# The Ribbon

A Newsletter of the Cornell University Program on Breast Cancer and Environmental Risk Factors in New York State (BCERF)


**Volume 5, Number 3, Early Fall 2000**

## Mouse Mammary Tumor Virus

***Introduction to this issue of the Newsletter by***
***Suzanne Snedeker, Research Project Leader, BCERF***

*When I was a post-doctoral student 15 years ago, there was a renewed interest in trying to identify human viruses that might cause breast cancer. How viruses may affect breast cancer risk is actually one of the oldest areas of breast cancer research, ever since a mouse (Murine) Mammary (breast) Tumor Virus (MMTV) was identified over 60 years ago. One of the articles on viruses and breast cancer risk that has recently gotten the attention of the popular press is an article by Stewart et al. (2000). These researchers proposed (hypothesized) than a certain type of wild mouse that resides mostly in Europe and North America, but not in Asia, may be transmitting the MMTV to human populations, and hence explain why breast cancer rates are lower in Asian compared to Western countries. I thought it was important to not only give The Ribbon readers a critique of this paper (see Research Commentary by Dr. Barbour Warren), but to also give our readers a perspective on the long history of research that has sought to identify and explore whether certain viruses may affect the risk of breast cancer. We are very fortunate to have the perspectives of two prominent scientists, Dr. Susan Ross from the Cancer Center, University of Pennsylvania Medical Center, Philadelphia, PA and Dr. Bonnie Asch from Roswell Park Cancer Institute Corp., Buffalo, NY.*

## Is There a Breast Cancer-Causing Virus in Humans?

***Susan R. Ross, Ph.D., Professor of Microbiology, Cancer Center,***
***University of Pennsylvania Medical Center, Philadelphia, PA***

Exciting advances have been made in recent years in understanding the genetics of breast cancer in humans. Scientists have identified several inherited genes that predispose women to breast or ovarian cancer, but these probably only account for five to 10 percent of the cases (Szabo and King, 1997). Understanding the causes of sporadic breast cancer is therefore of major importance. It has long been known that an infectious virus, called mouse mammary tumor virus (MMTV), causes breast cancer in mice. Recent findings have implicated a similar type of viral agent in human breast cancer. In this article, I discuss what is known about how MMTV is transmitted and causes cancer in mice and whether it is likely that a similar agent exists in humans.

MMTV was first identified in the 1920's, when it was found that a breast cancer-causing agent was passed through milk from mothers to daughters in mice (Bittner, 1936). This transmissible agent caused almost 100% of these daughters to develop breast cancer. But when the newborn daughters of mice from a strain with a high rate of breast cancer were nursed by foster mothers from a

strain that had a low rate of breast cancer, the virus was not transmitted and the daughters did not develop breast cancer. The only clearly established mode of transmission of infectious MMTV is through milk. Even when housed in the same cage, the virus is not passed from MMTV-infected to uninfected mice. MMTV is probably transmitted most efficiently via breast milk because of the extremely high levels of virus found in milk in comparison with, for example, blood, saliva or seminal fluid and with the increased susceptibility of newborns to infection with viruses in general.

MMTV was the first virus shown to cause cancer in mammals. Many other viruses have now been shown to cause cancer in mammals, including humans. Some of the more common viruses are Epstein Barr virus, which causes infectious mononucleosis in Western populations, but is associated with human nasopharyngeal carcinomas in Asia and lymphomas in Africa; the recently discovered Kaposi's Sarcoma Herpes Virus found in individuals with AIDS; and leukemia viruses, such as feline leukemia virus (FeLV) and human T cell leukemia virus I (HTLV I). These latter viruses (FeLV and HTLV I), as well as MMTV and HIV (human immunodeficiency virus), all belong to the class of viruses called retroviruses. Retroviruses share the same replication pathway; that is, their genes are encoded in ribonucleic acid (RNA) molecules in the virus. After they infect a cell, this RNA molecule gets converted into a deoxyribonucleic acid (DNA) molecule which then integrates into or becomes part of the cell's chromosomes. The DNA codes for viral proteins that are needed to make more viruses. With retroviruses like HIV, which gets into the white blood cells that are needed to fight off infections, there are virus proteins made in infected cells that have deleterious effects, leading to their death. Many other retroviruses, however, have little or no effect on the cells when they integrate into the chromosome, although the cells now become virus-producers. In some cells, when the virus integrates into the chromosome near genes involved in the regulation of cell growth, it activates those genes. The normal cell then turns into a cancer cell, and starts dividing until it becomes a tumor.

For MMTV, this type of cancer-causing integration into the chromosome occurs only in mice that are highly infected and produce very large amounts of virus. This is because the likelihood that the virus will integrate next to and activate a gene involved in the cell's growth regulation is very small. We know, for example, that some mice are genetically resistant to MMTV infection and as a result, never get MMTV-induced breast cancer even though small amounts of virus are found in their mammary glands. In mice that do develop breast cancer caused by this virus, almost every mammary gland cell gets infected.

