Structural, Antigenic, and Biological Characteristics of Precancerous Mastopathy

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Summary

A biologically significant relationship between precancerous mastopathy (PCM), in situ carcinoma (ISC), and invasive breast carcinoma is evidenced by the following: (a) PCM and ISC are commonly associated with each other and with invasive breast cancer; (b) Stage 0 PCM and ISC are both associated with an increased risk of subsequent breast cancer; (c) patients with Stage 0 PCM and ISC are more similar to invasive breast cancer patients than to patients with normotypic breast lesions in regard to family history of breast cancer and parity characteristics; (d) PCM and ISC resemble invasive breast cancers and differ from normotypic mammary epithelium in regard to epithelial cell-lymphoreticuloendothelial relationships; (e) PCM and, particularly, ISC are associated with distinctive immunogenic components; (f) two immunogens of invasive breast cancer tissues are related to and antigenically similar to immunogenic components of associated ISC; (g) immunogenic cancers (ISC and invasive) from different patients are antigenically similar to one another; (h) immunogenic cancers (ISC and invasive) from different patients are antigenically similar to some component of murine mammary tumor virus.

Our understanding and control of invasive breast cancer would be significantly advanced by additional knowledge regarding the development and characteristics of precursor lesions (PCM and ISC) and the ISC-invasive transition. ISC provides a uniquely valuable resource for studies of viral carcinogenesis and prognostically significant immunogenicity. The available data are consistent with the use of ISC-associated immunogen and/or antigenically similar components of murine mammary tumor virus to provoke prognostically favorable hypersensitivity responses, i.e., immunophrophylaxis. Such an experiment in immunoprophylaxis is supported by the unusually favorable stage and survival characteristics of breast cancers arising subsequent to the removal of precursor lesions.

Introduction

Since invasive breast cancers are derived from parenchymal cells within the mammary duct system, there can be no question regarding the existence of preinvasive (in situ) carcinoma. Rather, the questions of conceptual and practical significance are concerned with: (a) recognition of the early phases of intraepithelial malignant transformations, (b) identification of variables favoring and/or deterring the progression from in situ to invasive breast cancer, and (c) applying such information for prevention, early detection, and improved treatment of breast cancer, in situ and invasive. While many pertinent questions cannot as yet be answered in a satisfactory fashion, available data do permit at least partial answers. Equally important, they suggest that further investigations, with available techniques, would significantly increase our understanding and control of breast cancer.

Structural Characteristics

Invasive breast cancers are commonly associated with or preceded by intraductal proliferations which include normotypic hyperplasia and varying degrees of atypia. The most extreme form of the latter change (i.e., ISC2) may manifest all the cytological and histological characteristics of carcinoma except for invasion. It is not uncommon to find transitions from in situ to invasive breast cancer (microinvasion). In fact, it may sometimes be difficult to decide on the presence or absence of microscopic invasion. A relationship also seems to exist between invasive carcinoma and proliferative lesions that show less pronounced atypia than ISC. Thus, a prior history of so-called “fibrocystic disease” seems to be associated with an increased risk of breast cancer (34). This risk is magnified when the precursor lesions exhibit atypical changes, particularly when the reference lesion is ISC (36). However, such “guilt by association” tells us little of the biological relationship between putative precursors and invasive carcinomas.

In 1969, this laboratory initiated an investigation of PCM with particular reference to its biological aspects. An initial requirement was the development of a system for grading the degree and location of mammary duct atypia in a reproducible fashion. Accordingly, a grading system was devised which assigned numbers to the degree of atypia: Grade 1, normal; Grade 2, normotypic hyperplasia;

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1 The abbreviation used are: ISC, in situ carcinoma(s); NG, nuclear grade; PCM, precancerous mastopathy; I/P, invasive/precursor; L-RE, lymphoreticuloendothelial; U, lymphoid cellular infiltration; VPI, perivenous infiltration; SH, sinus histiocytosis; ILA, intraepithelial lymphocyte accumulations; MuMTV, murine mammary tumor virus.
Grade 3, distinct but minimal atypia; Grade 4, atypia suggestive of ISC; and Grade 5, atypia consistent with ISC. Such values are assigned to each of 4 subdivisions of the duct system designated as A, B, C, and D which are defined as follows: A, major subdivisions of interlobular ducts; B, terminal portion of interlobular ducts; C, entering portion of intralobular ducts; and D, terminal intralobular ducts. This system provides for a semiquantitative evaluation of the degree and location of atypical changes in breasts with and without invasive breast cancer.

