Tumour related inhibition of macrophage chemotaxis in patients with colon cancer

A HERMANOWICZ, P R GIBSON, AND D P JEWELL
From the Gastroenterology Unit, Radcliffe Infirmary, Oxford

SUMMARY The chemotactic migration in vitro of peripheral blood, intestinal mucosal, and mesenteric lymph node mononuclear cells has been assessed in patients with colorectal carcinoma. Peripheral blood mononuclear cells of patients exhibited normal chemotaxis. For control patients with non-malignant, non-inflammatory intestinal disease, the chemotaxis of mucosal mononuclear cells was similar to that of autologous peripheral blood mononuclear cells. The chemotactic migration of mucosal mononuclear cells, however, isolated distant from a colon cancer was less than that of autologous peripheral blood mononuclear cells. Chemotactic migration was progressively impaired with increasing closeness to the tumour itself. Chemotaxis of mucosal mononuclear cell was independent of the site of tumour and the Dukes' grading. Mononuclear cells from mesenteric lymph nodes, however, exhibited impaired migration only in patients with Dukes' C tumours. Supernatants of the collagenase digestion of either tumour or adjacent mucosa contained macrophage directed inhibitors of chemotaxis and these inhibitors were not produced by tumour mononuclear cells. The presence of such inhibitors in the digestion supernatants and the demonstration that proximity to the tumour was associated with impaired mononuclear cell motility suggest that the production of macrophage directed chemotactic inhibitors is by colon cancer cells and that this may be occurring in vivo.

Macrophages are often a prominent part of the inflammatory response to tumours and may play an important role in the immunopathogenesis of neoplasia.1 The major mechanism by which they accumulate in and around tumour tissue is probably by the chemotaxis of macrophages from surrounding tissue and of monocytes from peripheral blood. Over one half of patients with various forms of cancer have depressed chemotaxis of peripheral blood monocytes.2 In animal models, such a depressed chemotactic migration is reflected in a diminished capacity to mobilise macrophages into inflammatory infiltrates.3 5 One mechanism of such inhibition appears to be via macrophage directed inhibitors of chemotaxis which have been shown to be synthesised by murine tumour cells6 9 and are present in human malignant pleural effusions.10

Little is known of the motility of monocytes and macrophages of patients with colon cancer which now ranks as the second most common cancer in the Western World. The purpose of this study was to examine the relationship between the chemotaxis of macrophages and colon cancer by determining (a) the chemotactic migration of peripheral blood monocytes and macrophages isolated from histologically normal intestinal mucosa from patients with colon cancer, (b) the effect of proximity to tumour tissue on macrophage chemotaxis, and (c) whether tumour related macrophage directed inhibitors of chemotaxis are present.

Methods

Patients and specimens
Specimens of intestinal mucosa, tumour, and mesenteric lymph nodes were obtained from patients undergoing intestinal resection for colorectal carcinoma. The tumours were staged according to...
Dukes’ classification.11 For all specimens, mucosa was removed distant from the tumour but, for some studies, tissue adjacent to the tumour was also examined. This was dissected leaving a clear margin of at least 5 cm of macroscopically normal mucosa around the tumour. Histological examination of adjacent mucosa revealed no evidence of infiltration with malignant cells. One half of each mesenteric lymph node studied was also examined histologically. For some patients with tumour involving the ascending colon, ileal tissue was obtained.

A small number of histologically normal intestinal specimens from patients with non-malignant non-inflammatory intestinal disease was also examined. The reasons for surgery were diverticulosis coli (two), multiple tubular adenomata (one), angiodysplasia (one), redundant sigmoid loop (one), and penetrating benign gastric ulceration (one).

Peripheral blood was taken from all patients pre-operatively and from healthy volunteers. Details of patients and controls are presented in Table 1. Of the patients with colonic carcinoma, four were receiving digoxin therapy and one was taking phenytoin. None of the control patients were receiving medication apart from the patient with gastric ulceration who was receiving ranitidine.

