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DYNAMICS OF IMMUNOLOGIC AND CYTOCHEMICAL MARKERS DURING INDUCED DIFFERENTIATION OF CELL LINE K-562

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KEY WORDS: differentiation; monoclonal antibodies; tetradecanolphorbol-13-acetate; cell line K-562.

The process of differentiation of hematopoietic cells has been the subject of close attention by many investigators [1, 10], for knowledge of the laws of differentiation under normal conditions and their change during malignant transformation may provide a new approach to the treatment and diagnosis of various forms of leukemia and lymphosarcoma. Thanks to the production of monoclonal antibodies (MAB) to differential antigens, it has now become possible to make a detailed study of the process of cell maturation.

The aim of this investigation was to study changes in the immunologic phenotype of the K-562 cell line under the influence of the differentiation inducer tetradecanolphorbol-13-acetate (TPA) [1], by means of a panel of Soviet-produced antibodies, obtained at the All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR.

EXPERIMENTAL METHOD

A panel of ICO MAB was used: ICO-1 against the monomorphic determinant of Ia-like antigens; ICO-02 against antigen of undifferentiated blast cells; ICO-10 against early thymocyte antigen (Thy-1); ICO-11 against function-associated antigen; ICO-GM1 against myelomonocytic antigen; ICO-12 against granulocytic antigen [2]. MAB of the HAE series, specific for erythroid differential markers also were used: HAE3 against glycophorin-A and HAE9 against erythroblast antigen [3]. Expression of the antigens was determined in the indirect immunofluorescence test (IFT), carried out in a cell suspension in plastic test tubes. The results of the IFT were read on a Leitz luminescence microscope, with magnification of 400×. Simultaneously preparations were made from the cell culture on a cytocentrifuge for morphological and cytochemical investigations. The morphological investigations were carried out after staining of films by Romanovsky's method, and the following enzymes were studied cytochemically: peroxidase, AS-D-chloroacetate esterase, α-naphthyl acetate, and butyrate esterase, with inhibition by sodium fluoride, acid phosphatase [4], and also siderophilic granules.

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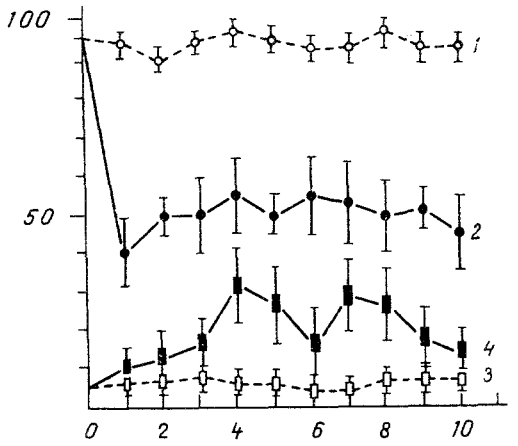

Fig. 1. Time course of erythroid markers. Abscissa, time of observation (in days); ordinate, number of cells (in %) expressing erythroid markers. 1, 2) Cells expressing erythroblast antigen, detected by MAB HAE9, in control and experiment respectively; 3, 4) cells expressing glycophorin-A, detectable by MAB HAE3, in control and experiment respectively.

The K-562 cell culture was maintained on medium RPMI-1640 with the addition of 15% bovine serum, 1% glutamine, and 1 μg/ml of gentamicin. The K-562 cells were introduced into 24-well panels (Falcon Plastics) in a concentration of $0.5 \cdot 10^6$/ml, and subcultured every other day. In the experimental group the cells were incubated in the same medium as in the control, with the addition of TPA in a dose of 20 ng/ml, with an initial cell concentration of 10^6/ml. The cells were incubated until 12 days, after which 50% of the medium in the wells was changed daily for fresh medium containing the same quantity of TPA, the cell concentration and the percentage of living cells being monitored each time. In the intact culture the number of dead cells did not exceed 5-10%. Expression of antigens was determined with an interval of 24 h, and the cytochemical and morphological investigations were undertaken after 1, 2, 4, 7, and 10 days.

EXPERIMENTAL RESULTS

The intact culture consisted of a population of blast cells, peroxidase-negative, not containing siderophilic granules. AS-D-chloroacetate esterase was present (++) in all the cells, as well as isozymes of nonspecific esterase: butyrate esterase (+), inhibited by NaF; α-naphthyl acetate esterase (++), not inhibited by NaF; all cells contained acid phosphatase (+++). Cytochemical investigations are evidence in support of the myelomonocytic nature of the cells.

During immunologic investigations neither late antigens of the myelomonocytic series nor the monomorphic determinant of la-like antigens, nor antigens of undifferentiated blast cells, nor antigens of natural killer cells were detected on the surface of the cells (ICO panel [2]). Meanwhile 90-95% of cells expressed erythroblast antigen (MAB HAE9) and 5-10% of cells carried glycophorin-A (MAB HAE3). These immunologic data are evidence of the erythroid nature of the cell population studied. Thus the subline of cell line K-562 which we studied can be regarded as precursor cells, possessing features of both erythroid and myelomonocytic series. Since the immunologic markers of the late stages of differentiation (GM1 and G2) characteristic of the monomyelocytic series were not found, and since cytochemically the cells were characterized as predominantly myelo-monocytic, the immunologic parameters were evidence of the erythroid trend of the cell line studied. Consequently, it can be postulated that cells of the K-562 line are polypotent.

It follows from data in the literature that during induced differentiation in a K-562 cell culture it might be expected that cell maturation would proceed along one of the possible pathways: myeloid, monocytic, erythroid, or megakaryocytic [1, 5, 6, 9, 10].