Breasts containing invasive breast cancer were found to have concomitant foci of duct atypia (Grades 3 to 5), in most instances. These lesions were characteristically located in close proximity to the invasive cancer, multicentric foci being the exception rather than the rule. A biological relationship between Grade 3 to 5 atypia and invasive breast cancer is also suggested by the relationship between age and the location of atypia within the mammary duct system. Thus, the median age of patients with intralobular atypia is the same, regardless of the presence or absence of associated invasive breast cancer, namely 47 years. Similarly, the median age of patients with extralobular (ductal) atypia is the same regardless of the presence or absence of associated invasive breast cancer, namely, 57 years.

It is readily demonstrable that there is a structural continuum in atypical lesions Grades 3 through 5; i.e., foci of Grade 5 atypia are typically associated with Grade 3 and 4 changes. An extension of this continuum to include invasive lesions is suggested by the similarity in the nuclear appearance in concomitant in situ (Grade 5) and invasive lesions. As reported previously, invasive breast cancers may be classified in terms of the degree of differentiation of their nuclei (NG) (11, 19). In contrast to histological patterns, the NG changes. An extension of this continuum to include invasive lesions as well as the primary tumor. Moreover, NG has been shown to be prognostically significant; i.e., the prognosis varies inversely with the NG (23, 26).

The above observations suggest that duct Grades 3 to 5 represent structural manifestations of a biological continuum which is qualitatively different from normotypic hyperplasia but qualitatively similar to invasive breast cancer. In short, Grade 3 to 5 lesions may be grouped together under the designation of PCM or incipient carcinoma (12). The precancerous nature of Grade 5 lesions (in situ carcinoma) is well documented by studies that demonstrate a 10-fold increase in risk of in situ and invasive breast cancers following such lesions (36). In a test of the precancerous potential of Grade 3 and 4 lesions, Black et al. (10) found that such lesions are associated with a 5-fold increase in risk of subsequent breast cancer, in situ and invasive. In contrast, no increased risk is associated with normotypic hyperplastic lesions.

The ability to identify structurally distinct lesions that have a statistically defined precursor relationship to invasive breast cancer allows for a more detailed comparison of their biological and chemical characteristics. It also makes possible investigations of the factors involved in the precursor-to-invasive progression.

**Clinical Characteristics**

Breast cancer arises as a nonrandom event which is correlated with age, family history of breast cancer, and parity characteristics (3, 27, 35, 40). It is therefore of interest to examine the association of such risk factors with different types of breast lesions derived from the same source (Flower and Fifth Avenue Hospitals, New York, N. Y.) over the same time period (January 1970 to August 1975). Table 1 presents the data regarding the relative frequency of family history of breast cancer, nulliparity, and late 1st parity (28+ years) by diagnostic category and age.

**Family History of Breast Cancer.** Only 13% of patients with normotypic breast lesions had a family history of breast cancer. Furthermore, the frequency of such a family history was the same in patients 30 to 44 years of age and in older patients. In contrast, 33% of invasive breast cancer patients 30 to 44 years of age had a family history of breast cancer. Among the older invasive breast cancer patients, the family

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**Table 1**

**Clinical characteristics of patients with different types of breast lesions**

<table>
<thead>
<tr>
<th>Invasive</th>
<th>In situ carcinoma</th>
<th>PCM</th>
<th>Normotypic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Family history</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30-44 yr</td>
<td>28/86 (33)</td>
<td>8/15 (54)</td>
<td>7/24 (29)</td>
</tr>
<tr>
<td>45+ yr</td>
<td>59/335 (18)</td>
<td>10/29 (35)</td>
<td>2/32 (6)</td>
</tr>
<tr>
<td>Total</td>
<td>87/422 (23)</td>
<td>18/44 (41)</td>
<td>9/56 (16)</td>
</tr>
<tr>
<td>Nulliparous</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30-44 yr</td>
<td>19/86 (22)</td>
<td>3/15 (20)</td>
<td>5/24 (21)</td>
</tr>
<tr>
<td>45+ yr</td>
<td>114/355 (34)</td>
<td>10/29 (34)</td>
<td>10/32 (31)</td>
</tr>
<tr>
<td>Total</td>
<td>133/442 (31)</td>
<td>13/44 (30)</td>
<td>15/56 (27)</td>
</tr>
</tbody>
</table>