Another important factor in the ability of this virus to cause breast cancer is the number of times the mouse gets pregnant. MMTV first infects the mammary gland during puberty, getting into a number of target cells that are dividing under the influence of hormones such as

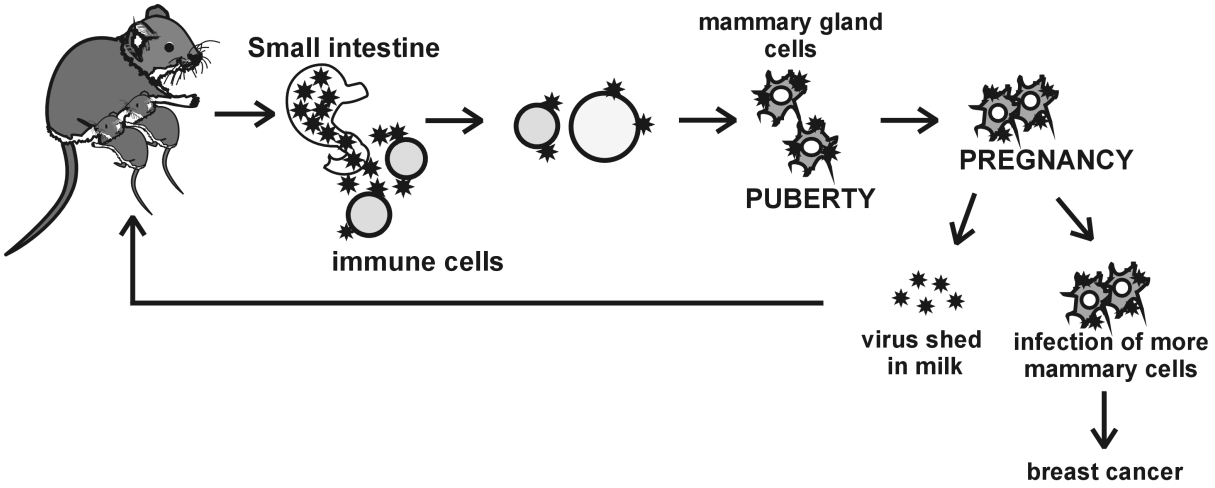


*Figure 1. MMTV infection. Virus is produced by the mammary gland and secreted into milk. Newborn animals that nurse on this milk acquire the virus. The virus first infects antibody-producing and other cells of the immune system. These cells bring the virus to the mammary gland. Infection first occurs during breast development associated with puberty. The mammary gland gets more infected with each subsequent pregnancy. The more infected the mammary gland gets, the greater the likelihood that the virus will cause cancer.*

estrogen. If infected mice never get pregnant, they have about a 50% chance of developing MMTV-induced breast cancer. However, if they are allowed to go through several pregnancies, this chance increases to almost 100%. This is because MMTV best infects cells that are dividing and the cells of the mammary gland divide every time an animal becomes pregnant to maximize milk production after birth. So mice that go through several pregnancies have many more MMTV-infected cells than virgin mice. As a result, there is a greater likelihood that the virus will make a cancer-causing insertion into the chromosome.

Might a similar type of virus exists in humans? After the identification of the actual MMTV virus particle in the 1970's, there were a number of publications that reported finding MMTV-like proteins in breast cancer biopsies and antibodies against the mouse virus proteins in human breast cancer patients (Day et al., 1981; Levine et al., 1984). However, there were no reports of finding genetic material in human breast milk that was similar to MMTV's RNA nor could any viral DNA be found in the tumor cells that was unique to breast cancer patients.

In recent years, very sensitive techniques have been developed that allow the detection of minute amounts of either DNA or RNA. These techniques have been used to reexamine the issue of whether MMTV-like genetic material is present in human breast cancer. Several research groups have reported that more than 30% of human breast cancer samples contain DNA that highly resembles MMTV (Wang et al., 1995; Etkind et al., 2000). In contrast, normal mammary gland and other tissues did not have this DNA. This has sparked some renewed interest in the idea that some human breast cancer is caused by a virus similar to that found in the mouse.

How likely is it that breast cancer is caused by a MMTV-like virus in humans? Based on what we know about the mouse virus, we would expect that pregnancy should increase cancer rates. However, in epidemiological studies, pregnancy has been shown to have a protective effect on the development of human breast cancer (Kampert et al., 1988). Another prediction is that breast-fed daughters of mothers who went on to develop breast cancer would themselves be at higher risk. Instead, breast cancer rates have gone up at the same time that breast feeding rates have decreased (MacMahon et al., 1973). Moreover, several epidemiological studies have shown no increased incidence of breast cancer in daughters who were nursed by mothers that later developed breast cancer (Titus-Ernstoff et al., 1998; Ekbom et al., 1993). However, these studies all rely on self-reported data, which in the case of breast-feeding that occurred decades before the development of cancer, may be inaccurate. Additionally, if we draw analogies from MMTV, virus transmission would occur only early on in breast feeding; the time period of nursing was not addressed in the studies. Nonetheless, we know that similar types of virus can be transmitted in humans by breast feeding. In Japan, it has clearly been established that breast milk is the major source of HTLV I transmission even though not all children get infected (Ichimaru et al., 1991; Saji et al., 1990). Indeed, HTLV I infection rates have been dramatically reduced by encouraging infected mothers to bottle-feed their children (Hino et al., 1997).

