Murine and Rat Mammary Tumors as Models for the Immunological Study of Human Breast Cancer

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Summary

The human breast cancer process and some aspects of experimental mammary cancer are compared in the light of Huxley's hypothesis that each neoplastic cell line may be viewed as a new obligate parasitic species derived from metazoan cells. Sufficient correlations are found to justify the hope that the viral-induced mouse mammary oncogenic process and the carcinogen-induced rat mammary system may serve as reasonable models of the human disease for immunological studies.

My assignment in this conference is to present an analytical comparison of clinical and experimental mammary neoplasms aimed at estimating the degree of similarity of the oncogenic process and the disease course in these systems. Implicitly, the question I have been asked is whether the laboratory models of this disease are in sufficient degree relevant to the immunological understanding of human breast cancer to encourage their use in immunological investigations intended for the control of that disease. In approaching this task, I should like to begin with some general reflections on the phenomena of neoplasia.

Huxley, in his review of the biology of neoplasia (26), proposed that from the standpoint of biological systematics, each tumor cell population has sufficient marks of individuality and heritability of character to be properly considered as a unique species within a new phylum of obligate parasitic eukaryotes derivable from metazoan cells. As such, a tumor cell population would be expected in principle to pursue the evolutionary adaptations characteristic of species generally: (a) multiplication (tumor growth); (b) increasing autonomy (tumor "progression") (17); (c) increasing competitive efficiency (invasiveness); (d) extension of ecological range (metastasis).

Members of this proposed new phylum would be restricted in their fundamental biological properties by the genetic information available in their cells of origin. Tumors should be classifiable, therefore, according to the biological kingdoms, phyla, and species of the organisms in which they appear. Plant and insect neoplasms, for example, would always have fundamental properties belonging to the cells of their respective kingdoms and phyla and would accordingly differ from neoplasms of other kingdoms and phyla even in cell culture.

Neoplasms would also be diversified according to the epigenetic development of the cells from which they were derived. Consequently, neoplasms would be grouped according to the embryogenesis of their cells of origin and according to the specific organ from which they had arisen as well. Thus a large number of species-specific, organ-specific, and tissue-specific antigens would be retained in them.

However, since each individual neoplasm is postulated to be a new species, the individual neoplastic cells constituting a particular neoplasm would also be expected to have functionally significant epigenetic differences from other neoplasms, even those derived from the same organ and species. There would be some antigens individually specific for each tumor (4, 23, 27, 28, 37). Even in the same animal, no 2 tumors of 1 organ would be identical, although they would share, as would also the tumors of isogeneic hosts, a wider range of properties and antigens than would those of unrelated hosts (23, 37). Huxley finds that the unique character of neoplasms, in contrast to other parasitic species, is their lack of opportunity to persist beyond the death of their original host. Only through the assistance of the experimentalist can this opportunity be provided.

The murine mammary neoplasms, in this hypothesis, could be thought of as parasitic cell populations which often coexist with their host in a state of commensal and temperate parasitism for long periods. They tend to remain confined to a narrow ecological niche, defined by the boundaries of the mammary fat pad. In this respect they differ from human breast cancers, which regularly colonize other organs and tissues shortly after their growth has begun and while they are still small in comparison to many nonmetastatic but nevertheless locally invasive murine breast cancers.

The proliferative advantage of neoplastic transformants over adjacent mammary epithelial cells of normal character seems to consist in their relative multiplicative independence from the need for high concentrations of trophic hormones. In some instances where intensive hormonal stimulation has been used to induce mammary tumors, persistent growth does require the continuance of that hormonal support for many transplant generations (9, 16, 18, 29). Even in these cases, however, the proliferative response of the neoplastic cells is far superior to that of their normal counter-
parts and the rule holds that the former make better use of a given hormonal stimulus than do the latter.

Whereas normal mammary epithelium needs the stimulus of pregnancy for sustained additive growth, HAN\(^2\) and tumors do not (13, 36). Neoplastic mammary cells, of both HAN and tumors, are also independent of the influence of the more distantly acting mutual growth regulations exerted by vicinal mammary epithelial cells on one another. These latter influences, which determine with great regularity and precision the exact spacing of ducts and alveoli from one another in the mammary fat pad, and that channel and limit the growth of the mammary epithelial anlage during normal development of the gland and in adult life, are not effective for the restriction of neoplastically transformed cellular populations. These populations occupy the mammary fat pad densely, compress and engulf normal glandular structures, and, when cancer supervenes, apparently replace them altogether. The neoplastic cells multiply continuously in the absence of the hormonal milieux required to stimulate growth or secretion in normal breast cells, and in the presence of larger concentrations of mammary-trophic hormones, they outstrip the growth of the normal cells.