| P28+/TP | |
|----------|-----|-----|-----|
| 30-44 yr | 6/54 (11) | 3/11 (27) | 2/18 (11) | 45/240 (19) |
| 45+ yr | 66/183 (36) | 6/19 (32) | 6/20 (30) | 49/207 (24) |
| Total | 72/237 (30) | 9/30 (30) | 8/28 (29) | 94/447 (21) |

* Numbers in parentheses, percentages.
history of breast cancer was similar to that in the normotypic series. The family history of breast cancer in ISC and PCM patients resembles that of the invasive breast cancer patients in regard to the relatively high frequency in young patients and a decreased frequency in older patients. These findings suggest that "familial factors" influence the developmental phase of the disease in young women but lack any selective effect on the in situ-to-invasive progression in young or old patients.

**Nulliparity.** Although nulliparity is considered to be a breast cancer risk factor, the overall percentage of nulliparity is remarkably similar in each of the diagnostic categories. Among young breast cancer patients, the percentage of nulliparity was less than among young women with normotypic lesions, i.e., 22 and 30%, respectively. In contrast, older breast cancer patients had a greater percentage of nulliparity than did older patients with normotypic lesions, i.e., 34 and 25%, respectively. In each age group, patients with ISC and PCM resembled the cancer patients in regard to the percentage of nulliparity. It appears that the influence of nulliparity on mammary carcinogenesis is age dependent and operable in the developmental phase of the disease.

**Late First Parity.** The overall percentage of parous patients whose first parity occurred at 28+ years of age was approximately 30% in the invasive cancer, ISC, and PCM series and 21% in the normotypic series. As with the percentage of nulliparity, late first parity was proportionately more frequent in the older patients with invasive, ISC, and PCM lesions than in the younger women. Among older women in each of these diagnostic categories, the percentage of late first parity was greater than in women with normotypic breast lesions, namely, 30 to 36% in the former and 24% in the latter. It appears that the influence of late first parity on mammary carcinogenesis is exerted on the developmental phase of the disease.

Considered in toto, the clinical characteristics of patients with PCM are similar to patients with ISC and both are more similar to invasive breast cancer patients than to patients with normotypic breast lesions. It further appears that the risk potential associated with a family history of breast cancer, nulliparity, and late first parity is age dependent and related to the development of precursor lesions rather than the in situ-to-invasive progression. While the relative frequency of risk factors is similar among patients with PCM, ISC, and invasive breast cancer tissue, there is a difference between the incidence of invasive and precursor lesions by age. As shown in Table 2, increasing age is associated with a proportionately greater increase in invasive lesions as compared with precursor (ISC and PCM) lesions. Thus, the I/P ratio is 5.5 in patients 45+ years of age, as compared with a ratio of 2.2 in patients 30 to 44 years of age. Similar values are maintained in patients with a family history of breast cancer, in nulliparous patients, and in patients with late first parity. It thus appears that these risk factors do not account for the preferential age-related increase in invasive breast cancer.

**L-RE Responses**

Reports from this and other laboratories have described a variety of L-RE responses to invasive breast cancer (1, 4, 5, 20, 22, 30). Such responses are seen in the primary tumor and in the regional lymph nodes. In the primary tumor, L-RE responses include diffuse lymphoid cellular infiltrations (LI) and PVI. LI is characterized by prominent infiltrations of varying sized lymphocytes and plasma cells among the invasive cancer cells and/or at its peripheral margin. While the prototype of this type of response is seen in medullary carcinomas with lymphoid infiltrate, it is by no means limited to cancers having a medullary growth pattern. However, LI is essentially limited to breast cancers having poorly differentiated nuclei (NG I). PVI is characterized by accumulations of small and medium-sized lymphocytes around venules draining cancerous foci. In contrast to LI, this reaction may be observed in association with breast cancers without regard to their NG.

