Impaired local natural killer cell activity in human colorectal carcinomas

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Summary. The present study was undertaken to study natural killer (NK) cell activity in patients with colorectal cancer at peripheral and local levels. Mononuclear cells were isolated from uninvolved colorectal mucosa, tumor tissue and peripheral blood, and tested against the colon carcinoma cell line CaCo-2 and the erythroleukemia cell line K-562. Peripheral blood NK cell activity from the patients showed similar levels compared with healthy controls, whereas, mononuclear cells of tumor tissue were found to have a significantly decreased NK cell activity compared to the normal intestinal mucosa ($P < 0.01$). No relation was found between the NK cell activity and the advancement of the disease according to the Duke’s stage. Interferon-y (IFN-γ) stimulated the NK cell activity of the mononuclear cells from blood, mucosa and tumor. However, the increase of NK cell activity after IFN-γ stimulation was lower in the tumor compared to the mucosa ($P < 0.02$). The lectin, phytohaemagglutinin, increased the cytotoxicity of mononuclear cells from blood, mucosa and tumor to a similar level. These results suggest that patients with colorectal tumors exhibit a normal NK cell activity in peripheral blood and intestinal mucosa; however, a diminished NK cell activity exists at the tumor level. Although mononuclear cells isolated from the tumor have a normal response to lectin stimulation they show hyporesponsiveness to IFN-γ stimulation with regard to their NK cell activity.

Introduction

Natural killer (NK) cells play an important role in many physiological functions including defence against tumor [11]. There is a considerable controversy in the literature with regard to NK cell activity of peripheral blood determined in cancer patients. Depressed NK cell activity has been related to advanced disease [15, 17, 19]. In contrast, other investigators have reported normal NK cell activity independent of the Duke’s grading in patients with colon cancer [23]. When circulating numbers of NK cells were studied with HNK-1 (Leu-7) in patients with colon cancer they were found to be reduced in comparison to control subjects [2, 3b].

Intestinal immunity can also play a role in the local immune surveillance. Although low percentages of NK cells are found in the normal intestinal mucosa, functional natural killer and lymphokine-activated killer cells are both present in the human gut mucosa [7, 9, 12, 13, 18]. At the tumor level, the NK cell activity has been reported to be absent or not measurable [3b, 4, 16]. However, the existence of an increased number of NK cells has been shown in tumor-infiltrating lymphocytes in patients with colon cancer [6]. Also it has been reported that human and animal tumors contain a high proportion of cytotoxic T lymphocytes [6, 16, 24].

The purpose of this study was to analyse the NK cell activity under optimal conditions in peripheral blood, intestinal mucosa and tumor from patients with colorectal carcinomas. We have also evaluated the response of these NK cell populations to the immunomodulator IFN-γ and the lectin phytohaemagglutinin.

Materials and methods

Patients. Fresh heparinized blood was obtained from 15 patients with colorectal cancer prior to surgery and from 15 healthy controls. Specimens of colorectal tumors and intestinal mucosa were obtained from 11 patients undergoing surgical resection of large-bowel adenocarcinoma (1 cecum, 4 colon ascendens, 3 colon transversum, 2 colon sigmoid and 1 rectum). The Duke’s stages of these patients were: A ($n = 1$), B ($n = 4$), C ($n = 4$), and D ($n = 2$). The adjacent intestinal tissue was taken at least 5 cm from the tumor and was examined histologically to ensure the absence of tumor cells.

