In Vivo Nonspecific Macrophage Chemotaxis in Cancer Patients and Its Correlation with Extent of Disease, Regional Lymph Node Status, and Disease-free Survival

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ABSTRACT

We have used a test to evaluate macrophage migration in vivo, derived from the technique of Rebucket. The test is based on counting the number of macrophages that migrate into an inflammatory site determined by a standardized superficial skin abrasion. It was applied to the study of macrophage migration in cancer patients in different clinical situations.

Macrophage migration was virtually abolished in patients with metastatic cancer as compared to healthy controls. In patients with resectable breast and lung tumors, the test performed preoperatively correlated closely with lymph node status as determined by pathological examination after operation. Patients without lymph node involvement showed a significantly stronger response than did controls, whereas those with lymph node involvement had a diminished or even an abolished response. Distinctive subgroups were characterized among patients both with and without lymph node involvement on the basis of their macrophage response, and these subgroups proved to have distinctly different prognoses, particularly the patients without lymph node involvement with a poor macrophage response who had an unusually poor prognosis. It is concluded that this test shows potential for predicting the prognosis among categories of patients hitherto considered as homogeneous, although further evaluation in larger numbers of patients is necessary.

INTRODUCTION

It has long been known that the status of the regional lymph nodes is fundamental in determining the prognosis of all resectable epithelial tumors. Indeed, disease-free postoperative survival is far longer in patients whose regional lymph nodes are not involved by the malignant process (designated N−) than in those who have involved nodes (designated N+). This has been particularly well documented in patients with breast cancer (4) and lung cancer (17) who constitute the patient series of patients, we have chosen to study in vivo macrophage chemotaxis to a nonspecific stimulus, using a modification of the technique proposed by Rebucket and Crowley (20) for studying neutrophil polymorphonuclear leukocytes and since used by others (3, 5).

METHODS

Skin Window Test. A hairless area of the forearm was cleansed thoroughly with ether. The skin was abraded superficially by means of a small battery-operated drill fitted with a spherical bit 2 mm in diameter. The eroded area corresponded to the projection of the spherical bit on the skin, i.e., 2 sq mm. Abrasion was continued until micropoints appeared, corresponding to capillaries in the papillary layer of the dermis, but overt bleeding was not allowed to occur. In this case, the procedure was recommenced. The abraded area was then wiped clean with a sterile gauze dressing to remove desquamated cells.

A sterile glass coverslip (No. 1) was placed over the abraded area. The coverslip was numbered and marked previously with a circle delineating the abraded area. The coverslip was covered with a gauze dressing and held in position by adhesive tape. Three hr later, coverslip 1 was removed, air dried, allowed to dry overnight, and fixed to a glass slide by means of Scotch glue in one corner with the cell-bearing surface uppermost. Immediately upon removal of Coverslip 1, a fresh coverslip (No. 2) was placed in position as above. Three hr after placing Coverslip 2 in contact with the abraded skin (i.e., 6 hr after the start of the test), it was removed, treated as above, and immediately replaced by Coverslip 3, which was replaced by Coverslip 4 6 hr later. Coverslip 4 was removed 12 hr later, i.e., 24 hr after the start of the test. This marked the end of the test. All material that came into contact with skin was sterilized (drill bit, coverslip, gauze dressings).

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Examination of Slides and Analysis. After a series of preliminary experiments (22), it was found that the early specimens (Coverslips 1 and 2) contained few macrophages and many polymorphonuclear leukocytes, the number of which did not correlate with the extent of disease or lymph node status, and hence their study was abandoned. The 12-hr specimen (Coverslip 3) contained both neutrophils and macrophages in approximately equal numbers, and analysis revealed that macrophage numbers did correlate with the extent of disease and lymph node status, but the study of this specimen provided no additional information beyond that derived from Coverslip 4 (24-hr specimen) where macrophages were largely predominant (Fig. 1). Consequently, in the present study, only the absolute number of macrophages migrating at 24 hr (Coverslip 4) was considered and will be referred to henceforth as the "macrophage response."

The coverslips were stained with May-Grunwald-Giemsa, which permitted easy identification of macrophages (Figs. 1 and 2) and their distinction from the few polymorphonuclear leukocytes present. The total number of macrophages on the coverslip was counted using a ×40 objective and a ×10 eyepiece (Fig. 2). Although only Coverslip 4 was examined, coverslips continued to be changed at 3, 6, 12, and 24 hr as described above for 2 reasons: to eliminate the majority of neutrophils that migrate essentially during the first 12 hr; and to ensure that the test was performed in comparable fashion in all patients. Compared to the technique described by Rebuck and Crowley, and also used by Dizon and Southam (3), our technique included 3 essential changes.