Lymph nodes draining invasive breast carcinoma exhibit a variety of reactive and degenerative changes. Of particular interest is a pattern of response characterized by paracortical pulp hyperplasia and distended sinusoids filled with large histiocytes having a granular eosinophilic cytoplasm. This pattern, designated as SH, is correlated with PVI responses in the primary tumor. In contrast, LI responses in the primary tumor are commonly associated with follicular hyperplasia in the regional lymph nodes.

The structural characteristics of the above L-RE responses are consistent with cell-mediated immunity to invasive cancer (7, 8, 21). This conclusion is supported by direct immunological measurements (15-17). In short, a significant proportion of invasive breast cancers possess antigens that are lacking in the normotypic breast tissues. Moreover, such antigens provoke prognostically favorable cell-mediated immunity responses. It is therefore of interest to determine whether similar antigenic changes are demonstrable in noninvasive precursor lesions. However, before considering reactive changes, it is appropriate to comment on the diminution in a normal lymphocyte-epi-
Precancerous Mastopathy

Lymphocytes are regularly seen within a variety of epithelial structures, e.g., intestine, skin, Fallopian tubes, and gallbladder, a phenomenon that has been termed emperipolesis (33). Black and Chabon (12) noted that such ILA are regularly seen in normal and hyperplastic mammary duct epithelium but are characteristically decreased or absent in foci of precancerous atypia. While the exact biological significance of ILA is still unknown, its diminution in precancerous lesions suggests that changes in lymphocyte-epithelial cell relationships are concomitant with the earliest stages of carcinogenesis. Diminished ILA in foci of PCM and ISC is not associated with similar changes in immediately adjacent normotypic mammary parenchyma. It would therefore be inappropriate to equate diminished ILA with diminished immunological surveillance of either a systemic or an organ-specific type.

In contrast to the reduction in ILA in PCM and ISC, such lesions are commonly associated with extraductal accumulations of L-RE cells. The cellular responses include mast cells, LI, and PVI. In addition, the draining axillary lymph nodes may show distinctive types of reactive and degenerative changes.

**Mast Cell Responses.** Mast cells are readily demonstrable in cryostat sections of freshly removed breast tissues after staining with polychrome methylene blue. In such preparations, mast cells are regularly found scattered in the stroma of benign breast tissues of control and cancerous breasts. In cancerous breasts, however, hypertrophied mast cells are commonly found in close proximity to areas of ISC and atypical ducts (7). Increased numbers of mast cells are also commonly found in the axillary lymph nodes of breast cancer patients, particularly in association with SH. However, mast cells are not commonly seen in proximity to invasive breast cancers. Simpson (38) reported that mast cells accumulated in the region of developing, carcinogen-induced skin carcinomas but disappeared with the advent of invasive carcinomas. Our lack of knowledge regarding the exact function of mast cells limits a specific interpretation of the above findings. Nevertheless, these observations, like the changes in ILA, do indicate that the earliest phase of human mammary carcinogenesis is associated with alterations in epithelial cell-L-RE cell relationships and suggest a biological continuity between duct Grade 3 to 5 lesions.

**Lymphoid Cellular Responses.** In situ breast carcinomas (duct Grade 5) without associated invasive breast carcinomas commonly [37 of 45 (82%)] show local accumulations of lymphoid cells in the form of LI and/or PVI responses (6). LI responses are seen as accumulations of plasma cells as well as large and small lymphocytes surrounding the involved ducts. As with invasive breast cancers, LI responses to in situ breast cancers are almost exclusively associated with lesions having anaplastic-appearing nuclei (NG I). PVI responses are characterized by the accumulation of small lymphocytes around venules in close proximity and at some distance away from the involved ducts. As with invasive breast cancers, such responses are not limited to ISC that have a particular NG. Moreover, PVI responses may also be seen in association with Grade 3 and 4 lesions, namely, 15 of 64 (23%) of such lesions unassociated with invasive breast cancer. In contrast, PVI and/or LI responses are essentially nonexistent to normotypic hyperplasias.