Nandi (36) reported that HAN may fail to distinguish between ovine prolactin and somatomotropin, a distinction accomplished by the cells of normal mammary ducts and alveoli. The latter multiply in hypophysectomized animals in response to prolactin only, and not in response to somatomotropin. Whether this type of alteration explains the proliferative advantage of HAN and tumors over normal mammary cells is uncertain, since differing strains also have genetically determined differences in their capacity for hormonal response and recognition (37, 45).

The classical studies on the oncogenic potentiality of individual hyperplastic mammary epithelial cell populations performed at the Cancer Research and Genetics Laboratories of Berkeley through the past 20 years (13, 37) fully support the hypothesis that the cell populations of individual isogeneic HAN should differ demonstrably from one another. The behavior of individual HAN transplants during serial transplantation in cleared fat pads shows that they do indeed possess a species-like individuality of growth pattern and of specific enzymatic content and that the efficiency with which they give rise to invasive tumors is a stable character of individual HAN populations (21, 37).

Long-term serial transplantations of individual HAN in syngeneic hosts show that the appearance of invasive neoplasms from them is not dependent upon the presence of demonstrable, infective MMTV. But these hyperplastic populations are susceptible to MMTV infection, and the rate of selection for, or evolution of, more independent and invasive subpopulations (i.e., tumor occurrence) is increased by MMTV infection. MMTV can be multipled within and secreted from HAN cells. Related studies also show a wide variance in the hormonal support requirements for rapid proliferation among individual nodules. Carcinogens and immunosuppressants also accelerate the rate and increase the frequency of appearance of invasive lesions from HAN transplants. Modulation of the oncogenic behavior of HAN can be achieved through dietary alteration (25) and by the physiological changes accompanying pregnancy and pseudopregnancy.

Whether neoplasms arise directly from the dominant cell population of HAN is disputed. In some experiments it has been possible to reduce the frequency of HAN in the breast without reducing the frequency of tumor formation (7, 54). But HAN are neoplasms by the criterion of sustained growth during transplantation. It is certain that invasive tumors will arise from the cells of serially transplanted HAN and that the frequency of tumor occurrence is increased in an animal whenever the formation of HAN is promoted.

Does an analog to the HAN exist in man? Are there hyperplastic, noninvasive, mammary epithelial cell proliferative foci, the frequency of which is correlatable with the frequency of mammary cancer in a given woman or a given population? Wellings' studies (52) are consistent with the hypothesis that such hyperplastic foci do occur in the lobules and prelobular ductules. Cancer-associated focal epithelial hyperplasias in small ducts have long been described in some women with cystic mastopathy (12, 22, 41). Wellings believes that the lesions he has characterized account for these earlier descriptions.

Fibrocystic disease of the breast has many of the same epidemiological associations as does breast cancer, and MacMahon et al. (32) state that it connotes a 2 to 3 times higher risk of breast cancer than that which obtains for the population generally. Black and Cutter (3) were able to identify morphologically a particularly ominous type of fibrocystic disease in which florid focal epithelial hyperplasia occurs.

In man, as in the mouse then, there is epidemiological and histopathological evidence that multiple foci of noninvasive mammary epithelial hyperplasia occur in the breast in which cancer development is occurring. As in the mouse (37), the form of these hyperplastic foci may be variable from one subject to another, and there is some doubt whether tumors must necessarily take their origins within them. Present evidence does not prove that a hyperplastic benign proliferative transformation is an obligatory step in the genesis of mammary cancer cells from normal mammary epithelium.

The proposal of Wolfe that xeroradiographic alterations (which he designates as the "prominent duct" and the "dysplastic" patterns) may identify patients at very high risk for subsequent breast cancer development, implies that a generalized subgross preneoplastic structural change in the mammary gland precedes human breast cancer development. The histopathological basis of Wolfe's radiographic patterns is not yet established, but the distribution and dimension of the linear nodularities he describes fit well with the topography and size of the lobular and ductular hyperplastic foci found by Wellings. Detailed correlation studies remain to be done, but if they confirm preliminary findings, analyses of the endocrinology of the hyperplastic foci that may precede breast cancer in man will be facilitated and sources of material for useful organ and cell culture studies will be provided.

