Is There a Breast Cancer Virus?

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The murine mammary tumor virus (MMTV) has caused much controversy over the years and has fascinated virologists, geneticists, immunologists, and clinicians alike. The diverse biology of MMTV has impacted on our understanding of several previously unappreciated processes such as superantigen activity, transmission of endogenous retroviruses, oncogene expression, and viral carcinogenesis, to name a few. Despite our vast fund of knowledge of MMTV, the existence of a human breast cancer virus has been the source of acrimonious debate for decades. In frustration with the recent claims of detecting a nucleotide sequence analogous to MMTV in human breast cancer tissues by the sensitive polymerase chain reaction technique, British virologist Mark Boyd stated: "In my opinion, a retrovirus is not going to cause breast cancer in humans. If there was something there, someone who knows what they are doing would bloody well have found something by now." (1) So why has the viral induction of human breast cancer generated such controversy for 30 years?

To answer this question, we should review the history of MMTV research (Table 1). At the turn of the century, genetic and chromosomal alterations were considered the most likely cause of carcinogenesis based on the irreversible and predictable course of most tumors. However, this view was soon challenged by the discovery of Rous Sarcoma Virus in 1911, which proved to be an infectious carcinogen that reproducibly caused sarcoma in chickens. Since then, the concept of viral carcinogenesis has steadily gained support, and a variety of tumors has now been linked to viral infection in animals and humans alike. In fact, there is substantial epidemiologic and biologic evidence that several families of viruses, including retroviruses, herpes viruses, and hepatitis viruses, represent a major cause of cancer on a worldwide basis.

There is little doubt that the majority of breast cancers in mice are caused by MMTV. It was originally thought that the familial occurrence of breast cancer was indicative of a hereditary component in the development of murine breast cancer. This was demonstrated by crossover breeding studies in low incidence and high incidence strains where back crossing with the high tumor line resulted in a higher incidence in breast tumors. However, some of these studies were inconsistent, and similar cross breeding experiments supported the hypothesis that an environmental factor was associated with breast cancer as well. Evidence for an infectious etiology came from foster parent studies in which newborn mice from low incidence mammary cancer strains were shown to have a higher incidence of breast cancer when they were fed by high incidence strain mothers. Subsequently, Bittner discovered that an extra-chromosomal factor, passaged in milk, was in fact the mammary tumor virus (2).

This retrovirus has an unusual life cycle. It can be transmitted horizontally as an infectious particle containing viral RNA or vertically in the murine germline as an endogenous proviral DNA genome. MMTV replicates in the mothers' mammary glands where the infectious B-type particles accumulate in milk. Subsequently, the weanlings ingest the virus, which invades the gut-associated lymphoid tissues and infects B lymphocytes. As viral replication is somewhat limited and tissue specific, MMTV uses a superantigen, encoded by the *orf* gene in the viral long terminal repeat (LTR) region, to activate specific T lymphocyte V β subsets. This serves to stimulate viral replication as well as clonal amplification of T lymphocytes, which provides a vehicle for viral passage from the gut-associated tissue to the breast. Although viral replication is maximal in the breast epithelium, hormone stimulation is still required to activate the corticosteroid response elements in the retroviral LTR. During pregnancy, increased viral replication is associated with hyperplastic nodule formation that can eventually lead to tumor formation.

MMTV can also be transmitted to offspring as proviral DNA in a Mendelian fashion. In fact, in some strains of mice, the proviral DNA can serve as a template to form infectious particles. In other strains, the creation of mutations and deletions in the endogenous retroviral genes prevents the

Table 1. Discovery and characterization of murine mammary tumor virus (MMTV).

