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Lymphatic invasion and metastasis

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Summary. The phenomenon of experimental lymphatic invasion and metastasis has been reviewed. The invasion of lymphatics must be dependent on the same factors as are involved in neoplastic invasion in general – cell motility, lack of adhesiveness, release of lytic enzymes, increase in cell population and tissue pressure and active migration of cells.

The process of lymphatic metastasis consists of a) penetration of the peripheral lymphatic wall, b) embolism lodgement and growth in the draining lymph node. The study of lymphatic metastasis in experimental animals requires precise and defined models. It seems in the only model studied that initial penetration occurs by reverse diapedesis of tumor cells through open interendothelial junctions. It seems at present that lymphocytes and macrophages migrate into lymphatic vessels in a similar way. It is likely but not certain that in some way the cells induce opening of lymphatic endothelial junctions. Whether this process is specific or non-specific and whether it involves release of chemical mediators is uncertain. There is some evidence that in one model there is induction selection of one tumor subtype for successful metastasis, though it is far from certain that this is universally true. The cellular reactions in the draining lymph node include sinus macrophage proliferation, proliferation of cells in germinal centres, and migration of cells from post capillary-venules. A definable burden of tumor cells in the node is necessary for successful metastasis; this burden is much smaller than that necessary for growth in the primary inoculation site, indicating a degree of immunological privilege within the node. A lymphoreticular and therefore presumably immunological reaction in either primary injection site or node may result in tumor rejection.

The influence of the lymph node on the process of metastasis is dubious. Lymph nodes are probably not effective barriers against tumor. There is much controversy both on the effect of the lymphoreticular response (enhancing or retarding growth) as well as on the effect of regional lymph node removal. While the 2 major methods of dissemination of neoplasm by blood and by lymph are closely related there may be differences in sensitivity to chemotherapy; in one model lymph node metastases have been shown to be more sensitive to chemotherapy. There is a great need for experimental work on lymphatic metastasis to provide guidelines for new approaches to the treatment, and even prevention of metastasis in human cancer.

One human being in five in an advanced society dies of cancer. While the outcome of the disease is usually ultimately determined by massive haematogenous metastasis, the initial mode of spread of many common human cancers is metastasis by lymphatics – and therefore lymphatic metastasis of tumors is of great human importance, yet its mechanism is poorly understood. A major cause of this lack of understanding has been the lack of suitable animal experimental models – because unlike human tumors most experimental animal tumors either kill the animal without metastasizing or metastasize by the blood stream. Lymphatic metastasis tends to be uncommon and late. This implies that lymphatic invasion rarely occurs. Recent excellent reviews on both neoplastic invasion and metastasis[1,2] do not cover the relationship between neoplastic in-

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vasion and lymphatic metastasis. The purpose of the present review is to discuss briefly experimental lymphatic invasion and metastasis and the role of host defensive mechanisms in obstructing it.

Many mechanisms have been postulated to account for neoplastic invasion in general[1]. These will be enumerated only briefly here. It has been suggested that tumor cells release lytic enzymes of various types; for instance many varieties of human and animal malignant tumors have a much higher content of collagenolytic enzymes than benign tumors or the corresponding normal tissues[3,4]. It is possible that lymphoreticular cells infiltrating the tumor release such enzymes. There may be induced anoxia in the surrounding tissue due either to pressure on blood vessels or competition by the tumor cells for oxygen. In addition or alternatively there may be competition by the tumor cells for essential nutrients such as aminoacids[5]. Tumor cell motility may be important; tumor cells have been described as migrating through normal tissues at rates of 50–100 μm per day[6]. There may be correlation between metastatic potential and abnormalities in intercellular adhesion (at least in the case of carcinomas[7]) or lack of immunogenicity[8–10].

Tumor cell division may be important. The excessive cell proliferation in tumors is probably due to shortening of interphase, rather than to any change in the length of mitosis itself. As tumors become more anaplastic during transplantation passage there is an increase in metastasis[11,12]. Conversely well-differentiated human mammary cancers grow more slowly[13] and the slow growth is associated with prolonged survival[14]. There are exceptions; chondrosarcomas rarely and basal cell carcinomas, very rarely metastasize, irrespective of their degree of differentiation. Clearly progressive multiplication of tumor cells in a restricted space could bring about an increase in tissue pressure with disruption in blood supply and ischaemia, obstruction of lymphatics and local oedema and possibly direct pressure on specialized cells. Direct pressure by neoplastic cells on their adjacent counterparts may cause cell degeneration or the tumor may spread due to the increased total hydrostatic pressure of the tumor mass pushing its way down tissue planes. Evidence for this derives from experiments with elastic jelly models[15,16].

