Monocyte-mediated Antibody-dependent Cell-mediated Cytotoxicity and Spontaneous Cytotoxicity in Normals and Cancer Patients as Assayed by Human Erythrocyte Lysis

Michael I. Bernhard, Ronald C. Pace, Stephen W. Unger, and Harold J. Wanebo

Division of Surgical Oncology, University of Virginia Medical Center, Charlottesville, Virginia 22908

ABSTRACT

The monocyte-macrophage system has long been recognized as a necessary accessory to the immune response. Recently, however, monocyte-macrophages have been shown to be important effectors of cell-mediated cellular cytotoxicity (both antibody dependent and antibody independent). In this study, monocyte-macrophage mediated cytotoxicity of both types was assessed on 51Cr-labeled human erythrocytes (type B+) using autologous and standardized AB serum, and monocytes from 57 normal controls, 16 women with benign breast disease, and 175 patients with cancers of the breast (44 patients), colorectum (46 patients), head and neck (33 patients), lung (13 patients), and melanoma (39 patients). Although results were variable, many of the patients had depressed antibody-dependent cellular cytotoxicity suggesting decreased ability of their monocyte-macrophage to lyse the sensitized erythrocytes. Enhanced antibody-dependent cellular cytotoxicity was observed in patients with localized colorectal cancer, but this effect was reversed in patients with advanced disease. Serum factors did not significantly affect responses in most cases. The clinical relevance of this assay remains to be determined.

INTRODUCTION

Traditional studies of CMI control of cancer in humans have concentrated on lymphocyte-tumor cell interactions with little reference to the role of the MO. In addition, most considerations of immune surveillance have ignored MO as effector cells. Early references to MO involvement in tumor regulation or destruction concentrated on the accessory or helper-cell functions of MO; it was assumed that effective antitumor CMI was an exclusive potential to play a central as well as an accessory role in the CMI control of human cancers.

The type of target cell used in ADCC assays has been shown to determine which effector cell population is directly cytolytic: lymphocytes appear to be responsible for tumor cell cytolysis (21, 25); MO and PMN, for HRBC lysis (14, 15, 21, 25); and MOs, PMNs, and lymphocytes, for chicken erythrocyte lysis (21, 25). Recent data have shown that PB MOs are capable of ADCC directly on tumor cell targets, including human tumor cells (11, 16, 19, 20, 32). In addition, tumor-derived MOs have been shown to have ADCC activity on tumor targets and HRBCs (36) as have cultured promonocytes (4). No alterations in ADCC were demonstrated with autologous or allogenic sera (36).

In this study, we have chosen to measure MO ADCC and SCC in controls and patients with cancer using plastic adherent, Ficoll-Hypaque-purified MO and HRBC targets. We have also investigated the effects of autologous serum on both ADCC and SCC. Patient groups studied included women with benign and malignant breast disease and patients with carcinoma of the colorectum, head and neck, lung, and melanoma.

MATERIALS AND METHODS

Study Populations. The control population consisted of 57 laboratory and hospital workers in good health and not receiving medication, with a mean age of 36. A total of 114 separate tests were performed; some individuals were tested repeatedly. In no cases did any individual's data overrepresent reactivity or nonreactivity relative to the group as a whole.

The cancer patients tested included 44 women with breast cancer (for a total of 48 tests) with a mean age of 56; 33 patients with head and neck cancer (no repeat tests) with a mean age of 59; 39 patients with melanoma (a total of 58 tests) with a mean age of 48; 46 colorectal cancer patients (a total of 70 tests) with a mean age of 62; 13 lung cancer patients (a total of 14 tests) with a mean age of 62; and 16
women with benign breast disease (no repeat tests) with a mean age of 46. Cancer patients were generally sampled prior to their first surgery and (on repeat tests) on follow-up clinic visits. Patients were not sampled within 30 days of chemotherapy or radiation therapy.

