Concanavalin A Agglutination of Cells from Primary Hepatocellular Carcinomas and Hepatic Nodules Induced by N-2-Fluorenylacetamide

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SUMMARY

A previous study demonstrated that cells of transplantable hepatocellular carcinomas were agglutinated by the plant lectin concanavalin A, while normal hepatocytes were not. In the present experiments, 95% or more of cells obtained from primary hepatocellular carcinomas which resulted from exposure of rats to N-2-fluorenylacetamide were agglutinated by this lectin. Exposure to this carcinogen also produces grossly visible foci of morphologically and biochemically altered hepatocytes which have been termed hepatic (hyperplastic, premalignant, neoplastic) nodules. Although these hepatocyte aggregates are generally accepted as precursors of the hepatocellular carcinomas, no agglutination was detected when their cells were exposed to concanavalin A. These results indicate that concanavalin A agglutinability is not acquired as a result of tumor transplantation. Furthermore, they suggest that significant alterations must occur in the cells of hepatic nodules prior to the manifestation of malignant behavior.

INTRODUCTION

Numerous reports have described various alterations of the surface membranes of malignant and transformed cells, when compared to normal cells (7, 8, 13, 19). It has been suggested by a number of investigators that the pattern of activities characteristic of malignant cells results from these surface alterations. Among the most interesting has been the demonstration that many malignant and transformed cells are agglutinated by various plant lectins (2, 7, 15, 16, 21), a finding originally reported by Aub et al. (1). In some instances, the normal cells from which such tumors arose or the untransformed, cultured cells are not agglutinated, although the latter phenomenon may be highly selective (22). The comparison of lectin susceptibility between normal and malignant cells derived from intact tissues has been performed only rarely (2, 13). In a previous publication (2) it was reported that fetal hepatocytes and cells obtained from a variety of THC were agglutinated by Con A while normal, adult hepatocytes were unaffected. These results suggested that susceptibility to this effect of a lectin might represent a reexpression of a fetal-surface alteration (15). Furthermore, it served to emphasize the potential importance of this characteristic in neoplastic development and in malignant behavior in in vivo circumstances.

The chemical carcinogen 2-FAA induces a sequence of hepatic alterations prior to the appearance of PHC which has been examined extensively (3, 4, 9–11, 18, 23, 24). Particular attention has been focused upon grossly visible aggregates of morphologically and biologically altered hepatocytes which have been termed variously hepatic, hyperplastic, premalignant or neoplastic nodules. It is generally accepted that these nodules bear a significant relationship to the evolution to cancer. Briefly, livers bearing such nodules are at a greatly increased risk for malignancy, and in some instances carcinomas have apparently arisen within the nodule(s) (9, 23). These aggregates therefore offer a unique opportunity to examine hepatocytes for lectin agglutination during and subsequent to the administration of carcinogens.

Further, although THC demonstratedly possess many of the characteristics of the PHC from which they were derived, they may differ in other characteristics, such as the selection for a specific chromosome composition (5, 6). It is possible therefore that the selection for cells that are capable of growing after transplantation accounts for their response to Con A.

For these reasons the present study was aimed at examining 2 questions. (a) Is Con A agglutinability induced in nonmalignant hepatocytes and, in particular, in hepatic nodules, by exposure to 2-FAA? (b) Are the cells of PHC susceptible to lectin agglutination or is this phenomenon acquired during transplantation?

MATERIALS AND METHODS

Male ACI rats (Microbiologic Associates, Walkersville, Md.) were received at 115 ± 10 g; they were accustomed to a meal regimen for 1 week and placed on carcinogen. The preparation and administration of the diet has been published in detail (23, 24). The 2-FAA was mixed into a standard laboratory meal (Wayne Meal; Frederick Feed & Supply, Inc., Spring Valley, N. Y.) at 0.06% and fed
RESULTS

The sequence of alteration induced in rat livers by the diet used in this study has been described in detail (3, 9, 11, 18, 23, 24). However, in view of the importance of identifying the tissues tested, some description must be presented. In many of the 2-FAA-exposed livers, particularly with increasing time following cessation of the carcinogen, areas without gross abnormality (or at most, small cysts) were evident. These were characterized histologically by fibroductular septa and ductular cysts with areas of relatively normal hepatocytes in normal trabecular arrangements. They were termed “nonnodular liver.” “Cirrhotic nodule(s)” was the term used to describe nodular aggregates of histologically normal hepatocytes circumscribed by fibroductular septa. Some alteration of trabecular structure was frequently present. These nodules were invariably less than 0.5 cm in diameter and liver colored.