Thus, there is no epidemiological support for a milk-borne, MMTV-like-induced breast cancer in humans. Recently it has also been suggested that this virus could be transmitted directly from mice to humans. This "zoonotic" mode of infection was proposed because it was shown that geographic areas of high breast cancer incidence, namely Western Europe and North and South America, overlap with the distribution of the Mus domesticus species of house mouse (Stewart et al., 2000) (see *Research Commentary*). Other regions of the world, where breast cancer incidence is lower, have other species of wild mice, such as the Mus musculus species predominantly found in Eastern Europe. Both Mus musculus and Mus domesticus carry infectious MMTV, however (reviewed in Stewart et al., 2000). The authors conclude that if there was zoonotic transmission of MMTV, only the Mus domesticus version could jump the species barrier.

Can we conclude from these various studies that there is a MMTV-like virus associated with human breast cancer? As it currently stands, this conclusion is problematic. Given that in mice, the species in which the virus is found, the only major mode of transmission is through milk, it is hard to imagine the mechanism by which the virus would jump from mice into humans. Furthermore, we know from laboratory studies that MMTV does not readily infect human cells (Howard and Schlom, 1980; Golovkina et al., 1998; our own unpublished observations). Because there is no indication from the epidemiological studies that there is passage of the virus from mothers to daughters through nursing, such a zoonotic transmission would have to be occurring at a very high rate to account for the presence of a MMTV-like virus in greater than 30% of human breast cancers. Thus, this means that not only would the virus have to mutate to be infectious in humans, but it would have to be transmitted in a completely novel mode.

However, one of the surprising observations made about the MMTV-like viruses found in human breast cancer

samples was how closely related they are to the mouse virus (Wang et al., 1995; Etkind et al., 2000). What is even more surprising is that the part of the virus that was found in the human breast cancers is the gene for a viral protein (the envelope protein) that determines whether the virus can enter a cell. If a mouse virus had mutated so that it now was capable of infecting human cells, one would expect the greatest differences between the mouse and the human virus to be in the envelope protein and yet, the published human virus envelope gene is almost identical to that of the mouse virus.

Furthermore, if a MMTV-like virus were involved in human cancer, we would expect it to be readily detectable not only in the tumor tissue, but in the normal breast tissue. This is because MMTV causes cancer by infecting many cells until it accidently integrates into a gene involved in cell growth regulation, as described above. Yet, human breast cancer samples have such low levels of the MMTV-like virus that only extremely sensitive methods could detect it (Wang et al., 1995; Etkind et al., 2000). In the few cases where normal breast tissue was obtained from the same individual who had a tumor, no MMTV-like virus DNA could be detected at all in the normal tissue (Wang et al., 1995). Finally, we know that the only way that MMTV can infect mammary gland cells in mice is if their white blood cells get infected first (see Fig. 1) (Tsubura et al., 1988; Golovkina et al., 1998) and yet no MMTV-like sequences were detected in the blood cells of breast cancer patients.

So, while the recent data implicating a human MMTV-like virus in breast cancer is tantalizing, the current evidence argues against such an agent. If there were such a virus, it would have to infect humans in a dramatically different way than does MMTV in mice. In this case, it is hard to explain the high degree of similarity between the recently identified human virus and MMTV. Much more data are needed to show that such an agent exists.

## References


Bittner, J.J. "Some possible effects of nursing on the mammary gland tumor incidence in mice". *Science* 84 (1936):162.

Day, N.K., S.S. Witkin, N.H. Sarkar, D. Kinne, D.J. Jussawalla, A. Levin, C.C. Hsia, N. Geller, and R.A. Good. "Antibodies reactive with murine mammary tumor virus in sera of patients with breast cancer: geographic and family studies". *Proc. Natl. Acad. Sci. USA* 78 (1981):2483-2487.

Ekbom, A., C.C. Hsieh, D. Trichopoulos, Y.Y. Yen, E. Petridou, and H.O. Adami. "Breast-feeding and breast cancer in the offspring". *British J. Cancer* 67 (1993):842-845.

Etkind, P., J. Du, A. Khan, J. Pillitteri, and P.H. Wiernik. "Mouse mammary tumor virus-like ENV gene sequences in human breast tumors and in a lymphoma of a breast cancer patient". *Clin. Canc. Res.* 6 (2000):1273-1278.

Golovkina, T.V., J.P. Dudley, and S.R. Ross. "Superantigen activity is need for mouse mammary tumor virus spread within the mammary gland". *J. Immunol.* 161 (1998):2375-2382.

Golovkina, T.V., J.L. Dzuris, B. van den Hoogen, A.B. Jaffe, P.C. Wright, S.M. Cofer, and S.R. Ross. "A novel membrane protein is a mouse mammary tumor virus receptor". *J. Virol.* 72 (1998):3066-3071.

Hino, S., S. Katamine, H. Miyata, Y. Tsuji, T. Yamabe, and T. Miyamoto. "Primary prevention of HTLV-1 in Japan". *Leukemia* 11 Suppl 3 (1997):57-59.

Howard, D.K. and J. Schlom. "Isolation of a series of novel variants of murine mammary tumor viruses with broadened host range". *Int. J. Cancer* 25 (1980):647-654.

Ichimaru, M., S. Ideda, K. Kinoshita, S. Hino, and Y. Tsuji. "Mother-to-child transmission of HTLV-1". *Cancer Det. Prev.* 15 (1991):177-181.

Kampert, J.B., A.S. Whittemore, and R.S. Jr. Paffenbarger. "Combined effect of childbearing, menstrual events, and body size on age-specific breast cancer risk". *Am. J. Epidemiol.* 128 (1988):962-979.

