Mammary Carcinogenesis in Foster-nursed X/Gf Mice

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SUMMARY

Newborn X/Gf mice were foster nursed by mothers of the DBA/212 mouse strain. [The X/Gf mice are highly resistant to spontaneous development of mammary tumors, whereas DBA/212 mice produce mammary tumors in high instances and contain an abundance of the mammary tumor virus (MMTV).] Varied numbers of F1 to F7 generations of foster-nursed females produced mammary tumors at ages ranging from 12 to 24 months. Sections of the tumors stained with hematoxylin and eosin and studied by light microscopy showed the characteristic appearance of mammary carcinomas, although differing in degree of differentiation. Electron microscope studies revealed the characteristic type MMTV particles in the tumors. The budding process of type B virus particle formation on cell membranes is illustrated in the electron micrographs. Morphologically, the virus particles in X/Gf mammary tumors appeared identical to those seen in spontaneous mammary tumors of the DBA/212 foster mothers. Separate biochemical studies on molecular level of growing X/Gf tumor extracts revealed viral-specific high-molecular-weight 70 S RNA and reverse transcriptase; the putative viral complex was localized in sucrose gradients at a density of 1.05 g/ml, the position characteristic of RNA MMTV. Thus, the electron microscope and biochemical data on a molecular level provide documentary evidence of the infectivity of the MMTV in the host cells.

X/Gf mice, challenged with MMTV by foster nursing, produced mammary tumors in early generations but failed to develop tumors after the seventh generation. This suggests that the X/Gf host, known to generate high levels of antibodies against MMTV, may be responsible for the cessation of mammary tumor development and not the virus. Thus, the significance of the immune competence of the host in neoplasia becomes apparent.

INTRODUCTION

Bittner (5), in his pioneering investigations, postulated that "an agent" (at that time a virus was suspected but not documented) is involved in the high incidences of mammary tumors appearing in females of certain strains of mice, and this agent is transmitted through the milk of mothers to offspring. This postulate was based on the high incidence of mammary tumors in females of low cancer strains that were suckled by foster mothers of high cancer strains. Numerous experiments along these lines were subsequently extended to mice of various strains with diverse susceptibility to the spontaneous development of mammary tumors (1, 23). The incidence of mammary tumors that developed in foster-nursed females of various strains served as the basic criterion in evaluating results. Subsequent electron microscope studies revealed virus-like particles in mammary tumor cells. Bernhard (4) classified the VP's of mammary tumors on the basis of their morphology. Excellent reviews on this subject are available (2, 9, 22, 27).

Previous communications from this laboratory described the origin and several endogenous characteristics that had been revealed in inbred X/Gf mice. In brief, mice of this strain which were inbred since 1953 proved to be resistant to the spontaneous development of neoplasms as well as to several oncogenic viruses and showed low susceptibility to X-irradiation and to chemical carcinogenic agents (13, 14, 17). It was of interest to test the response of X/Gf mice to the MMTV transmitted by foster nursing by DBA/212 mice, a strain which is known to carry MMTV in abundance (16). The results obtained constitute the substance of this report. To the knowledge of the authors, no electron microscope studies have been reported on tumors that had developed in ensuing generations of mice that initially had foster mothers.

MATERIAL AND METHODS

Mice of the following inbred strains were used: (a) DBA/212; these mice are known to produce mammary tumors spontaneously in high instances and the tumors carry the MMTV in abundance (16); (b) X/Gf mice, mentioned earlier.

Each nursing DBA/212 mother was placed in a cage containing newborn X/Gf mice, after the X/Gf mother was removed from the cage. All of the mice were kept in stainless steel cages and housed in animal quarters at a regulated temperature of 75°F, and were fed Purina laboratory pellets and water ad libitum. In addition, the nursing mothers received bread soaked in milk every 2nd day. At about 4 weeks of age, the foster-nursed X/Gf mice were weaned and designated F1 generation, foster nursed. Most mothers to offspring. This postulate was based on the high incidence of mammary tumors in females of low cancer strains that were suckled by foster mothers of high cancer strains. Numerous experiments along these lines were subsequently extended to mice of various strains with diverse susceptibility to the spontaneous development of mammary tumors (1, 23). The incidence of mammary tumors that developed in foster-nursed females of various strains served as the basic criterion in evaluating results. Subsequent electron microscope studies revealed virus-like particles in mammary tumor cells. Bernhard (4) classified the VP's of mammary tumors on the basis of their morphology. Excellent reviews on this subject are available (2, 9, 22, 27).

