at 1500*g* for 20 min. The content and glycosylation of two TF isoforms were estimated by the method based on electrophoretic separation of plasma proteins [2]. Horizontal electrophoresis in 3.5% polyacrylamide gel was performed in a Multiphor apparatus (model 2117, LKB) in 0.2 M Tris-glycine buffer, pH 8.9 [2]. Protein bands were stained with amido black (Serva) or Schiff reagent [2]. Since commercial mouse TF preparations necessary for TF localization are practically unavailable, we used two indirect approaches to the identification of TF on the plasma protein electrophoregrams: 1) comparison of the electrophoretic pattern of plasma proteins and relative mobility of mouse TF with those of human

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plasma proteins and 2) removal of all plasma globulins except TF by sequentional sedimentation with rivanol (final concentration 0.31%) and ethanol (final concentration 25%) [9]. After this procedure TF remains in the solution, and its band is clear cut on electrophoregram. The content and glycosylation of TF were quantified by densitometry of electrophoregrams; the contents of TF and carbohydrates in the TF fraction were measured in relative units, i.e., the TF area on the densitograms (cm^2) standardized to one microgram of total plasma protein. Plasma proteins were measured after staining with Coomassie G-250 [13]. The amount of total protein applied onto electrophoretic lane (100-600 μg) strongly correlated with the contents of TF and carbohydrates in the TF fraction (r=0.968 and 0.990, respectively). The absolute plasma TF concentration was calculated from a calibration curve constructed with the aid of parallel electrophoresis of varied concentrations of commercial human TF (Fluka). Glycosylation (rel. units) was calculated as a ratio of carbohydrate content in TF fraction (rel. units) to the total TF content (rel. units).

From three to six mice were used for each experimental point. The data were analyzed using the Student—Fisher t test.

RESULTS

Analysis of electrophoregrams of human and mouse plasma proteins stained with amido black or Schiff reagent shows that human and mouse TF have different electrophoretic mobilities (Fig. 1). Therefore, the location of mouse TF can be only approximately determined by extrapolation of the electrophoretic pattern of human serum proteins. However, successive rivanol—ethanol precipitation proved that the assumed fraction represents TF, since no other β-globulin and immunoglobulin bands were seen on electrophoregrams after this procedure, while the TF fraction, which did not precipitate under these conditions [9], was the only protein fraction (Fig. 1, *a*, *b*). This was clearly seen on electrophoregrams stained with Schiff reagent (Fig. 1, *b*).

Analysis of the electrophoregrams revealed a higher degree of glycosylation in human TF compared with that of mouse TF (Fig. 1, *b*). This is consistent with the observation that the sialic acid content of human plasma TF is higher than that of rodent TF [11,14]. It should be emphasized that on electrophoregrams stained with amido black the TF fraction appeared as two separate subfractions: slow TF1 and fast TF2. In rodents, the degree of glycosylation of these subfractions is different: TF1 contains 2 sialic residues per molecule, while TF2 contains approximately 3 residues [14].

After a dose of 825 rad, the total plasma content of TF decreased. An increase in the irradiation dose to 900 rad led to further decrease in TF content: it dropped to 29% of the control. The fast TF2 fraction was more sensitive to irradiation: its content dropped

