Correction of Testosterone-Induced Changes in a Population of Hemopoietic Precursors by a Bone-Marrow Inhibitor of the Proliferative Activity of a Hemopoietic Stem Cell

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A factor inhibiting the proliferation of splenic colony-forming units, injected both *in vivo* and after preincubation of mouse bone marrow cells *in vitro*, had a dose-dependent effect on the increased proliferative activity of splenic colony-forming units from the bone marrow of mice treated with testosterone propionate. This was associated with a reduction in the number of early hemopoietic precursors of mouse bone marrow. The counts of clonogenic granulocytic-macrophagal and macrophagal colony-forming units decreased and that of burst-forming units in murine bone marrow increased after exposure to the hormone. Testosterone propionate promoted a decrease of the repopulating potential of bone marrow cells, which recovered after their preincubation with the factor inhibiting the proliferation of splenic colony-forming units.

Key Words: *hemopoietic precursors; differentiation; proliferation; testosterone propionate*

The stimulating effect of testosterone propionate (TSP) on the population of splenic colony-forming units (sCFU) is well known. The number of endogenous sCFU in the bone marrow of mice injected TSP has been shown to increase as early as 2 h postinjection [1]. That sCFU enter the proliferative pool under the influence of TSP has been proven: TSP increases the sensitivity of sCFU to "thymidine suicide" [5]. Some scientists have hypothesized that TSP shortens the sCFU cell cycle, a theory which has been confirmed by experimental findings [1].

There are reasons to think that the effects of androgens on sCFU are based on the presence of a specific receptor system in them [4]. Evidently, TSP has an impact on both the migration and proliferative activity of sCFU which are stimulated during a shortened cell cycle. It is the latter effect of the hormone that may underlie TSP induction of the differentiation of hemopoietic stem cells in the direction of erythropoiesis, judging from data on enhanced erythropoietin production under the action of TSP [9].

Previous experiments demonstrated the production of factors stimulating and inhibiting sCFU proliferation in the bone marrow of mice with normal proliferative activity of sCFU [7]. We showed that the factor inhibiting sCFU proliferation (FIP) is not produced in the bone marrow of mice with increased proliferative activity of bone marrow sCFU, even after TSP injection. This may cause an increase of the proliferative activity of early hemopoietic precursors [2]. In this work we assessed the effects of FIP on the proliferative activity of sCFU of mice injected TSP, studied the effects of FIP on the colony-forming activity of early (colony-forming

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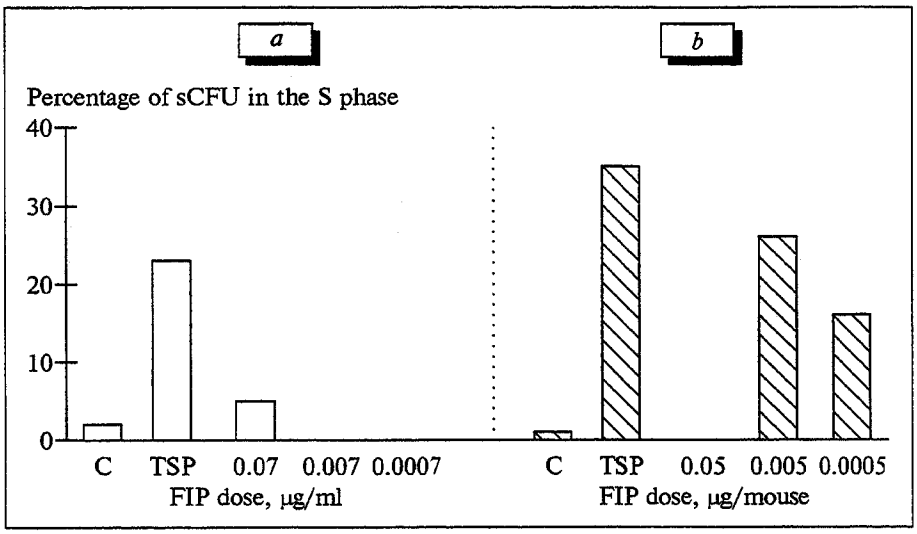