**MO Separation.** Approximately 40 ml of heparinized blood were diluted 1:1 with Earlie's balanced salt solution without Ca²⁺ and Mg²⁺ (Grand Island Biological Co., Grand Island, N. Y.), layered onto Ficol-Hypaque (Pharmacia Fine Chemicals, Piscataway, N. J.), and centrifuged at 400 × g for 30 min. The band containing monocytes (MO) and lymphocytes was removed by pipet, diluted with EBSS, and centrifuged for 15 min at 400 × g. The cell pellet was resuspended in 2 ml of EBSS, and the volume was adjusted to 50 ml with EBSS. The resuspended cells were spun at 400 × g for 15 min. This cell pellet was resuspended in 1 ml of 10% heat-inactivated serum from type AB donors (ABS) RPMI 1640 with L-glutamine, penicillin, streptomycin, and gentamicin (Grand Island Biological Co.). A single lot of ABS was used for these experiments. The separated cells (10 μl) were diluted with 190 μl of 0.5% crystal violet in 10% acetic acid and counted in a hemocytometer. This stain allowed us to see the nuclei of the cells and to identify the MOs from the lymphocytes. We defined the MOs to be cells which were generally larger than a lymphocyte and had a kidney-shaped nucleus.

**ADCC-SCC Assay.** HRBCs, obtained from a single donor throughout the study, were used as targets. The RBCs were resuspended 3 times (200 × g for 10 min) with EBSS 5% FCS. The RBC pellet was resuspended in 150 μl of 5% FCS EBSS, to which 150 μl of 51Cr (specific activity, 300 to 500 mCi/mg; New England Nuclear, Boston, Mass.) was added, and was placed in a water bath at 37°C for 1 hr with mixing every 15 min. After the hr, the cells were washed 3 times with 15 ml of 5% FCS EBSS to remove free 51Cr and were adjusted to 1 × 10⁶/ml in 10% ABS RPMI 1640 for the assay.

The MOs were adjusted to 5 × 10⁶/ml in 10% ABS RPMI 1640 (based on above count), and 1 ml of this solution was added to each of 3 plastic test tubes (Falcon 2054) per group. These tubes were incubated for 2 hr at 37°C. After 2 hr, the tubes were removed and gently washed 3 times with 5% FCS RPMI-1640 at room temperature. In experiments to determine the amount of adherent cells remaining, all washes were collected, these cells were spun down at 400 × g for 10 min, and the pellet was resuspended in 1 ml of 10% ABS RPMI-1640 and counted as described earlier. Results are shown in Table 1. Next, 1 ml of 10% ABS RPMI 1640 was added to tubes for ADCC in ABS, or 1 ml of 10% ATS RPMI 1640 was added to tubes for ADCC in ATS. Human type B typing serum (anti-B; Dade Chemical, Miami, Fla.), diluted 1:10 and 1:100 in EBSS without the diluted anti-B typing serum, was added in 0.1-ml amounts (final dilutions, 1:120 and 1:1200) to 2 tubes in each group, with the third tube receiving no typing serum (SCC) but 0.1 ml of 5% FCS EBSS. Tests were performed simultaneously in ATS and ABS. All tests were done in duplicate. After the addition of anti-B, 0.1 ml of the labeled HRBC (10⁹/ml) was added to each tube, followed by incubation at 37°C in a 5% CO₂ incubator with 90% humidity for 18 to 20 hr. The spontaneous release was determined by incubating an equal number of tubes containing only RBC with and without the diluted anti-B typing serum.

Following the incubation, tubes were centrifuged at 400 × g for 10 min at room temperature, and then 0.5 ml of the supernatant (one-half of the total supernatant) was removed and counted in Beckman HP Redi Solv scintillation fluid in a Beckman LS8000 scintillation counter (Beckman, Irvine, Calif.). The percentage of 51Cr release was calculated as:

\[
\text{Total cpm in supernatant} - \text{spontaneous release} \times 100 \\
\text{Total cpm in supernatant} + \text{cpm in pellet} - \text{spontaneous release}
\]

A control tube without MO was set up for each assay to assure spontaneous 51Cr release from the labeled erythrocytes was negligible. Data are given in Tables 3 and 4. Max ADCC refers to the maximum ADCC observed in ABS, whether at an anti-B serum dilution of 1:10 or of 1:100 (each done in duplicate). SCC is the value observed in the absence of anti-B serum.

