Liver Cancer in Transgenic Mice Carrying the Human Immunodeficiency Virus tat Gene

Jonathan Vogel, Steven H. Hinrichs, Laura A. Napolitano, Lien Ngo, and Gilbert Jay

ABSTRACT

Patients with the acquired immunodeficiency syndrome are at risk to develop a variety of different cancers. Based on epidemiological data, Kaposi's sarcoma and non-Hodgkin's lymphoma have been clearly associated with infection by the human immunodeficiency virus (HIV). Additional cancers such as basal cell and squamous cell carcinomas, melanoma, and hepatocellular carcinoma have also been reported to be associated with a diagnosis of acquired immunodeficiency syndrome. A direct causal role of HIV has yet to be established for any of these cancers. We now report that transgenic mice carrying the HIV tat gene develop a high incidence of hepatocellular carcinoma after a long latency and that these changes in the liver are likely to be initiated by extrahepatic growth signals from the tat expressing cells in these mice. We predict that as acquired immunodeficiency syndrome patients begin to respond to therapy and show prolonged survival, such "secondary" malignancies induced by HIV will become increasingly prevalent.

INTRODUCTION

In addition to the immunodeficiency and neurological complications associated with HIV infection (1) a variety of different cancers have also been described in individuals infected with HIV (2-4). An increased incidence of both KS and NHL have clearly been associated with HIV infection by epidemiological data (5, 6). Additional cancers such as basal cell carcinoma; squamous cell carcinomas of the rectum, skin, head, and neck; melanoma; and hepatocellular carcinoma have been reported to be associated with a diagnosis of AIDS based on epidemiological data, reviews of the hospital charts of AIDS patients, and case reports (2-8). However, the association of these cancers with HIV infection is not as striking as it is for KS and NHL. This uncertainty is due in part to the presence of other potential risk factors of oncogenesis such as the Epstein-Barr virus, cytomegalovirus, hepatitis B virus, and human papillomavirus in the HIV-infected population developing these additional cancers (9-14). Each cancer may have its own unique etiology in the overall setting of HIV infection. Uncertainty also results when different investigators analyzing different sets of data reach different conclusions about cancers such as the squamous cell carcinomas of the rectum, skin, head, and neck (2-8).

Due in part to better treatment and new therapeutic modalities, the latency period from the time of HIV infection until the onset of AIDS continues to lengthen, and increased numbers of people will be harboring HIV for longer periods of time. Thus, it is expected that those cancers which require a longer latency period than KS and NHL will appear with increased frequency.

As is also true for KS and NHL, a direct causal role of HIV has yet to be established for these additional cancers. Several different mechanisms could explain how HIV could contribute to or be responsible for these different cancers. Perturbations of varying severity to the immune system are likely to occur at different stages of HIV infection and contribute to the development of malignancies (1). Alternatively, HIV may act as a cofactor with environmental agents such as the viruses mentioned above and together enhance the ability to induce cancer. A third possibility is that HIV genes may induce cancer by interfering with important functions in cells and tissues where they are expressed. This interference may alter the differentiated state of a cell leading directly to proliferation of that cell, or it may induce growth factors or cytokines to be released from that cell which can then induce proliferation in other cell types in a paracrine or endocrine manner.

We have previously described a transgenic mouse model where the tat gene of HIV, under the control of the viral LTR, is expressed primarily in the dermal portion of the skin and induced skin lesions that resemble KS (15). These skin lesions are found only in males and consist of focal epidermal thickening and dermal proliferation of varying severity which progress to dermal tumors in some of the mice. The findings suggest that expression of the tat gene in the skin may alter the normal communication between cells by inducing epidermal cells to inappropriately release factors which stimulate the underlying cells of the dermis to proliferate (15). Prolonged expression of cytokines from epidermal cells may result in extensive dermal hyperplasia and selection of a second genetic event which will give rise to cancer.

We now describe a long term follow-up and analysis of these same transgenic mice which was conducted to determine if additional tumors are associated with expression of the tat gene. This analysis reveals that as the male mice age past 18 months a significant percentage develop liver tumors, lending support to epidemiological evidence that hepatocellular carcinoma may be associated with HIV infection in humans (3, 4, 7, 8, 16).

MATERIALS AND METHODS

Derivation of Transgenic Mice. The generation of transgenic mice containing the LTR-tat transgene has been described previously (15). The transgene in these mice consists of the HIV LTR-regulatory region directing expression of the HIV tat gene. Three independent founder lines designated C4, E10, and F2 were used in these studies.