Levine, P.H., R. Mesa-Tejada, I. Keydar, F. Tabbane, S. Spiegelman, and N. Mourali. "Increased incidence of mouse mammary tumor virus-related antigen in Tunisian patients with breast cancer". *Int. J. Cancer* 33 (1984):305-308.

MacMahon, B., P. Cole, and J. Brown. "Etiology of human breast cancer: a review". *J. Natl. Cancer Inst.* 50 (1973):21-42.

Saji, F., K. Ohashi, Y. Tokugawa, S. Kamiura, C. Azuma, and O. Tanizawa. "Perinatal infection of human T-lymphotropic virus type I, the etiologic virus of adult T-cell leukemia/lymphoma". *Cancer* 66 (1990):1933-1937.

Stewart, T.H.M., R.D. Sage, A.F.R. Stewart, and D.W. Cameron. "Breast cancer incidence highest in the range of one species of house mouse, Mus domesticus". *British J. Cancer* 82 (2000):446-451.

Szabo, C.I. and M.C. King. "Population genetics of BRCA1 and BRCA2". *Am. J. Hum. Genet.* 60 (1997):1013-1020.

Titus-Ernstoff, L., K.M. Egan, P.A. Newcomb, J.A. Baron, M. Stampfer, E.R. Greenberg, B.F. Cole, J. Ding, W. Willett, and D. Trichopoulous. "Exposure to breast milk in infancy and adult breast cancer risk". *J. Natl. Cancer Inst.* 90 (1998):921-924.

Tsubura, A., M. Inaba, S. Imai, A. Murakami, N. Oyaizu, R. Yasumizu, Y. Ohnishi, H. Tanaka, S. Morii, and S. Ikehara. "Intervention of T-cells in transportation of mouse mammary tumor virus (milk factor) to mammary gland cells in vivo". *Canc. Res.* 48 (1988):6555-6559.

Wang, Y., J.F. Holland, I.R. Bleiweiss, S. Melana, X. Liu, I. Pelisson, A. Cantarella, K. Stellrecht, S. Mani, and B.G.T. Pogo. "Detection of mammary tumor virus ENV gene-like sequences in human breast cancer". *Canc.Res.* 35 (1995):5173-5179.

# The Search for Viral Involvement in Human Breast Cancer

*Bonnie B. Asch, Ph.D., Division of Experimental Pathology,*
*Roswell Park Cancer Institute Corp., Buffalo, NY*

**The idea of a virus playing a role in human breast cancer originated with the discovery of Mouse Mammary Tumor Virus (MMTV)**. The suspicion that a virus might cause or in some way contribute to human breast cancer became firmly established with the discovery that a virus present in certain mice caused breast cancer in about 90% of the infected females. However, research to make the transition from suspicion to proven fact has been a long, arduous task that is still far from finished. The mouse virus was designated MMTV and has been under intensive study since the 1930's. This virus has many interesting properties and has been an invaluable tool for scientists who are investigating the cause and mechanism of breast cancer development. In infected mice, MMTV reproduces in breast epithelial cells and makes numerous copies of itself. Under standard conditions, infection by MMTV does not occur in adult mice. For example, uninfected adult female mice do not become infected with MMTV even when they are housed for many months with infected mice and are in constant, direct contact with them (Moore and Holben, 1978). Instead, MMTV is passed in the milk of an infected mother to her young during nursing. Infection can be prevented by transferring the mouse pups to an uninfected foster mother before they nurse from their birth mother. Once infection occurs, the animal remains infected throughout life. Tumors begin appearing when the mice are about 6 months of age or older (reviewed in Asch, 1996).

MMTV is a retrovirus, which is a RNA tumor virus that contains a protein called reverse transcriptase. This protein is essential for both the ability of the virus to infect a cell and the ability of the virus to cause a tumor (Asch, 1996). When MMTV invades a cell, it uses reverse transcriptase to make a DNA copy of itself. The DNA form of the virus becomes incorporated into the DNA of the infected cell. When it inserts into cell DNA, the virus causes a mutation by adding extra DNA and disrupting the normal sequence of the cell's genetic material. MMTV does not contain any oncogenes (genes that can cause cancer) in its DNA. The virus initiates the development of breast cancer by activating oncogenes present in normal breast epithelial cells of infected mice. This happens because the insertion of viral DNA into cell DNA often occurs near an oncogene (Asch, 1996). The activity of an oncogene is normally regulated very tightly by a cell so that cell growth is carefully controlled. If an oncogene becomes overactive in a cell, then uncontrolled growth can occur which eventually might lead to formation of a cancer. This is the proposed consequence of oncogene activation by MMTV in a cell.