There is conflicting information on whether metastasis is promoted by massage or exercise; some workers find that massage or exercise stimulate metastasis[17] while others using either immobilization or massage fail to confirm this[18–20]. Local pressure increase dislodges more tumor cells, but this does not affect the ultimate total number of metastases[21]. Clinically (e.g.[22]) there does not appear to be a precise correlation between the presence of malignant cells in the blood and metastasis. The importance of simple mechanical factors in promoting metastasis is probably limited. It is likely that these mechanisms operate to a different degree in different tumors and to a different degree at different sites. All of these mechanisms are not necessarily relevant in the highly individual process (or perhaps just fortuitous epiphenomenon) of lymphatic metastasis.

The first step in lymphatic metastasis is access to the lymphatic system: the penetration of a lymphatic vessel. Next tumor cells must pass up the lymphatic system to the regional lymph node where they settle and multiply. Finally, tumor cells detach from the secondary deposit and migrate further to secondary nodes and then into the blood stream. At all stages the tumor cells are potentially sensitive to inadequacies in nutrition and susceptible to attack by host lymphoreticular mechanisms.

When constructing a model of lymphatic metastasis in which these stages can be analyzed, it must be clear that the model is a good reflection of lymphatic metastasis in human cancer and is yet susceptible to experimental modification. It should be reproducible. The tumor dose should be measurable with some degree of accuracy, which implies the use of a cell suspension in which both numbers and tumor cell variability can be estimated. The possibility of direct intralymphatic injection should be eliminated and the process should be demonstrable as occurring in natural stages: penetration of lymphatic by tumor cells, detachment and embolus formation within the lymphatic, settling survival and growth within the node, persistent recruitment from the primary site and ultimate production of a lethal metastasis in the lungs and elsewhere. This model, it should be remembered, does not relate to the important human situations where haematogenous metastasis occurs independently and early.

The first critical step a tumor cell must take is across the lymphatic vessel wall. Before this it can be stopped by the lymphoreticular reaction. Studies of the relationship between the dose of administered tumor cells and the success of lymphatic metastasis have shown that at the level where the tumor may fail to take there is a florid local lymphoreticular reaction[23–25].

Before considering how tumor cells cross the lymphatic wall it is necessary to review briefly the way in which things other than cells cross the lymphatic wall. Lymphatic vessels[26] have very thin walls. The major route through them consists of open junctions. About 2% of the total length of cell junctions is open, 10% close and the remainder tight. The junctions tend to be open in oedema, being poorly supported by adhesion devices and basement membrane; inflowing fluid pushes the inner flap of cell aside and vesicular transport through the cytoplasm is of little significance.

The earliest account of the passage of tumor cells into lymphatic vessels is a description of the passage of tumor cells into the open diaphragmatic lacunae of the diaphragm and thence to mediastinal nodes as seen

by scanning electron microscopy[27]. These lacunae differ from peripheral lymphatics in having permanently widely patent intercellular junctions. Cells are initially intravasated, that is passively absorbed into the lacunae. Later the mesothelial cells round up exposing basement membrane, and tumor cells attach to the denuded areas and thence penetrate to the pleural surface of the diaphragm, a process of active infiltration possibly involving the production of lytic factors by the tumor cells.

The passage of tumor cells through ordinary lymphatic vessel walls has been studied using as a model the injection of Rd 3 tumor cells into the footpad of the rat[28,29]. The tumor cells concerned were characteristically circular in cross section in a situation such as a flank tumor where metastasis rarely occurred. The lymphatic junctions in control rat footpads are usually closed; it can be assumed that this means not more than 2% of the junctional area is open[26]. Tumor cells are seen migrating singly through between patent interendothelial junctions as 2 sets of profiles. Firstly, cytoplasmic processes are seen singly or in groups; secondly, the profiles of whole cells are seen between the endothelial cells (figures 1 and 2). It is interesting that in this experimental situation macrophages are seen migrating between the endothelial cells in precisely the same way as tumor cells; the cytoplasmic processes of the 2 types of cells are often clearly distinguishable only in serial section. The act of penetration of the vessel was a relatively rare one, occurring about once in 5 mm^3 of tumor tissue. In the experimental situation described it is clear that the cells are migrating into the vessel from without, and it seems likely that the cytoplasmic processes of the tumor cells act as probes in determining a preferred pathway. Channels of varying density probably exist within connective