To facilitate counting, we used a smaller abrasion area. To reduce variability in the size and depth of abrasion, we used a miniature drill with an abrasive bit. The area always corresponded to the projection of the drill bit on the skin, and depth was defined by the appearance of capillaries in the papillary layer rather than by overt capillary hemorrhage. The technique was highly reproducible in our hands.

Skin abrasion alone was used as the stimulus, thus simplifying the coverslip changing procedure and making the test feasible on an outpatient basis. Finally, the skin abrasion was performed by 2 nurses, and the results were analyzed for comparability. No significant difference was found. The coverslips were always read by the same technician who had no knowledge of the clinical status of the patients, and random samples were periodically checked by R. Samak.

Patient Population. The skin window test was performed in 35 healthy controls, aged 20 to 60 years, 50 untreated patients with various metastatic carcinomas, and 151 patients with localized operable lung and breast tumors. In this group of patients with operable tumors, the test was performed before surgery and 4 weeks after surgery. Pathological examination of regional lymph nodes permitted patients to be divided into 2 groups, those with and those without lymph node involvement (N+ and N−, respectively). There were 106 patients with non-oat cell bronchogenic carcinoma (65 N+ and 41 N−; 80 squamous cell, 16 adenocarcinoma, 9 mixed pattern, and one bronchioloalveolar) and 45 with breast cancer (29 N+ and 16 N−). Patients and controls were tested over the same period of time.

RESULTS

Reproducibility of the Macrophage Response

Potential variability in the area and depth of abrasion and possible inaccuracy in counting large numbers of cells may have been suspected of causing nonreproducibility of the test. To ensure that the test was reproducible, it was performed twice at 4-week intervals in 10 patients with various slow-growing tumors who were receiving no treatment during this period. Chart 1 shows the results of this study and illustrates that the macrophage response was highly reproducible.

Correlation between Macrophage Response and Extent of Disease

Healthy controls had a mean macrophage response of 3112 ± 864 (S.D.). Patients with metastatic cancer had a significantly lower response (Table 1).

Patients with localized operable tumors were tested preoperatively, and the results were analyzed according to the presence or absence of regional lymph node involvement as determined by pathological examination after operation. Both lung and breast cancer patients with uninvolved lymph nodes (N−) did not, as may have been expected, have a similar macrophage response to that of the control population but a significantly stronger response, whereas patients with involved nodes (N+) had a significantly weaker response than did controls (Table 1). There was no difference in macrophage response between lung cancer N− and breast cancer N−, nor between lung cancer N+ and breast cancer N+.

Chart 2 shows the individual values for N− and N+ lung and breast cancer patients. As can be seen from this chart, a minority of N+ patients had a "good" response, and a minority of N− patients had a "poor" response, the limit being defined as the mean value for controls minus 1 S.D. (approximately 2000 macrophages at 24 hr). These categories, henceforth referred to as good N+ and poor N−, were subsequently the object of distinct analyses with regard to disease-free survival data.

[Table 1]

<table>
<thead>
<tr>
<th>Disease</th>
<th>N−</th>
<th>N+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy controls</td>
<td>3112 ± 864</td>
<td>6422 ± 2112</td>
</tr>
<tr>
<td>Metastatic</td>
<td>841 ± 322</td>
<td>1820 ± 931</td>
</tr>
<tr>
<td>Lung N−</td>
<td>6784 ± 2112</td>
<td>1914 ± 893</td>
</tr>
<tr>
<td>Breast N−</td>
<td>6240 ± 1901</td>
<td>1820 ± 931</td>
</tr>
<tr>
<td>All N− combined</td>
<td>6422 ± 1851</td>
<td>1820 ± 931</td>
</tr>
<tr>
<td>Lung N−</td>
<td>6422 ± 1851</td>
<td>1820 ± 931</td>
</tr>
<tr>
<td>Breast N−</td>
<td>1914 ± 893</td>
<td>1914 ± 893</td>
</tr>
<tr>
<td>All N+ combined</td>
<td>1891 ± 1012</td>
<td>1891 ± 1012</td>
</tr>
</tbody>
</table>

Significance vs. controls (p)

- <0.01
- <0.001
- NS, not significant

Mean ± S.D.