**Table 1** Details of patients and control subjects

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>Colorectal carcinoma</th>
<th>Miscellaneous group</th>
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<tbody>
<tr>
<td>N</td>
<td>35</td>
<td>22</td>
<td>6</td>
</tr>
<tr>
<td>Age (range)</td>
<td>36 (22–44)</td>
<td>62 (27–81)</td>
<td>73 (66–82)</td>
</tr>
<tr>
<td>Male/female</td>
<td>27/8</td>
<td>17/5</td>
<td>3/3</td>
</tr>
<tr>
<td>Segment of intestine studied:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ileum</td>
<td>5</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Ascending</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Descending</td>
<td>3</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Rectum</td>
<td>10</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Dukes’ classification:</td>
<td>A</td>
<td>B</td>
<td>C</td>
</tr>
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</table>

Peripheral blood obtained from patients and controls was centrifuged over a discontinuous Ficoll-Paque gradient12 and the mononuclear cell population thus obtained washed three times in HBSS.

All mononuclear cell populations were suspended in Eagle’s medium with Earle’s salts (Gibco) supplemented with L-glutamine and antibiotics as above. No further purification steps were performed. The cells were counted in a haemocytometer (Neubauer chamber) and the cell viability assessed by 0.1% trypan blue exclusion. The proportion of macrophages or monocytes in the mononuclear cell populations was estimated in cyto-centrifuge preparations stained with May-Grunwald-Giemsa and non-specific esterase (using alpha-naphthyl acetate as substrate).11 The tumour mononuclear cell population was also characterised by examining toluidene blue stained 0.75 μm sections of cells fixed in 0.4% glutaraldehyde.

**ASSAYS OF CHEMOTAXIS AND RANDOM MIGRATION**

Chemotactic and random migration of mononuclear cells were determined by a modification of the Boyden chamber method10 as described by Wilkinson7 using the leading front technique.10 0.2 ml of mononuclear cells at a concentration of 5 million cells per ml was poured into the upper compartment of the modified Boyden chamber. The lower compartment was filled with either chemotactic factor (chemotaxis) or Eagle’s medium with Earle’s salts (random migration). Both upper and lower compartments were divided by cellulose ester filters with a mean pore size of 8 μm (Millipore). The chambers were incubated for two hours at 37°C in a humid atmosphere with 5% CO2, 95% air. The filters were then removed, fixed in ethanol, stained with Harris haematoxylin, washed in water, dehydrated in alcohol, cleared in butanol and xylol, and mounted on a glass slide. The distance in micrometres moved by the leading front was measured in 15 randomly
selected high power fields with the micrometre attachment of a Leitz Laborlux II microscope. Every

To ascertain whether the inhibitors of chemotaxis were produced by mononuclear cells, tumour and

The migrating cell was inferred to be a monocyte or macrophage on the following criteria: (a) it was

PREPARATION OF CHEMOTACTIC FACTORS

For most experiments, the chemotactic factor was prepared by activating autologous or heterologous

STATISTICAL ANALYSIS

Experimental values have been expressed as mean ± standard deviation where appropriate. Statistical analyses were performed by Student’s t test or by Wilcoxon’s rank-sum-test.

Results

CHARACTERISTICS OF ISOLATED MONONUCLEAR CELL POPULATIONS

The proportion of macrophages or monocytes was similar in peripheral blood, intestinal, tumour, and

CHEMOTACTIC AND RANDOM MIGRATION

The random migration of mononuclear cells from peripheral blood and intestine was similar in all

The collagenase supernatant from intestinal mucosa or tumour was examined for the presence of

In additional experiments, to elimination of residual debris contaminat-
Tumour related inhibition of macrophage chemotaxis in patients with colon cancer

Heolthy subjects

Miscellaneous

Colorectal carcinoma

Healthy subjects

Miscellaneous

Colorectal carcinoma

Fig. 1  Chemotactic migration of peripheral blood (●) and colonic (○) and ileal (□) mononuclear cells (MNC) stimulated with zymosan activated plasma in patients with colorectal carcinoma and miscellaneous intestinal diseases and in normal subjects. Bars represent mean±SD. *Student's t test and Wilcoxon's rank-sum-test.