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\(^2\) The abbreviations used are: HAN, hyperplastic alveolar nodules; MMTV, mouse mammary tumor virus; DMBA, 7,12-dimethylbenz(a)anthracene.
No means have been found for the extinction of HAN populations once well established, and some of them will persist even when normal mammary epithelia have become atrophic in the course of aging or as a result of ovariectomy, adrenalectomy, or hypophysectomy (37).

The frequency with which these populations occur in the breast of various inbred mouse strains in the absence of demonstrable MMTV infection is under genetic control (37). Susceptibility to an increased frequency of their development under the influence of MMTV, of chemical carcinogens, and of hormonal stimulation, whether with continuous high-dose estrogens or by supernumerary ectopic pituitary implantation (high prolactin levels) is similarly under genetic control (36, 37).

Familial concentrations of breast cancer in man suggest a genetic component in the human disease also (31), and cystic mastopathy is often found in breast cancer families. That the genetic characteristics related to mammary tumor development in the mouse are not entirely attributable to differences in the epithelium of the gland itself has long been suspected. Huseby showed that the ovaries of a high-tumor strain transplanted to spayed F1 hybrids yielded a higher incidence of mammary tumors than did ovaries from the low-tumor parental strain (37). Albert and Johnson (1) and Nandi and McGrath (37) have demonstrated evidence of the relative immunoincompetence of high-tumor strains. This is aggravated by milk-borne MMTV but is demonstrable also in its absence (36).

Nevertheless, the predominant role of the genetics of the mammary epithelial cell itself in determining susceptibility to neoplastic transformation by MMTV has been elegantly established by the fundamental embryological studies of Mintz and the classic transplantation experiments of Boot (37). In mosaics created by the fusion of blastulae from high- and low-mammary cancer strains, Mintz showed that the carcinogenic activity of MMTV is much stronger for cells derived from the high-tumor strain than for those derived from the low-tumor strain. Here there is an identical hormonal milieu and immunological context for the 2 cell types, so that the predominantly cellular genetic basis of susceptibility to viral mammary oncogenesis is clearly demonstrated. Boot, with transplants of high- and low-risk glands into F1 hybrids, found a similar restriction of virus-induced tumor development to the former.

Each inbred strain of mouse is analogous to 1 immortal infinitely recallable patient, in whom the process of mammary neoplastic development can be observed from its inception to its terminus, under any conditions desired. Just as the character and course of the neoplastic process differ from one patient to another, they differ markedly between strains. In some mouse strains neoplasia is rare without the stimulus of repeated pregnancy, and the tumors are initially hormone dependent. In others the tumors are apparently autonomous from the outset. In some, the accompanying focal hyperplastic lesions are lobulo-[alveolar (HAN) and in others they consist of “plaques” of a different histological character. In some strains virgins have few tumors; in others, virgins develop as many tumors as do breeders (13, 36, 37).

In all spontaneous mouse mammary tumors, a set of related RNA tumor viruses appear to be critical for the oncogenic process and are present at least as a characteristic nucleotide sequence in the neoplasms that appear, even if budding of viral particles is not demonstrable (42). In some strains this virus is transmitted exclusively through milk; in others it is transmitted to progeny from both sexes and nursing is not necessary for the infection of progeny (37).

Clearly, there is space within the mouse system for a range of endocrinological dependencies, genetic influences, and viral transmission modes sufficient to encompass the phenomenological spectrum of human mammary neoplasms.

The finding of B-particles (35, 43), and subsequently of enzymatic and polynucleotide components indicative of an MMTV-related RNA virus in human milk (2, 14, 42), has brought the hypothesis of an essential similarity in murine and human mammary oncogenesis to a new level of interest in the last 5 years. Evidence for the presence of related viral components in a human mammary cancer line (49) has been developed in our laboratory (33), and biochemical studies have revealed similar components in a number of human breast cancers (2).

The picture that emerges from a broad survey of mouse mammary carcinogenesis is that of a protracted process taking origin in very early life, aggravated by specific RNA viral infection of mammary epithelium, dependent on a mammotrophic hormonal milieu for its evolution, and yielding both hormone-requiring and independent neoplasms according to genetic determination. Invasive neoplasia does not appear until senescence has begun in most strains, although atypical hyperplasia is demonstrable in early reproductive life. There is evidence (K. B. DeOme, personal communication) from transplantation of cell suspensions of very young mammary gland cells that cellular transformants with altered hyperplastic growth properties are already hidden in the mammary epithelium at a few weeks of age, well before HAN appear.