For simplicity of identification in this study, the term hepatic nodule(s) was used to denote those aggregates of morphologically altered hepatocytes that have been suggested to signal a liver at high risk for cancer (9, 18, 23). The hepatic nodules were all larger than 1 cm in diameter, grey-liver colored and, in most instances, demonstrated prominent surface vascularity. Histologically, they demonstrated sharply demarcated margins with compression of surrounding hepatocytes and a pseudo-capsule of compressed stroma. Their hepatocytes were much larger than normal and generally eosinophilic with an increased cytoplasmic:nuclear ratio. The pattern of liver cords was atypical or difficult to delineate.

Six of the 8 PHC studied were yellow-white in color; 2 were grey or pale yellow admixed with areas of liver color. All were bossedlated and demonstrated focal areas of hemorrhagic necrosis. All had invaded the adjacent parenchyma histologically and 2 had metastasized to the lungs. Their histological classification is presented in Table 1.

Con A Agglutination (Table 1). Single cell suspensions were easily obtained from tumors and from nontumorous tissue (Fig. 1a). From 95 to 100% of the cells of each of the 8 PHC were agglutinated by Con A, in medium and large clumps. Three of the 8 PHC were poorly differentiated on histological examination. The cells isolated from these tumors varied widely in diameter in suspension, but Con A-induced aggregates were formed by random agglutination of cells of all sizes (Fig. 1b). Although the size of the clumps diminished with the concentration of Con A, even the lowest concentration used, 125 μg/ml, produced almost total clumping of tumor cells in small aggregates.

At least 3 hepatic nodules were obtained at each month from the 4th to 10th months. There was no agglutination of the cells of these 23 nodules, nor in 17 samples of cirrhotic nodules, nor in 14 samples of nonnodular liver, at any concentration of Con A tested. The control red blood cells were invariably agglutinated, while normal adult hepatocytes were not.

DISCUSSION

To our knowledge, this is the first comparison of Con A agglutination of cells of primary malignant tumors and their putative premalignant tissue. No agglutination of the cells of hepatic nodules, nor other nonmalignant hepatic cells, was detected with Con A exposure. This was true from the
Table 1

Con A agglutination of cells from PHC

<table>
<thead>
<tr>
<th>Diameter (cm)</th>
<th>Histology</th>
<th>Con A agglutination (%)</th>
<th>Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5</td>
<td>PD</td>
<td>98</td>
<td>L</td>
</tr>
<tr>
<td>2.25</td>
<td>WD</td>
<td>100</td>
<td>L</td>
</tr>
<tr>
<td>2.0</td>
<td>PD</td>
<td>95</td>
<td>M</td>
</tr>
<tr>
<td>1.5</td>
<td>WD</td>
<td>100</td>
<td>M</td>
</tr>
<tr>
<td>1.0</td>
<td>WD</td>
<td>98</td>
<td>L</td>
</tr>
<tr>
<td>0.7</td>
<td>WD</td>
<td>100</td>
<td>M</td>
</tr>
<tr>
<td>1.0</td>
<td>WD</td>
<td>95</td>
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</tr>
<tr>
<td>2.5</td>
<td>PD</td>
<td>100</td>
<td>L</td>
</tr>
</tbody>
</table>

| Hepatic nodules (23) | 1.2-2.1 |
| Cirrhotic nodules (17) | 0.2-0.5 |

- Tumor size is expressed as greatest diameter. For hepatic or cirrhotic nodules, the range of sizes is listed. In all samples of the cirrhotic nodules tested, some pooling was necessary to obtain sufficient tissue for cell suspension.
- PD, poorly differentiated; mainly sheets of cells with poor margins and nuclei which varied greatly in size. Necrosis was invariably present. WD, well differentiated, mainly trabecular or papillary areas with occasional solid foci. Most of these cells had definable borders and regular, hepatocyte-like nuclei.
- Con A was tested at several concentrations ranging from 125 to 500 µg/ml, but the results are expressed here at 250 µg/ml.
- The size of aggregates: L, large; M, medium.
- No agglutination with Con A.
- Numbers in parentheses, number of samples.