CHEMOTAXIS AND PROXIMITY TO TUMOUR

The chemotactic migration of mononuclear cells isolated from within tumour tissue was less than that from colonic mucosa adjacent to (less than 5 cm from) tumour which, in turn, was less than that from colonic mucosa distant (greater than 5 cm) from tumour (Fig. 2). Colonic mononuclear cells distant from tumour also moved significantly more slowly than that of autologous peripheral blood mononuclear cells. The Dukes' grading did not influence the chemotaxis of mucosal or tumour mononuclear cells. The chemotaxis of mononuclear cells from mesenteric lymph nodes draining the tumour depended, however, on the presence of metastases – in patients with Dukes' C cancer, chemotactic migration was significantly lower than that in Dukes' A and B cancer (p<0.02). No difference was observed in the random migration of mononuclear cells from the tumour itself (48.4±7.8 μm), from mucosa adjacent to 49.0±8.8 μm) or distant from tumour (43.2±7.8 μm), or from mesenteric lymph nodes (45.1±10.4 μm).

CHEMOTAXIS OF TUMOUR MONONUCLEAR CELLS TO LYMPHOCYTE DERIVED CHEMOTACTIC FACTOR

To assess if the depressed chemotaxis of mononuclear cells isolated from tumour was because of a loss of reactivity to complement-derived chemotactic factors, chemotaxis using lymphocyte derived chemotactic factor was compared with that using zymosan activated plasma as chemoattractant. As presented in Table 2, no difference in chemotactic migration was found in five experiments. No chemotactic activity was observed for phytohaemagglutinin in Eagle's medium alone or for supernatants from peripheral blood mononuclear cells cultured without phytohaemagglutinin.
Fig. 2  Relation of chemotactic migration of mononuclear cells (MNC) towards zymosan activated plasma to proximity to tumour. †Indicate lymph node metastases. *Student's t test.

PRESENCE OF MACROPHAGE DIRECTED INHIBITORS OF CHEMOTAXIS

Overnight incubation of normal peripheral blood mononuclear cells with the collagenase supernatant from mucosa distant from tumour or from mucosa of the miscellaneous group had no effect on chemotaxis (Fig. 3). Collagenase supernatant from tumour markedly inhibited the chemotactic migration of normal peripheral blood mononuclear cells, however, while that from mucosa adjacent to tumour had a smaller but still highly significant inhibitory effect (Fig. 3). Control experiments incubating mononuclear cells in supplemented RPMI containing collagenase had no effect on chemotaxis. 0.22 μm filtration of collagenase supernatants from tumour did not alter their potency to inhibit chemotaxis in two experiments.

No evidence was found for the production of chemotactic inhibitors by tumour or mucosal mononuclear cells even when stimulated with phytohaemagglutinin. Hence, the random and chemotactic migration of normal peripheral mononuclear cells was not impaired by prior incubation with the supernatants from phytohaemagglutinin stimulated or unstimulated cultures of either tumour or mucosal mononuclear cells.

Discussion

The chemotactic migration towards complement derived chemotactic factors of mononuclear cells from peripheral blood of patients with colon cancer is normal. This contrasts with previous reports that about 60% of patients with cancer of many different origins have depressed monocyte chemotactic responsiveness. It is unlikely that this discrepancy is related to the selection or the relatively small number of patients studied but does indicate that colon cancer affects the chemotaxis of peripheral blood monocytes less than many other types of cancer.

Abnormalities of the chemotaxis of mucosal mononuclear cells are, however, present in colon
Table 2 Chemotactic response of mononuclear cells isolated from tumour and from normal peripheral blood to lymphocyte-derived chemotactic factor and to zymosan activated plasma (um±SD)