Fig. 1. Effect of FIP on the proliferative activity of sCFU from the bone marrow of mice injected TSP. *a*) *in vitro* incubation of cells with FIP; *b*) *in vivo* injection of FIP to mice. *C*: control.

units, granulocytic-erythroid-monocytic-megakaryocytic, CFU-GEMM) and committed (granulocytic-macrophagal and macrophagal colony-forming units and burst-forming erythroid units) hemopoietic bone marrow precursors of mice treated with TSP, and examined the effect of FIP on the capacity of bone marrow cells of TSP-treated mice to repopulate the bone marrow of a lethally irradiated recipient.

MATERIALS AND METHODS

Hybrid mice (CBA×C57Bl/6) F_1 aged 2-3 months bred at the *Stolbovaya* Breeding Center, Russian Academy of Medical Sciences, were used in the study. FIP was isolated from the supernatant of fractionated bone marrow of porcine ribs. The product obtained by chromatography on reverse-phase adsorbent was homogenous, as was shown by polyacrylamide gel electrophoresis. The fraction containing the product with a molecular weight of 17 kD was used as an inhibitor of sCFU proliferative activity. *In vitro* FIP was used in a dose of 0.005 μg/ml per 20×10^6 bone marrow cells. *In vivo* FIP was injected to mice 1 day after TSP injection in a dose of 100 mg/kg; the number of sCFU was counted in the bone marrow 1 day after FIP injection.

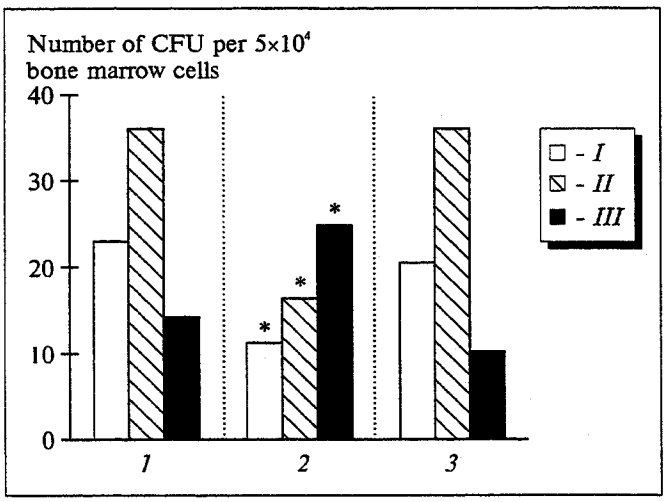

Fig. 2. Effect of FIP on the colony-forming activity of hemopoietic precursors in the bone marrow of mice injected TSP. *I*) granulocytic-macrophagal CFU; *II*) macrophagal CFU; *III*) burst-forming erythroid units. *$p<0.05$. Here and in Fig. 3: bone marrow cells from intact mice (*1*), after TSP (*2*), and after TSP, preincubated with FIP (*3*).

The number of sCFU in the bone marrow of animals was estimated by exogenous colony formation in the spleens of lethally irradiated recipients [11]. The number of sCFU in the S phase was assessed by the "thymidine suicide" method [3].

For assessment of the number of CFU-GEMM precursors, bone marrow cells released from macrophages by 2-hour adhesion to plastic were added (in a dose of 2×10^5/ml) to wells of 24-well plates in 0.5 ml culture medium containing 0.9% methylcellulose, 20% fetal calf serum, 5×10^{-5} M 2-mercaptoethanol, 1% bovine serum albumin, 2 units erythropoietin/ml, and 10% conditioned WEHI-3B cell medium as a source of interleukin-3. The colonies were counted on day 10 after incubation. Colonies with at least 50 cells, including at least 20% hemoglobinized cells, were considered [6].