HAN first appear after the reproductive period of life has begun. Events associated with sexual maturation and reproduction determine their frequency in any given genetic and viral setting favorable to their occurrence. Tumor frequency in later life correlates with HAN frequency in adult life. Both HAN and tumors are infrequent in the highly immunocompetent 1 strain (37). The shared antigenicities to tumor cells invoked by related viruses make possible an immune responsiveness directed generally against mammary tumors arising in a variety of strains.

In man, benign breast disease and benign as well as malignant breast tumors are uncommon until late adolescence or early adult life. As in the murine systems, age-specific cancer incidence rates rise acutely as the close of reproductive life approaches, and they continue to climb steadily on into senescence (32). Between adolescence and the late 20's, the human breast cancer process appears to be suppressible by the events of a full-term pregnancy (32). Attempts to understand this epidemiological correlation in terms of estrogen metabolism have so far been unsuccessful.

The role of progesterone and prolactin in the oncogenic process in women is not clear, and specific prolactin suppression with 2-α-bromoergocryptine has been disap-
pointing in the treatment of established human breast cancers. In the murine systems there is little doubt that prolactin is important for tumor development (29, 37), but in rodents prolactin is strongly luteotrophic so that account must also be taken of possible progesterone promotion of the disease. In the mouse, progesterone is not present except during pregnancy, whereas in the human it is secreted during the latter half of each menstrual cycle. These endocrine differences may be related to the differing effects of early parity on breast cancer evolution in the 2 species. It is well to recall, however, that a first pregnancy after age 30 appears to increase breast cancer risk in women, indicating that the 2 species are not altogether different with respect to the effect of pregnancy on breast cancer promotion (32).

There is a widespread assumption in breast cancer research that hypersecretion of hormones or hyperstimulation of the mammary gland is a cause of the disease. This has been due in part to the induction of animal tumors with large sustained doses of estrogen and the salutary effects of oophorectomy in some metastatic breast cancer patients. However, in the mouse as well as in the human it is quite clear that the disease ordinarily makes its appearance at the age of involution of the mammary gland, when mammothrophic stimulation is low.

The same age incidence patterns are also found in dogs, cats, primates, rats, and rabbits. It is true that breast cancer can and does occur during the reproductive period in all species, but this should not obscure the fact that the disease is typically one of older females (38).

One is tempted to suggest that neoplastic cell populations may in fact be hindered in their tumorigenic potential and proliferative activity by the presence of a full complement of healthy neighboring epithelial cells. Rather than searching for an excessively mammothrophic endocrine milieu to identify high-risk groups, it might be better to search for correlation of breast cancer occurrence with a hypothrophic endocrine condition (5, 8), a mammary presenesence. By reducing the competition of normal cells for mammary lebensraum, occupation of new niches by a neoplastic cell population might be facilitated and the growth and adaptation of Huxley’s “new eukaryotic parasitic species” aided. The larger the population of a species, the better chance there is for population genetics to succeed in broadening its phenotypic representation and its overall chance for acquisition of more advantageous properties for the exploitation of a variegated environment. Reduction of selection pressures results in an increased rate of appearance of variants in diploid organisms (47). Fundamentally, cancer cells have the genomic distributional features of diploid organisms.

Evidence in support of the concept that expression of transformation (tumor occurrence) may be impaired when normal cell populations are active is provided by hydrocarbon carcinogenesis experiments in rats (10). In this situation, as Dao has shown, pregnancy, and pretreatment or immediate posttreatment with large doses of estrogen, progesterone, or both, will block the carcinogenic action of a DMBA injection (11). To some extent this may be explainable by alteration of hepatic metabolism of the carcinogen, or of its retention time in the gland. However, Dao was able to show that mammary glands were not saved from carcinogenesis when promptly transplanted to an undisturbed new host. We then undertook to demonstrate that carcinogenesis in this system needed no adrenal damage or secretory alteration and no extramammary activation of the carcinogen by exposing glands in vitro for 20 min to DMBA, washing them well, and reimplanting them interscapularly in the animal of origin (6).

In this autochthonous system, utilizing a minimally disturbed host, we were able to show that the treated glands would regularly become neoplastic even though in no case did neoplasia develop in other mammary glands of the same animal.

