Immnomorphological Features of Prognostic Significance in
Dukes' Class B Colorectal Carcinoma1

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SUMMARY

Histological slides of primary tumors and regional lymph nodes from 134 unselected patients operated on for colorectal carcinoma of Dukes' Class B were assessed semiquantitatively for the presence of perivascular lymphocyte cuffing in the muscular layers and pericolic/subserosal fat immediately subjacent to the tumors and for paracortical hyperplasia in the regional lymph nodes. These two immunomorphological features related significantly to each other (p < 0.05), and their combined presence related significantly to favorable disease-free interval (p = 0.02) and to survival (p = 0.04), making possible the identification of a subgroup of approximately one-third of Dukes' B class patients with an estimated better than 85% chance for 5-year recurrence-free survival.

INTRODUCTION

The prognosis in patients with cancer of the large bowel has improved only marginally during the last 25 years in spite of earlier diagnosis (9). While it is well established that survival is inversely related to the extent of spread (3, 6, 8), factors that influence survival in patients within the same Dukes' class are incompletely known.

In previous studies we were able to demonstrate that in vitro cell-mediated antitumor cytotoxicity correlated inversely with the degree of spread and directly with tumor differentiation (19). Further, we found a good correlation between paracortical-cortical hyperplasia, tumor differentiation, and in vitro antitumor cytotoxicity in the regional lymph nodes (20).

The aim of the work reported here is to assess to what extent such regional lymph node hyperplasia would be reflected in immunocytic cell infiltration at the primary tumor site and whether these immunomorphological expressions could be used for prognostic purposes. The latter would be of value in the selection of a poor prognosis subgroup of patients for tumor immunological monitoring (16) and adjuvant therapy (13).

MATERIALS AND METHODS

Patient Material. The material consisted of hematoxylin-eosin-stained histological slides from 143 consecutive patients of Dukes' B class as originally defined (6), operated on at the M. D. Anderson Hospital and Tumor Institute between 1963 and 1973 and surviving the 1st 2 postoperative months. Cases where the tumor had invaded into but not through the muscular layers were not included in the study. A previous report on this material failed to show prognostic significance of vascular tumor invasion (11). After the completion of the immunomorphological analysis as detailed below, it was found that 134 of these patients had adequate information and follow-up for evaluation.

Histological slides were available from the primary tumors of all 134 patients and from the regional lymph nodes (mean, 14) of 128 patients. In the majority of cases, insufficient or no information was available about the topography of individual lymph nodes in relation to the tumor. Seventy-two of the patients were females, 62 were males; their mean age was 64.2 years. Sixty-three patients had rectosigmoid primaries, 6 had multicentric tumors, and 65 had primaries proximal to the rectosigmoid part of the large bowel.

Histological Assessment. Both conventional and immunomorphological procedures were used. All tumor and lymph node sections were examined by Dr. E. Pihl and reviewed by Dr. N. Khankhanian.

Tumor infiltration through the muscular layers was confirmed; i.e., there were no cases where the tumors were confined to the wall. There were no lymph node tumor deposits. Tumor differentiation was classified as good or poor, the latter also including tumors of intermediate differentiation according to conventional histopathological criteria as previously applied (19).

The lymph nodes were graded according to: (a) degree of development of cortical follicles and germinal centers, hereafter referred to as cortical areas; and (b) the degree of development of the paracortex (Fig. 1). Classical criteria for defining these zones were used (4) as applied previously (20). If either of these zones in the majority of the lymph nodes in each case occupied 15% or more of the sections, the nodes were classified as showing cortical or paracortical hyperplasia. Presence or absence of such hyperplasia could be either concordant or discordant. Initial measurements were made using an integrating micrometer-disc turret (Zeiss, Oberkochen, West Germany) equipped with 25-, 100-, and 400-point standardized square graticules.
were made to analyze the relationship between histological and immunomorphological factors that did not seem to relate to survival data (Table 1), nor were any attempts made to analyze the relationship between the former and the rectosigmoid or colonic site.

