DIFFERENCES IN "ANTIONCOGENE" p53 EXPRESSION IN HUMAN MONOCYTES AND LYMPHOCYTES IN VITRO

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Nuclear phosphoprotein p53 was found originally in transformed cells and was identified as an oncogene in several model systems [1, 5]. Evidence has been obtained that p53 can behave as an "antioncogene" in various types of cells, including hematopoietic [8] and lymphoid [3]. However, the mechanisms of regulation and action of p53 have not been adequately studied It has been shown that p53 mRNA is induced after stimulation of blood lymphocytes by phytohemagglutinin (PHA) [9, 12]. A marker of monocyte and lymphocyte activation is early transcription of the gene of tumor necrosis factor α (TNF-α) The data in this paper relate to expression of p53 and TNF-α genes in resting and activated monocytes and lymphocytes, isolated under inactivating conditions on a continuous Percoll gradient. Considering the action of cycloheximide (CHX) on accumulation and stability of mRNA of different oncogenes [12] and cytokines [14], we compared the action of CHX on expression of p53 and TNF-α in resting and activated human blood monocytes and lymphocytes in vitro.

EXPERIMENTAL METHOD

Mononuclear leukocytes (MNL) were isolated from fresh heparinized healthy human blood by the standard method [2] on a stepwise Ficoll–Verografin gradient at 4°C, after which they were fractionated on a continuous Percoll ("Pharmacia") gradient at 4°C [16]. The isolated MNL fraction contained more than 80% of cells giving a positive reaction for α-naphthyl acetate esterase (α-NAE; monocytes; kit for determination of α-NAE from "Sigma"). Monocytes and lymphocytes (2 million/ml) were incubated in medium RPMI-1640 ("Gibco") with 5% fetal calf serum (FCS) and antibiotics in plastic cultural Petri dishes ("Flow Laboratories") in an atmosphere containing 5% CO_2 at 37°C. Monocytes were activated with a suspension of Staphylococcus aureus Cowan I (SAC) [6] in a concentration (v/v) of 0.001%. To activate lymphocytes, PHA-P ("Serva") was used in a concentration of 30 μg/ml. To inhibit protein synthesis, CHX ("Calbiochem") was added in a concentration of 10 μg/ml.

The cDNA of gene p53 was generously provided by Dr. M. Cline (University of California, Los Angeles, USA). The EcoRI-SalGI fragment of this DNA was subcloned into phage vector M13mp18. The test for TNF-α was described previously [11]. The single-stranded probe was labeled with α-^{32}P-dNTP to specific activity of more than 10^9 fissions/μg by the primer lengthening method.

Total RNA was isolated from the cells by lysis in a solution of guanidine isothiocyanate ("Fluka") [4]. After spectrophotometric determination of the RNA concentration 15 μg of total cellular RNA was applied to a denaturing MOPS-formaldehyde 1.4% agarose gel, and after electrophoresis, it was transferred to a nylon membrane ("Bio-Rad") as described in [10]. Application of the specimens and the quality of RNA were tested by staining with ethidium bromide. Hybridization with the labeled probe was carried out in a solution containing 50% formamide,

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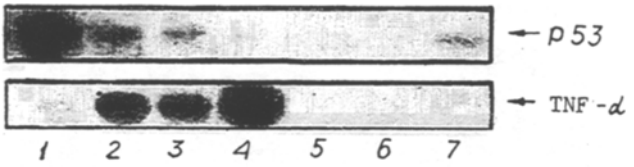

Fig. 1

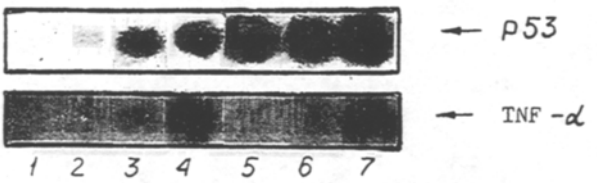

Fig. 2

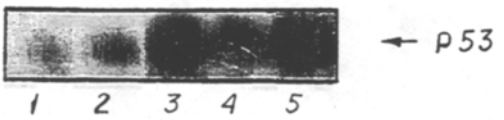

Fig. 3

Fig. 1. Northern blot analysis of mRNA of genes p53 and TNF-α in isolated human blood monocytes in presence of SAC and/or CHX. 1) Freshly isolated monocytes, 0 h; 2) stimulation by SAC, 3 h; 3) stimulation by SAC, 6 h; 4) stimulation by SAC 6 h, CHX added after 3 h of incubation; 5) monocytes cultured for 18 h; 6) stimulation by SAC, 18 h; 7) monocytes cultured with CHX, 18 h.