Tissue Analysis. The male and female mice included in this study were autopsied and histologically examined when they either died of natural causes or were sacrificed for other reasons past the age of 12 months.

Histological Procedures. Tissue samples from autopsied mice were placed in a 10% buffered formalin for 24 h, embedded in paraffin, sectioned, and stained in hematoxylin and eosin.

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2 To whom requests for reprints should be addressed.

3 The abbreviations used are: HIV, human immunodeficiency virus; KS, Kaposi's sarcoma; NHL, non-Hodgkin's lymphoma; AIDS, acquired immunodeficiency syndrome; LTR, long terminal repeat; PCR, polymerase chain reaction; HA, hepatic adenoma; HCC, hepatocellular carcinoma.

PCR Amplification of RNA. RNA was prepared from tissues using the standard guanidinium isothiocyanate-CsCl procedure. Optimal concentrations of specific upstream and downstream primers for the tat gene were added to the RNA in a standard PCR reaction buffer. The RNA was then reverse transcribed for 20 min at 42°C by 5 units of avian myeloblastosis virus reverse transcriptase, followed by 20 cycles of PCR amplification with Taq DNA polymerase (1 min at 95°C, 1 min at 55°C, and 1.5 min at 72°C). The PCR-amplified products were visualized by liquid hybridization to a 32P-end labeled internal oligonucleotide (5 min at 95°C followed by 1.5 h at 55°C), followed by electrophoresis on an 8% polyacrylamide gel. After drying, the gel was exposed to Kodak XAR film for 1–16 h. All primer and probe concentrations were optimized and multiple dilutions of RNA were performed to ensure that the results were within the linear range (17). Identical procedures are performed with β-actin primers to control for the amount of RNA used.

RESULTS

Development of Liver Lesions in tat Transgenic Mice. A long term follow-up and analysis of these transgenic mice reveals that in addition to the KS-like skin lesions previously described, a high percentage of the male transgenic mice also develop liver lesions as they age.

Table 1 includes data from three independent transgenic lines and shows the incidence of liver lesions in each line and the breakdown of the liver lesions into different histological grades. Since only three liver tumors were found among 105 female mice (<3%), only data from the male cohort are presented. While the percentage varied somewhat between different transgenic lines (33.8–51.7%), the overall incidence of liver lesions among males is 42.2% (78/185). Control CD-1 males which were littermates of the transgenic mice were similarly analyzed and only 8% (4/50) had liver lesions; this is comparable to the published incidence in multiple surveys of CD-1 mice (18–21). The incomplete penetrance of disease among the male transgenic mice may be explained by the long latency from birth to the development of the liver lesions.

Histological Characterization of the Liver Lesions. Based on histology, the liver lesions could be divided into three different, but overlapping, categories: liver cell dysplasia, HA, and HCC (22). The dysplastic lesions were diffusely present throughout the liver and did not alter the overall liver architecture. When compared with normal liver histology (Fig. 1A), they consisted of considerable hepatocyte pleomorphism in both nuclear appearance and cell size (Fig. 1F). The large cells in the dysplastic mixture have large irregular nuclei and proportionately increased amounts of cytoplasm, while the small cells have an increased nuclear cytoplasmic ratio and a dense chromatin pattern (23, 24). The cytoplasm is usually finely vacuolated and basophilic but occasionally can be eosinophilic. These preneoplastic lesions were found in all histological categories but were present without accompanying HA or HCC tumors in only 13% (10 of 78) of those animals with liver lesions (Table 1).

In this dysplastic background, HAs of variable size and histological appearance were frequently present. The size ranged from small nodules which appeared grossly as pearly white areas to tumors which involved entire lobes. The lesions were often multifocal and involved multiple lobes. In a typical nodule, the proliferating hepatocytes would compress the adjacent normal liver parenchymal tissue resulting in a sharp border (Fig. 1C). The architecture of the liver lobule was generally maintained even though involved with HA. Histologically, the HA hepatocytes were usually basophilic, sometimes with finely vacuolated cytoplasm (Figs. 1, B and C). Occasionally cells in the HA nodule would have an eosinophilic cytoplasm, but clear lesions were not seen. These HA lesions were seen without accompanying HCC in approximately 23% (18 of 78) of those animals with liver lesions (Table 1).

