Effects of Thymidine and Phorbol 12-Myristate 13-Acetate on Erythroid Differentiation of K562 Cells and Their Sensitivity to Nonspecific Lysis by Rat Splenocytes

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Induction of hemoglobin synthesis in K562 cells by thymidine (2 mM) *in vitro* did not significantly enhance major histocompatibility complex antigen-unrestricted lysis of splenocyte-exposed K562 cells. Inhibition of thymidine-induced hemoglobin synthesis by simultaneous incubation of cells with thymidine and phorbol 12-myristate 13-acetate (100 nM) decreased cytolytic activity of splenocytes against K562 cells. Preincubation of tumor cells with phorbol ester alone did not affect major histocompatibility complex antigen-unrestricted lysis induced by rat splenocytes but decreased the basal level of hemoglobin synthesis.

Key Words: *nonspecific cytotoxicity; erythroid differentiation; phorbol 12-myristate 13-acetate*

Some tumor cells, including human erythromyeloleukosis K562 cells [10], are sensitive to major histocompatibility complex antigen-unrestricted lysis (nonspecific cytotoxicity, NCT lysis) induced *in vitro* by human and animal natural killer cells. During *in vitro* incubation, K562 cells express a low level of hemoglobin, but its synthesis considerably increases in the presence of some substances, in particular sodium butyrate [6], hemin [16], adriamycin [12], and 1-(β-D-arabinofuranosyl)cytosine [13]. Enhanced synthesis of hemoglobin and expression of glycophorin A [11] are the markers of erythroid differentiation, which is also observed in mouse erythromyeloleukemia MEL cells treated with dimethylsulfoxide (DMSO), sodium butyrate, and N,N'-hexamethylene-bis-acetamide [8]. On the other hand, incubation of K562 cells with phorbol 12-myristate 13-acetate (PMA) inhibited basal synthesis of globin and glycophorin A and induced expression of megakaryocyte markers [9]. In experiments on MEL cell, PMA inhibits DMSO-induced synthesis of hemoglobin [15].

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Inductors of erythroid differentiation, in particular DMSO, induce DNA breaks [18] and, therefore, can modulate cell sensitivity to NCT lysis mediated by natural killer cells and proceeding through of apoptosis based on DNA fragmentation [3].

Here we studied changes in the sensitivity of K562 cells to NCT lysis mediated by natural killer cells under conditions of induction of erythroid markers and after PMA-inhibition of induced erythroid differentiation.

MATERIALS AND METHODS

Experiments were performed on Wistar rats weighing 180-220 g. Spleen fragments were forced through a metal grid to obtain splenocytes. Erythrocytes were lysed with distilled water.

K562 cells (All-Russian Collection of Cell Cultures, Institute of Cytology) were grown in RPMI-1640 medium (Institute of Poliomyelitis and Viral Encephalitis) containing 11% fetal bovine serum (N. F. Gamaleya Institute of Epidemiology and Microbiology), 2 mM L-glutamine, 40 μg/ml gentamicin sulfate, and 5×10^{-5} M 2-mercaptoethanol (Ferak).

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K562 cell cultures (0.5 ml, 10^5 cells) were placed into 24-well plates (Nunc), and thymidine and/or PMA (100 nM) were added. The initial solution of PMA was prepared using DMSO and, therefore, DMSO was added to control cultures to a final concentration of 0.06%. After incubation, the number and viability of cells (estimated by trypan blue exclusion), hemoglobin concentration, and sensitivity of control and treated K562 cells to NCT lysis by rat splenocytes were determined.

NCT reaction of splenocytes against ^{3}H-uridine-labeled K562 cells was performed as described elsewhere [1]. The effector/target cell ratio was 50:1. Cytotoxic index (CI) representing the ratio between lysed target cells and the total number of target cells added to the sample was calculated by the formula: CI (%)= [1-(cpm) treated samples/(cmp) control samples]×100%. Cultures of ^{3}H-uridine-labeled K562 cells without splenocytes served as the control.

Intracellular hemoglobin concentration was measured by benzidine reaction [7]. The calibration curve was constructed with horse hemoglobin (Reanal).

Thymidine (Reanal), PMA (Sigma), ^{3}H-uridine (Izotop), trypan blue (Sigma), and other reagents (Russia) were used.

The results were analyzed by Student's *t* test.

RESULTS

The treatment of K562 cell with increasing concentrations of thymidine for 3 days led to a dose- and time-dependent stimulation of hemoglobin synthesis (Fig. 1). Trypan blue exclusion test showed considerable decrease in cell viability on days 2-3 and 3 of incubation with 3 mM and 2 mM thymidine, respectively (data not shown). Therefore, in further experiments we used 2-day incubation with 2 mM thymidine.

Results of 2-day incubation of K562 cells with 100 nM PMA and PMA+thymidine were compared to analyze the effects of PMA on thymidine-induced hemoglobin synthesis; cells incubated with 0.06% DMSO and DMSO+thymidine, respectively, served as the control. PMA alone and in combination with thymidine considerably decreased the concentration of hemoglobin (Fig. 2). DMSO stimulated hemoglobin synthesis, and after addition of thymidine an additive effect was observed (Fig. 2).

After 2-day incubation of K562 cells in all variants (except for cultures containing 0.06% DMSO) cell proliferation was completely inhibited (Fig. 3). The presence of PMA in 2-day-old cultures increased cell permeability for trypan blue, while cell viability remained above 90% (data not shown).

