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# Oval Cell Proliferation in Early Stages of Hepatocarcinogenesis in Simian Virus 40 Large T Transgenic Mice

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***In transgenic mice bearing the Simian Virus 40 large T antigen under the control of the human antithrombin III regulatory sequences, a stepwise progression toward hepatocellular carcinoma is observed. We have used two monoclonal antibodies (A6 and G7) developed against a surface antigen expressed in oval cells from dipin-treated mice, to analyze the emergence of such preneoplastic populations in the livers of antithrombin III Simian Virus 40 T transgenic mice. We show that a unique population of small heterogenous epithelial cells, which probably corresponds to oval and/or transitional cells according to their morphological features, consistently appears at approximately the 10th week after birth and proliferates thereafter. This oval cell-like population stained positively for A6 and G7 monoclonal antibodies. Furthermore, different subpopulations usually recognized as possible precursors of carcinoma cells including hyperplastic foci and neoplastic nodules as well as carcinoma cells, were also positive for A6 but not G7 monoclonal antibodies. Stimulation of cell proliferation by partial hepatectomy performed at the time of emergence of the oval-like cells resulted in a rapid increase in the number of oval/transitional A6-positive cells. Our findings support the view that a common mechanism may be involved in the development of carcinomas that are induced by chemical carcinogens and in transgenic mice expressing a potent oncogene under the control of a hepatic specific promoter. In addition, our findings demonstrate a specific precursor-product relationship between the appearance of the oval/transitional cells and the development of neoplastic hepatocytes in this transgenic model. (Am J Pathol 1993, 143:1326–1336)***

Many recent observations support the idea of a liver stem cell population in normal liver[2] (for review see ref. 3) that retains broad developmental capacities. Exposure of rats and mice to a number of chemical carcinogens that induce hepatocellular carcinoma (HCC) and hepatocholangioma results in the proliferation of small epithelial cells, morphologically defined as oval cells.[4–9] These cells are also stimulated to proliferate after severe injury.[10,11] Although the origin and localization of oval cells still remain controversial, they are thought to be closely related to epithelial cells of terminal bile ductule[2] or to even less mature periportal stem cells[3] and are considered by some authors to be the equivalent of liver stem cell.[12–14]

The oval cell population has been shown to be composed of heterogeneous subpopulations, some of which have the capacity to differentiate into hepatocytes or ductular cells.[3,15–17] However, whether tumor cells originate from this cell population still remains unclear. Although recent observations have suggested a direct relationship between oval cells and hepatoma cells in the rat,[18,19] their precise role in the development of HCC is not yet fully established.

We and others have recently developed another approach to study oncogenesis, utilizing transgenic mice that carry an oncogene placed under the con-

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trol of a tissue-specific promoter. This has proved to be a powerful strategy for the study of the development of carcinogenesis in various tissues. Several new tumor models have been established, especially those using the large T antigen of the simian virus 40 early region (SV40 TAg)[20,21] whose expression has been directed to a wide variety of tissues when linked to heterologous regulatory regions.

In general, SV40 TAg expression has been found in the cell population in which the promoter used is normally expressed. Several hybrid transgenes have been directed to the liver by using different hepatic regulatory sequences, including metallothionein,[22] a major urinary protein,[23,24] $\alpha$-1-antitrypsin, and albumin regulatory elements.[21,25] In all instances, transgene expression has led to the development of liver tumors that are primarily HCCs. However, to date no evidence has been available concerning the presence of oval cells in transgenic mice, except in mice that are transgenic for hepatitis B virus and have been exposed to hepatocarcinogens.[26]

Several transgenic lines bearing the SV40 large T oncogene directed to be expressed in the liver by antithrombin III (ATIII) regulatory elements have been developed in the laboratory.[1] ATIII is a serum protein mainly synthesized in the liver, and these transgenic mice develop HCC within 6 to 8 months after birth, depending on the mouse line.[1]

The present study was undertaken to determine whether hepatocarcinomas that arise from these transgenic mice exhibit the characteristic changes that occur in chemically induced HCCs,[27] in the course of their development. In this context, the initial question we addressed was whether oval cells can proliferate during the early stages of the progression to HCC.

Using oval cell fraction from livers of dipin-treated mice,[28] specific monoclonal antibodies (MAbs) were raised that react against surface antigens common to mouse biliary epithelial cells and oval cells in the liver (MAbs A6 and G7). In normal adult mouse livers, A6 and G7 react with membranes of epithelial cells in bile ducts and ductules. They are not present on hepatocyte cell surface. Utilizing these antibodies, we demonstrated that large TAg-specific expression induces the appearance of a premalignant cell population that expresses the A6 and G7 membrane antigens. These positive cells have morphological and ultrastructural features resembling those of oval and/or transitional cells. After 12 weeks, proliferation of these cells was increased and was stimulated when livers from transgenic mice were subjected to partial hepatectomy. We also found that those neoplastic foci that are positive for TAg coexpress A6 antigen simultaneously.