**Lymph Node Responses.** As reported by Black and Chabon (12, 13) lymph nodes draining in situ breast cancers show SH responses in approximately 70% of the cases, a level that exceeds that found among patients with invasive breast cancer. The lymph node studies, as well as the local L-RE responses to Stage 0, Grade 3 to 5 lesions suggest that they are antigenically different from normotypic mammary duct hyperplasia and control duct epithelium. Since LI, PVI, and SH are prognostically favorable responses, it follows that prognostically significant immunogenicity is an early consequence of mammary carcinogenesis.

While the qualitative similarity in L-RE responses to in situ and invasive carcinomas suggests that both types of lesion possess immunogens, this does not, per se, demonstrate that the immunogens of in situ and invasive breast cancers are necessarily the same.

**In Situ-Invasive Relationship**

**Immunogenicity**

Information regarding the relationship between immunogenicity of in situ and invasive breast cancers may be obtained by examining breasts having both types of lesions. In a consecutive series of 250 invasive breast cancers treated by mastectomy and axillary dissection, 175 (70%) had foci of ISC. As shown in Table 3, L-RE responses were found associated with 106 (61%) of the 175 foci of ISC. This level of response is lower than that against ISC that were not associated with invasive breast cancer (94%) but is greater than...
that against associated invasive breast cancers, i.e., 102 (41%) of 250 cases. Table 3 also demonstrates that local L-RE responses to in situ and invasive breast cancers are more frequent in those lesions having anaplastic nuclei (NG I), compared with analogous lesions having more differentiated nuclei (NG II and NG III). As noted above, L-RE responses to NG I nuclei include LI as well as PVI responses. The former type of response is rarely associated with NG II or NG III lesions, in situ or invasive. Qualitative differences between the LI and PVI responses are also manifest in regard to associated changes in the axillary lymph nodes; PVI is commonly associated with SH in the lymph nodes while LI is associated with follicular hyperplasia (7). As shown in Table 3, the percentage of invasive breast cancer patients having SH-positive lymph nodes is not correlated with the nuclear grade.

Further data regarding in situ-related immunogenicity in invasive breast cancer cases are shown in Table 4. It is evident that breast cancers lacking L-RE responses to foci of in situ carcinoma are characterized by a low level of response to associated invasive breast cancer. In contrast, breast cancers with L-RE responses to in situ foci have an increased level of local response to associated invasive breast cancer in each of the NG groups. However, the NG I cases maintain their distinctive immunogenicity, compared with NG II and NG III invasive breast cancers. While SH is also increased among the in situ-positive groups, the level is essentially similar in each NG.

It appears from the data of Table 4 that the immunogens of invasive breast cancers are antigenically similar to the immunogens of ISC in the same breast. The decreasing proportion of L-RE responses in the sequence in situ, Stage 0 > in situ, Stage I+ > invasive cancer, might be due to loss of antigenicity, loss of specific hypersensitivity, circulating blocking factors, or a combination of such variables. The finding that approximately 20% of NG I invasive breast cancers and 50% of NG II and NG III invasive breast cancers are L-RE-negative despite simultaneous L-RE responses to in situ foci indicates that a loss of immunogenicity occurs as an independent variable in the course of progressive disease.

The biological significance of NG and L-RE responses to invasive breast cancer is exemplified by the data in Table 5. These data are consistent with previous studies that demonstrated that postoperative survival is a function of the aggressive potential of the cancer, correlated with NG and the tumor-retarding influence of the host, manifest in L-RE responses. More specifically, the tumor-retarding responses of the host are dependent on the retention of in situ-associated immunogens and specific hypersensitivity to such immunogens. It follows that the progressive characteristics of breast cancer are associated with, or due to, a loss of in situ-associated immunogenicity and/or loss of specific hypersensitivity.

**Immunological Measurements**

Further insight into the relationship suggested by the structural studies are provided by direct immunological measurements using in vivo and in vitro procedures.