Date	Investigators	Findings
1854	Crisp	Mammary tumors found in wild mice
1918	Lathrop and Loeb	Genetic influence on development of breast cancer
1933	Jackson Laboratories	Mammary tumors related to extra-chromasomal factor
1936	Bittner	Transmission of filterable factor in milk
1948	Bittner and Graff	Electron microscopy detection of B-type virus particles in milk
1959	Nandi and Blair	Mammary tumors observed to be hormone-responsive
1960s	(multiple groups)	Characterization of inbred mouse strains
1971	Schlom, Spiegelman, and Moore	Discovery of MMTV reverse transcriptase
1972	Varmus	Detection of an endogenously encoded provirus
1973	Festenstein	MLR observed in MHC-compatible strains
1976	Cohen and Varmus	Molecular characterization of endogenous retroviruses
1981	Buetti, Majors, and Ucker	Initial cloning and sequencing of MMTV genome
1984	Varmus and Bishop*	Discovery of protooncogenes activated by MMTV
1986	Prakash	Cloning and genetic mapping of endogenous provirus
1991	Acha-Orbea and Choi	Superantigen encoded by orf in MMTV LTR responsible for MLR

* Both investigators awarded Nobel Prize for discovery of oncogenes.
MLR = mixed lymphocyte response
MHC = major histocompatability complex
LTR = long terminal repeat

formation of viral particles and the development of cancer. This picture of both exogenous and endogenous transmission of infection clearly complicated the analysis of the relative contribution of genes versus environment in the early crossbreeding experiments for breast cancer formation. However, the story does not end there. In some instances, the hosts, to their own benefit, had subverted the use of the endogenous MMTV sequences for protection against exogenous MMTV infection. During the development of the immune system, expression of the endogenous MMTV *orf* gene results in the expression of the viral superantigen and the deletion of specific T lymphocyte V β subsets. During infancy, the mouse is then protected from infection with the exogenous virus that has an absolute requirement for the deleted subset of T lymphocytes.

The majority of breast cancers in mice are associated with MMTV. However, MMTV does not contain an oncogene and acts as a slowly transforming virus. It has been shown that MMTV proviral DNA can integrate close to cellular protooncogenes and enhance their activity. Several protooncogenes associated with viral integration have been described such as *int1* and *int2*, which belong to developmental and growth factor families, respectively. Increased expression of these protooncogenes has been found in human breast cancer tissues as well. It is possible that other features of MMTV biology are also responsible for generating tumors in mice, as transgenic mice models have demonstrated that the overexpression of some protooncogenes is insufficient for tumor development. In one model, the expression of the superantigen *orf*-encoded protein alone has been reported to be sufficient for the production of tumors.

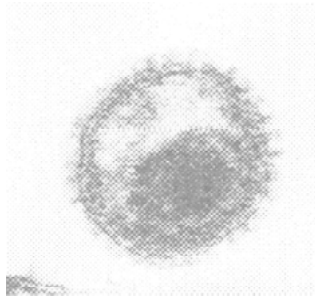


Figure 1. Etectron micrograph of the murine mammary tumor virus demonstrating classical B-type morphology with an eccentric oval nucleus (mouse mammary tumor virus). (Reprinted with permission from Plenum Publishing Corporation. Coffin JM. Structure and classification of retroviruses. In: The Retroviridae. Levy JA, editor. New York: Plenum Press 1992; 22)

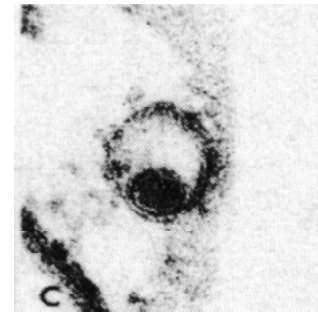


Figure 2. Similar B-type viral particles found by electron microscopy in Parsi milk. (Reprinted by permission from Nature. Moore DH, Charney J, Kramarsky B, et al. Search for a human breast cancer virus. Nature 1971; 229:612. Macmillan Magazines, Ltd.).

