Effect of Vitamin A Alcohol on the Surface Coat and Charges of L1210 Leukemic Cells

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SUMMARY

L1210 leukemic cells were treated with either vitamin A alcohol or neuraminidase. Ascites cells in vivo and cultures of an L1210 cell line in vitro were used. In untreated cells stained with ruthenium red, the glycoprotein surface coat appears as a thick, uninterrupted, electron-dense band. Positive colloidal iron staining results in deposition of particles in similar continuous fashion. No gaps were detected with both stains. After negative colloidal staining, no particle deposition was seen on the cell surface.

Treatment with vitamin A resulted in the following changes. Ruthenium red staining showed a thinner, discontinuous surface coat. Positive colloidal iron binding was markedly decreased, and particle deposition occurred in sparse clusters separated by extensive gaps. Negative colloidal particle binding was enhanced, with the appearance of patchy clusters separated by gaps. These results indicate a loss of surface coat material, reduction in negative cell surface charge, and exposure of positive changes induced by vitamin A. The striking similarities between the effects of the vitamin and neuraminidase suggest that release of lysosomal neuraminidase by vitamin A may play a role in the surface changes. A direct effect of the vitamin on the cell coat cannot be disregarded.

INTRODUCTION

Vitamin A alcohol, a surface-active compound (see reviews in Refs. 15, 19, and 30) enhances in vivo the antitumor effect of various chemotherapeutic agents and X-rays (1, 5, 6, 8). Under in vitro conditions, the vitamin exerts growth-inhibitory effects on cultured murine leukemic cells (7). Extensive investigations have been conducted to elucidate the biochemical mechanisms and subcellular target sites of the vitamin that may contribute to the understanding of its effects on malignant cells (4, 20, 21).

Because of the action of vitamin A on membrane structures, it appeared of interest to examine the cell surface of malignant cells exposed to vitamin A and to compare this with neuraminidase treatment under similar conditions. The latter substance alters the composition and electric charge of cell surfaces that may influence certain biological properties of cells (2, 10, 18, 24, 27, 31, 32). Surface coat characteristics of L1210 leukemic cells, both in vivo and in vitro, and the effects of vitamin A and neuraminidase are described in this work.

MATERIALS AND METHODS

In Vivo Studies. L1210 leukemia was carried as the ascites in DBA mice. Experiments were conducted in C57BL/6J × DBA/2J mice by transplanting 1 × 10⁶ L1210 cells/mouse (average body weight, 20 g) via i.p. injection. Animals were used 4 days after inoculation.

In Vitro Studies. L1210 leukemia cells were obtained from Associated Biomedic Systems, Buffalo, N. Y. Cells were grown in spinner culture using Roswell Park Memorial Institute 1640 supplemented with 10% fetal calf serum (inactivated) and penicillin-streptomycin (Grand Island Biological Co., Grand Island, N. Y.). Incubation was at 37° under an atmosphere of 95% air and 5% CO₂. Before use, log-phase cells were collected by centrifugation at 240 × g for 10 min and diluted with fresh medium to 5 × 10⁶ cells/ml. All manipulations were conducted using 37° equilibrated medium. Cell viability was monitored by nuclear exclusion dye tests using trypan blue or erythrocin B.

Vitamin A Treatment. Vitamin A (Trans Retinol, crystalline synthetic type X) was purchased from Sigma Chemical Co., St. Louis, Mo. L1210 ascites cells in vivo were treated by i.p. injection of 1 mg vitamin A in 0.1 ml of nitrogen gas-saturated dimethylsulfoxide per mouse. After 2 to 24 hr treatment, the ascites fluid was harvested to collect the cells.

For the in vitro experiments, the cultured cells were treated continuously for 2 to 12 hr with vitamin A that was dissolved in a small amount of dimethyl sulfoxide and diluted to the final concentration of 10 μg/ml medium. Untreated and dimethyl sulfoxide-treated cells were used as control materials.

Neuraminidase Treatment. Ascites cells were incubated in vitro with neuraminidase (Sigma type V, 5 units/ml) at 37° for 2 hr.

After the various treatments, the ascites cells or the cultured cells were washed in balanced salt solution and processed for the various staining techniques. Untreated cells or solvent-treated cells were handled in identical fashion.
Ruthenium Red Stain. Cells were resuspended for 60 min at 4°C in 2% glutaraldehyde in 0.067 M cacodylate buffer (pH 7.4) with 1000 ppm ruthenium red (16). After fixation, cells were washed 3 times in cacodylate buffer and subsequently postfixed for 2 hr at room temperature in 1.3% osmium tetroxide in 0.067 M cacodylate buffer with 1000 ppm ruthenium red.