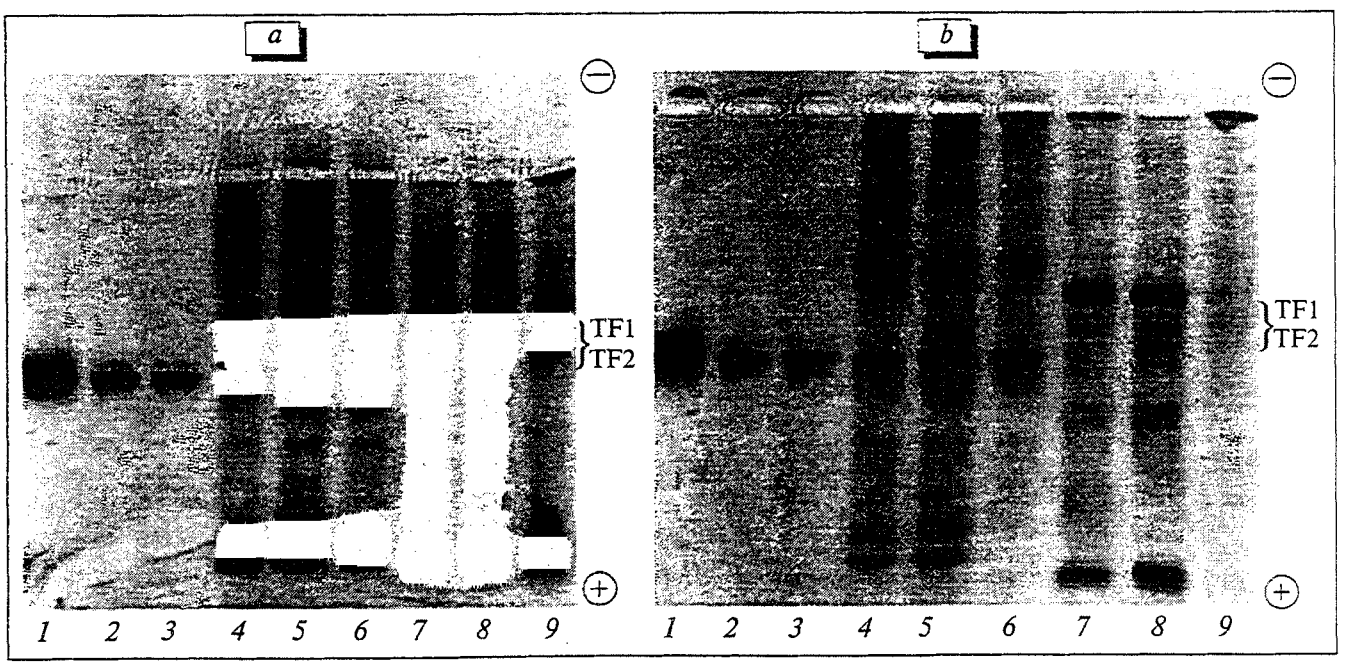

Fig. 1. Polyacrylamide gel electrophoresis of human serum and mouse plasma at different stages of transferrin (TF) purification. Staining with amido black (*a*) and Schiff reagent (*b*). *1-3*) standard human TF, *4-6*) human serum, *7-9*) mouse plasma. *1*, *4*, *7*) initial preparations; *2*, *5*, *8*) rivanol treatment; *3*, *6*, *9*) ethanol treatment.