## Materials and Methods

### Animals

Transgenic mice for the hybrid ATIII SV40 TAg were established as described previously.[1] In the line used, the transgene was integrated on the Y chromosome (data not shown), which facilitates the screening procedure. The time course of tumor development was identical to ASVA1 mice.[1] The transgene was transmitted as a single allele. Both normal and transgenic animals were B6/D2 F1 hybrids.

Animals were sacrificed at various ages ranging from 4 to 24 weeks. Some animals were partially hepatectomized between 10 to 14 weeks. After avertine anesthesia, two lobes corresponding roughly to a 50% hepatectomy were removed. Up to 12 weeks of age, three animals were examined at each developmental point, ie, 4, 6, 8, and 10 weeks. From 12 weeks of age, six mice were examined at each age tested (12, 14, 18, and 20 weeks) and for hepatectomy experiments.

### Tissue Preparation and Sectioning

Liver samples were fixed either by simple immersion or perfusion followed by immersion in a 4% paraformaldehyde solution buffered with 0.1 mol/L sodium cacodylate, pH 7.4, and added with 0.05% saponin. For direct fixation by immersion, the liver was removed from the mouse and sections approximately 1 to 2 mm thick were cut with a surgical blade; they were fixed for 4 hours at 4 C. When samples were fixed by perfusion, phosphate-buffered saline (PBS) was injected into the portal vein for 30 seconds to 1 minute and followed by perfusion with the fixative for 15 minutes at a flow rate of 4 ml/minute at 15 C. The livers were then removed, and 1- to 2-mm slices were cut and fixation was continued by immersion for 4 hours at 4 C as for immersion procedures.

After fixation time in cold fixative, the samples were washed several times in PBS for 2 to 3 hours. Slices were then frozen in isopentane in liquid nitrogen and stored at −80 C. For light and electron microscopy, 5- to 10-μ sections were cut with a freezing microtome (Shandon, U.K.) and revealed for A6 or G7 antigens.

Cryostat sections from unfixed livers were also prepared and fixed for 10 minutes in cold acetone,

permeabilized for 5 minutes in 1% Triton X 100/PBS, and then washed before revealing TAg. In some experiments, slices were fixed with 8% paraformaldehyde in PBS containing 0.05% saponin to reveal α-fetoprotein (AFP) antigen.

For electron microscopic studies, liver sections were postfixed in 1% osmium tetroxide in 0.1 mol/L cacodylate buffer, pH 7.4, following the immunocytochemical reaction and embedded in epon following ethanol dehydration.

Anti-AFP antibodies were a gift from Dr. A. Gleiberman (Cancer Center, Moscow). Anti-TAg antibodies were a gift from Dr. D. Hanahan (University of California at San Francisco, CA). MAbs A6 and G7 were used as rat hybridoma supernatant as previously described.[28] They recognize subpopulations of oval cells as well as biliary epithelial cells but not hepatocytes.

## *Immunocytochemistry*

Sections or freely floating fixed sections were preexposed for 1 hour to nonspecific antiserum at room temperature. They were then exposed to either monoclonal rat anti-mouse A6 (diluted 1:60 with PBS), monoclonal rat anti-mouse G7 (diluted 1:100 with PBS), polyclonal rabbit anti-mouse AFP (diluted 1:100 with PBS), polyclonal rabbit anti-mouse vimentin (diluted 1:2,000 with PBS), or polyclonal rabbit anti-mouse TAg (diluted 1:5,000 with PBS) for 18 hours at 4 C. Sections were exposed to a nonimmune serum as a control.

Bound polyclonal antibodies were detected using fluorescein-conjugated goat anti-rabbit antibodies for TAg and avidin-biotin peroxidase complex (Vector Laboratories, Burlingame, CA) for AFP and vimentin. Sections exposed to monoclonal A6 were treated with sheep anti-rat polyvalent immunoglobulin G, peroxidase-linked F(ab) fragments (Amersham, U.K.) (dilution 1:50). Incubation time was 30 minutes at room temperature.

Peroxidase activity was subsequently revealed by incubating 3-3′ diaminobenzidine in 0.1 mol/L TRIS-HCl, pH 7.6, for 20 minutes at room temperature.[29] After visualization of the reaction, sections were counterstained with hematein for light microscopy analysis. Other sections were processed for electron microscopic examination.

