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Abstract

The composition of total lipid extracted with chloroform-methanol from the AV12-induced tumor was investigated by thin-layer and paper chromatography. The content of lecitin and sphingomyelin was somewhat decreased and a cerebroside was characteristically detected in this tumor.

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LIPID COMPOSITION OF ADENOVIRUS-INDUCED TUMOR

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The present report deals with the results obtained in the study conducted on the problem whether or not there is a specificity in the lipid composition of AV12-induced tumor (1) which morphologically possesses a specificity as the neuroectodermal origin (2) differing from many other virogenic tumors.

MATERIALS AND METHODS

Virus and animals

At the two-week latent period after the intraperitoneal inoculation of 0.1 ml of $10^{2.5}$TCID 50/0.1 ml of adenovirus type 12 (AV12) (Huie strain) into newborn Syrian hamsters of less than 24 hours old, tumor developed in the peritoneal cavity were removed and used for the experiment.

Preparation of lipid fraction

This was conducted by FOLCH's method (3): namely, 25.6g of the extirpated tumors were suspended in 500 ml of chloroform-methanol (2 : 1, v/v) and homogenized by Waring blender, allowed to stand at room temperature with stirring for 4 hrs. The extract was obtained after filtration through the sintered glass funnel. The extraction was repeated twice, the first being at room temperature overnight and the second at 37°C overnight. The whole series of filtrates was pooled together and brought to dryness by a rotary evaporator. The extract thus obtained was subjected to FOLCH's partition dialysis (4) in order to remove inorganic materials and other organic comtaminants.

Thin-layer chromatography

The thin-layer chromatography was carried out on glass plate with a layer of silicic acid (Silica Gel G, Merck). The isolation was conducted mainly for phospholipids, using the solvent system of chloroform-methanol-water (70 : 25 : 4, v/v/v) for the primary chromatography and chloroform-methanol-7N ammonia (60 : 35 : 5, v/v/v) for secondary. As for the coloration iodine vapor was used for organic compounds, DITTMER reagent (5) for general phospholipids, ninhydrine reagent (6) for lipids containing amino-group, DRAGENDORFF reagent (6) for lipids containing choline and anthrone reagent (7) or diphenylamine reagent (6) for glycolipids.

Mild alkaline hydrolysis of phospholipids

The water soluble components obtained by a mild alkaline hydrolysis of lipid according to DAWSON's method (8) were chromatographed on Whatman No. 1 paper using the mixture of phenol-water-acetic acid-ethanol (80 : 20 : 10 : 12, v/v/v/v) as the solvent system. Each spot was detected with ninhydrin reagent and HANES-ISHERWOOD reagent (9). As reference compounds for comparison of the RF value, hydrolyzates of phosphatidyl glycerol and phosphatidyl ethanolamine from E. coli, cardiolipin from bovine and lecithin from egg were used.

RESULTS

By exposing the thin-layer chromatogram to iodine vapor 17 spots were obtained as shown in Fig. 1. Six of them ware identified as lysophosphatidyl choline, sphingomyelin, phosphatidly ethanolamine, cardiolipin

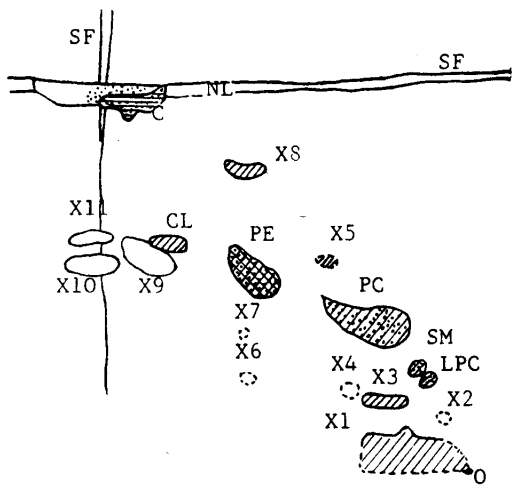

Fig. 1 Two-dimentional thin-layer chromatography of lipid extracted from the AV12-induced tumor.

: Positive for Dittmer reagent
: Positive for ninhydrine reaction
: Positive for Dragendorff reagent
: Positive for anthrone reagent

The chromatogram was primary developed from right to left with chloroform-methanol-water (70 : 25 : 4, v/v/v) and then in the vertical direction with chloroform-methanol-7N ammonia (60 : 35 : 5, v/v/v). O, origin ; SF, solvent front ; LPC, lysophosphatidyl choline ; SM, sphingomyelin ; PC, phosphatidyl choline ; PE, phosphatidyl ethanolamine ; CL, cardiolipin ; C, cerebroside ; NL, neutral lipid ; and unknown substances listed as X1—X11. Dashed lines indicate faint spots. X3, X5, X8 and X9 have been tentatively identified as phosphatidyl inositol, phosphatidyl grycerol, n-n-acethyl-phosphatidyl ethanolamine and fatty acid, respectively.

and cerebroside, and another 4 spots were tentatively identified as phosphatidyl inositol, phosphatidyl grycerol, n-n-acetyl-phosphatidyl ethanolamine and fatty acid, respectively. The remaining 7 spots could not be identified by the methods employed.

In order to make further identification of these compounds, their deacylated products of mild alkaline hydrolysis were characterized by means of the comparison of their RF values on a paper chromatogram with those of the reference compounds. The hydrolyzates derived from the major components were identified by the color detections and the quantitative relationship in addition to the comparison of RF values.

DISCUSSION

In the present experiment with AV12-induced tumor the composition ratio of each component in the phospholipids did not show any appreciable difference as in other tumors, (10, 11, 12, 13) but there can be observed a decrease in lecitin and sphingomyelin content.

What appears to be somewhat specific in the AV12-induced tumor was the presence of a fairly large quantity of glycolipid. Judging from the color reaction and the RF values of that glycolipid, it seemed to contain an abundant neutral sugar, which gave a suspicion of cerebroside. These findings seem to be interesting in view of the fact that AV12-induced tumor is morphologically said to have originated from the undifferentiated neuro-ectodermal tissue (2).

SUMMARY

The composition of total lipid extracted with chloroform-methanol from the AV12-induced tumor was investigated by thin-layer and paper chromatography. The content of lecitin and sphingomyelin was somewhat decreased and a cerebroside was characteristically detected in this tumor.

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