Fig. 2. Northern blot analysis of mRNA of genes p53 and TNF-α in isolated human blood lymphocytes in presence of PHA and/or CHX. 1) Freshly isolated lymphocytes; 2) stimulation by PHA, 3 h; 3) stimulation by PHA, 5 h; 4) stimulation by PHA, 5 h, CHX added after 3 h after incubation; 5) stimulation by PHA, 20 h; 6) stimulation by PHA 20 h, CHX added ab initio; 7) stimulation by PHA 20 h, CHX added after 18 h of incubation.

Fig. 3. Northern blot analysis of mRNA of p53 gene in human blood MNL. 1) Freshly isolated MNL, 2) MNL activated by PHA for 5 h; 3) activation by PHA for 20 h; 4) incubation for 20 h with PHA and CHX; 5) stimulation by PHA for 20 h, CHX added after 18 h of incubation.

0.25 M NaCl, 0.25 M Na-phosphate buffer, pH 7.2, 0.25% Na-pyrophosphate, 1% SDS, 1 mg/ml heparin, and 100-200 μg/ml of some DNA at 50°C for 12 h. The sample was washed successively in solutions of 2 SSC + 0.2% SDS and 0.2 SSC + 0.2% SDS at room temperature and 0.1 SSC + 0.2% SDS at 65°C. The autoradiographs were developed after exposure overnight with intensifying screens at −70°C.

EXPERIMENTAL RESULTS

Human monocytes, freshly isolated under inactivating conditions on a continuous Percoll gradient, contain distinct amounts of mRNA of gene p53, but not mRNA of TNF-α (Fig. 1). To prevent possible induction of RNA synthesis in monocytes during isolation, actinomycin D (AcD) ("Serva") was added to the blood samples before isolation of the cells in a concentration of 10 μg/ml. It was found that early treatment of the cells with AcD does not affect the mRNA p53 level in freshly isolated monocytes, although it effectively blocks induction and synthesis of mRNA of TNF-α (not shown). The mRNA p53 level in resting monocytes also was determined after incubation for 18 h. Stimulation of the monocytes by the powerful activator SAC caused the p53 mRNA level to fall sharply after

3 h, and to disappear completely after incubation with SAC for 18 h At the same time, induction of a considerable number of transcripts of the TNF-α gene was observed after incubation for 3 h with SAC (Fig. 1).

Addition of CHX to the monocyte culture did not prevent disappearance of p53 mRNA in activated cells, but caused disappearance of p53 mRNA in resting monocytes in culture after incubation for 18 h. On the addition of CHX 3 h before harvesting of the cells, superinduction of TNF-α mRNA was observed after 6 h in a monocyte culture activated by SAC (Fig. 1).

To compare these results with those obtained previously [9, 12] expression of the p53 gene was analyzed in unfractioned human peripheral blood mononuclears, consisting of lymphocytes and monocytes (Fig. 3). Results showed that freshly isolated unfractionated MNL contained p53 mRNA, in the same way as monocytes freshly isolated on Percoll. Starting with the 3rd hour the pattern of expression of p53 in MNL, stimulated by PHA, became similar to that observed in purified human lymphocytes.

The presence of p53 mRNA in freshly isolated monocytes suggests that the p53 gene is transcriptionally active at least in some fractions of circulating human blood monocytes. The observed disappearance of p53 mRNA during activation of the cells can be explained either by cessation of transcription of the p53 gene or by the action of mechanisms of specific degradation of p53 mRNA, activated during monocyte stimulation.

A similar fall of the p53 mRNA level was described previously in cells of an embryonic teratocarcinoma and virus-induced mouse erythroleukemia, induced to differentiate in vitro [7, 13].