# *Results*

The histological liver changes induced by SV40 TAg expression are identical to those previously reported for other lines carrying the same transgene (ASVA1).[1] Separate stages can be distinguished histologically, including 1) dysplastic hyperplasia and replacement of part of the original hepatocyte population that becomes apoptotic (in preparation), 2) formation of multifocal hyperplastic foci composed of small cells with dense nuclei (12 to 16 weeks), 3) appearance of neoplastic nodules (16 to 20 weeks), and finally 4) maturation to HCCs. A similar sequence has been observed in response to the expression of SV40 TAg[23] under the control of the major urinary protein promoter.

## *Histological Properties of Transgenic Livers*

Histological analyses of liver sections from these transgenic mice of different ages using hematein staining revealed several cell types emerging approximately at the 10th week after birth. Among these populations, small cells are clearly visible (Figure 1A). They have an oval basophilic nucleus with scant cytoplasm.

These cells were identified as small epithelial cells radiating from the portal spaces in rows or clusters along the plates of hepatocytes, then forming structures outside the portal spaces at 12 weeks of age. They seem to be morphologically similar to the small cell population described as oval cells by a number of investigators in the liver of carcinogen-treated rats and mice.[4–9,28]

Another cell type present was a population of small hepatocytes that were distinguished from normal hepatocytes solely on the basis of size. They differed from oval cells by their shape and contained prominent nucleoli and condensed chromatin. In addition, cells in these foci (Figure 1B) do not have the regular sinusoidal arrangement found in normal liver. At this stage and in the following weeks, apoptotic hepatocytes were present and were distributed throughout the parenchyma.

## *Immunolocalization of A6 Antigen*

### *Light Microscopy*

Liver sections from transgenic mice and normal mice of different ages were treated with MAb A6 to determine whether it could react against preneoplastic populations with oval cell-like structures. MAb A6 detects an antigen shared by mouse biliary epithelial cells in normal liver and by oval cells induced by a single injection of dipin following partial hepatectomy.[28] Figure 2A shows a 8-μ section from

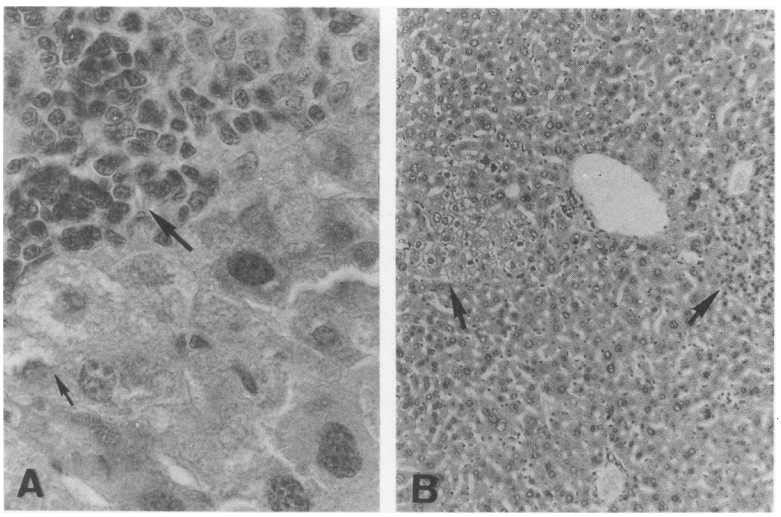


Figure 1. *Light microscopy of H&E-stained section of liver from an ATIII TSV40 mouse, showing a group of oval/transitional cells (arrow) at 12 weeks* (**A**, *280×*) *and microfoci at 16 weeks (arrows)* (**B**, *82×*). *Apoptotic cells are visible at the vicinity of the cluster of oval cells (arrow).*

a normal liver stained with A6 MAb. A few cells located around portal areas and corresponding to bile ductular cells were reactive.

In transgenic mice younger than 10 weeks, scattered positive cells were present, located around the periportal areas. This pattern was similar to that observed in normal livers. After 10 to 12 weeks of age, which corresponds to a stage of dysplastic hyperplasia in transgenic mice, and before the appearance of multiple hyperplastic foci, there was an increase in the number of A6-positive cells. These cells correspond to oval cells, as already demonstrated by hematein staining. We also observed another population of cells with size and structure intermediate between those of oval cells and mature hepatocytes. They have a round nucleus, more cytoplasm, and larger size than oval cells. From 12 weeks of age, clusters of A6-positive cells are located around periportal areas cells but also infiltrate the tissue from these areas (Figures 2, B and C, and 3A). The microfoci visible at this stage are also A6-positive. They are neither encapsulated nor sharply delineated but are also morphologically different and easily distinguishable from the original hepatocyte population. These foci consist of small cells and might represent a population with a transitional phenotype as they differ from hepatocytes in their antigenic properties: they react strongly with MAb A6 (Figures 2, D and E, and 3B).