Non-specific esterase activity was also examined (38). Briefly, 5 ml of a solution of lymphocytes in 10% ABS RPMI 1640 (2 × 10⁸ cells/ml) were added to a 60 × 15-mm Petri dish (Falcon 3002) and incubated for 2 hr. The plates were then washed with 5% FCS EBSS to remove the nonadherent cells. The adherent cells were removed by placing 1 to 2 ml of wash media into the dish and gently scraping the surface of the dish with a rubber policeman. The plates were washed twice more, and cell media were collected. The cells were spun at 400 × g for 10 min and resuspended in 1 ml of 10% ABS RPMI 1640 and 10-μl aliquots. Samples (10 μl) were deposited on glass slides in triplicate. The slides were then air-dried; fixed in cold phosphate buffer acetone/formaldehyde, pH 6.6, for 1 min; and rinsed with H₂O. They were then stained for 45 min with α-naphthyl acetate. This was prepared by mixing ethyleneglycol monomethyl ether (8 mg/ml), 18 ml of phosphate buffer, and 0.6 ml of 4% sodium nitrite, and this was reacted with 0.6 ml of p-rosanilin hydrochloride in 2 n HCl for 1 min before adding to the above mixture. The pH was adjusted to 6.1 with 1 M NaOH. After 45 min, cells were counterstained with 1% methyl green (chloroform-extracted) for 5 min, washed, dried, and examined for PMN contamination (PMNs show green nuclear staining only) and MO uptake of p-rosanilin hydrochloride (red).

**Statistical Analysis.** Data were analyzed by type of cancer and by stage, i.e., localized (Stages I and II) or advanced (Stages III and IV) disease. Student’s t test was used to analyze overall changes, and shifts within quartiles were analyzed by goodness of fit to the control quartile distribution. In addition, 10th and 90th percentile cutoff values were determined based upon the control population. The percentages of the patient populations above the 90th percentile and below the 10th percentile were determined and analyzed by Fisher’s exact test and χ² in comparison to controls.

### RESULTS

**Effect of Plastic Adherence on the Mononuclear Cell Counts**

A 2-hr incubation of the Ficol-Hypaque-separated leukocytes on plastic dishes with subsequent washing and removal of the nonadherent cells resulted in recovery of 64% of the nonattached cells in controls. Recovery of the nonadherent cells in different cancer groups ranged from 54% in the lung cancer patients examined to 65% in the colon cancer patients (Table 1). These recovery rates are not significantly different from those of controls. The remaining “attached cells” not recovered by the washing consisted primarily of MOs, adherent T-cells, and other temporarily attaching cells. The results of esterase staining of adherent cells remaining after 2 hr showed quite similar cell counts in a separate study of 12 cancer patients and 11 normal persons (Table 2). Esterase staining in cancer patients and controls. The remaining “attached cells” not recovered by the washing consisted primarily of MOs, adherent T-cells, and other temporarily attaching cells.

### Table 1

<table>
<thead>
<tr>
<th>Type of cancer</th>
<th>No. of patients</th>
<th>% of nonadherent cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>25</td>
<td>63.94 ± 22.65</td>
</tr>
<tr>
<td>Breast</td>
<td>14</td>
<td>62.23 ± 18.93</td>
</tr>
<tr>
<td>Head and neck</td>
<td>18</td>
<td>54.89 ± 20.38</td>
</tr>
<tr>
<td>Colon</td>
<td>26</td>
<td>64.60 ± 24.83</td>
</tr>
<tr>
<td>Melanoma</td>
<td>12</td>
<td>57.64 ± 21.75</td>
</tr>
<tr>
<td>Lung</td>
<td>6</td>
<td>53.80 ± 23.66</td>
</tr>
</tbody>
</table>

* Mean ± S.D.
**Patient Studies**

**Colorectal Cancer.** Patients with localized colorectal cancer showed an overall enhancement of Max ADCC \( (p < 0.05; \text{Chart 1}; \text{Table 3}) \) and increased numbers of high responders (interquartile shift) in ADCC 1:100 and ATS-SCC \( (p < 0.05; \text{Table 4}; \text{Chart 2}) \). Patients with advanced disease showed the opposite interquartile shift in Max ADCC, showing increased numbers of low responders \( (p < 0.05; \text{Table 4}; \text{Chart 2}) \).