Colloidal Iron Stain. Cells were fixed with 2.5% glutaraldehyde in 0.1 M phosphate buffer at pH 7.4 for 45 min at 4°C. The cells were washed with distilled water and stained for 20 min at room temperature with either positive or negative dialyzed colloidal iron solution. The positively or negatively charged colloidal ferric oxide solutions were prepared according to the technique described by Gasic et al. (9).

After immersion in the colloidal solutions, the cells were washed with acetic acid or acetate buffer, following the original method, and postfixed for 30 min at 4°C in 1% osmium tetroxide in 0.1 M phosphate buffer. After postfixation, cells were washed in 0.1 M phosphate or cacodylate buffer and preembedded with 1.5% agar. The cell containing agar gel was cut into small blocks, dehydrated in ethanol, and embedded in Epon 812 via propylene oxide.

Thin sections were cut on a Porter Blum MT-2 Ultramicrotome, stained with saturated uranyl acetate in 50% ethanol, and examined with an RCA EMU-4 electron microscope.

RESULTS

Characteristics of the Cell Surface Coat In Vivo

Effects of Vitamin A on Ruthenium Red Staining. In untreated L1210 ascites cells (Fig. 1) the surface coat appears delineated by a continuous electron-dense deposit of ruthenium red. In cells from mice pretreated for 4 hr with vitamin A, stainability with ruthenium red is almost completely abolished (Fig. 2). The differences in binding of the dye to the surface coat between the untreated controls (Fig. 3) and vitamin A-pretreated cells in vivo (Fig. 4) are more readily seen at the higher magnifications.

Similarities between the Effects of Vitamin A and Neuraminidase on Positive Colloidal Iron Staining. In untreated L1210 ascites cells (Fig. 5) positive colloidal iron particles are adsorbed uniformly to the surface coat. In the leukemic cells pretreated in vivo with vitamin A for 4 hr (Fig. 6), positive colloidal iron deposits are seen as discontinuous patchy granules attached to the surface coat. This is seen clearly at higher magnifications. The untreated controls (Fig. 7) show linear arrays of positive colloidal iron particles, bound almost uninterruptedly to the surface coat. After treatment of the ascites cells in vitro with neuraminidase (Fig. 8), binding of colloidal iron-positive particles to the surface coat is almost completely abolished (Fig. 8). In the ascites cells from mice treated in vivo with vitamin A (1 mg/mouse, for 4 hr), binding of positive colloidal iron particles to the surface coat appears discontinuous and only occasional patchy precipitates are visible (Fig. 9).

Similarities between the Effects of Vitamin A and Neuraminidase on Negative Colloidal Iron Staining. In untreated ascites L1210 leukemic cells (Fig. 10) only occasional sparse aggregates of negative colloidal iron particles can be seen on the surface coat. After treatment with vitamin A in vivo (1 mg vitamin A per mouse, i.p., 4 hr treatment), deposition of negative colloidal iron particles on the surface coat is increased (Fig. 11). The number of clusters of particles is not only increased, but each cluster consists of a larger number of granules than in the untreated ascites cells. Similar changes are noted in ascites cells pretreated in vitro with neuraminidase (5 units/ml of incubation mixture for 2 hr). Fig. 12 shows a representative area of the surface coat of a control untreated ascites cell; the area shows occasional clusters of negative colloidal iron particles. In the neuraminidase-treated cells (Fig. 13) the number of clusters of negative colloidal particles bound to the surface coat is increased, and each cluster contains more individual particles than do the control cells. Fig. 14 is from a vitamin A-pretreated cell, printed at the same magnification as Figs. 12 and 13, and is intended to compare the distribution of clusters of negative colloidal iron particles on the surface coat between untreated (Fig. 12), neuraminidase-treated (Fig. 13), and vitamin A-treated (Fig. 14) L1210 leukemic ascites cells.

Characteristics of the Cell Surface Coat of L1210 Leukemic Cells In Vitro

Effects of Vitamin A on Surface Coat Stainability with Ruthenium Red. The untreated L1210 cultured lymphocytes reveal a surface coat of uniform thickness, without any visible gaps (Fig. 15). In leukemic lymphocytes pretreated with vitamin A in vitro (10 µg/ml, for 5 hr), the surface coat, as revealed by ruthenium red staining, is thinner and shows abundant gaps of unstained areas (Fig. 16).

Effects of Vitamin A on Surface Coat Deposition of Positive Colloidal Iron Particles. In untreated cultured cells (Fig. 17), positive colloidal iron particles are deposited uniformly on the surface coat, without visible intervening gaps. After treatment with vitamin A in vitro (10 µg/ml, for 5 hr), deposition of positive colloidal iron particles is discontinuous (Fig. 18). The clusters of particles are less dense than in the untreated cells and are separated by gaps lacking colloidal iron deposits.