These cells proliferate and give rise to large hyperplastic foci. After 26 weeks, each liver of transgenic mice is composed of multiple nodules that promote the progression to HCC. These different cell populations are largely A6-positive (Figure 2F). After 28 weeks, all the transgenic mice exhibited several tumors that satisfy the criteria of malignancy. In a few cases, lung metastasis also appeared, and these metastatic cells were A6-positive (data not shown).

### *Electron Microscopy*

Electron microscopy was performed on sections from 12- to 15-week-old mouse livers that had been previously labeled with MAb A6. Cells of different sizes but smaller than typical hepatocytes were stained. By analogy to the cell populations that have been described in carcinogen-treated rats, they may be considered as transitional forms between oval cells and hepatocytes. Compared to typical oval cells, they seem to have a more differentiated morphology with a number of organelles (mitochondria and endoplasmic reticulum) and a lower nucleus/cytoplasm ratio. The labeling was restricted to the plasma membrane that was uniformly stained. Bile canaliculus structures were also intensely stained (Figure 4).

## *Oval Cells Express a Marker of Normal Adult Bile Duct Epithelium*

Another MAb, G7, developed by one of us, reacts strongly with an antigen contained in bile duct epithelium in normal mouse and in oval cells in the dipin-treated mouse model.[28] In ATIII TAg transgenic mice, this antibody revealed a similar distribution pattern in biliary epithelial and oval cells. By contrast, hyperplastic foci as well as hepatocellular nodules were negative at any stages examined, as in dipin-treated mice (data not shown).