RESULTS

Tumor Differentiation. No significant differences were found in disease-free interval or survival, whether the primary tumors were well or poorly differentiated (Table 1). Eight patients of 34 (24%) with well-differentiated tumors had recurrences, as compared to 26 recurrences in 99 patients (26%) in the poorly differentiated group. Twenty-nine of 35 (83%) patients with well-differentiated tumors were still alive at the time of analysis, as compared to 75 of 98 (77%) with poor tumor differentiation.

Cortical Lymph Node Hyperplasia. Cortical lymph node hyperplasia, defined as cortical follicles and germinal centers occupying 15% or more of the node sections, was seen in 105 of the 128 (82%) cases whose lymph nodes were available for examination (Table 1). No statistically
significant differences were noted in disease-free interval or overall survival.

Paracortical Lymph Node Hyperplasia. Paracortical lymph node hyperplasia, defined as the paracortical areas occupying 15% or more of the lymph node sections (Fig. 1), was seen in 76 of 128 (59%) patients (Table 1). There were 16 recurrences and 13 deaths among these patients (21% and 17%, respectively), while the corresponding figures for those without hyperplasia were 17 recurrences and 15 deaths in 52 patients (33% and 29%, respectively). While the difference in disease-free interval was only marginally significant ($p = 0.09$), the difference in overall survival was significant ($p = 0.04$). These data indicate a favorable association between paracortical hyperplasia and these clinical parameters.

Sinus Histiocytosis. No favorable association was noted between sinus histiocytosis and survival data. There were 7 recurrences and 5 deaths (18% and 14%, respectively) among patients with sinus histiocytosis, as compared to 26 recurrences and 23 deaths among those without (29 and 25%, respectively).

Perivascular Lymphocyte Cuffing. There was a statistically significant correlation between perivascular lymphocyte cuffing (Fig. 2) occupying more than 5% of a section through the muscular layers and pericolic/subserosal fat subjacent to the tumor and disease-free interval (Table 1). Twelve of 60 patients (20%) with this reaction had recurrences, as compared to 22 of 73 (30%) of those without this reaction ($p = 0.04$). A favorable trend, which did not reach statistical significance ($p = 0.12$), was noted in relation to overall survival (Table 1).

Combined Paracortical Hyperplasia and Perivascular Lymphocyte Cuffing. There was a clear association between paracortical hyperplasia in the regional lymph nodes and perivascular lymphocyte cuffing in the primary tumor (Table 2). Forty-two of 128 patients (33%) manifested both phenomena, and 34 (27%) showed neither. Seventy-six of 128 patients (60%) were concordant, i.e., manifested either both phenomena or neither, while 52 patients (40%) were discordant and manifested either 1 or the other ($p < 0.05$). We therefore combined paracortical hyperplasia and perivascular cuffing and examined whether the presence of both reactions in the same patient had any correlation with clinical course. We found significantly fewer recurrences and deaths among those who showed this combination (Table 1). Only 6 of 40 patients (15%) with paracortical hyperplasia-perivascular cuffing had recurrences, as compared to 27 of 88 (31%) without ($p = 0.02$). The corresponding figures for tumor deaths were 6 of 41 (15%), as compared to 22 of 87 (25%) ($p = 0.04$). The corresponding disease-free interval and survival curves are shown in Charts 1 and 2. At the 75th percentile, the disease-free interval was only 39 months for those without paracortical hyperplasia-perivascular cuffing, as compared to 118 months for those with these immunomorphological characteristics (Chart 1). The corresponding figures for survival were 48.5 and 120 months, respectively (Chart 2).
vascular cuffing and all single factors listed in Table 1 were subjected to a multivariate regression analysis (5) to assess their relation to disease-free interval and to survival. Only the combined factor was found to have a statistically significant relationship to disease-free interval ($p = 0.024$).

**DISCUSSION**

Our group has previously demonstrated that tumor-free regional lymph nodes with paracortical hyperplasia, with sinus histiocytosis, and (to lesser extent) with cortical hyperplasia are significantly more often cytotoxic to autologous tumor cells in vivo (20). Our data reported here would seem to indicate that only paracortical, *i.e.*, T-lymphocyte, hyperplasia (4) relates significantly to favorable survival data. We have also presented evidence that blood lymphocyte antitumor cytotoxicity related to favorable clinicopathological features (19), and early data indicated that such cytotoxicity tends to occur in patients whose primaries show perivascular lymphocyte aggregates (17), a feature that, in the present series, has been shown to relate to favorable survival data.