Results showing absence of p53 mRNA in freshly isolated human lymphocytes and its accumulation after stimulation of the cells are in agreement with those described previously [9, 12], except that we demonstrated the presence of p53 mRNA in freshly isolated MNL. Technical differences in the method of isolating the cells and the Northern blot analysis may perhaps have played an important role. It seems evident that the presence of p53 mRNA in MNL was due to the presence of a fraction of monocytes in them.

In our experiments addition of CHX did not cause superinduction of p53 mRNA in monocytes; moreover, disappearance of p53 mRNA was observed after treatment of the cells with CHX, accompanied by simultaneous superinduction of TNF-α mRNA. In activated lymphocytes CHX had a stabilizing effect on the p53 mRNA level, comparable with the observed superinduction of TNF-α mRNA in several experiments.

The results of this investigation suggest that regulation of transcription of the p53 gene differs in human monocytes and lymphocytes. Bearing in mind data on differences in the action of CHX on the p53 mRNA content, it is reasonable to suggest also that there are differences in post-transcription regulation. Considering results slowing terminal differentiation of mouse erythroleukemia cells under the influence of hexamethylene-bis-acetamide [7], it can be tentatively suggested that the observed difference in expression of transcripts of the p53 gene by monocytes and lymphocytes is attributable to the fact that, after stimulation of phagocytosis, monocytes undergo differentiation [15], whereas lymphocytes, after stimulation by PHA, embark upon a cycle of proliferation. The possibility cannot be ruled out that these differences in regulation are caused by definite differences in the function of the p53 gene product in monocytes and lymphocytes, and also, possibly, in its oncogenic/antioncogenic action in human tumors of monocytic and lymphocytic origin.

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PHYSICOCHEMICAL AND FUNCTIONAL PROPERTIES OF HEMOGLOBIN IN MICE WITH EHRLICH'S CARCINOMA

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A special role in the functional system of the blood is played by its hemoglobin component. Hemoglobin is a hemoprotein with an exceptionally wide range of functions; it determines the oxygen balance of the body, and it can respond accurately to various factors in the microenvironment that induce changes in the properties and functional activity of the respiratory protein of the erythrocytes, aimed at directing oxygen-dependent pathological states.

The determinative factors of modulation of the oxygen-binding properties of hemoglobin include the specific features of metabolism in organs and tissues, changes in the endocrine background, etc. Definite changes have been shown to take place in the oxygen transport system in several oncologic diseases [10]. Meanwhile, in order to obtain some idea of the mechanisms of the compensatory reaction of the blood to pathological changes in the body, research embracing both cellular and molecular levels of hemoglobin is essential.

It was accordingly decided to study physicochemical and functional properties of freely soluble and membrane-bound hemoglobin in whole erythrocytes and erythrocytes fractionated in a sucrose density gradient.

EXPERIMENTAL METHOD

Experiments were carried out on mice weighing 18-20 g (50 mice kept under animal house conditions). The animals were divided into two groups: 1) intact, 2) animals with Ehrlich's carcinoma. Inoculation of Ehrlich's tumor cells was carried out by injecting 1 million cells per mouse, suspended in physiological saline. The cells were counted in a Goryaev's chamber. The cells were diluted so that 0.1-0.2 ml of physiological saline contained 1 million cells. Blood was taken from the mice on decapitation on the 10th day after inoculation of the cells. The resistance of the erythrocytes was determined by the acid erythrogram method [9]. Hemoglobin was obtained by Starodub's method [7]. Heparinized blood, after decapitation of the animals, was centrifuged at 1000*g* for 5 min; the plasma was removed and the blood cells washed 3 times with physiological saline. The erythrocytes were hemolyzed with 5 volumes of water, and 2 volumes of 0.1 M Tris-HCl-EDTA buffer, pH 8.9, made up in 1.5 M NaCl, compared with the volume of the erythrocytes, was added to the hemolysate. The mixture was shaken for 20 min and allowed to stand for hemolysis at 4°C for 1 h. The hemolysate was then centrifuged at 15,000*g* for 20 min at 4°C. The soluble hemoglobin fraction was withdrawn by suction with a syringe, and the residue was treated 3 times with cold water to remove the weakly bound and readily soluble hemoglobin; each time, washing was followed by centrifugation at 15,000*g* for

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