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F₁ females were mated with their littermate brothers, 3 females and 1 male to a cage. All females were allowed to breed throughout their reproductive period. A number of F₁ females and F₂ males were placed in separate cages (6 to 10) and kept as virgins. This procedure was used throughout successive generations. Both foster-nursed X/Gf breeders and virgins were carefully observed. The breeding females, as well as the virgins, were inspected weekly, and the appearance of a tumor was recorded.

RESULTS

The incidence of mammary tumors that developed in the foster-nursed breeding and virgin X/Gf females, the age at which their tumors appeared, and other details were reported previously (15). Several pertinent points, however, are mentioned. Among the 1st generation (F₁) of breeding females, approximately 46% developed mammary tumors at an age ranging from 10 to 18 months (most were more than 1 year old) whereas, among the F₂ generation virgins, 21% developed mammary tumors at an age ranging from 12 to 24 months. A rapid decrease in the incidence of spontaneous mammary tumors occurred in the ensuing generations of breeding as well as virgin females. Among 25 breeding females of F₂ generation, only 1 developed a mammary tumor at the age of 12 months. No mammary tumors have developed among breeders or virgins of the F₃ generation. All mammary tumors that developed in females of each generation were subjected to microscopic studies. The mammary tumors that developed in the foster-nursed X/Gf mice were diagnosed adenocarcinomas and were of the type noted in various strains of mice belonging to the same classifications as described in Refs. 10–12. A number of photographs of these tumors appeared in a previous publication (15).

Electron Microscope Observations. It would be too lengthy to describe here the ultrastructural features of each tumor that developed in females of the same foster-nursed generations (F₁ to F₇). A wide variation in cytological appearance was noted even within cells of the same tumor. For example, the shape of the nucleus differed from cell to cell (Fig. 1). Generally, the integrity of the cell cytoplasm was well preserved, although the number of mitochondria and other cytoplasmic organelles differed among the cells of the same tumor section. Whether the divergence is due to the difference in the stage of cellular activity at time of fixation cannot be ascertained. The principal interest in this study was to reveal the response of the X/Gf mice to the transmitted MMTV. Accordingly, an intensive search was made for detection of VP’s when viewing the tumor sections in the electron microscope. Generally, the tumor cells, as seen in the electron microscope, were (a) those showing only the characteristic type A VP’s within the cell cytoplasm (intracytoplasmic), (b) cells showing both type A in the cytoplasm and budding VP’s from the plasma membrane and from the bordering membranes of microvilli, (c) cells showing only budding VP’s from the plasma membrane, and (d) mature type B VP’s lying free within cytoplasmic vacuoles and within the intercellular spaces. Morphologically, they belong to the same type of VP’s, described by Bernhard (4), that have been seen in mammary tumors of mice of various cancer strains and in those of DBA/212 mice (16). Tumor cells free of virions predominated in the tumors that developed in the foster-nursed X/Gf females (Fig. 1).

Intracytoplasmic Type A VP’s. As indicated earlier, this type of VP was designated type A by Bernhard (4). Dalton (7) termed these intracytoplasmic type A to distinguish them from intracisternal type. Their size may vary from 60 to 75 nm in diameter. They are of a doughnut-like shape consisting of an outer shell of low density and of an electron-lucent center. The origin or the formation of the type A VP’s is still obscure. An elaborate study on the nature of type A particles was recently published by Tanaka et al. (30). These authors detected 2 antigens in MMTV. One antigen is characteristic of the intracytoplasmic type A particles, and the other is shared by the B particles. On this basis the authors inferred that the intracytoplasmic A particles are immature forms of MMTV, and further state that “Although the intracytoplasmic A particles are now known to be the immature forms of MTV, it should be emphasized that their accumulation in the cytoplasm is not essential for the B particle formation because the latter can steadily occur at the cell surface without the former. Furthermore, accumulation of A particles does not assure production of B particles.” The incorporation of type A particles into the formation of B particles has been noted by other investigators [Dalton and Potter (8), Imai et al. (21), and Sarkar et al. (25)]; however, this phenomenon is not always observed, e.g., Dalton (6), and also in the study reported herein.

In viewing numerous cells in the tumors of the foster-nursed X/Gf mice, only type A VP’s were noted. For example, at low magnification, in 1 of 8 cells, an aggregate of type A VP’s is seen in the center of the cytoplasmic matrix (Fig. 1). In another cell, at high magnification, the characteristic type A VP’s are seen in abundance (Fig. 3); their size ranges from 62 to 71 nm in diameter; they are adjacent to each other, forming large groups, mainly in the central portion of the cytoplasmic matrix; they are also seen along the limiting membrane of a cytoplasmic vacuole. Numerous vesicles of ER, ribosomes, and polysomes constitute the rest of the cytoplasmic matrix (Fig. 3). It may be assumed that the abundance of these cytoplasmic constituents provided a favorable medium for their existence.