**Breast Cancer.** Women with breast cancer showed no overall differences in mean erythrocyte lysis as compared to controls \( (\text{Chart 1}; \text{Table 3}) \). However, there were increased numbers of low responders (interquartile shift) in Max ADCC tests within the overall group \( (p < 0.05; \text{Table 4}; \text{Chart 2}) \), although this shift was not demonstrable when patients were analyzed by stage of disease \( (\text{Table 4}) \). There were also increased numbers of very low responders and decreased numbers of very high responders among those women with localized breast cancer when assayed in ABS \( (p < 0.05; \text{Table 4}; \text{Chart 2}) \), and in ADCC in ATS at 1:10 \( (p < 0.05; \text{Table 4}; \text{Chart 2}) \).

Although normal donors are an appropriate control population for our cancer patients in general, women with benign breast disease may provide a more relevant control group for the breast cancer patients. In comparison to women with benign breast disease, women with breast cancer showed overall depression of ADCC \( (\text{ADCC in ABS, 1:10}; p < 0.05; \text{Max ADCC, } p < 0.02) \), as did women with localized disease \( (\text{Max ADCC, } p < 0.05) \). SCC was enhanced in these patients, however, when compared to women with benign breast disease \( (p < 0.05) \). Thus, women with breast cancer showed depressed ADCC when compared to normal controls or to women with benign breast disease. SCC was enhanced in women with localized disease when compared to women with benign breast disease.

**Melanoma.** Patients with advanced disease exhibited an overall depression of ADCC at an anti-B dilution of 1:10 \( (p < 0.02) \) and in Max ADCC \( (p < 0.05) \); patients with localized disease showed depressed ADCC at an anti-B dilution of 1:100 \( (p < 0.05) \). Interquartile shifts showed increased numbers of low responders for all melanoma patients in ADCC at an anti-B dilution of 1:10 \( (p < 0.025; \text{Table 4}; \text{Chart 2}) \) and Max ADCC \( (p < 0.01; \text{Table 4}; \text{Chart 2}) \), and for patients with advanced disease \( (\text{Max ADCC, } p < 0.05; \text{Table 4}; \text{Chart 2}) \). No significant differences from the control population were found for SCC. Two patients with multiple dermal metastases who had depressed ADCC were treated with intralesional BCG. Both showed restoration to normal or above-normal responsiveness after 1 month of therapy. To summarize, significantly depressed ADCC erythrocyte lysis was a frequent finding in the melanoma patients by different methods of analysis.

**Head and Neck Cancer.** Only patients with localized disease showed altered erythrocyte lysis. An overall depression of ADCC was found at an anti-B dilution of 1:100 \( (p < 0.025; \text{Table 3}) \).

**Lung Cancer.** Although only 13 lung cancer patients were studied, there were distinctive changes in reactivity. Patients with localized disease showed depressed ABS-SCC \( (p < 0.005) \), and patients with advanced disease showed an overall depression of ADCC \( (p < 0.05; \text{Table 3}) \).