**Autologous Breast Cancer Tissue.** The use of cryostat sections of autologous breast cancer tissue to test skin hypersensitivity responses has been described previously (15, 16). Of particular pertinence to this report is the finding that approximately 70% of autologous Stage 0, in situ cancer tissues provoked a positive skin window response (6). In contrast, such responses were uncommon (<25%) when L-RE-negative, Stage II autologous breast cancer tissues were
used as the target. Similar stage-response relationships are found when an in vitro leukocyte migration technique is used as an index of hypersensitivity responses to cryostat sections of autologous breast cancer tissues (Table 6), namely, in situ cancer tissue (Stage 0) = in situ cancer tissue (Stage I) > in situ cancer tissue (Stage II) = invasive cancer (Stage I) = invasive cancer (Stage II) (17). Such differences suggest a stage-related decrease in antigenicity and/or specific hypersensitivity. A more direct evaluation of differences in antigenicity between in situ and invasive breast cancer tissues is provided by simultaneous measurements of the response of autologous leukocytes to in situ and invasive foci from the same breast. As shown in Table 7, positive responses were found in 8 (53%) of 15 tests against the in situ targets in contrast to 5 (33%) positive tests against the invasive targets. Of particular interest is the finding that, in 8 instances in which the in situ tissue was “seen” by the autologous leukocytes, the invasive cancer tissue from the same breast was not seen in 4 cases. The latter observation provides in vitro confirmation of the microscopic studies which suggested that immunogenicity varies, independent of specific hypersensitivity, and that decreased immunogenicity is a concomitant of the in situ-invasive progression.

The above studies support the immunological basis of L-RE responses to breast cancer tissues and the particular immunogenicity of Stage 0, in situ carcinomas. Each procedure demonstrates qualitative similarities between the antigenic properties of in situ and invasive breast cancers and each indicates that prognostically significant immunogenicity and specific hypersensitivity are most regularly demonstrable in Stage 0 breast cancer patients. However, these observations do not provide direct evidence that in situ-associated immunogens are the same in different patients. If such immunogens were “private” and dissimilar in different patients, the prospects for immunoprophylaxis would be poor indeed. On the other hand, if similar immunogens were shared by most, if not all, in situ breast cancers, then immunoprophylaxis would be feasible. Information bearing on this biologically important point may be obtained by simultaneous tests of the antigenic characteristics of different breast cancer tissues, namely, hypersensitivity responses to homologous in situ breast cancer tissues in relation to responses to autologous breast cancer tissues.

### Table 6

<table>
<thead>
<tr>
<th>Target</th>
<th>Stage of disease</th>
<th>No.</th>
<th>MI &lt; 0.80*</th>
</tr>
</thead>
<tbody>
<tr>
<td>In situ carcinoma</td>
<td>0</td>
<td>27</td>
<td>18 (67)</td>
</tr>
<tr>
<td></td>
<td>I</td>
<td>9</td>
<td>6 (67)</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>11</td>
<td>4 (36)</td>
</tr>
<tr>
<td>Invasive carcinoma</td>
<td>I</td>
<td>110</td>
<td>46 (42)</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>97</td>
<td>32 (33)</td>
</tr>
</tbody>
</table>

* Migration index (MI) < 0.80 = positive response.
* Numbers in parentheses, percentages.

### Homologous Breast Cancer Tissue

Other laboratories have reported that similar antigens may be present in some extracts of breast cancer tissues from different patients (2, 28, 31, 32). Unfortunately, such studies have ignored the clinicopathological characteristics of the tissues providing the antigen and the patients providing the leukocytes. In our studies, leukocytes were derived from patients who were specifically characterized as to such variables as age, stage of disease, and microscopic classification of tumor and L-RE responses. Such leukocytes were tested against cryostat sections of microscopically defined breast tissues from clinically defined patients. These studies demonstrated that breast cancer patients’ leukocytes were more regularly cross-reactive to in situ as compared with invasive homologous breast cancer tissues (18, 25). It was also found that leukocytes that are responsive to L-RE-positive autologous breast cancer tissues are more regularly responsive to L-RE-positive homologous as compared with L-RE-negative homologous breast cancer tissues (24). These findings suggest that L-RE-positive breast cancer tissues, in general, and in situ breast cancer tissues, in particular, commonly possess components having similar antigenic characteristics.