Here again, in chemical carcinogenesis as in viral carcinogenesis, the conclusion would seem justified that the carcinogenic process does not require any hormonal milieu other than the normal one consistent with normal mammary gland development, reproductive capacity, and longevity. The atrophy of the excised and reimplemented mammary gland consequent to the interruption of its blood supply during the ex vivo treatment and transplantation to a new site, together with the application of a potent carcinogen, were sufficient together to allow the induced neoplastic cell species to evolve rapidly into a gross tumor. No host alteration was needed. Also in this system, estrogen administered 24 hr before the maneuver, or merely added to the carcinogenic bath, was able substantially to impede the transformation, raising the question on the one hand of chemical competition for the sites critical to transformation and, on the other, of the possibility that a growth stimulus to the target cell population interfered with the carcinogenic process.

While endocrinological studies have generally been based on the presupposition of endocrine hyperactivity as the probable promoter of mammary cancer development, immunological research has tended to expect to find immunological hyporesponsiveness responsible for the disease. The findings reviewed above with regard to the absence of evidence for need of any special endocrine state to explain mammary tumorigenesis in the murine or the rat systems can also be used to argue against the need for an immune deficit.

The normal mouse organism tolerates the syngeneic or histocompatible HAN or mammary tumor implant and allows its progressive growth in all but exceptional instances (e.g., the l strain mouse). The normal rat immune system tolerates genetically compatible carcinogen-induced mammary tumor transplants (10). Not only that, but it tolerates the genesis in vivo of new carcinogen-induced tumors in circumstances where carcinogen alteration of the immune apparatus has not occurred (6, 11).

These findings, that immunodeficiencies need not be present to allow for development or growth of experimental tumors, fit well with the negativity of clinical attempts to demonstrate a regular immunological deficit in women with operable breast cancers.

The hormonal dependency of the DMBA-induced tumor systems is well known (10, 24). These neoplasms often replicate very closely the behavioral spectrum of dependent human breast cancers with respect to administered hormones and endocrine ablations. In this they are said to be
better models of human breast cancer than the mouse tumors. But since at least 60% of human breast cancers are clinically autonomous with respect to the endocrine system, this claim seems excessive (34, 46).

These neoplasms also often regress spontaneously and are not readily transplantable for more than a few passages. Many assume that this raises serious question of their neoplastic nature and contravenes their relevance for the understanding of human breast cancer. It is widely assumed that human mammary neoplasms always grow progressively, and it is known that clinical lesions rarely regress spontaneously.

But the frequency of occult cancer in the human breast at autopsy (40, 41) and the clearly excessive frequency of “microscopic” contralateral cancer when routine biopsies are made at surgery (39, 51), in comparison to the observed frequency of 2nd primaries in large series of breast cancer patients (22, 30), make it appear that subclinical neoplastic cell populations in the breast may not only fail to progress but may also regress spontaneously.

If each neoplasm is composed of a new species of cells attempting to occupy ecological niches already filled (48), and having no hope for survival and growth other than a promptly successful evolutive adaptation in the face of substantial competitive pressure (and possibly even of specific predator attack by immunocytes), failures of progressive growth and regressions would not be unexpected. In the mouse and the rat, as in women, the frequency of mammary hyperplastic foci far exceeds that of successful neoplastic foci in tumor-prone glands (15, 19, 20, 50). What knowledge there is of breast cancer in the dog points in the same direction.

Conclusions

Biological experiments in mammary cancer induction and observations of the natural course of the disease in both the virus-related murine and hydrocarbon-induced rat experimental tumor systems reveal many correspondences within these systems with features observed in clinical breast cancer.

When a theoretical biological approach to the understanding of all these types of mammary neoplasia consistent with that proposed by a great biologist is followed, their commonalities appear to be substantial and the relevance of these 2 models to the search for immunological understanding and methods useful in the human disease seems strong. Recent findings and ongoing research indicative of a viral participation in the induction of breast cancer in women make the mouse system especially interesting from an immunological standpoint.

The hypothesis that tumor initiation may fail for biological reasons not concerned with immunology or endocrinology deserves consideration. There are good reasons for doubting that disorders of these systems are requisite to either the genesis or support of mammary neoplasia in man or in experimental animals.

Nevertheless, the manipulation of endocrine and immunological factors may serve to alter the course of breast cancer development and growth in useful ways. Given the more specific immunological agents and responses that would be elicitable were a related set of human mammary cancer viruses to be isolated and made available in large quantity, the practical contribution of immunology to clinical breast cancer control would be substantial.

References

25. Hui, Y. H., DeOme, K. B., and Briggs, G. M. Inhibition of Pituitary-Induced Nodular Hyperplasia in Mammary Glands of C3H Mice Fed a
Breast Cancer in Comparative Medicine


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