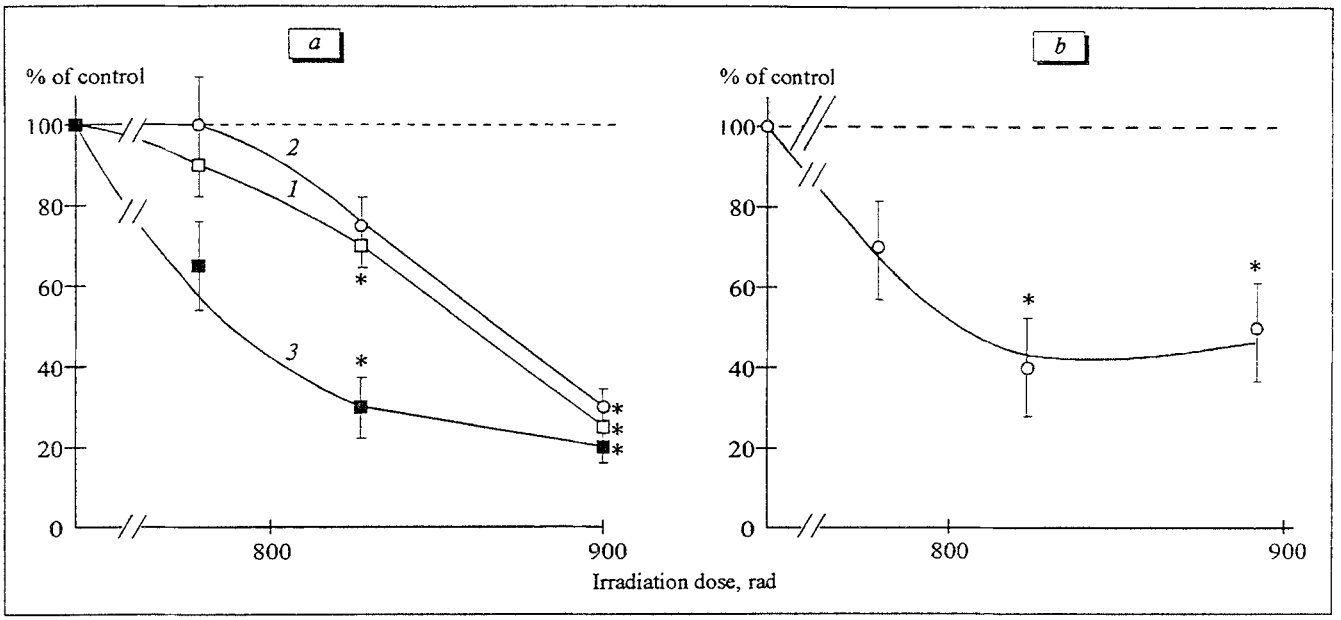

Fig. 2. Content of transferrin (TF, *a*) and its glycosylation (*b*) in mouse plasma 30 days after irradiation. *a*) total TF (*1*), TF1 (*2*), and TF2 (*3*); *b*) glycosylation of total TF fraction (since glycosylation was equally reduced in both TF subfractions, only one common curve is presented). Control: total TF = 100±6, TF1 = 100±5, TF2 = 100±8, glycosylation of total TF = 100±10; *$p<0.05$ compared with the control.

by 35% after exposure to a dose of 775 rad, while that of TF1 remained unchanged. However, after the maximum dose (900 rad) the content of both fractions dropped to 29% of the control. It should be noted that the total plasma protein content decreased to a much lesser extent in comparison with TF (by 19% of the initial level after 900 rad). This suggests that TF is much more sensitive to irradiation than other plasma proteins.

In addition to a decrease in the total TF content, irradiation markedly lowered the level of TF glycosylation (Fig. 2, *b*). However, despite different sensitivity of TF1 and TF2 to irradiation, glycosylation of both subfractions decreased to the same extent: after exposure to 775 rad it was 71% for TF1 and 63% for TF2, while after 825 rad these values dropped to 37 and 40%, respectively.

Thus, we have demonstrated for the first time that ionizing radiation induces considerable changes in TF glycosylation which persist over 30 days after irradiation. The mechanism of the suppressive effect of irradiation on TF glycosylation remains unknown. However, it was reported that the content of hexosamines, the precursors of GP oligosaccharide chains, in γ-irradiated rats is decreased [4], while the activity of proteases during the postirradiation period increases, which is accompanied by tissue accumulation of low-molecular-weight GP fragments [3]. It can be hypothesized that reduced glycosylation is a consequence of a decrease in the content of GP precursors and activation of proteases and glycosidases which degrade the carbohydrate chains of GP. On the other hand, inhibition of glycosyltransferases, the key enzymes in the synthesis of glycane chains of GP, cannot be ruled out. However, irrespective of the mechanisms underlying disturbances in the GP synthesis and degradation, the unequal changes in the TF content and glycosylation are of particular interest. Indeed, as seen from Fig. 2, 30 days after exposure to a dose of 825 rad the content of TF decreased by 28%, whereas its glycosylation dropped more than 60%. Hence, the reduction of TF glycosylation (or its restoration during the postirradiation period) is more pronounced than inhibition of polypeptide synthesis (or its restoration). Similar shifts were observed with nonradiation factors. For instance, we have previously shown that experimental vitamin A deficiency [7] and administration of zinc-metallothionein markedly enhances the synthesis of some proteins, their glycosylation being delayed. These data can be regarded as additional evidence in favor of a relatively independent regulation of the synthesis of polypeptide and glycane chains [5], the glycosylation systems being more sensitive to various damaging factors and/or more rapidly respond to metabolic changes than the protein synthesis systems. Further investigations are required to evaluate the physiological and/or pathological significance of the dissociation between the rate of the synthesis of TF polypeptide and glycane chains.

It should be noted that reduced glycosylation of TF may at least partially account for the delayed postirradiation disturbances. For example, it was

demonstrated that desialylation of TF changes its iron transport capacity [11]. The level of TF glycosylation is higher during pregnancy, i.e., when iron metabolism is changed, and returns to normal after delivery [11]. On the other hand, the content of colony-forming units in mouse bone marrow remains decreased over several months postirradiation [1]. In dogs, secondary γ-induced immunodeficiency observed during the late postirradiation period may also result from hemopoietic disturbances [6]. Since all these disorders may be related to iron metabolism, changes in the iron transport caused by decreased glycosylation of TF may play an important role in their development.

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