Effects of Vitamin A on Negative Colloidal Iron Particle Deposition. When stained with negative colloidal iron, the untreated cultured cells show almost a complete absence of negative colloidal iron particles on the surface coat (Fig. 19). After in vitro treatment of cultured cells with vitamin A (10 µg/ml for 5 hr), numerous areas of negative colloidal iron binding appear in the surface coat (Fig. 20). The negatively charged colloidal iron particles appear as irregular clusters separated by gaps devoid of particle deposition. In the groups treated with vitamin A alcohol, the changes in the surface coat became readily detected after 4 hr of treatment. The illustrations in this paper are from animals pretreated for 4 hr, whereas the cultures were preincubated 5 hr in the vitamin A-containing medium.
DISCUSSION

A carbohydrate-containing surface coat has been shown on the outer aspect of the plasma membrane in L1210 leukemic cells, both under in vivo and in vitro conditions. A surface coat of similar morphological appearance has been demonstrated in numerous cell types, including normal thymus and spleen lymphocytes (24).

Staining with ruthenium red and binding of positive colloidal iron can reveal the presence of acidic mucopolysaccharides (3, 17), glycoproteins, and glycolipids (23, 28, 29). Decrease or abolition of these reactions by neuraminidase shows the presence of neuraminic acid in the acidic glycoproteins of the surface coat (3, 17). Binding of positively charged colloidal iron particles by these cells reflects the presence of negative charges on the leukemic cell surface, which can be ascribed mostly to the carboxylic groups of neuraminic acid (11, 12, 17, 22). When the latter was digested with neuraminidase, the negative cell-surface charges decreased or disappeared, and little if any binding of positive colloidal iron particles occurred. However, discontinuous clusters of negatively charged colloidal iron particles were observed in the neuraminidase or in the vitamin A-treated leukemic cells. As no binding of negative colloidal iron particles occurs in the surface coat of untreated cells, this suggests that vitamin A and neuraminidase produce a shift from uniformly distributed cell surface negative charges to a pattern of discontinuous positive charges (17, 25).

The above changes, when due to treatment with neuraminidase, can be explained, at least partly, on the basis of the enzymatic digestion of neuraminic acid from the cell surface coat (14, 17, 24, 25). No definite explanation is presently available to account for the surface changes induced by vitamin A, and their striking similarity to those induced by neuraminidase. Vitamin A is a membrane-active compound with well-established lysosomal labilizing properties (15, 19, 21, 27). It has been shown that vitamin A can interact with, and produce alterations of, lysosomal membranes, resulting in the release of acid hydrolases (15, 16, 19, 21). The enzymes may shift from a condition of particle-bound to apparently free activities into the cytosol, but they may also leak from the cell. This vitamin also interacts with and alters the plasma membrane and can cause leakage of intracellular material (15, 19, 30).

Consequently, changes in surface coat-staining properties and electric charges induced by vitamin A, as shown in this study, may result from a direct action of the vitamin on the cell membrane, or the effect may be indirect and mediated by acid hydrolases released by lysosomes. Studies in this laboratory have indicated a marked release of lysosomal hydrolases from L1210 leukemic cells by vitamin A treatment, under experimental conditions similar to those in this report (21). Preliminary observations include lysosomal neuraminidase as part of the acid-hydrolytic complement released by vitamin A. The lysosomal nature of neuraminidase has been demonstrated in Ehrlich ascites tumor cells and in normal liver cells (13), and this supports the assumption that this enzyme may be responsible for surface coat changes produced by vitamin A.

Pretreatment with vitamin A has been shown to enhance the therapeutic effectiveness of X-rays and chemotherapeutic agents such as Cytoxan and 1, 3-bis(2-chloroethyl)-1-nitrosourea, both under in vivo and in vitro conditions (1, 5–8). To explain this phenomenon, it has been postulated that release of lysosomal degradative enzymes by this vitamin may produce subcellular alterations that increase susceptibility of tumor cells to X-rays and chemotherapeutic drugs (1, 5, 6, 8). The results described in this work point to the glycoprotein surface coat as a possible target that may account for the enhancement of the antitumor effects of X-rays and alkylating agents by vitamin A. A correlation between the synergistic effect of cyclophosphamide and vitamin A treatment and a reduction in tumor cell glycosaminoglycans and release of lysosomal enzymes has been observed in rats with the AH-130 ascites tumor (26).

The surface coat plays a role in many of the properties of the cells, such as surface charge, antigenic properties, contact inhibition, and recognition by macrophages (2, 10, 18, 24, 31, 32). Alterations of some of these properties by vitamin A may be responsible, at least partly, for the apparent increase of sensitivity of tumor cells to subsequent X-rays or chemotherapeutic agents, as shown in previous studies (1, 5, 6, 8, 9).

REFERENCES


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