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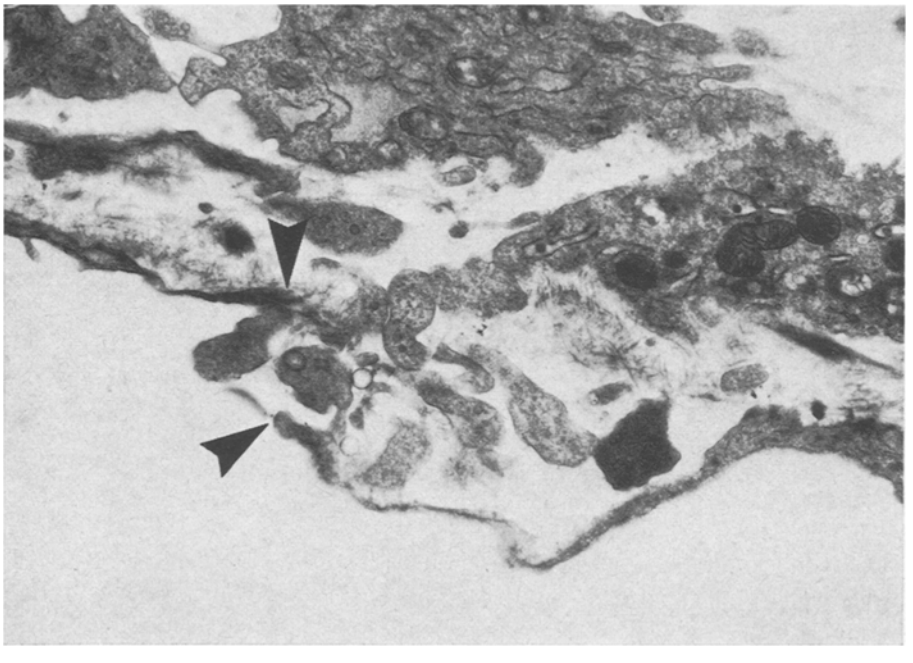

Fig. 1. A lymphatic vessel from the footpad of a rat 5 days after the local injection of 20 million Rd/3 tumor cells. Fine cytoplasmic processes from a tumor cell are penetrating an open gap between endothelial cells. The edges of the gap, that is the ends of the endothelial cells, are arrowed. The lumen of the lymphatic vessel lies at the bottom of the picture. ×8,700.

tissue which form prelymphatic pathways[26] allowing preferential movement of fluid and it is possible that cells follow such pathways.

It may be that once a cell is in such a preferred pathway, tissue hydrostatic pressures will force it in the direction of an endothelial junction in a lymphatic wall. The presence of cytoplasmic processes of both tumor cells and macrophages in patent interendothelial spaces however suggests that these processes may have a role either in seeking out the very few spaces which are already patent or in causing contraction of endothelial cells and opening of a close junction. The protrusion of cytoplasmic processes by tumor cells has been shown to play an important part in neoplastic invasion in several sites, notably in epidermal carcinogenesis[30–32] and liver[33–36]. Comparison of the behavior of lymphoma and carcinoma cells showed that the latter even more than the former protruded pseudopods into hepatocyte cytoplasm[35,36]. Under some circumstances tumor cell processes apparently protrude between intact endothelial cells as the tumor cell leaves the sinus. The view that cell processes can have an exploratory function is suggested by the demonstration that when 3T3 tumor cells were allowed to spread on a surface of heterogeneous surface characteristics, the filopodia located preferentially on areas of one particular type[37]. If the earliest stage in access to a lymphatic is access to a preferred tissue pathway, it is reasonable to ask whether there is any particular subtype of tumor cell gains this access more readily. There are a number of pertinent facts. Trypsinization in some cases allows a metastasizing tumor to metastasize more readily, but does not make a nonmetastasizing tumor metastasize[38]. This probably means that detached single cells have a greater chance of access. Usually the cells in lymph node deposits are similar in nuclear dimensions to those in the primary lesion[39]; this is