**DISCUSSION**

The major objective of this study was to evaluate monocyte function in cancer patients by measuring the capacity of their...
Monocyte ADCC-SCC in Cancer Patients

Table 3

<table>
<thead>
<tr>
<th>Type of cancer</th>
<th>Mean age of patient</th>
<th>ADCC</th>
<th>SCC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>ABS (1:10)</td>
<td>ATS (1:10)</td>
</tr>
<tr>
<td>Controls</td>
<td>36 (57)</td>
<td>29.7 (111)</td>
<td>22.2 (58)</td>
</tr>
<tr>
<td>Benign breast</td>
<td>46 (16)</td>
<td>38.1 (16)</td>
<td>19.3 (14)</td>
</tr>
<tr>
<td>Breast</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>56 (44)</td>
<td>27.2 (40)</td>
<td>19.0 (30)</td>
</tr>
<tr>
<td>Localized</td>
<td>59 (27)</td>
<td>27.7 (23)</td>
<td>19.6 (16)</td>
</tr>
<tr>
<td>Advanced</td>
<td>54 (13)</td>
<td>23.8 (12)</td>
<td>19.1 (9)</td>
</tr>
<tr>
<td>Colorectal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>62 (46)</td>
<td>30.5 (63)</td>
<td>22.2 (36)</td>
</tr>
<tr>
<td>Localized</td>
<td>62 (17)</td>
<td>35.5 (25)</td>
<td>22.6 (13)</td>
</tr>
<tr>
<td>Advanced</td>
<td>62 (29)</td>
<td>25.7 (38)</td>
<td>23.9 (20)</td>
</tr>
<tr>
<td>Melanoma</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>48 (39)</td>
<td>22.2 (53)</td>
<td>17.5 (38)</td>
</tr>
<tr>
<td>Localized</td>
<td>47 (28)</td>
<td>25.5 (27)</td>
<td>19.4 (19)</td>
</tr>
<tr>
<td>Advanced</td>
<td>49 (11)</td>
<td>19.0 (23)</td>
<td>15.1 (19)</td>
</tr>
<tr>
<td>Head and neck</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>59 (33)</td>
<td>27.7 (32)</td>
<td>22.6 (20)</td>
</tr>
<tr>
<td>Localized</td>
<td>45 (4)</td>
<td>35.3 (7)</td>
<td>29.6 (5)</td>
</tr>
<tr>
<td>Advanced</td>
<td>63 (17)</td>
<td>29.4 (22)</td>
<td>18.1 (12)</td>
</tr>
<tr>
<td>Lung</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>62 (13)</td>
<td>31.6 (14)</td>
<td>23.9 (7)</td>
</tr>
<tr>
<td>Localized</td>
<td>58 (6)</td>
<td>35.6 (7)</td>
<td>19.3 (3)</td>
</tr>
<tr>
<td>Advanced</td>
<td>73 (3)</td>
<td>18.3 (4)</td>
<td>19.3 (3)</td>
</tr>
</tbody>
</table>

<sup>a</sup> Serum dilutions.
<sup>b</sup> Max ABS, the higher of the ADCC ABS values (1:10 or 1:100 dilutions) in each individual assay.
<sup>c</sup> Numbers in parentheses, number of patients.
<sup>d</sup> Interquartile shifts compared to controls.
<sup>e</sup> p < 0.05; determined by Student's t test compared to control populations.
<sup>f</sup> p < 0.02; determined by Student's t test compared to control populations.
<sup>g</sup> Numbers in parentheses, number of tests.

No significant interquartile shifts were seen in the remaining groups of cancer patients, patients with shift toward decreased reactivity (p < 0.01) with head and neck cancer, and 13 patients with lung cancer.

Table 4

<table>
<thead>
<tr>
<th>Type of cancer</th>
<th>ADCC</th>
<th>SCC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ABS (1:10)</td>
<td>ATS (1:10)</td>
</tr>
<tr>
<td>Benign breast</td>
<td>0 (16)</td>
<td>0 (14)</td>
</tr>
<tr>
<td>Breast</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>0 (40)</td>
<td>0 (46)</td>
</tr>
<tr>
<td>Localized</td>
<td>0 (23)</td>
<td>0 (26)</td>
</tr>
<tr>
<td>Advanced</td>
<td>0 (12)</td>
<td>0 (9)</td>
</tr>
<tr>
<td>Colorectal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>0 (63)</td>
<td>0 (36)</td>
</tr>
<tr>
<td>Localized</td>
<td>0 (25)</td>
<td>0 (13)</td>
</tr>
<tr>
<td>Advanced</td>
<td>0 (36)</td>
<td>0 (20)</td>
</tr>
<tr>
<td>Melanoma</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>- (53)</td>
<td>0 (38)</td>
</tr>
</tbody>
</table>