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Correlation between Preoperative Macrophage Response and Disease-free Survival

Among the 94 Patients with Involved Lymph Nodes. Of the N+ patients, 17 had an adequate macrophage response comparable to the control population or even the N— patients (good N+ group). These 17 patients consisted of 8 with breast cancer and 9 with bronchogenic cancer. It is interesting to note that all 8 breast cancer patients had only one involved node. Disease-free postoperative survival in these breast cancer patients ranged from 2+ to 39+ months. Similarly, of the 9 good N+ lung cancer patients, 6 had only one involved lymph node. Disease-free postoperative survival for these patients was significantly better than for poor N+ patients (p < 0.01, log rank test) (Chart 3).

All the N+ breast cancer patients were treated postoperatively by a combination of melphalan and 5-fluorouracil and randomly assigned to receive Corynebacterium parvum or not (National Surgical Adjuvant Breast Protocol B 10).

Lung cancer patients with involved nodes were treated by actinomycin D, Adriamycin, and methotrexate until February 1980 and by a combination of bleomycin and cis-platinum since that date.

Among the 57 Patients without Lymph Node Involvement. Of the N— patients, 12 had a macrophage migration pattern similar to that of patients with metastatic disease (poor N— group). These 12 patients included 2 breast cancer patients currently surviving disease free for 9+ and 22+ months and 10 lung cancer patients, 9 of whom died of metastases between 2 and 16 months after surgery. One patient experienced recurrence after 3 months but is still alive. All 45 good N— patients (both lung and breast) are currently surviving disease free from 3+ to 43+ months. All the N— patients were followed postoperatively without treatment.

Charts 3 and 4 show the projected actuarial disease-free interval in the 4 subgroups individualized by this analysis for lung cancer and breast cancer patients, respectively.

DISCUSSION

The results of this study confirm the nonspecific deficit of macrophage chemotaxis in patients with advanced cancer, demonstrated previously by several authors (6, 15, 22). In patients with resectable non-oat cell lung tumors and breast tumors, there is a close correlation between preoperative macrophage response and lymph node status determined postoperatively. Patients with lymph node involvement have a depressed response compared to controls.

The deficit is partially or totally reversible when the tumor is removed and therefore appears to be related to either products secreted by the tumor or substances synthesized by the host in response to the presence of the tumor as suggested by a number of studies (12–14). We did not observe any difference in macrophage response between breast cancer and lung cancer patients for a given node status, suggesting that the correlation may be a general one. This will have to be confirmed by studying other tumors.

Table 2 shows that, after removal of the primary tumor and regional nodes, the values for all groups tended to come together at a level above control values with one exception, that of the poor N— group, for which values remained very low. It is interesting to note that the poor N+ patients improved their score to above control values. Since there was no difference in preoperative values for breast and lung cancer patients of given node status (N+ or N—), these 2 sites were analyzed together for postoperative values.

Macrophage Response 4 Weeks Postoperatively

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Macrophage migration assayed by the skin window test appears to be able to single out a subgroup of patients who, despite the absence of lymph node involvement, have a poor prognosis. Indeed, of 10 lung cancer patients without lymph node involvement but with a poor macrophage response (poor N−), 9 died of metastases between 2 and 16 months after surgery, and one patient had recurrent disease in the chest 3 months after surgery but is still alive. The 2 poor N− breast cancer patients are currently disease free 9+ and 22+ months after surgery.

Among the 31 good N− lung patients, all are currently surviving disease free from 3+ to 42+ months after surgery. All 14 good N− breast cancer patients are currently disease free 9+ and 22+ months after surgery.

References

2. Di Luzio, N. R., McNamee, R., Olcay, I., Kitahama, A., and Miller, R. M.


Fig. 1. Cells migrating onto a coverslip over an abraded area (skin window) between 12 and 24 hr after the abrasion was produced. Macrophages (m) are easily distinguishable from polymorphonuclear leukocytes (pmn) without specific staining. Note that the vast majority of cells are macrophages. May-Grünwald-Giemsa, x 1000.

Fig. 2. Low-power view of the same preparation as in Fig. 1. This magnification was always used to count the macrophages. m, macrophages; pmn, polymorphonuclear leukocytes. x 400.
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