Investigation of myelo-monocytic markers, identified from cytochemical data, and of erythroid markers, identified immunologically, was undertaken. Their time course is shown in Figs. 1 and 2. It will be clear from Fig. 1 that by the 1st day there was a decrease in

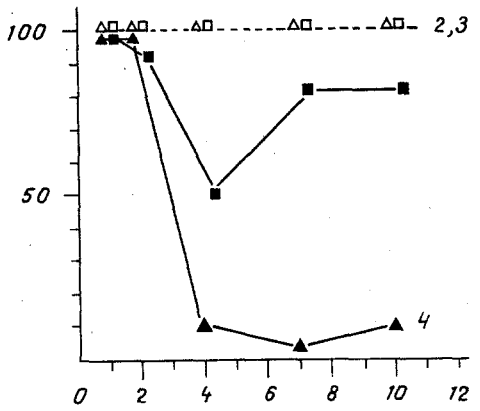

Fig. 2. Time course of cytochemical parameters. Abscissa, time of observation (in days); ordinate, cytochemical parameters of cells (in %). 1, 2) Butyrate esterase activity inhibited by NaF, in control and experiment respectively; 3, 4) α-naphthyl acetate esterase activity, not inhibited by NaF, in control and experiment respectively.

the percentage of cells carrying erythroblast antigen, detectable by MAB HAE9, from 90 to 50. The number of cells carrying antigen detectable by MAB HAE3 (glycophorin-A), on the other hand, increased from 5-10%, to reach something of the order of 30% by the 4th day. A decrease in esterase activity was observed at these same times: butyrate from 100 to 10% and α-naphthyl acetate from 100 to 50%. Activity of the latter was restored by the 7th day.

Thus intact cells of the K-562 subline characterized by two directions of differentiation (myelo-monocytic and erythroid) demonstrated its ability under the influence of TPA to mature in the erythroid direction. Starting with the 4th day, the percentage of dead cells in the culture increased, to reach something of the order of 50% by the 10th-12th day.

To give a biological interpretation of these data the complex action of TPA must be taken into consideration [1, 7-9]. We know that it has the following action of K-562 cells: it blocks proliferation and induces differentiation [1]. In the experiment there was no increase in the number of cells (in the culture incubated with TPA), which does not contradict the fact that proliferation was blocked. The time course of the markers, illustrated in Figs. 1 and 2, is evidence of differentiation in the erythroid direction. In fact, the marker of the early stages (HAE9) was reduced, and this was accompanied by an increase in the late marker (HAE3). It can be tentatively suggested that during induced differentiation in cell culture under the influence of TPA as promotor, the final stages of maturity are not reached, and for that reason cells of the K-562 line begin to die at a certain stage of differentiation, as is confirmed by coincidence of the peak of expression of the marker identified by HAE3 with the beginning of an increase in the number of dead cells (4th day). Butyrate esterase activity also decreases at this same time, and this was interpreted as loss of the properties of the granulocytic trend. It can be concluded that cells possessing polypotency in an intact culture had progressed through the stages of differentiation in the erythroid direction under the influence of the differentiation inducer TPA.

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EFFECT OF INJECTION OF *Cryptococcus* HETEROPOLYSACCHARIDE INJECTION INTO BONE MARROW DONORS AND RECIPIENTS ON STRUCTURE OF A HETEROTOPIC FOCUS OF HEMATOPOIESIS

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KEY WORDS: heterotopic focus of hematopoiesis; polysaccharide.

The number of known preparations with an inhibitory or, still less, a stimulating action on hematopoiesis in vivo is still extremely limited. However, glycosaminoglycans, which have an appreciable effect on hematopoietic cell proliferation [3-5], are one of the components of the hematopoietic microenvironment.

It was accordingly decided to study the action of a *Cryptococcus* heteroglycan on the formation of a heterotopic focus of hematopoiesis.

EXPERIMENTAL METHOD

The extracellular polysaccharide (PS) formed by the yeast *Cryptococcus luteolus* strain 228, is a branched heteropolymer containing α-1,3-bound mannan in its main chain and xylose and glucuronic acid residues in the side chain, connected to the main chain by β-glycoside bonds [1].

Experiments were carried out on 120 male (CBA × C57B1)F_1 mice weighing 18-20 g. Heterotopic transplantation of mouse bone marrow was carried out by the method in [2, 3]. The polysaccharide was injected intraperitoneally into the mice which had received the bone marrow in the course of 30 days after the operation in doses of 25 mg/kg once or twice a week and of 200 mg/kg once a week. Retransplantation of the ectopic focus was carried out 7 days after the first implantation. In the course of these 7 days the polysaccharide was injected into intermediate recipients once or twice in a dose of 25 mg/kg. Control animals received injections of physiological saline. The donor mice were given intraperitoneal injections of solutions of the polysaccharide (25 mg/kg) parallel with physiological saline twice a week with an interval of 3 days, and daily for 7 days immediately before implantation. Bone marrow, isolated from the femora of these animals, was then implanted into intact recipients. The mice were killed 30 days after primary implantation and after retransplantation, by compression in the suboccipital region, and the dimensions of the heterotopic foci of hematopoiesis which had formed were estimated on the basis of the number of cells and the weight of the bony capsule.

EXPERIMENTAL RESULTS

Injection of PS into the experimental animals in a dose of 25 mg/kg once a week for 30 days after implantation of bone marrow caused a significant increase in the number of cells and in the weight of the bony capsule of the ectopic foci compared with the control. When the dose of polysaccharide was increased to 200 mg/kg the dimensions of the ectopic foci in the experimental and control animals were identical (Fig. 1a). To find out why the number of cells and the weight of the bony capsule increased, PS was injected once into intermediate recipients of the transplanted bone marrow. In this case the dimensions of the

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