The number of committed precursors was assessed by the agar cultures method in the presence of various differentiation factors. Bone marrow cells in a concentration of 5×10^4 per well were cultured in semisolid agar cultures according to methods described previously [10].

Conditioned media of the following cell lines were the sources of colony-stimulating activities: WEHI-3B was used to detect the units forming granulocytic-macrophagal colonies and L-929 to detect macrophage colonies; the number of colonies was counted after 7 days. The units forming

erythroid "bursts" were estimated with WEHI-3B with erythropoietin, and the results were assessed after 10 days. The repopulating capacity of bone marrow cells was judged by intravenous injection of a suspension to lethally irradiated recipients in a dose of 10^6 cells; after 14 days the mice were sacrificed and colony-forming activity was assessed by the semisolid agar cultures method [8]; the number of colonies on the 0.1, 0.05, and 0.02 fraction of the femur was counted.

The data were processed using Student's *t* test.

RESULTS

FIP injected to mice *in vivo* reduced the TSP-induced high proliferation of bone marrow sCFU. However, such an effect was observed only with an FIP dose of 0.05 μg/mouse; tenfold lower doses of FIP were ineffective (Fig. 1, *b*). *In vitro* preincubation of bone marrow cells of TSP-injected mice with FIP for 4 h also led to a reduction of the proliferative activity of sCFU (Fig. 1, *a*).

According to our data, the FIP-effected reduction of the proliferative activity of early hemopoietic precursors influences the colony-forming activity of CFU-GEMM: 4-hour incubation of macrophage-free bone marrow cells of mice injected TSP led to a reliable ($p<0.01$) decrease of the number of CFU-GEMM (8.2±0.35 *vs.* 12.0±0.5 in the control).

In order to assess the differentiation potential of hemopoietic bone marrow stem cells from TSP-treated mice, we investigated the ability of bone marrow cells to form colonies of hemopoietic precursors of the granulocytic-macrophagal, macrophagal, and erythroid direction *in vitro*. The number of functionally active committed precursors in the bone marrow of mice injected TSP was observed to change (Fig. 2). The number of granulocytic-macrophagal and macrophagal CFU dropped reliably after TSP injection in intact mice. The content of burst-forming erythroid units in mouse bone marrow increased after exposure to the hormone in comparison with the control. The results indicate the predominance of erythropoiesis in the bone marrow following injection of TSP, this agreeing with the results of other scientists who revealed enhanced production of erythropoietin, increased incorporation of iron, and elevation of the hematocrit under the influence of TSP [1,9]. Figure 2 shows that preincubation of bone marrow cells with FIP led to a reliable reduction of the number of burst-forming erythroid units and to an increase of the number of granulocytic-macrophagal and macrophagal CFU. The myeloerythrocytic coefficient, calculated as the ratio of the sum of granulocytic-macrophagal and macrophagal CFU to the number of burst-forming erythroid units, reflects an appreciable increase of the share of early erythroid precursors (a coefficient of 1.5) in animals injected TSP in comparison with intact animals (a coefficient of 4). Preincubation of bone marrow cells with FIP led to a normalization of the myeloerythrocytic coefficient.

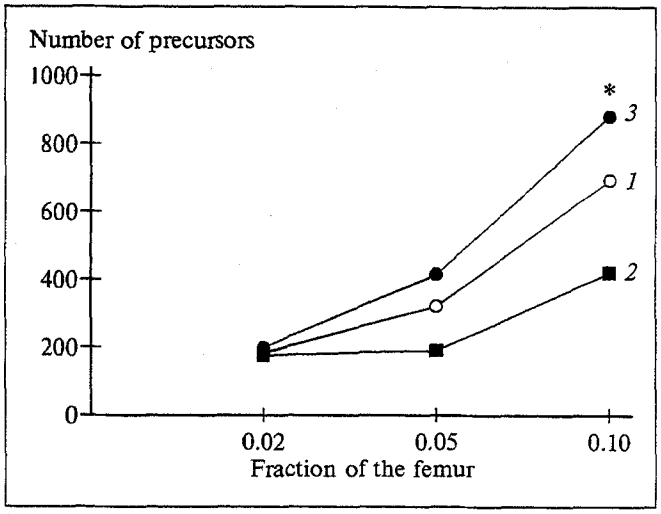

Fig. 3. Effect of FIP on the repopulating activity of hemopoietic precursors in the bone marrow of mice treated with TSP. *$p<0.01$.