Since PMA could inhibit hemoglobin synthesis in native and thymidine-treated K562 cells, we compared

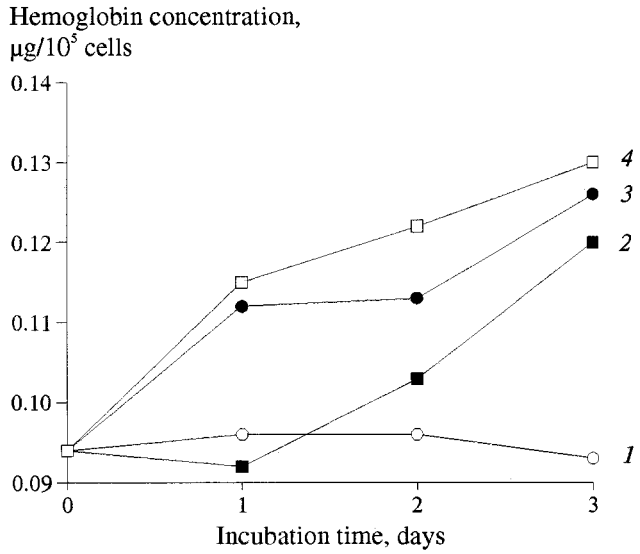

Fig. 1. Kinetics of hemoglobin synthesis in K562 cells treated with thymidine: control K562 cells (*1*) and K562 cells incubated with 1 mM (*2*), 2 mM (*3*), and 3 mM thymidine (*4*).

the effects PMA on these target cells in the NCT test. Combined treatment of K562 cells with thymidine and PMA markedly decreased NCT activity of rat splenocytes (24.0±4.4 *vs.* 45.6±5.1% after incubation with thymidine, $p<0.02$). Preincubation with 0.06% DMSO did not affect K562 cell sensitivity to NCT lysis by rat splenocytes (data not shown).

PMA alone (without thymidine) did not decrease the sensitivity of K562 cells to nonspecific lysis by rat splenocytes. Thymidine inhibited K562 cell proliferation and induced hemoglobin synthesis (the marker of erythroid differentiation). Under the effect of thymidine, the tendency toward sensitization of K562 cell

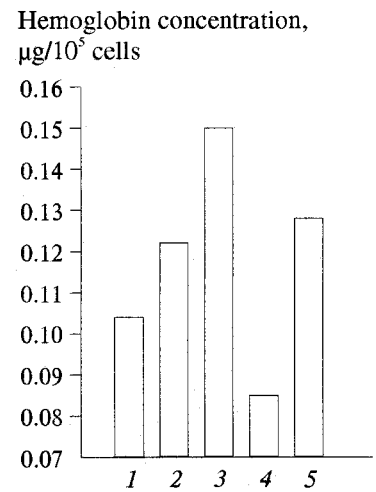

Fig. 2. Hemoglobin synthesis in K562 cells over 2-day incubation under various conditions: control cells (*1*) and cells incubated with 0.06% DMSO (*2*), 2 mM thymidine and 0.06% DMSO (*3*), 100 nM phorbol 12-myristate 13-acetate (*4*), and 100 nM phorbol 12-myristate 13-acetate and 2 mM thymidine (*5*).

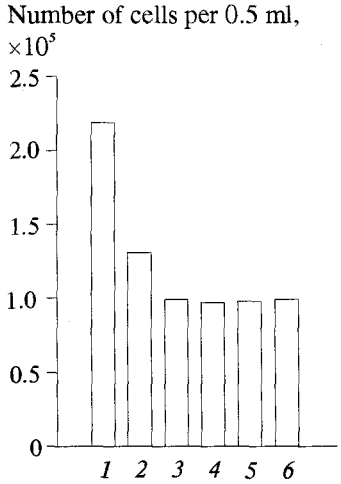

Fig. 3. Proliferation of K562 cells over 2-day incubation under various conditions: control cells (*1*) and cells incubated with 0.06% DMSO (*2*), 2 mM thymidine (*3*), 2 mM thymidine and 0.06% DMSO (*4*), 100 nM phorbol 12-myristate 13-acetate (*5*), and 100 nM phorbol 12-myristate 13-acetate and 2 mM thymidine (*6*).

to lysis by rat splenocytes ($0.1<p<0.2$) was probably due to a high degree of DNA destabilization (increased number of DNA breakpoints). Two-parameter fluorescence [5] with DNA-tropic dyes, ethidium bromide (EtBr) and 4',6-diamidino-2-phenylindole (DAPI), showed that the ratio between fluorescences of EtBr and DAPI in DNA samples of thymidine-synchronized K562 cells 2-fold surpassed this parameter in freshly isolated rat thymocytes (data not shown). This probably attests to a higher degree of DNA relaxation (in the nucleotide form) in synchronized K562 cells due to accumulation of single-stranded and double-stranded DNA breaks [5] induced by damaging factors [2] or various biological processes (including DNA reparation).

Since double-stranded DNA breaks, including internucleosomal DNA fragmentation, form the basis for apoptosis (programmed cell death) [3], while single-stranded DNA breaks can be transformed to double-stranded breaks [4], the degree of thymidine-induced DNA damage is probably responsible for increased K562 cell sensitivity to NCT lysis by rat splenocytes.

The effect of PMA can be interpreted as follows: since PMA inhibits DNA fragmentation in thymocytes [14] and ceramide-induced apoptosis in human leukemia cells [17], it cannot be excluded that this effect of PMA contributes to low sensitivity of K562 cells treated with PMA and thymidine to NCT lysis by rat splenocytes (in comparison with sensitivity of cells treated with thymidine and 0.06% DMSO). Thymidine probably causes DNA damage or increases cell sensitivity to such damage induced by rat splenocytes, because PMA alone (without thymidine) has no effects on NCT lysis of K562 cells.

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