The literature on the prognostic significance of paracortical lymph node hyperplasia is contradictory. Patt *et al.* (18), in a small series of sigmoidal carcinomas, demonstrated a positive correlation between survival and paracortical lymph node hyperplasia. Grahame and Sprout (21), grading the inflammatory reaction around the primary tumor area would initially reach the primary tumor; a cytotoxic paracortical lymphocytes would then reach postnodal lymphatics (afferent arm). If this hypothesis was correct, strong tumor antigenicity and/or host immunocompetence would be reflected in paracortical reaction. We are not aware of any publications dealing with the perivascular lymphocytic cuffing adjacent to colorectal tumors, which (as used in this work) is more easily defined and can be expressed in quantitative terms.

The prognostic significance of sinus histiocytosis is difficult to evaluate. Patt *et al.* (18), in 18 patients with sigmoidal carcinoma, found a favorable association with survival. Although Murray *et al.* (14) found significantly better 5-year survival in Dukes' Classes B and C combined, a statistically significant association in only Dukes' Class B could not be demonstrated, a finding of our study also (Table 1).

Several studies have reported on the significance of "stromal inflammatory reaction" in cancer of the large bowel. MacCarty (12) reported on a favorable association between lymphocyte infiltration in the primary tumor and survival, but did not define qualitative or quantitative criteria. Yoon (24) found a favorable association between semi-quantitatively graded eosinophilia and survival in gastrointestinal cancer, but did not give any breakdown between gastric and large bowel tumors in his analysis. Spjut and Sprout (21), grading the inflammatory reaction around the cancer from none to intense, found a doubling of the 5-year survival in the latter category. This was confirmed by Murray *et al.* (14), who found 46% 5-year survival in Dukes' B cases "without local inflammatory reaction" and 89% in those with such a reaction. We are not aware of any publications dealing with the perivascular lymphocytic cuffing adjacent to colorectal tumors, which (as used in this work) is more easily defined and can be expressed in quantitative terms.

On the basis of previous findings by our group (15–19, 20) and results presented here, we hypothesize that paracortical lymph node hyperplasia and perivascular lymphocyte cuffing in the primary tumor are expressions of a chain of events initiated by the recognition of tumor antigen in the regional lymph node, followed by proliferation of cytotoxic lymphoid cells in the paracortex (Chart 3). Cytotoxic paracortical cells would then reach postnodal lymphatics (afferent arm). It follows, then, that only a fraction of these lymphoid cells proportional to the blood supply of the tumor area would initially reach the primary tumor; a larger number would reach other organs and circulate as cytotoxic blood lymphocytes (efferent arm). If this hypothesis were correct, strong tumor antigenicity and/or host immunocompetence would be reflected in paracortical reaction.

**Table 2**

*Association between paracortical lymph node hyperplasia and perivascular lymphocyte cuffing subjacent to colorectal tumors of Dukes' B class*

Concordance is shown between paracortical lymph node hyperplasia and perivascular lymphocyte cuffing at the primary tumor in 76 of 128 cases (60%); *i.e.*, both features are present in 42 cases and absent in 34.

<table>
<thead>
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<th>No. of cases with paracortical hyperplasia</th>
<th>Perivascular cuffing</th>
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<th>Absent</th>
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<td>18</td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>34</td>
<td>34</td>
<td></td>
</tr>
</tbody>
</table>

* $\chi^2 = 4.49; p < 0.05$. 

**Chart 1.** Recurrence-free period (surgery to present time or relapse) in patients with Dukes' B class carcinoma, with and without combined paracortical lymph node hyperplasia-perivascular lymphocyte cuffing.

**Chart 2.** Overall survival period (surgery to present time or death) in patients with Dukes' B class carcinoma, with and without paracortical lymph node hyperplasia and perivascular lymphocyte cuffing.

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REFERENCES

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