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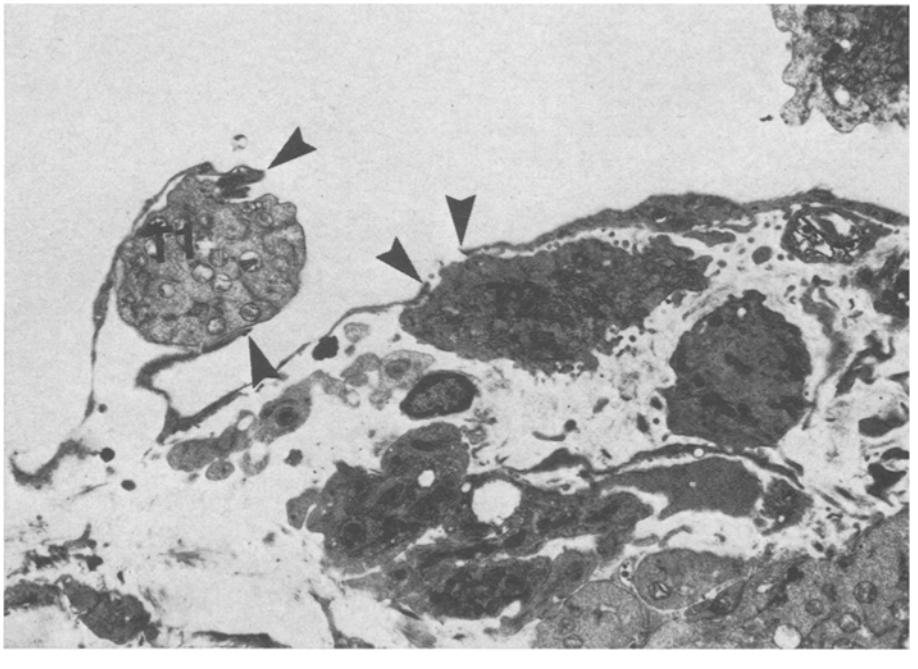

Fig. 2. A lymphatic vessel from the same rat as figure 1. A mass of tumor cell cytoplasm (T1) is passing through an open gap between endothelial cells. The edges of the gap are arrowed. Another tumor cell (T2) is just protruding through a similar patent gap. ×3,300.

not always the case however and the nuclear ploidy of the metastatic deposit may be higher[40]. Such differences are accountable by differential proliferation in the different and possibly more favorable milieu of the lymph node. The best evidence that some cells in a tumor possess characteristics which allow them to metastasize more readily than the rest comes from a study of a metastasizing tumor in Syrian hamsters[41]. The prevalent cell type in the metastasis was different as shown by chromosomal analysis, having 2 long submetacentric chromosomes and was more globular in shape and showed decreased cellular adhesiveness. This was explained by postulating the induction of a metastasizing variant by derepression of genes with resultant phenotypical alteration. Since some of these altered cells were present in the primary tumor it is reasonable to assume that the phenotypical alterations conferred advantage in gaining access to the lymphatic; it is equally possible that they led to a better chance of survival in the environment of the lymph node. This study however requires confirmation.

The role of secretion by tumor cells in gaining access to the lymphatic is uncertain. There is little evidence of bulk secretion of large amounts of material. By immunofluorescence small amounts of cathepsin have been shown to be present on tumor cell surfaces[42], and it is by no means impossible that some type of short range histiolytic process is at work in breaking down connective tissue matrix around the lymphatic or indeed in opening the lymphatic junction. This however remains to be demonstrated.

The essentials of an adequate model of lymphatic metastasis have been described. A number of the described models involve features such as direct intralymphatic injection which render them incomplete as models and will not be considered.