**MMTV-related material is detectable in many human breast cancers**. Two possibilities have been explored to determine MMTV's potential role in human breast cancer. The first theory is that humans are often exposed to MMTV because infected house mice live very close to us and occasionally, the virus is able to infect a person, eventually enter breast epithelial cells, become integrated into the cell's DNA, and start the sequence of events which will change the normal cells into cancer cells. The second possibility is that a human version of MMTV exists (human mammary tumor virus) which is similar, but not identical, to MMTV, and can cause cancer by the same method as the mouse virus (Wang et al., 1995). To investigate these possibilities, several approaches have been taken, including studies to identify MMTV-like virus particles in human breast cancer tissue and cells, surveys to detect MMTV proteins in breast cancer tissues and cells and to detect antibodies against these proteins in breast cancer patients, analyses to find DNA sequences of such a virus in DNA of human breast cancers (Wang et al., 1995), and most recently, an epidemiological study to compare the distribution of MMTV-infected mice with the incidence of breast cancer in various regions of the world (Stewart et al., 2000) (see *Research Commentary*). The results of these studies have been provocative (reviewed and discussed in Stewart et al., 2000). MMTV-like virus particles have been identified in breast cancer tissues and cells, MMTV proteins and antibodies against them were found in breast cancer patients, DNA sequences closely related to MMTV sequences have been detected in about 40% of breast cancer DNA samples examined (Wang et al., 1995), and a high breast cancer incidence correlated with geographical regions where house mice populations are high (Stewart et al., 2000). MMTV-related sequences and proteins have not been found in normal breast tissue near the positive tumors.

**Is the evidence for a MTV conclusive or circumstantial?** While the above findings justify continued studies on MMTV as a suspect in the origin of some breast cancers, they do not provide conclusive evidence that a MMTV or its human equivalent has a causal role in the human disease. The key question then

is, what would constitute such evidence? The gold standard for determining if a particular biological agent, whether it is bacterial, fungal, viral, etc., is responsible for causing a certain disease, is that criteria called "Koch's postulates" must be fulfilled. Koch's postulates consist of the following requirements for the agent: 1) it must be present in every case of the disease; 2) it must be isolated from the diseased tissue, e.g. breast cancer, and grown in culture to obtain sufficient quantities for testing; 3) when a sufficient amount of a pure preparation of the agent is inoculated into a healthy test animal, the disease must develop; 4) it must be reisolated from the diseased tissue that develops in the test animal (the test animal, of course, is not going to be human). With certain agents like viruses, these criteria are difficult or impossible to meet. All viruses require living cells to grow, so they cannot grow alone in pure culture. Even with a culture of living cells, many viruses, including MMTV, grow poorly or not at all. If a virus were isolated, grown in cell culture, and sufficient quantities were obtained and inoculated into a test animal, it might not produce the disease if it is a virus that can only infect and grow in humans. The cause of more than 90% of breast cancers is unknown, but many scientists think there is probably more than one cause of the disease. Some cancers might result from exposure to a particular environmental chemical, others from some type of hormonal abnormality, and perhaps others from a virus. If this were true, then the virus would not be present in every case of the disease. Thus, there are problems with all of Koch's postulates in applying them to investigating the role of MMTV or a human MTV in breast cancer. For the present, then, the evidence is circumstantial.

**Other viral candidates that might have a role in breast cancer**. In addition to MMTV, other viral suspects are also under investigation, including Epstein-Barr virus (EBV) and bovine leukemia virus (BLV). EBV is a human tumor virus associated with various types of lymphomas and some nasopharyngeal carcinomas. The first report suggesting a possible link of EBV with human breast cancer was published in 1995 by British scientists (Labrecque et al., 1995), who detected EBV sequences in DNA from 21% of 91 different breast cancers and found EBV RNA in several of them as well. Two subsequent studies by other investigators failed to find evidence of EBV in their series of breast cancers, but a more recent study done in France (Bonnet et al.,1999) detected EBV DNA sequences in 51% of breast cancers analyzed. EBV sequences were not detected in normal breast tissue of the patients. BLV causes leukemia in cows, and can infect human cells in culture. Research on BLV's potential relationship with breast cancer has been pursued by Dr. Gertrude Buehring and her collaborators (Buehring et al., 1994; Buehring et al., 1998). This virus is present in many cattle and in beef and dairy products consumed by humans. Dr. Buehring has found BLV protein in epithelial cells of some human breast tissue samples and antibodies specific for a BLV protein in blood samples of about half of the humans tested (Buehring et al., 1994; Buehring et al., 1998). The mechanisms by which EBV and BLV cause cancer are different from that of MMTV and are poorly understood. The evidence suggesting either EBV or BLV initiates or has some other type of role in human breast cancer is even weaker than that supporting such a role for MMTV or its human counterpart.

**In summary**. Currently available information puts each of these viruses at the "crime scene" of breast cancer. Proof beyond a reasonable doubt that one or the other of them had anything to do with the "crime", i.e. development of breast cancer, however, will require substantial additional evidence. This evidence can only be obtained through further research.

## References


Asch, B.B. "Tumor viruses and endogenous retrotransposons in mammary tumorigenesis." *J. Mammary Gland Biol. Neoplasia* 1 (1996):49-59.

Bonnet, M., J-M. Guinbretiere, E. Kremmer, V. Grunewald, E. Benhamou, G. Contesso, I. Joab. "Detection of Epstein-Barr virus in invasive breast cancers." *J. Natl. Cancer Inst.* 91 (1999):1376-1381.

Buehring, G.C., P.M. Kramme, R.D. Schultz. "Bovine leukemia virus (BLV) in breast tissue and antibodies to BLV in human sera." *Anticancer Res.* (1998).

Buehring, G.C., P.M. Kramme, R.D. Schultz. "Evidence for bovine leukemia virus in the mammary epithelial cells of infected cows." *Lab. Invest*. 71 (1994):359-365.