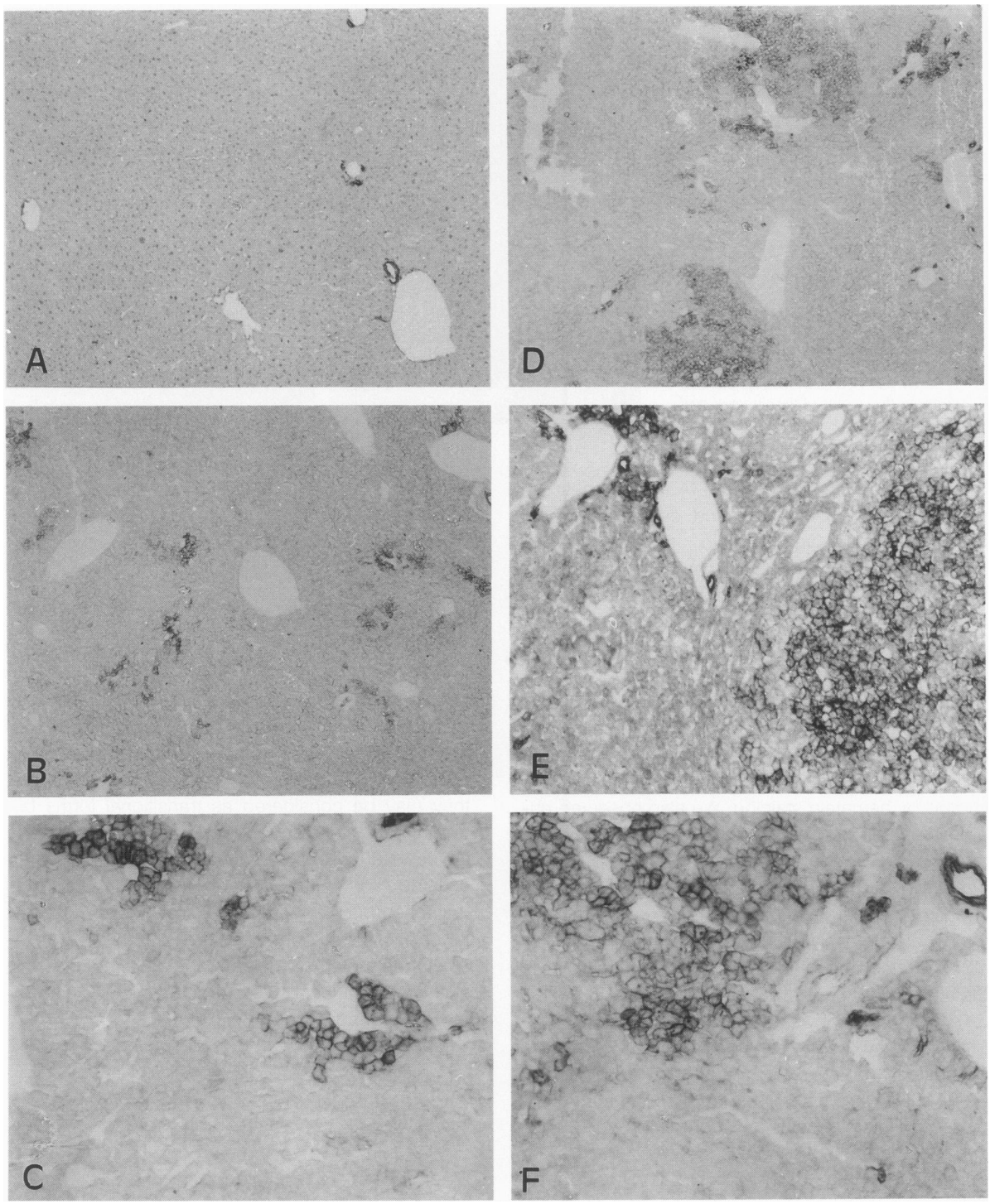


Figure 2. *Liver sections from normal and ATIII TAg transgenic mice killed at different stages of hepatocarcinogenesis. Immunoperoxidase stain using A6 monoclonal antibodies showing positively stained cells in close association with small ducts in normal liver of 4-week-old transgenic mice* (A, 45×); *the same stain shows progression of oval cells as clusters of cells within the parenchyma at 12 weeks* (B, 45×), (C, 72×) *and foci formation at 16 to 20 weeks* (D, 45×), (E, 72×). *Note the heterogeneity of A6 staining* (F, 72×).

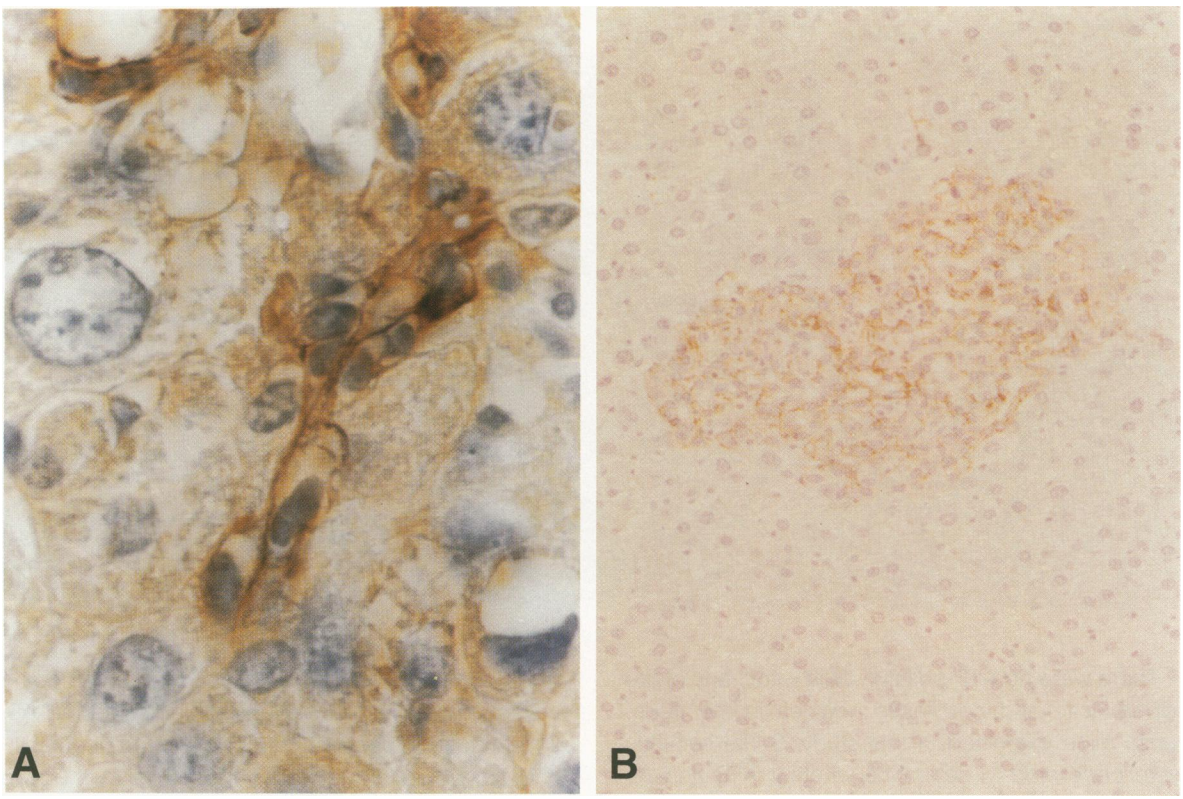


Figure 3. *Light microscopic immunolocalization of A6 antigen in oval cells* (A, *624* ×) *and microfoci* (B, *200* ×).

The two MAbs used in these studies have different specificities as in dipin-treated mice. MAb G7 recognizes only the bile duct cells and oval cells, whereas MAb A6 detects a marker present on oval cells and cells at different stages of differentiation in the course of hepatocarcinogenesis. Thus, the two markers reveal the existence of different subpopulations of oval cells.