The most common histological finding in these mice by a large margin was HCC, being found in 64% (50 of 78) of liver lesions. In some cases, the HCC lesion clearly arose in a preexisting HA lesion (Fig. 1F), while more commonly the HCC lesion was quite extensive and occupied multiple liver lobes. The normal architecture of the liver lobules was usually obliterated by the HCC and both solid and prominent trabecular patterns were present with the trabeculae three or more cells thick (Fig. 1, D and E). The HCC had frequent mitoses, multinucleated cells of variable size, and cytoplasm that is vacuolated and contained inclusion bodies.

Progressive Nature of the Liver Lesions. The distinct impression derived from the histology is that there is a progression through the different histological stages, with diffuse dysplasia as the initial alteration of hepatocytes. Out of this dysplastic background, focal and multifocal HA nodules are noted which sometimes progress to involve the entire lobe where they are found. Foci of HCC are often noted inside these larger HA lesions (Fig. 1F), while HCC lesions in other livers are multifocal and involve entire lobes. Additional histological findings in the livers of these transgenic mice included both occasional lymphocytic infiltrates in the earlier dysplastic lesions and periportal oval cell proliferations in all three histological categories, but most prominently in advanced HCC (25, 26).

Role of tat Gene Expression in the Development of Liver Tumors. In order to determine the role of the HIV tat gene in the development of this phenotype, the expression of tat mRNA in the liver was examined. Previously, we had determined by Northern blot hybridization that tat was expressed only in the skin of these animals and not in the liver. However, the possibility remained that tat was expressed at a level undetected by Northern blot analysis in a subset of liver cells, such as the Kupffer cells, and could initiate cellular events leading to hepatocyte dysplasia and proliferation. In order to increase the sensitivity of detection, PCR following a reverse transcriptase intermediate step was used to determine if low levels of tat mRNA were present in the liver (Fig. 2). Both the F2 (Fig. 2B) and the C4 (data not shown) founder lines did not have detectable expression of tat mRNA. In this transgenic model, the expression of the HIV tat gene is required for the development of liver lesions, and the progression from dysplasia to carcinoma is, at least in part, dependent on tat expression.
induce a variety of different growth factors and cytokines which could have far reaching effects on other target tissues besides the dermis. The skin is more than just a passive integument providing protection for the body. This master organ is involved in a variety of immunological, endocrinological, and metabolic functions that may have widespread effects, especially since even small amounts of growth factors or cytokines released on a per cell basis may be quite significant given that skin is the largest organ in the body (27). Factors known to be released by different cell types in the skin, such as keratinocytes and Langerhans cells, include interleukin 1α and 1β, interleukin 6, basic fibroblast growth factor, transforming growth factor α and β, α-interferon, granulocyte-macrophage colony-stimulating factor, and prostaglandin E2 (28-30).

The liver may be a preferred target of the tat-induced factor(s) and respond with hepatocyte dysplasia and proliferation leading to tumor formation. The liver is a complex biochemical organ and has the ability to respond to a wide variety of hormonal signals including: (a) polypeptide signals such as epidermal growth factor, fibroblast growth factor family, hepatocyte growth factor, insulin, growth hormone, and glucagon; (b) steroid hormones such as cortisol, progesterone, androgens, estrogens, and vitamin D; and (c) tyrosine derivatives such as thyroxine, epinephrine, and acetylcholine (31-36). The role that these hormonal signals play in both normal liver proliferation and regeneration, as well as in abnormal processes leading to tumor formation, is being intensely investigated. The livers of these transgenic mice may be responding to a changing hormonal milieu, due in part to factors released by the skin.

Extensive research on animal models of hepatocarcinogenesis has helped define and distinguish between promotional and initiator events used to induce liver tumors (37, 38). Based on the histology alone, it is difficult to characterize the role of tat in this mouse model as either a promoter of a predisposition to form liver tumors or an initiator capable of inducing new genetic events leading to hepatocyte proliferation. In either case, this model would suggest that the role of tat is an indirect one mediated through other factors and events.

Likewise, the extreme sexual dimorphism of developing liver tumors that we see in our model is reminiscent of the male preponderance that is seen in all strains of mice as well as in humans (39, 40). The causes for this dimorphism are complex and may involve environmental and hormonal influences. The same factors likely provide a background in our model in which tat exerts its effects.

Despite potential mechanistic differences in murine and human liver carcinogenesis, the observed similarities suggest that the transgenic mouse model has much relevance for the development of human liver tumors. Our model predicts that the
development of HCC will be increasingly associated with HIV infection as the mean time of infection to development of AIDS continues to lengthen. Furthermore, our model suggests that HIV plays a critical role in the etiology of these liver tumors, albeit indirectly.

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