Cells Showing Budding and Mature Type B VP’s. In contrast to those cells showing type A VP’s, there were cells that showed only budding and mature B particles (Fig. 2). The portions of the cytoplasm seen in this figure show an abundance of polysomes, ribosomes, vesicles, membranes of ER, etc. No type A particles are noted in this figure. The size of the type B particles within the intercellular space ranged from 89 to 100 nm. The size of these B particles appear to be smaller than those of the foster mothers, which ranged from 90 to 125 nm (16). Further, the B particles of the X/Gf tumors contained only 1 nucleoid, whereas some B particles of the foster mothers contained 2 nucleoids (16).

Ultrastructural Features of Tumor Cells Free of Virus. At low magnification, adjacent tumor cells, apparently free of
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VP's, predominated in each microscopic field. Some of these cells were bridged together by desmosomes. A variation in size and shape of nuclei prevailed (Fig. 1). The cytoplasm was usually filled with apparently well-preserved organelles, such as mitochondria, vesicles, membranes of ER, Golgi apparatus (when visible), and numerous polysomes and ribosomes. The reason these cells did not show virus particles remains to be elucidated. They may carry a part of the virus genome, but it is not expressed, or they were not virus infected.

Electron Microscopy of Mammary Tumors Passed in Isogenic X/Gf Mice. One of the tumors that developed spontaneously in a foster-nursed female was used for s.c. implantation into X/Gf females. Electron microscope study revealed the characteristic MMTV particles in the cells of this tumor. The small particles of the intact tumor tissue that were s.c. inoculated into X/Gf females developed mammary tumors. Since then, this tumor has been carried by serial transplants to isogenic hosts; it grows well in X/Gf mice of both sexes. It continuously contains secretory material, but to a lesser extent than during the earlier passages. It was of interest to detect whether the VP's were present in tumors of ensuing passages. This tumor is at present in its 203rd passage of transfer. Sections of passed tumors showed the characteristic MMTV particles. The cytoplasmic type A VP's predominated. However, most of the cells in the passed tumors showed no VP's.

The Absence of MMTV in Regressing Passaged Tumors. A significant number of X/Gf mice of both sexes were sent to the F8 generation of foster-nursed X/Gf mice. Two aspects may shed light on this question, (a) Recent studies at a molecular level provided evidence of the intimate interaction of the transmitted MMTV with the genetic material of the host cell. Also, the morphology of the VP's herein described, is identical with those noted in the tumors of the nursing mothers (16), with those described by Bernhard (4), and with those more recently described by Sarkar and Moore (24) in mammary tumors of RII1 mice. (b) The gradual formation of MMTV at the cell-plasma membrane and at the bordering surfaces of the tumor cells, the mature type B VP's in the intercellular spaces, and the presence of the intracytoplasmic type A VP's, all serve as credible evidence of the interaction of the transmitted MMTV with the genetic material of the host cell. Also, the morphology of the VP's herein described, is identical with those noted in the tumors of the nursing mothers (16), with those described by Bernhard (4), and with those more recently described by Sarkar and Moore (24) in mammary tumors of RII1 mice.

DISCUSSION

The results obtained from the present experiments are discussed from 3 aspects: (a) the true induction of the mammary tumors in foster-nursed X/Gf females, (b) the fate of the MMTV transmitted to newborn X/Gf mice by foster nursing, and (c) the possible cause of the gradual decrease in ensuing generations and the eventual cessation of development of mammary tumors in the F2 generation of foster-nursed X/Gf mice.

The True Induction of Mammary Tumors by MMTV. As indicated earlier, the X/Gf females very rarely produce mammary tumors spontaneously. Only in 4 females had mammary tumors developed over 20 years of inbreeding. Electron microscope studies of these tumors failed to detect the characteristic MMTV. Therefore, the occurrence of mammary tumors in the foster-nursed X/Gf females and the presence of the characteristic MMTV in the tumors may serve as documentary evidence of the specific and intimate involvement of MMTV with the epithelial cells of the host mammary glands as their suitable targets for replication and transformation. The actual presence of the budding particles from the bordering membranes and from microvilli of the tumor cells, the mature type B VP's in the intercellular spaces, and the presence of the intracytoplasmic type A VP's, all serve as credible evidence of the interaction of the transmitted MMTV with the genetic material of the host cell. Also, the morphology of the VP's herein described, is identical with those noted in the tumors of the nursing mothers (16), with those described by Bernhard (4), and with those more recently described by Sarkar and Moore (24) in mammary tumors of RII1 mice.