## *Influence of Partial Hepatectomy on the Appearance of A6-Positive Cells*

Partial hepatectomy was performed on transgenic mice of different ages as well as on normal mice to examine the effect of transient proliferation on the emergence of A6-positive oval or transitional-like cells. Up to the 12th week, neither transgenic livers nor normal livers showed any change in their phenotype or A6 positivity after 60% hepatectomy, and small cells remained located around periportal areas. By contrast, in transgenic livers but not in normal livers, after 12 weeks of age, at a time when oval cells started to spread throughout the livers, resection of lobules resulted in a dramatic increase of A6-positive cell populations. Numerous clusters of oval and/or transitional cells were found to infiltrate livers from transgenic mice. In addition, hyperplastic microfoci were also induced compared to the same liver before hepatectomy; the lobes that were resected were used as a control (not shown). Hepatectomized livers were studied between 3 to 5 days after operation. We also performed PCNA antibody staining on resected and hepatectomized livers from 14-week-old mice. The increase in positively stained cells after hepatectomy strongly suggested that A6-positive cells induction correspond to proliferation of new cellular populations.

## *Expression of AFP*

We examined the expression pattern of AFP in the transgenic livers of different ages. AFP is an abundant serum glycoprotein highly expressed in fetal livers and in most liver tumors but is undetectable in normal adult rat or mouse liver. We detected AFP in a small population of transitional-like cells in the transgenic livers at 12 to 18 weeks after birth. After 18 weeks of age, positive cells were mainly visible at the periphery of hyperplastic nodules in atypical cells morphologically different from hepatocytes (not shown).

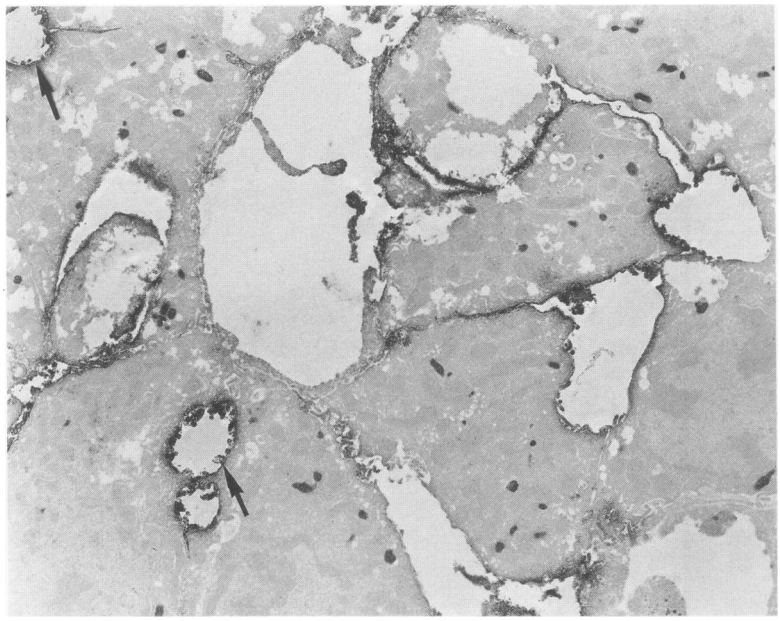

**Figure 4.** *Light microscopic immunolocalization of A6 antigen in oval cells* (**A**, *624×*) *and microfoci* (**B**, *200×*). *Electron microscopy picture of transgenic liver from a 15-week-old mouse after immunoperoxidase staining with A6 monoclonal antibodies* (*5,900×*). *Note that bile canaliculi are stained* (*arrows*).

There were no AFP-positive cells within any of the hyperplastic and neoplastic nodules in the transgenic mice examined in this study. Serial sections were performed to determine whether AFP and A6 antigens were present within the same population of cells. The results were always negative in our transgenic model. In normal liver, no AFP-positive cell was detected.

## *Expression of Large T Antigen*

In livers from 12-week-old transgenic mice, large TAg could not be detected in the clusters of oval cells from the transgenic liver at 12 weeks of age. However, in the populations of transitional cells, a weak staining for large T could be seen in some cells that were also positively labeled with MAb A6. By contrast, hyperplastic foci as well as neoplastic nodules were strongly positive for large TAg. Figure 5A shows a liver section from a 18-old-week transgenic mouse, in which the nuclei of transitional cells are weakly positive for large TAg at the vicinity of an intensely-stained hyperplastic focus.