Immunogenic breast cancer tissues are not only antigenically similar to one another, they are also antigenically similar to some component(s) of MuMTV (18, 24, 25). As shown in Table 8, breast cancer patients’ leukocytes that are responsive to MuMTV-containing RIII mouse milk are clearly more responsive to homologous in situ breast cancer tissues than RIII-negative leukocytes, i.e., 61 and 17%, respectively. Similarly, breast cancer patients’ leukocytes that are responsive to autologous breast cancer tissues cross-react with homologous in situ breast cancer tissues approximately 3 times as frequently as autologous-negative leukocytes, i.e., 57 and 20%, respectively. Of particular significance is the finding that those breast cancer patients’ leukocytes that are simultaneously responsive to both autologous breast cancer tissue and RIII milk cross-react with homologous in situ breast cancer tissues as frequently as leukocytes responsive to only one of these reference targets. These data provide strong support for the thesis that in situ breast cancer tissues from Stage 0 and Stage I+ cases possess components that are antigenically similar to one another and to prognostically significant immunogens.
Precursor-Invasive Relationship

It is well known that, after age 30 years, the average annual incidence rate of breast cancer rises rapidly with increasing age. In most Western countries this increase continues through the postmenopausal years, while in Eastern countries such as Japan the incidence rate may plateau or decrease during the postmenopausal period. Table 9 demonstrates that the number of primary invasive breast cancers increases progressively with increasing age. In contrast, the number of both types of precursor lesion is similar in each of the age categories. Only one-third of the precursor lesions are found after age 54 years. In contrast, 50% of invasive breast cancers are found after this age. Thus the I/P ratio increases progressively with increasing age. It would be of clinical and conceptual importance to determine how age acts as a preferential risk factor on the incidence of invasive breast cancer.

The age-related differences in incidence of invasive and precursor lesions might have one or more explanations: (a) precursor and invasive breast carcinomas are the result of different etiological factors, and their structural resemblance and frequent association are only coincidental; (b) invasive breast cancers do occur in fact arise from Grade 3 to 5 precursor lesions, but the biological progression depends on secondary promoting factor(s); (c) the biological progression from precursor lesion to invasive cancer is impeded by mechanisms (immunological?) which become less efficient with advancing age; and (d) there exists a combination of promoting factors and diminished efficiency of impeding mechanisms.

The antigenic and anatomic similarities, as well as the clinical association between precursor and invasive lesions, suggest that they are etiologically related and representative of different phases in a step-wise progression. This interpretation is supported further by the finding that risk factors (family history of breast cancer, nulliparity, and late first parity) show a similar association with invasive breast cancer and precursor lesions. While the latter point suggests that precursor lesions and invasive breast cancer are etiologically related, it provides no explanation for the age-related differences in incidence.

As shown above, immunogenicity and specific hypersensitivity are correlated with the biological behavior of breast cancer. It is therefore of interest to determine whether age-related changes in L-RE responses might be correlated with age-related increases in invasive breast cancer. As seen in Table 10, L-RE responses to invasive breast cancer do show a diminution with increasing age, e.g., <45 years, 52%; 45 to 54 years, 41%; 55+ years, 30%. An age-related decrease in SH is also evident. However, minimal SH values are approached in the 45 to 54 age group.

Table 10 also demonstrates that the number of patients having both L-RE responses in their primary tumor and SH responses in their lymph nodes was not influenced by age. In contrast, the number of L-RE-negative, SH-negative invasive breast cancers increased linearly with increasing age. The relative constancy in the incidence of L-RE-positive, SH-positive invasive breast cancers in the different age groups is reminiscent of the minimal effect of advancing age on incidence rate of breast cancer in Japanese women. It is pertinent that Japanese breast cancer patients have an unusually high frequency of ISC's. Moreover, their invasive breast cancers are associated with an unusually high frequency of L-RE responses locally and SH responses in their lymph nodes (29, 37).