A number of satisfactory models have been produced in recent years[1,23,38,43–50]. These suffer in the main from the disadvantage that reactions following injection of tumor cells are being studied. Despite the intrinsic disadvantages of this, such models provide the best practical manner of studying a difficult problem. An early and excellent model involved the study of 2 similar hamster lymphomas one of which did and one of which did not metastasize ([51–53], summarized in[1]). The lymphoma which did not metastasize evoked a paracortical cell proliferation in the draining node with some follicular enlargement and activation of sinus macrophages; the metastasizing lymphoma on the other hand evoked medullary plasma cell proliferation and immunoglobulin production[1]. The cells of the metastasizing lymphoma often contained small stacks of endoplasmic reticulum[54] and had localized caps of surface immunoglobulin, as shown by an EM peroxidase technique[55]. The attractive though not the only explanation for this latter finding is that the immunoglobulin secreted by the tumor cells may potentiate metastasis. In another satisfactory model[43,44] a hepatoma was injected into skin and metastasized to regional lymph nodes; the injection of BCG into the tumor before excision under appropriate circumstances eradicated metastasis. The process of lymphatic metastasis has been roughly quantitated using a metastasizing anaplastic chemically induced tumor[23,38,45–47]. The injection of 5×10^6 cells into the footpad led to the presence of tumor cells in the popliteal node; after 1 day about 1.5×10^2 cells were present in 1 node, after 2 days 2.3×10^4, after 3 days 4.5×10^4 and after 4 days 5.0×10^5. The histological stages of metastasis seen here were similar to those described in other reports. Firstly, tumor cells passed across the lymphatic wall; this was difficult to identify. Tumor cells were then seen singly or in clusters, often accompanied by macrophages in the lymphatics in the foot, in the afferent lymphatics in the popliteal node and then in the subcapsular sinus. Occasional dividing cells occur in the pre-nodal lymphatics, numerous divisions occur in the subcapsular sinus, and single cells and clumps of cells spread down the radial and the medullary sinusoids, distending them as they go. The tumor cells then migrate out of the sinusoid, proliferating and destroying the lymph node and replacing it entirely with tumor. Before this final stage occurs tumor cells can already be seen in considerable numbers in efferent lymphatic vessels. The critical dose of tumor cells necessary in the node for successful metastasis seemed to be about 2.5×10^2 cells; it seemed fairly certain that tumor cells could be destroyed both in the footpad and the lymph node. The tumor cell burden necessary for successful growth in the node (2.5×10^2 cells) is much smaller than that necessary for successful growth in the primary injection site, suggesting that a degree of immunological privilege exists in the node. BCG injection often prevented metastasis. Metastasis occurred similarly with several other tumors but was not a universal property of all tumors as indicated by the failure of most mouse mammary tumors to metastasize.

It seems likely that lymphatic metastasis may be conditioned by local lymphatic density or even by variations in lymphatic vessel structure from one topographical site to another, but there is little information on this.

None of these tumors correspond precisely to the common human metastasizing carcinomas. A useful model of carcinoma of the breast has been produced in the mouse by injection of tumor cells into the footpad and examination of popliteal and inguinal nodes, and lungs[56]. Only 1 of a series of 9 tumor systems initially studied uniformly showed early lymph node and late lung metastases. This mammary carcinoma was more fully characterized and was found to be non-antigenic following s.c. and i.v. challenge tests. When adjuvant chemotherapy was applied to this

system, lymph node metastases and lung metastases showed a striking difference in sensitivity to chemotherapy. When a dose of a chemotherapeutic agent which gives a significant prolongation of post surgical survival is administered, only 23% of the treated animals show lymph node metastasis at death, as opposed to 100% of controls. Lower doses of chemotherapeutic agent which do not significantly affect survival still reduce lymph node metastasis by as much as a half (unpublished results). Clearly therefore, the lymph node metastases are more sensitive to chemotherapy in this situation. These results indicate that although the 2 systems of dissemination may be closely related (figure 3), lymphogenous and haematogenous metastases may markedly differ in their response to chemotherapy. Presently it is not clear, however, whether this is because more cells are killed in the lymph node metastasis, or because recurrence patterns are different. The possibility that the lymph node bed is a poorer area for regrowth after damage to the lymphatic system by drug treatment cannot be excluded.

The idea that the regional lymph node may act as an effective barrier to the spread of cancer[57] is an old one, and is obviously suggested by the way in which their anatomy converts the flow of lymph into percolation[58]. While the barrier may be temporarily effective ([59,60] see Strauli[61] for review) it is clear from other studies that the protection is at best only temporary since tumor cells may stream through lymph nodes with relatively little seeding[62–65]. As with normally circulating leukocytes relatively rapid exchange may occur in both directions between lymph stream and blood stream[66,67]. (This is summarized in figure 3).