Labrecque, L.G., D.M. Barnes, I.S. Fentiman, B.E. Griffin. "Epstein-Barr virus in epithelial cell tumors: A breast cancer study." *Canc. Res.* 55 (1995):39-45.

Moore, D.H., J.A. Holben. "Observations on the question of horizontal transmission of mouse mammary tumor virus." *Canc. Res.* 38 (1978):2455-2457.

Stewart, T.H.M., R.D. Sage, A.F.R. Stewart, D.W. Cameron. "Breast cancer incidence highest in the range of one species of house mouse, Mus domesticus." *British J. Cancer* 82 (2000):446-451.

Wang, Y., J.F. Holland, , I.J. Bleiweiss, S. Melana, X. Liu, I. Pelisson, A. Cantarella, K. Stellrecht, S. Mani, B.G.T. Pogo. "Detection of mammary tumor virus ENV gene-like sequences in human breast cancer." *Canc. Res.* 35 (1995):5173-5179.


# FACT SHEETS

Single copies available at no cost. For multiple copies please contact BCERF (address below).

## General Information on Breast Cancer

__# *3*—Understanding Breast Cancer Rates

__# *5*—The Biology of Breast Cancer

__# *6*—Tumor Suppressor Genes - Guardians of Our Cells

__# *9*—Estrogen - What is the Relationship?

__#*10*—Estrogen - What Factors Affect a Woman's Exposure to Estrogen?

__#37Consumer Concerns about Hormones in Food

## Diet and Lifestyle

__*# 1*—Phytoestrogens and the Risk of Breast Cancer--*Revision*

__# *8*—Childhood Life Events

__#*13*—Alcohol

__#*18*—Fruits and Vegetables

__#*19*—Exercise

__#*27*—Dietary Fat

__#*29*—Breast Feeding

__#*33*—Dairy Foods and the Risk of Breast Cancer

__#36—Whole Grains and Fiber

__#39—Meat, Poultry and Fish

**CRITICAL EVALUATIONS OF PESTICIDES AND BREAST CANCER**

Critical Evaluations are available on the BCERF web page (see address below) as portable document files (pdf).

If you would like to order a hard copy please indicate below and send your check payable to Cornell University for **$3.00 each**, to cover the cost of reproduction and mailing.

__#1 2,4-D
__#2 Lindane
__#3 Heptachlor and Heptachlor Epoxide
__#4 Chlordane
__#5 Simazine
__#6 Cyanazine
__#7 Dichlorvos
__#8 Atrazine
__#9 Chlorpyrifos
__#10 Diazinon

## Pesticides and Breast Cancer Risks

__# *2*—DDT, DDE and the Risk of Breast Cancer

__#*11*—An Evaluation of Chlordane

__#*12*—An Evaluation of Heptachlor

__#*14*—An Evaluation of 2,4-D

__#*15*—An Evaluation of Lindane

__#*16*—An Evaluation of Simazine

__#*17*—An Evaluation of Cyanazine

__#*20*—An Evaluation of Dichlorvos

__#*23*—An Evaluation of Atrazine

__#*26*—An Evaluation of Chlorpyrifos

__#*28*—An Evaluation of Diazinon

__#*32*—An Evaluation of Alachlor

__#34—An Evaluation of Phosmet

__#38—An Evaluation of Mancozeb

## Pesticide-Related Issues

__# *4*—Reducing Pesticide Exposure in the Home and Garden: Alternatives and Proper and Legal Use Resource Sheet --*Revision*

__#*7A*—Reducing Potential Cancer Risks from Drinking Water--*Part I: Contaminant Sources and Drinking Water Standards*

__#*7B*—Reducing Potential Cancer Risks from Drinking Water--*Part II: Home Water Treatment Options*

__#*21*—Avoiding Exposure to Household Pesticides: Protective Clothing

__#*22*—Safe Use and Storage of Hazardous Household Products

__#*24*—Consumer Concerns About Pesticides in Food

__#*25*—Pesticide Residue Monitoring and Food Safety

__#*30*—Resources for Information on the Health Effects of Pesticides and Responding to Pesticide Poisonings

__#*31*—Integrated Pest Management Around the Home and Garden

**Cornell University**
***Program on Breast Cancer***
***and Environmental Risk Factors in New York State***
112 Rice Hall, Ithaca, NY 14853-5601
Phone: (607) 254-2893; FAX: (607) 255-8207
E-Mail: breastcancer@cornell.edu
http://www.cfe.cornell.edu/bcerf/

—add me to your mailing list

__remove me from your mailing list

NAME_____

Address_____

_____

Telephone _____

Fax _____

Email _____

*"We Need to Know"*

# Ad Hoc Discussion Group

*"Learning Together"*

Almost 40 people gathered in the Carriage House at the Bayard Cutting Arboretum in Oakdale, Long Island on June 21 for the most recent meeting of the BCERF Ad Hoc Discussion Group. Cornell Cooperative Extension of Suffolk County hosted participants in this beautiful setting.

Ann Lemley, the Associate Director of BCERF, facilitated the meeting and provided the group with an update and information on the transition between directors (see last issue of *The Ribbon*). Rodney Dietert, new BCERF Director, will facilitate the coming meeting. Ann updated the group on the progress made on Critical Evaluations and print materials, as well as the BCERF Education Tool Kit. The five modules of the Tool Kit were completed in draft form and "unveiled" at the Cornell Cooperative Extension System conference in the first week of June. Field testing will begin in September.