Serial sections performed on livers from 5- to 6-month-old transgenic mice demonstrated that neoplastic cells could coexpress large T and A6 antigens (Figure 5B). However, even at this stage, heterogenous labeling could be noticed for both antigens, because in the same focus some cells were negative for MAb A6 and other cells were negative

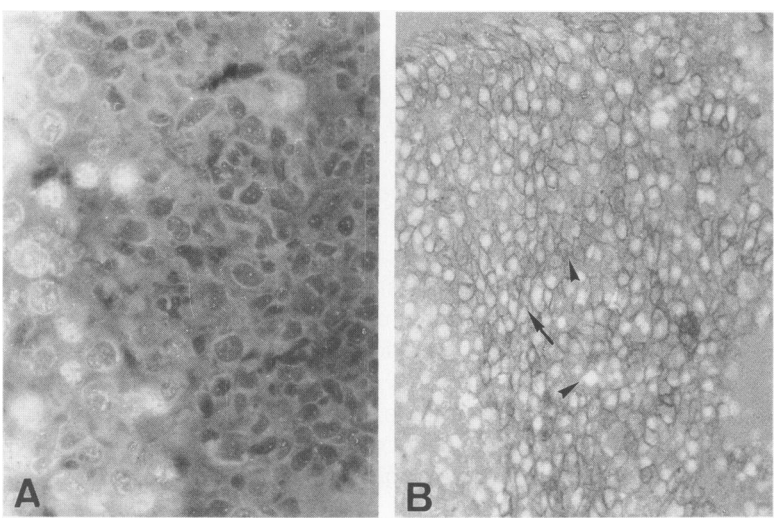


**Figure 5.** *Liver section from a 18-week-old transgenic mouse. Immunofluorescence produced by reaction of TAg antibodies showed strong staining in transformed hepatocytes and weak staining in adjacent small transitional cells* (**A**, *273×*). *Double-labeling using TAg* (*immunofluorescent stain*) *and A6 antibodies* (*immunoperoxidase stain*) *reveals the heterogeneity of a focus with cells expressing both antigens* (*arrow*) *and cells expressing either one or the other* (*arrowheads*) (**B**, *104×*).

for large T, whereas a large proportion were positive for both antigens.

### *Vimentin Staining*

In both normal and transgenic mice, hepatic distribution of vimentin, as expected, was observed in sinusoidal endothelial and Kupffer cells. Some clusters of oval and transitional cells proliferating during the early stages of carcinogenesis displayed apparent staining for vimentin (not shown). Some foci were also found to be vimentin-positive. Some of these foci did not react with MAb A6. This result reflects the characteristic of proliferation displayed by such transitional cells, which is, for this marker, independent of neoplastic development.

## *Discussion*

The study of cell lineage development during carcinogenesis has been hampered by the paucity of appropriate markers specific for preneoplasia. The most successful approach has been the use of monoclonal or polyvalent antibodies that recognize hepatic differentiation markers[8,30,31] and the use of MAbs against the surface components of bile ductular epithelial cells and hepatocytes.[32–37] These antibodies have been selected and utilized for their ability to distinguish clearly between these different types of cells. This approach has been widely used with rats exposed to carcinogenic diets.

To date, however, in mice livers transformed by chemical carcinogens or that express an oncogenic transgene, no specific marker has been identified that selectively detects preneoplasia subpopulations, except A6 and G7. In the present study, we have used these specific MAbs to investigate the proliferation of oval cells and to trace the cell lineage leading to HCC using a transgenic mouse model in which a powerful oncogene is specifically expressed in liver. In precancerous livers from dipin-treated mouse, A6 antigen, besides biliary epithelial cells, is present on the membrane of oval cells and of a fraction of newly formed hepatocytes usually located in close association with oval cells. A fraction of transformed hepatocytes also express A6 antigen. By contrast, G7 antigen is not found in newly formed and transformed hepatocytes but only in biliary epithelial and oval cells.

We have found that populations of nonparenchymal cells, which are essentially not detected in normal adult liver, proliferate in response to ATIII SV40 TAg stimulus. Light and electron microscopic analyses have revealed that a portion of these populations was positive for MAb A6, which recognizes a membrane marker specific to bile ducts in normal liver. In the transgenic liver, the A6 marker was present in hyperplastic foci and nodules where it was coexpressed with TAg, which is controlled by a differentiated hepatocyte-specific promoter (ATIII). This is the first evidence in mice of a marker that is contained in specific subpopulations during the early stages of preneoplastic development. Our results with A6 and G7 antigens coincide well with those obtained in dipin-treated mice. They show a close parallelism in the dynamics of the different cell populations and A6 and G7 expression in both systems. They can be compared to those of Dunsford and Sell[34] who, using the OV6 antigen as a marker, raised a number of MAb from rat livers induced by AAF and was able to follow neoplastic progression from positive oval cells to positive carcinoma cells.