Host defensive mechanisms may affect tumor growth in either non-immunologic or immunologic ways[68,69]. Only reactions that relate to lymphatic metastasis will be dealt with here. There have been many studies of reactive changes in lymph nodes draining human malignant tumors[69–73] and it has been proposed that reactive changes can be related to immunological function[74]. It seems clear that the major reaction in lymph nodes draining experimental tumors is sinus macrophage proliferation and an early hyperplasia of the paracortical area which has been shown to be a thymus-dependent area[82,83] followed by an increase in the size and number of cortical follicles[75–80]. Similar appearances are found in human lymph nodes and may be of prognostic importance[81–89].

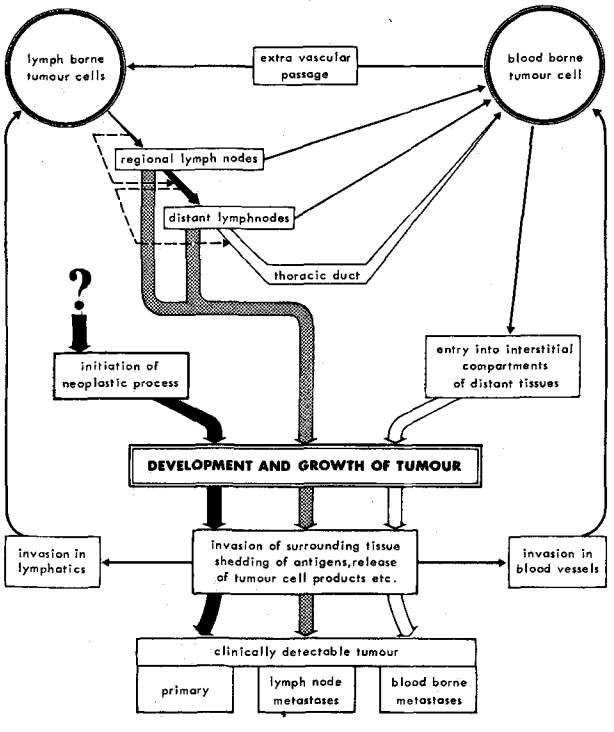

Fig. 3. A diagram of metastasis.

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The clinical and experimental evidence just described indicates that at certain stages in the development of a tumor, the draining lymph nodes respond to it and that these early responses are temporarily inimical to the progressive growth of the tumor. Indeed experimental findings confirm that cellular (T-cell) responses are relevant to the rejection of the tumor[90] and humoral (B-cell) responses may allow enhancement[91]. However, conflicting data exist showing that weakly antigenic tumors can lead to a cell-mediated response which stimulates rather than inhibits tumor growth[92,93]. It is not even quite clear what part of the reactivity of the lymph nodes must be ascribed to such non-specific factors as cell necrosis and inflammation in the tumor.

Usually the lymph node reaction is either ineffective, or whatever effect it has is exerted at a distance and not within the lymph node, because usually a given type of experimental tumor either metastasizes progressively or not at all. There is evidence however that tumor can regress within a lymph node, when its antigenicity is modified by viral infection[94], when immunological mechanisms are stimulated by BCG injection or when the initial dose of a metastasizing tumor given is at the critical border line level necessary for successful metastasis[43,44,23,25].

This background of conflicting information makes it uncertain whether the regional lymph nodes possess properties that make them different from the rest of the lymphoreticular system and whether they should perhaps be preserved rather than removed with a primary tumor. Much experimental work has been carried out on experimental lymph node removal with conflicting results. It seems that lymph node removal reduces survival only in some experimental models and only at an early phase of tumor growth[18,20,95–110]. In man clinical trials comparing simple mastectomy (without regional lymphadenectomy) with radical mastectomy (with regional lymphadenectomy) for early cancer of the breast did not show significant differences in survival[111–119]. Moreover, it is likely that an immune response, even though initiated in the regional nodes, has disseminated throughout the immune system by the time that most patients first present clinically.

It is important in improving the cure rates in treatment of solid tumors to integrate chemotherapy with local surgical excision and radiotherapy, since surgical treatment and radiotherapy are local modalities which eliminate tumor cells only locally. Even when they remove all the tumor visible or detectable, they fail to cure many patients because of presence of disseminated microscopic foci at the time of local therapy. Chemotherapy, when used optimally has the potential for eradicating the metastatic foci of early disease[120]. Many clinical and experimental studies have shown the value of combination chemotherapy along with other types of treatment[121]. The resulting need to determine the best schedules of drug administration in situations free from the logistic and ethical problems of clinical experimentation makes the use of suitable animal tumor models involving lymphatic metastasis of real medical as well as biological interest.

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