### What's Going on with West Nile Virus?

Dr. Lois Levitan, Program Leader for the Environmental Risk Analysis Program in the Cornell Center for the Environment, provided the first presentation, on the West Nile Virus. Lois' work addresses the simultaneous concerns of the dangers of the virus and the potential hazards of pesticide use in dealing with mosquitoes (one type of mosquito is the critical link in transmission to humans). She reviewed the transmission cycle of this virus, and the history of its arrival to this side of the Atlantic. She compared this season to date with last year, and credited the efforts of the New York State Department of Health (DOH) with its winter and early spring surveillance and prevention program. As of the time of her presentation, three diseased crows had been discovered. She noted that the probability of recurrence among humans is unknown, but that the elimination of mosquito breeding sites and larval control reduces this risk. Lois described the DOH guidance plan for counties, available on the world wide web and reachable through her program's web site: http://www.cfe.cornell.edu/risk/ Lois also emphasized the important contributions individuals can make to this effort, for example by cleaning up all standing water, and noted the critical role that citizens asking good questions has played in the conscientiousness with which NYS is addressing this problem.

### The Challenges of Historic Reconstruction of Pesticide Use: A Long Island Case Study

Dr. Ruth Allen, Environmental Epidemiologist and EPA Program Director for the Long Island Breast Cancer Study Project, provided the next timely and informative presentation. Dr. Allen's project involves collaborative, cross-disciplinary and public participatory efforts to bring us closer to understanding the extent and type of pesticide exposures that have occurred on Long Island. As the group is well aware, the period of the 1930's through the present has been a time of complex and changing pesticide use practices on Long Island. Dr. Allen's work – environmental exposure characterization – has been underway since about 1995, and fits into a trend in research in which large scale geographic patterns and scientific work such as microbiology are coming together. Her presentation provided a lot of context to help the group understand the progress which has been made in environmental epidemiology, and where the discipline is headed. For example, in her overview of the "four eras of epidemiology," she described the current era as that of ecoepidemiolgy and ecogenetics, in which gene-environment interactions are the focus. She described the criteria and methods for historic reconstruction of pesticide use, and methodological challenges. The group was very interested in detailed graphs Dr. Allen displayed on historical pesticide use; she described these as "a labor of love" by a colleague of hers, which were extremely difficult to construct. Dr. Allen can be reached at Allen.Ruth@epamail.epa.gov

### Pesticide Sales and Use Registry (PSUR)

Robert Haggerty of the NYS DEC Bureau of Pesticide Management and William Smith of Cornell's Pesticide Management Education Program (PMEP) brought the group up to date on PSUR. Available on the PMEP web site at http://pmep.cce.cornell.edu/regulation/psur/ are final reports for 1997 and 1998 and a preliminary report for 1999.

## Mark Your Calendars!!!

**Ad Hoc Discussion Group Meeting**
**Thursday, September 28, 2000**
**Faculty Commons Room,**
**Martha VanRensselaer Hall,**
**Cornell University, Ithaca, NY**
**11:00am to 3:30pm.**

*Ad Hoc Discussion Group meetings are open to any and all stakeholders to come together to discuss issues related to breast cancer and environmental risk factors.*

# Research Commentary

**Breast Cancer Incidence Highest in the Range of One Species of House Mouse, Mus domesticus.**

*Stewart, T.H.M., R.D. Sage, A.F.R. Stewart and D.W. Cameron. British Journal of Cancer 82 (2000):446-45.*

This study compared the incidence of human breast cancer to the geographical areas inhabited by different types of mice. Human breast cancer incidence varies to a large extent between different geographical areas. Mice living in different geographical areas may have different percentages of their populations infected with the mouse mammary tumor virus (MMTV). This virus causes mammary tumors in mice and these investigators propose that it can undergo several changes to allow it to cause breast cancer in humans. They also propose that the presence of MMTV infected mice explains the geographical differences in the incidence of breast cancer.

It is important to realize that this study was designed only to generate a hypothesis to be evaluated by subsequent investigations. The idea that certain types of mice give humans cancer was not examined directly but rather the investigators used data from other studies to put forth this hypothesis. In epidemiology, studies of this type are called ecological studies. Since they are designed to generate hypotheses, they are only required to set forth a possible explanation. No proof is expected or required.

The authors do not present a strong hypothesis. It is built on unestablished information and remotely possible phenomena. Central to this hypothesis is the idea that there are areas occupied by mice highly infected with MMTV and areas occupied by mice lowly infected by this virus. There is no foundation for such a proposal, as studies have not been conducted to directly determine locations occupied by mice with potentially active virus. Two types of mice do exist which seldom live in overlapping areas in various regions of the world. Most dramatically, there is a division in the living range of these mice between the western and eastern parts of Europe. Although both these types of mice can carry the MMTV gene, studies have shown that they can differ in the number of copies of the MMTV gene they do carry. However, the mice were from too few locations for the authors to make predictions about the presence of MMTV in mice in other areas. For example, the authors focus on differences in infected mice in Europe but the presence of MMTV in one of the types of mice has never been examined anywhere in Europe. The authors assume that North American mice, which have been examined, would be infected to the same extent as those in Europe because the North American mice are thought to have originated in Europe. Geographical localization of mice was not the focus of these studies and they were not designed to answer this question reliably. The authors have made worldwide predictions about the presence of MMTV in mice from studies of a few locations. The extent of infection of the different types of mice at different geographical locations is uncertain and should not be used as the foundation for a hypothesis.