<table>
<thead>
<tr>
<th></th>
<th>Normal mononuclear cells (n=5)</th>
<th>Tumour mononuclear cells (n=5)</th>
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<tbody>
<tr>
<td>Zymosan activated plasma</td>
<td>142.1±4.0 ±3.0</td>
<td>93.3±14.7 ±5.3</td>
</tr>
<tr>
<td>Lymphocyte derived chemotactic factor</td>
<td>119.8±14.2 ±4.9</td>
<td>93.3±15.8 ±4.9</td>
</tr>
<tr>
<td>Culture supernatant without phytohaemagglutinin</td>
<td>48.4±5.2 ±3.4</td>
<td>47.3±3.4 ±4.9</td>
</tr>
<tr>
<td>Eagle’s medium with 1% phytohaemagglutinin (vol/vol)</td>
<td>46.9±4.2 ±4.6</td>
<td>46.0±4.6 ±4.6</td>
</tr>
<tr>
<td>Eagle’s medium (random migration)</td>
<td>50.1±4.5 ±3.5</td>
<td>48.8±3.5 ±4.5</td>
</tr>
</tbody>
</table>

*p<0.001; t=0.02; †controls for lymphocyte derived chemotactic factor.

cancer. The small group of patients with non-malignant intestinal disease studied suggests that isolated intestinal mononuclear cells exhibit migration similar to that of autologous peripheral blood mononuclear cells. In patients with colon cancer, mucosal cells taken more than 5 cm from tumour demonstrate chemotaxis which is significantly slower than that of autologous peripheral blood mononuclear cells. No evidence that this may be caused by artefactual effects of the isolation process were found since collagenase, overnight culture, and collagenase supernatant from mucosa distant from tumour did not influence the chemotaxis of normal mononuclear cells. As autologous peripheral blood mononuclear cells migrated normally, this depression of mucosal mononuclear cells chemotaxis is not a manifestation of a generalized mononuclear phagocyte defect but is more likely secondary to local mucosal inhibition.

The origin of such inhibition was related to the tumour tissue. Proximity of mucosal mononuclear cells to tumour was directly related to depression of chemotaxis and tumour mononuclear cells exhibited grossly impaired motility. In addition, a macrophage directed inhibitor of chemotaxis was present in the supernatant from collagenase digestion of tumour and, to a lesser extent, mucosa adjacent to tumour. In preliminary experiments, no evidence for the production in vitro of an inhibiting factor from tumour mononuclear cells could be found. Thus it is more likely that the tumour cells themselves are producing the factors and that surrounding mucosal macrophage chemotaxis is suppressed by local diffusion of such factors. This is in accord with rodent studies in which potent tumour derived factors inhibiting tissue accumulation and in vitro chemotaxis of macrophages have been demonstrated.24 Characterisation of this factor has shown it to be a 19000 dalton protein which is physicochemically and antigenically related to a P15 (E) structural protein of retroviruses.† A protein of similar antigenic specificity and functional effect has been isolated from human cancerous effusions.25

Characterisation of the inhibiting factors in this study has been limited. It is present in the soluble fraction of collagenase supernatant and produces irreversible suppression of macrophage chemotaxis. It probably does not act via specific macrophage receptor inhibition as tumour mononuclear cells demonstrated equally depressed migration towards complement-derived (zymosan-activated plasma) and lymphocyte-derived chemotactic factors. The possibility, however, that the depressed chemotaxis of tumour mononuclear cells is caused by in vivo receptor desensitisation with the inflammatory mediators, C5a and lymphocyte derived chemotactic factor has not been excluded. Its mechanism of action may involve suppression of metabolic activity as impairment of adherence and cytotoxicity by mononuclear phagocytes has also been shown in patients with cancer.26 27 Non-specific toxic effects, however, were not evident as collagenase supernatant from tumour tissue did not reduce viability any more than
collagenase supernatant from mucosa distant from
tumour (data not shown). More precise characterisa-
tion of the inhibitory factors from colonic cancer and their relationship to the retroviral envelope protein,
P15 (E), have to be determined.

In conclusion, this study highlights the marked
effect colon cancer tissue may have on the motility of mucosal and nodal macrophages without a concomi-
tant effect on peripheral blood monocytes. The release of macrophage directed inhibitors of chemotaxis probably from tumour cells during the collagenase digestion of tumour and adjacent
mucosa, together with the association of proximity to
tumour with inhibition of macrophage chemotaxis,
support the hypothesis that the production by colon
cancer cells of such inhibitors is occurring in vivo.

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Health Authority.

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