Much has been learned about the biology of MMTV over the years, and it was not unreasonable to ask the question of whether this or a similar agent could cause breast cancer in humans. In fact, several scientists involved in describing the biology of MMTV were interested in pursuing the hypothesis that human breast cancer had a viral etiology as well. In 1971, Moore (who had been responsible for describing the MMTV reverse transcriptase activity) and associates reported that 60% of milk samples from breast cancer patients had electron microscopy evidence of B-type particles indistinguishable from MMTV as compared with a prevalence of 5% in the milk from the general population (3). They also reported that 39% of Parsi women of India, an inbred population with a twofold increased prevalence of breast cancer, had evidence of B-type particles using the same methodology (Figures 1 and 2). From these studies, Moore and associates suggested that it might be possible to immunize against human breast cancer using inactivated MMTV as a vaccine, but there was resistance in the medical community due to a lack of supportive data.

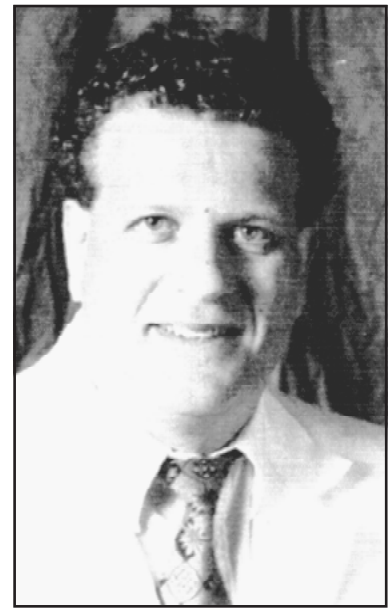
Following this initial electron microscopy report, an avalanche of studies documented a role of MMTV in breast cancer patients. Numerous studies revealed MMTV envelope-like antigens in human breast cancer tissues, cell lines, human milk, and serum that were reactive to anti-MMTV envelope antibodies. Electron microscopy studies revealed anti-MMTV envelope immunoreactivity to A-type particles (a precursor to B-type particles) in human breast cancer tissue. Furthermore, both humoral and cellular immunoreactivity to MMTV proteins were specifically demonstrated in breast cancer patients. In addition to the immunologic findings, molecular hybridization studies revealed nucleic acid sequences homologous to MMTV in breast cancer patients' lymphocytes and in breast cancer cells. In other words, evidence from numerous studies supported the hypothesis that patients with breast cancer had been infected with a virus that had antigenic cross reactivity, nucleotide homology, and an identical morphology with MMTV.

At that time, however, counter arguments to the existence of a human breast cancer virus appeared to be equally convincing. For example, it was demonstrated that human proteins could cross react with the MMTV envelope antibodies. Furthermore, there were numerous debates about whether the electron microscopy, molecular hybridization, and immunologic studies were an artifact of endogenous retroviruses rather than a demonstration of an exogenous human mammary tumor virus. In the 1970s, no exogenous human retrovirus had been discovered and interest in endogenous retroviruses was emerging. Subsequently, interest in a human breast cancer virus waned in the 1980s as the scientific community appeared to become increasingly intransigent to the hypothesis.

However, interest in a human breast cancer virus has been rekindled. Two separate groups have produced convincing data suggesting that a virus with a nucleotide sequence homology very close to MMTV can be found in humans. Using the sensitive polymerase chain reaction technique, Pogo and Holland detected MMTV sequences in approximately 40% of human breast cancer samples (4). In addition, Garry reported

that sequences homologous to MMTV RNA can be detected in approximately 80% to 90% of breast cancer tissues using the RNase protection assay (5), which is less prone to artifact and usually less sensitive than the polymerase chain reaction technique.

So the human breast cancer controversy lives on. Mark Boyd opined in the Journal of National Cancer Institute that "This field has a track record of amateurs who like the idea of poor quality research" (1). However, many of the pioneering MMTV researchers depicted in Table 1 were the scientists subsequently responsible for promoting the human breast cancer virus hypothesis. One can only hope that further studies will prove the skeptics wrong, so that vaccination programs and other pertinent therapies could be employed to prevent this devastating disease.



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Other Suggested Reading

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