Since the immunogenicity of invasive breast cancer seems to reflect the retention of in situ-associated immunogens, it is of interest that L-RE responses to in situ foci in breasts with invasive cancer are also influenced by age. As shown in Chart 1, the age-related decrease in percentage precursor lesions-precursor plus invasive breast cancer is paralleled by an age-related decrease in L-RE responses to invasive breast cancer and to in situ foci in breasts with...
Table 10
L-RE and SH responses to invasive breast cancer in relation to age

Prognostically significant L-RE and SH responses are proportionately less frequent with advancing age. While the incidence of L-RE-positive, SH-positive lesions remains similar, the incidence of L-RE-negative, SH-negative lesions increases sharply with advancing age.

<table>
<thead>
<tr>
<th>Age (yr)</th>
<th>L-RE+</th>
<th>SH-negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;45</td>
<td>20</td>
<td>8</td>
<td>29 (52)</td>
</tr>
<tr>
<td>45-54</td>
<td>18</td>
<td>15</td>
<td>35 (41)</td>
</tr>
<tr>
<td>55+</td>
<td>21</td>
<td>18</td>
<td>44 (30)</td>
</tr>
</tbody>
</table>

* Numbers in parentheses, percentages.

Chart 1. Relative frequency of L-RE responses to foci of ISC (Stage I+) and invasive breast cancers and proportion of precursor lesions among precursor + invasive breast cancers; variations with age. pos., positive.

invasive cancer. It appears that age-related changes in in situ-associated immunogenicity and specific hypersensitivity play a role in the in situ-invasive progression.

Comments

Structural, clinical, and immunological studies singly and collectively indicate that a biological continuity exists between intraepithelial Grade 3 to 5 lesions and invasive breast cancer. It further appears that the progression from in situ to invasive breast cancer, like the biological behavior of invasive breast cancer, is a function of specific characteristics of tumor and host. In both types of lesions, aggressive potential is correlated with NG, while the tumor-retarding potential of the host is dependent upon the presence of specific immunogen(s) in the cancer cells and specific immunological responsiveness of the host L-RE system. Of particular pertinence to the ultimate development of immunoprophylaxis and immunodiagnosis are data that indicate that prognostically significant in situ-associated immunogens are antigenically similar in different patients. Recent studies suggest that such immunogenicity is correlated with the presence of protein components having a molecular weight of approximately 50,000 and distinctive migration characteristics in polyacrylamide gel electrophoresis (24). Isolated in situ-associated immunogens might constitute a valuable reagent for the diagnosis and monitoring of breast cancer. Equally important, it should possess immunoprophylactic potential.

If, as seems to be the case, mammary carcinogenesis involves an immunogenic precursor phase that is antigenically similar in different patients, then the minimal requisites for immunoprophylaxis are attainable. Patients having hypersensitivity responses to such immunogens should exert biologically significant cross-reactivity against developing breast carcinomas. A natural test of this expectation is provided by breast cancers arising in women who have prior removal of precursor lesions. As shown by Black et al. (14), postprecursor breast cancers are in fact superior in stage and survival to breast cancers arising after prior removal of normotypic benign breast lesions or unselected invasive breast cancers. It would seem to be of practical as well as conceptual importance to test the ability of isolated in situ-associated immunogens to provoke specific hypersensitivity responses in control individuals and the influence of such induced hypersensitivity on the development and behavior of breast cancer.

The finding that prognostically significant, in situ-associated immunogens are antigenically similar to some component(s) of MuMTV suggests that MuMTV might provide a source of immunodiagnostic and/or immunoprophylactic reagents. This possibility is further supported by the finding that the distinctive protein found in immunogenic breast cancer tissues possesses physicochemical similarities to the gp52 protein of MuMTV (9). It should also be noted that molecular hybridization studies have demonstrated MuMTV-homologous RNA in intralobular carcinomas (39).

Individually and collectively, the structural, clinical, immunological, and physicochemical data suggest that in situ breast cancers provide a uniquely favorable resource for studies of the development of mammary carcinoma and the biological basis of its clinical behavior. The more general point to be emphasized is the need to relate analytic procedures to the clinicopathological characteristics of the target tissue.

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2603

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