<sup>a</sup> Dilution of anti-B typing serum used.
<sup>b</sup> Max ABS, higher of the ADCC ABS values (anti-B at 1:10 or 1:100) from each individual assay.
<sup>c</sup> No significant interquartile shifts: --, shift toward decreased reactivity (p < 0.05); ---, shift toward decreased reactivity (p < 0.01); +, shift toward increased reactivity (p < 0.05); ++, shift toward increased reactivity (p < 0.01).
<sup>d</sup> Numbers in parentheses, number of tests.

Monocytes to lyse sensitized and nonsensitized human RBC. Simultaneous testing in ATS and ABS was done to determine possible functional alterations by serum factors. Ficoll-Hypaque-purified, adherent PB leukocytes (2 hr of plastic adherence) were used as effector cells on HRBC. Although esterase staining of the adherent cells showed only 37% monocytes, these are presumably the major effectors of HRBC lysis as SCC (14, 15, 21, 25), and the PMN count was negligible. We initially were concerned that numbers of adherent MO might vary greatly from patient to patient following the 2-hr incubation in the assay tubes, even though cell concentration was adjusted to maintain the same number of monocytes (MO) from patient to patient. Examination of the cell counts and differentials of the adherent cells and the separated nonadherent cells resulting from this assay in a representative sample of patients and controls showed an acceptable variation. The variation in numbers of adherent cells was below the inherent experimental variation of cell-mediated assays and was not consistently increased or decreased in any study group. It was not possible to both quantitate adherent MO and still perform the assays in all patients because of the limited amount of blood and MO available.

Most studies generally use the concentration of specific antisem giving the highest mean response. We felt that the variation might be significant (especially considering our findings with optimal and suboptimal concentrations of mitogens on lymphocyte blastogenic responses) and, therefore, used anti-B erythrocyte serum at both 1:10 and 1:100 dilutions. It is impossible to predict which concentration of this already diluted commercial antiserum most closely approximates physiological levels of specific antibodies in patients. Although mean ADCC was consistently greater at a 1:10 dilution of anti-B erythrocyte serum than at 1:100 (Table 1), there were numerous exceptions in which the 1:100 dilution was associated with the highest response. Be-
cause of difficulty in correlating all of these factors and the need for population group statistics, we have included values obtained at both concentrations of anti-B sera in our tables.

The results were variable. The most clearcut changes were seen in patients with colorectal cancer and melanoma and in selected subgroups of other cancers. ADCC was augmented in patients with localized colorectal cancer, but was depressed in patients with advanced colorectal cancer and melanoma, and was selectively depressed in subgroups of patients with breast cancer, head and neck cancer, and lung cancer. Comparison of responses in ABS versus ATS showed no consistent pattern. The overall results with MO ADCC or SCC failed to show the stage-related correlations frequently seen in studies of lymphocyte function (39) or other assays of MO function in cancer patients (25, 34).

Several recent reports have addressed the subject of MO ADCC on human and animal tumor cell targets and HRBC targets. Vose (38) has shown that adherent cells from human tumors have the ability to lyse autologous tumor cells, and 14 of 15 preparations showed ADCC on HRBC (2 of 20 demonstrated SCC on HRBC). He was not able to demonstrate effects due to serum factors, which we corroborate.

Several investigators (16, 17, 19, 20, 32) have shown that human PB MOs have the ability to lyse human tumor cells in an antibody-dependent manner. In addition, Mantovani et al. (23) and Pehamberger et al. (28) have demonstrated depressed MO ADCC in patients with melanoma by using HRBC targets. Mantovani et al. (23) found enhanced MO ADCC in patients with Hodgkin’s disease and no alteration in MO ADCC in patients with multiple myeloma or mycosis fungoides. Pehamberger et al. (28) found that, although melanoma patients showed depressed MO ADCC on HRBC, BCG therapy restored MO ADCC to normal levels. The data presented here confirm depressed ADCC in melanoma patients. In addition, 2 of the melanoma patients who initially had depressed ADCC received intralesional BCG therapy and showed enhanced ADCC following therapy.