What is the Fate of MMTV Transmitted to Newborn X/Gf Mice by Foster Nursing on DBA/212 Mothers? One wonders about the fate of the MMTV after it was introduced to newborn X/Gf mice. Two aspects may shed light on this question. (a) Recent studies at a molecular level provided evidence of the intimate interaction of the genetic material of the MMTV with the genetic material of the host target cell (20). Briefly, extracts of the actively growing mammary tumors in the X/Gf mice contained viral-specific high-molecular-weight 70 S RNA and reverse transcriptase (26); the putative viral complex was localized in sucrone gradient at a density of 1.16 g/ml, the position characteristic of RNA tumor viruses (19). (b) The gradual formation of MMTV at the cell-plasma membrane and at the bordering surfaces of the tumor cells, the mature type B VP's in the intercellular spaces, and the presence of the intracytoplasmic type A VP's, all serve as credible evidence of the interaction of the transmitted MMTV with the genetic material of the host cell. Also, the morphology of the VP's herein described, is identical with those noted in the tumors of the nursing mothers (16), with those described by Bernhard (4), and with those more recently described by Sarkar and Moore (24) in mammary tumors of RII1 mice.
membranes of microvilli, and the release of the virus into the intercellular space, where it matured and assembled an electron-dense nucleoid, can be reconstructed from the electron microscope observations (Fig. 2). Thus, the observations by electron microscopy and studies at the molecular level provide apparent credible evidence of the potent infectivity of MMTV. There were, however, cells free of virus. These cells may carry a part of the viral genome that is not expressed, or perhaps not all mammary gland cells became infected with the virus.

Decrease and Cessation of Mammary Tumor Development in Foster-nursed X/Gf Mice. The presumable cause of gradual decrease with eventual cessation of development of mammary tumors in ensuing generations of the foster-nursed X/Gf mice mentioned earlier may be explained, at least partially, by (a) the action of antigens against the induced MMTV that the X/Gf mice are known to have in their blood serum (3), (b) their high immune competence (28), and (c) their high phagocytic activity (29). The fact that only single tumors had developed in foster-nursed X/Gf females at ages ranging from 12 to 24 months, whereas the nursing DBA/212 mothers developed multiple tumors at ages ranging from 7 to 10 months (16), supports this inference. Furthermore, DBA/212 mice have been and are continuously developing mammary tumors since inception of their inbreeding in 1918 (18), whereas the foster-nursed X/Gf females produced mammary tumors only to F7 generation, and in decreasing instances.

It is significant to note the persistence of the VP’s in the passaged X/Gf tumors. This occurrence may indicate that once the viral genetic information was acquired by the first infected cells, it is continuously expressed. If this were not the case, the genetic information would be diluted out after several passages. Incidentally, this interpretation is in agreement with the provirus theory of Temin (31). With respect to the structural integrity of the cells having the virus, these cells may possess all the components of normal cells and are of usual appearance (see Fig. 6 in Ref. 13). This is not surprising: if these cells had not preserved their structural integrity, they would be unable to function. Numerous well-preserved mitochondria, ribosomes, vesicles, etc., in the virus-infected cells presumably provide adequate metabolic activity and energy needed for the maintenance of normal physiological function for the cell per se and for the replication of the virions that became a part of the cell.

Generally, from previous and present studies, it appears that the X/Gf mice constitute a suitable host-animal system for investigation on the etiology of neoplasia and factors involved in this process.

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REFERENCES


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Fig. 1. Low-power view of 9 adjacent tumor cells. One cell shows cytoplasmic type A VP’s (upper arrow). Type B VP’s are seen within a vacuole or in the intercellular space (lower arrow). The cytoplasm of each cell contains an abundance of ribosomes, polysomes, vesicles of ER, and mitochondria. x 9,500.

Fig. 2. Portions of 3 tumor cells. Two are connected by desmosomes (D). The cytoplasm of these cells contains numerous polysomes, ribosomes, vesicles of ER, etc. Budding particles from the plasma membranes and from microvilli, and type B particles within the intercellular space are seen. x 44,400.

Fig. 3. A portion of a cell cytoplasm; note the intracytoplasmic type A particles and the type A particles lined up at the bordering membrane of a cytoplasmic vacuole (upper arrow); numerous ribosomes, polysomes, vesicles of ER; mitochondrion filled with cristae (lower arrow). x 66,000.

Fig. 4. Central portion of a regressing tumor that consisted mainly of soft, presumably necrotic material. The pale structures may represent type A VP’s in a degenerating state that failed to take up the stain (uranyl acetate). Their size ranges from 52 to 66 nm in diameter. For detailed explanation, see text. x 66,000.
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