The earliest gross appearance of these aggregates after 4 feeding cycles and throughout the period when cancers were most frequently detected at 8 to 10 months. Indeed, 5 of these hepatic nodules were isolated from livers bearing PHC, thus eliminating external factors as a possible influence.

There is strong presumptive evidence that the hepatic nodule is a precursor of hepatocellular carcinomas (9, 18, 23). These lesions appear in the livers of animals exposed to all hepatocarcinogens prior to the appearance of cancer. Those livers in which they do not appear, despite exposure, do not develop tumors and, in a number of instances, histologically malignant foci have been identified within the confines of the nodules. However, despite these findings, and biochemical and morphological similarities between hepatic nodules and hepatocellular carcinomas, no alteration has been identified which marks the transition of the nodule to cancer. One explanation for this dichotomy has been the possibility that the nodule is a continually evolving lesion and, further, that the final transition to cancer is probabilistic, occurring in a subpopulation(s) of the nodules' cells (3, 9, 10, 11). Indeed, the long lag period between the appearance of hepatic nodules and final appearance of the carcinoma, the progressively changing pattern of biological activity of cellular populations within the nodules (4, 10, 11), and the presence of persistent and active cell division within them (3, 9), as well as other factors (9), have strongly suggested that significant alterations must occur sequentially in the nodules' cells before a malignant behavior is evident (9, 23).

It has been previously suggested that the loss of susceptibility to Con A agglutination demonstrated by hepatocytes during neonatal development occurs as a result of cell division. It is possible, therefore, that Con A agglutinability reappears abruptly after a single cell division(s) which occurs during the evolution of the nodules. Whether this resumption of agglutinability reflects a crucial functional alteration, obligatory for malignant activity, or a parallel and unrelated manifestation, is unknown. However, it appears that this characteristic is at present the most definitive method of distinguishing the cells of the nodule from those of carcinomas. To date, the only other "marker" with which to distinguish these tissues has been the lack of transplantability of the nodules.

The results of this study also demonstrate that agglutination of the cells of hepatocellular carcinomas is not the result of an adaptation acquired during transplantation (5, 6). The overwhelming majority of the cells of each PHC examined were susceptible to Con A agglutination. Furthermore, several of these tumors were relatively small when examined, which suggests that this characteristic was present at the onset of their appearance. From previous studies of the correlation between histological appearance and chromosomal composition (5), it is possible to suggest that both diploid and aneuploid tumors were present in the group of PHC tested. Diploid and aneuploid THC have shown great variation in growth rate (5, 6, 14) production of α-fetoprotein (5, 6, 20) and expression of fetal isoenzymes (12, 17, 25, 26). The uniformity of the phenomenon of Con A agglutination in all of the PHC studied and its presence during neonatal development occurs as a result of cell division. It is possible, therefore, that Con A agglutinability reappears abruptly after a single cell division(s) which occurs during the evolution of the nodules. Whether this resumption of agglutinability reflects a crucial functional alteration, obligatory for malignant activity, or a parallel and unrelated manifestation, is unknown. However, it appears that this characteristic is at present the most definitive method of distinguishing the cells of the nodule from those of carcinomas. To date, the only other "marker" with which to distinguish these tissues has been the lack of transplantability of the nodules.

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early in the growth of these tumors serves to emphasize its usefulness in a study of tumor biology.

REFERENCES


Fig. 1. In a, a cell suspension prepared from PHC 8 by the collagenase technique (see Table 1) after 30 min of incubation in buffer without Con A. Note the variation in cell size, good cell preservation and lack of clumping. Phase microscopy at a final magnification of approximately × 225. In b are cells of PHC 8 obtained as in a and exposed to a concentration of Con A of 250 μg/ml for 30 min. Almost every tumor cell was in large cell aggregates as depicted. The variable sizes of cells comprising these clumps are evident. Phase microscopy at a final magnification of approximately × 275.
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