Our results differ from those reported by Sepulveda et al.[25] They did not find clusters of oval cells in their transgenic mice that express SV40 large T antigen under the control of $\alpha$-1-antitrypsin regulatory elements. However, despite the fact that their model seems to be similar to ours, the level of TAg expression might be slightly different, influencing the level of oval cell proliferation; it is conceivable that, in their case, a small percentage of oval cells could emerge during a short period of time and thereafter differentiate into more mature cells. We believe that the proliferation of oval cells is a general phenomenon for TAg transgenic mice, and we are currently testing this hypothesis with other oncogenes.

The oval cell population from dipin-treated mice permitted the production of two MAbs, A6 and G7, that defined different antigens contained in bile ducts and Hering canals. However, A6 displayed heterogeneous staining of canals, thus defining two subpopulations of G7-positive cells and revealing heterogeneity of oval cell populations. This heterogeneity was also found in our transgenic model even at the stage of neoplasia because not all the cells within the nodules were A6-positive. Moreover, G7 antigen was not expressed in neoplastic cells, which suggests that G7 is a marker of immature precursor cells only and/or of ductular cells. Similar patterns of subsets of cells that react against different monoclonal antibodies have also been documented in carcinogen-fed-rats[34] where uneven distribution of oval cell markers also occurred.

In the rat, oval cells have been shown to proliferate when the cytotoxic effects of chemical carcinogens on hepatocytes prevent normal tissue renewal that occurs after hepatectomy. Similarly, oval cells

proliferate during the regenerative process that occurs after liver injury induced by D-galactosamine. In ATIII SV40-expressing transgenic livers, the oval cells that proliferate are morphologically and phenotypically similar to oval cells found in rat liver during carcinogen-induced hepatocarcinogenesis and in liver injury induced by D-galactosamine. The oval cells also showed a similar distribution within the liver lobule in which they seemed to radiate out from the portal areas. In our transgenic mouse model, the appearance of such oval cell populations was coincident with the emergence of neoplastic progenitor cells.

The burst of oval cells occurred after large TAg-induced cytolysis and after the peak of apoptosis that we found between weeks 4 and 10. At this stage, oval cells do not express SV40 large T, probably because the ATIII promoter that drives its synthesis is activated in more differentiated cells. It is known that the ATIII expression reaches only 50% of its normal expression at day 17 in the rat.[38] Thus, this burst of oval cells may correspond to an immature population that is induced to proliferate because of the cytotoxic effect of large T on mature hepatocytes.[39] It is noteworthy that the absence of cholangiocarcinoma in such mice can also be explained by the lack of large T expression in the immature oval cells and then in the ductular cells.

Weakly positive cells could correspond to a transitional, more differentiated phenotype committed toward hepatocyte lineage and that could not lead to the proliferation of transformed bile ducts; rather, they could give rise to neoplastic foci of transformed hepatocytes through a multistep process. In that respect, it is noteworthy that partial hepatectomy, which in normal liver induces proliferation of normal hepatocytes, resulted in the transgenic livers in a rapid increase of oval/transitional cells as well as foci formation. Although we suggest the existence of transitional cells in our model in view of electron microscopy results, a precise distinction between oval and transitional cells has not been attempted. We then cannot exclude the possibility of A6 reexpression in unrelated cell populations that do not derive from oval cells.

The expression of A6 and G7 antigens at the surface of normal bile duct cells is in agreement with the hypothesis of Fausto and Marceau for rat liver,[15,17] which suggests that at least some cells in the oval compartment are dual lineage progenitors and might constitute a facultative stem cell population. These cells would express new developmental properties as a consequence of hepatic damage.

In transgenic mice, at the stage of hyperplastic foci, AFP-positive cells were found in the periphery of microfoci in large cells, which probably correspond to hepatocytes with a dedifferentiated phenotype. Our model resembles the rat Solt Farber regimen model in which carcinoma cells are also AFP-negative.[39]

In summary, we conclude that, during carcinogenesis in our transgenic mice model, small epithelial cells with progenitorlike properties and transitional morphologies reflecting neoplastic development proliferate. This work identifies several similarities between the stepwise process of cell transitions in carcinogenesis induced by azodye carcinogens in rats and by a powerful oncogene in mice. Based on these findings, we propose that at least a portion of transformed hepatocytes is derived from oval/bile ductular cells, which indicates, in mice as in rats, the role of bipotential liver progenitor cells and supports the theory of liver cancer development as a multistep process[40,41] that arises from periportal stems cells via a number of preneoplastic lesions. The exact origin of cancerous hepatocytes is still unclear. The experimental system described here may be useful in the further characterization of oval cells and in the determination of their role in carcinogenesis. Work is in progress to characterize biochemically A6 antigen.

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