The finding of enhanced ADCC and SCC in patients with localized colorectal cancer is of interest. Urač et al. (37) have also reported enhanced ADCC on HRBC targets in patients with colorectal cancer, although their patients were unstaged. The ultimate depression of ADCC in the patients with advanced colorectal cancer does coincide with the usually expected depression seen in other cancer patients with advanced stage disease. 39

MO SCC is dependent upon MO recognition of cell surface characteristics, and MO SCC directed toward erythrocyte targets may bear no direct relationship to tumor cell recognition. However, target recognition is only part of the process, and HRBC targets allow determination of the cytotoxic potential of MO. SCC on erythrocyte targets also provides data on the role of serum factors in MO SCC in cancer patients when performed simultaneously in ABS and ATS. SCC in ATS was consistently greater than in ABS, although not at significant levels. This rules out serum-inhibitory factors. Enhanced ATS SCC is explainable on the basis of serum antibody, i.e., our standard ABS was unreactive to HRBC, whereas ATS from patients of non-B and non-AB blood types may exhibit antibody activity to type B erythrocytes, thus making ATS-SCC a modified ADCC assay in some instances. The decreased SCC in ABS observed in lung cancer patients (p < 0.005) is the opposite to our findings in other groups and is not caused by antibodies to type B RBC and, therefore, reflects depressed MO function. Although this may indicate a role for serum factors, the sample is too small to make conclusions.
In general, ADCC was slightly greater in ABS than in ATS. This is probably an artifact of our data analysis, since SCC values were subtracted from total ADCC figures to derive a final number representing only ADCC. When this calculation is omitted, ADCCs in ATS and ABS were nearly identical, again ruling out serum-inhibitory factors. The depressed ATS ADCC observed in patients with localized lung cancer (not significant) is most probably due to the enhanced ATS SCC that was observed in these patients. Patients with breast cancer, however, showed depressed ADCC, whether in ABS or ATS, indicating truly depressed ADCC in these patients.

As summarized in Table 5, patients with breast cancer, melanoma, and advanced colorectal cancer showed depressed ADCC, as did some of the head and neck and lung cancer patients. These data indicate decreased MO ability to kill target cells. Patients with localized colorectal cancer were the only group which demonstrated enhanced ADCC, a condition which was reversed in patients with advanced colorectal cancer.

Although our control population was younger than the cancer patients, none of the data indicated that either ADCC or SCC activity changed markedly in the age groups involved. In fact, women with benign breast disease were 10 years older than the normal control population and showed enhanced reactivity, a shift which would be unexpected if MO function were to decline in these age brackets and which may make depressed values more meaningful.

The in vivo significance of these findings is unclear. However, the observations indicate alteration of at least certain specific MO functions in the specified cancer patient groups and the absence of inhibitory serum factors. It is likely that ADCC is truly depressed in patients with breast cancer, melanoma, and advanced colorectal cancer, and enhanced in patients with localized colorectal cancer. Because of the smaller size of the head and neck and lung cancer groups, we hesitate to make comments on the changes in ADCC seen in these patients. Serum factors may have played a role in SCC in lung cancer patients.

ACKNOWLEDGMENTS

We wish to thank Donald L. Kaiser, DPH (Department of Internal Medicine, University of Virginia Medical Center), for his assistance in the statistical analysis of these data. We also wish to acknowledge the technical assistance of Mark Adelman and David Marshall.

REFERENCES

23. Mantovani, A., Bar Shavit, Z., Perl, G., Polentarutti, N., Bordignon, C., Sessa,
M. I. Bernhard et al.


Monocyte-mediated Antibody-dependent Cell-mediated Cytotoxicity and Spontaneous Cytotoxicity in Normals and Cancer Patients as Assayed by Human Erythrocyte Lysis

Michael I. Bernhard, Ronald C. Pace, Stephen W. Unger, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/43/9/4504

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.