In view of the close relationship between the proliferative activity of early hemopoietic precursors and their capacity for self-maintenance, we investigated the repopulating ability of bone marrow cells of mice treated with TSP. The results on the ability of cells to repopulate the bone marrow of lethally irradiated recipients are presented in Fig. 3. Evidently TSP reduces the repopulating capacity of bone marrow cells and incubation with FIP normalizes it.

Hence, the increase of the proliferative activity of sCFU caused by TSP injection alters the functional status of hemopoietic precursors. We observed an increase of the share of early erythroid precursors in the bone marrow in parallel with a reduction of the granulocyte and macrophage precursors and a decline of the repopulating potential of the bone marrow. The FIP-promoted decrease of the proliferative activity of sCFU leads to a normalization of the colony-forming activity of the bone marrow, to a recovery of the myeloerythrocytic coefficient, and to an enhancement of the repopulating activity of bone marrow cells.

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Enzyme Immunoassay of Lipoprotein(a)

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Sheep polyclonal antibodies to human lipoprotein(a) were used for the development of sandwich enzyme immunoassay. These antibodies virtually do not interact with human plasminogen, apoB-100, or fibrinogen in this assay. The method permits measurements of lipoprotein(a) in a wide range of concentrations: from 2 to 500 mg/dl. The variability of analyses within the range of 5 to 180 mg/dl is approximately 4.5% in a plate and 10.5% in different tests. Correlation analysis of the results of the enzyme immunoassay modification developed in our laboratory and of its foreign analogs showed a high similarity of the methods, the correlation coefficient being 95%.

Key Words: *enzyme immunoassay; lipoprotein(a); atherosclerosis*

Lipoprotein(a), or Lp(a), is a lipoprotein particle containing a protein apo(a) unique to the lipoprotein family; it is bound to molecule apoB-100 with a covalent disulfide bond, and this molecule is the principal protein component of a corpuscle of low-density lipoprotein (LDL). Lp(a) was discovered by Berg in 1963 as a new antigen of human plasma and as a variant of LDL corpuscles [2]. Further studies showed that Lp(a) differs from LDL not only in composition, molecular weight, and electrophoretic mobility, but also in physiological role and metabolism [12].

Active studies of Lp(a) were started after it was shown to be associated with the risk of atherosclerotic involvement of the heart [4]. Further investigations clearly demonstrated that a high level of Lp(a) in the blood is closely related to the appearance and development of atherosclerotic lesions in various arteries [7,12].

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Some scientists believe that Lp(a) may act as a bond in the processes of thrombosis and atherogenesis [10] (due to the high homology of the primary structure of apoprotein(a) with the plasminogen molecule [5]) and that it is involved in fibrinolysis, competing with plasminogen for fibrin binding [6].

At present, various immunological methods for measuring Lp(a) have been described: radial immunodiffusion, electroimmunodiffusion, immunonephelometry, and immunoturbidimetry [1,9], and different types of enzyme immunoassay (EIA) exist [3,9, 13]. Commercial kits for measuring Lp(a), manufactured by Biopool, Terumo, etc., are available. Still, because of the specific structure of an Lp(a) particle, its heterogeneity, and its mobility, there is a need for new methods facilitating its study.

MATERIALS AND METHODS

Lp(a) and LDL were isolated from the blood plasma of patients with elevated concentrations of

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