MMTV is a retrovirus and during infection adds its gene to the genes of the host mouse. Mice can carry a number of copies of this gene. How active MMTV becomes depends not on the number of copies the animal carries but rather where the MMTV gene is inserted, that is, what genes it has as its neighbors. The studies, which looked at the presence of MMTV in the two mouse populations, found that about 85% of one type of mice carry MMTV, whereas, 40% of the other type of mice carry MMTV. This is a two-fold difference and does support the author's idea that one type of mice would be more likely to be infected. However, the authors were not satisfied with this size of difference. They devised their own criteria, based on the number of copies of MMTV the mice carried, to artificially increase the infection difference of the two types of mice. Since the mice that had the gene less frequently also had fewer copies, this division based on copy number dramatically changed the comparison of the mice. There was no biologically proven basis for this division and it was misuse of the data.

MMTV, like all members of its class of viruses, is able to undergo recombination. This means that MMTV could potentially pick up from other viruses the ability to infect humans. Such a change would be very rare but is possible. A recombination allowing MMTV infection of other species has not been observed, no other species exhibits breast cancer with MMTV-like biology. Most importantly, breast cancer in humans behaves differently from MMTV-induced cancer in mice. The spread of MMTV between mice has only been demonstrated to occur through breast milk. Infected mothers produce virus in their breast milk, which is transferred to nursing pups. Virus in the breast milk infects the pup's white blood cells and breast epithelial cells. The potential for breast feeding as a route for human breast cancer has been examined directly by a large case-control study involving 8300 women with breast cancer (Titus-Ernstoff et al., 1998). This study found that transmission of breast cancer through breast milk was unlikely (see discussion in accompanying article by SR Ross). Differences also exist in the tissues infected. Unlike MMTV infected mice, traces of the virus are not found in normal breast tissue and white blood cells of women with breast cancer who do have MMTV-like DNA in their breast tumors (Wang et al., 1995). The behavior of the virus during pregnancy in mice also differs from human breast cancer. MMTV induced mammary tumors in mice are increased by pregnancy whereas, pregnancy decreases the risk of breast cancer in humans. MMTV would thus have had to

undergo changes in its route of transmission, tissues infected and its activation by pregnancy to behave in a manner compatible with human breast cancer biology. Such major changes in the behavior of the virus would be very unlikely.

The authors finally separate first Europe and then the rest of the world based on the ranges of the two types of mice. They perform a statistical analysis of human breast cancer incidence in the European areas, which coincides with east and west Europe. They find a significant difference in breast cancer incidence between the two regions. There is also an area in Europe where both types of mice live. Here, they find intermediate breast cancer rates. Breast cancer incidence in other parts of the world was also presented relative to their mouse populations. The results were shown to agree somewhat with the area's mouse distributions. The authors attribute these differences to the presence or absence of MMTV infected mice but other well established explanations exist. The dissimilarity, between these regions, which is accepted by most epidemiologists, is that of affluence. Women living in Western Europe have a much higher standard of living than women in Eastern Europe. Breast cancer has been associated with affluence through the independent development of a number of risk factors. Breast cancer risk is higher in women who: do not have children; have children at a later age; have more education or income; do not breastfeed; have early menarche or late menopause, and; have a taller stature. Affluence impacts all of these factors. It should, nonetheless, be kept in mind that these risk factors and other risk factors could explain at best half the cases of breast cancer. Hypotheses, such as this one, serve the important functions of pointing to areas where knowledge is incomplete and stimulating thought and discussion.

Presentation of this paper in the popular press gave the impression that mice had been demonstrated to be possible carriers of breast cancer. It was not made clear that this was a hypothesis put forth by one group of investigators in an ecological study. One can identify ecological studies by the fact that the investigators are comparing an environmental factor to the incidence of some disease and producing a hypothesis.

## References

Titus-Ernstoff L., K.M. Eagan, P.A. Newcomb , J.A. Baron, M. Stampfer, E.R. Greenberg, B.F. Cole, J. Ding, W.M. Willett, D. Trichopoulous. "Exposure to milk in infancy and adult breast cancer risk." *J Natl Cancer Inst* 90 (1998):921-924.

Wang, Y., J.F. Holland, I.R. Bleiweiss, S. Melana, X. Liu, I. Pelisson, A. Cantarella, K. Stellrecht, S. Mani, and B.G.T. Pogo. "Detection of mammary tumor virus ENV gene-like sequences in human breast cancer." *Cancer Res*. 35 (1995):5173-5179.

*Barbour S. Warren is a Research Associate for the BCERF program; he received his Ph.D. in Pharmacology and Toxicology from the University of Louisville Medical School.*

**Cornell University**
***Program on Breast Cancer and Environmental Risk Factors in New York State***
112 Rice Hall, Cornell University
Ithaca, NY 14853-5601

Phone: (607) 254-2893
FAX: (607) 255-8207
E-Mail: breastcancer@cornell.edu.
WWW: http://www.cfe.cornell.edu/bcerf/

Cornell Cooperative Extension
*Helping You Put Knowledge to Work*