Mononuclear cell isolation. We have applied a modified technique from Bull and Bookman [5], as described previously [22], for the separation of mononuclear cells from intestinal mucosa and colorectal tumors using enzymatic dispersion and separation on the basis of density and size by Ficoll-Hypaque. In brief, after resection, tumor and intestinal mucosa tissues were processed immediately under sterile conditions. Necrotic and connective tissue was removed from the tumor and the remaining tissue was cut into small pieces of 5 mm$^2$ and washed twice with calcium/magnesium-free HBSS (Gibco Europe). Normal mucosa was dissected free of submucosa, washed and cut into pieces of
by transferring 1:3 twice a week. Single-cell suspensions for microscopy. The total numbers of cells obtained after enzymatic dispersion were for adjacent intestinal mucosae $33 \pm 8 \times 10^6$ cells/g tissue and for the tumors $34 \pm 6 \times 10^6$ cells/g. From these initial cell suspensions, the recovery of mononuclear cells from heparinized peripheral blood were obtained by Ficoll/Hypaque density gradient centrifugation. The interface of mononuclear cells. IFN-γ was diluted in culture medium with the normal mucosa as illustrated in Table 1. No relation was observed between NK cell activity of the mononuclear cells infiltrating the tumor and 49% (29%–69%) in the case of those cells infiltrating the tumor showed a similar level of NK cell activity, compared with 8%–69%) in the case of those cells infiltrating the tumor mucosa yielding respectively $9 \pm 2 \times 10^6$ cells/g and $15 \pm 3 \times 10^6$ cells/g. The mononuclear cell from the tumors contained $52 \pm 4\%$ lymphocytes and those from the intestinal mucosa $63 \pm 4\%$, contamination with epithelial/malignant cells was less than 10%. The viabilities of the mononuclear cells of intestinal mucosa and of tumors were respectively $89 \pm 1\%$ and $84 \pm 3\%$.

**Results**

**Intestinal cell isolation**

The total numbers of cells obtained after enzymatic dispersion were for adjacent intestinal mucosae $33 \pm 8 \times 10^6$ cells/g tissue and for the tumors $34 \pm 6 \times 10^6$ cells/g. From these initial cell suspensions, the recovery of mononuclear cells from heparinized peripheral blood were obtained by Ficoll/Hypaque density gradient centrifugation. The interface of mononuclear cells. IFN-γ was diluted in culture medium with the normal mucosa as illustrated in Table 1. No relation was observed between NK cell activity of the mononuclear cells infiltrating the tumor and 49% (29%–69%) in the case of those cells infiltrating the tumor mucosa yielding respectively $9 \pm 2 \times 10^6$ cells/g and $15 \pm 3 \times 10^6$ cells/g. The mononuclear cell from the tumors contained $52 \pm 4\%$ lymphocytes and those from the intestinal mucosa $63 \pm 4\%$, contamination with epithelial/malignant cells was less than 10%. The viabilities of the mononuclear cells of intestinal mucosa and of tumors were respectively $89 \pm 1\%$ and $84 \pm 3\%$.

**NK cell activity**

Peripheral blood mononuclear cells from the patients showed a similar level of NK cell activity, compared with those from the controls, against both targets (K-562, 52±11 vs 58±2 and CaCo-2, 25±4 vs 28±3).

Mononuclear cells of the intestinal mucosa and of the tumor showed a low NK activity against CaCo-2 and K-562 tested with the ratio 50:1 (9±2 vs 4±1 and 2±3 vs 1±1 respectively). Cytotoxicity against the CaCo-2 cell line showed a significant difference between mononuclear cells from the intestinal mucosa and from the tumor (P < 0.05) (Fig. 1). With an effector:target ratio of 500:1, the cytotoxicity of the mononuclear cells of the intestinal mucosa and tumor increased significantly against the two cell lines tested, CaCo-2 and K-562 (P < 0.02). Using this ratio, the NK cell activity of the mononuclear cells infiltrating the tumor was found to be markedly decreased compared to the that of the adjacent intestinal mucosa (P < 0.01) (Fig. 1). No relation was observed between NK cell activity and the Duke's stage with the tumors nor with the normal mucosa as illustrated in Table 1.

Phytohaemagglutinin increased significantly the cytotoxicity of the mononuclear cells from blood, mucosa and tumor (P < 0.02). However, there was no difference in the
cytotoxicity levels after phytohaemagglutinin stimulation between peripheral blood mononuclear cells from patients and controls nor between mononuclear cells from mucosa and tumor. Also no difference was found in the increase in the cytotoxicity of blood mononuclear cells between patients and controls nor between those of intestinal mucosa and tumor (Fig. 2).

IFN-γ stimulated significantly the cytotoxicity of mononuclear cells from blood, tumor and normal adjacent intestinal mucosa (P < 0.02). However, the increase of the cytotoxicity after IFN-γ stimulation of the tumor was significantly less when compared to the intestinal mucosa (4±1 vs 10±1, P < 0.02), and no difference was found with the peripheral blood mononuclear cells.

**Discussion**

This study shows that patients with colorectal carcinoma have a defect of the NK cell activity at the tumor level compared to the adjacent intestinal mucosa, which is unrelated to the Duke's stage, with a normal peripheral blood NK cell activity.

Although the number of NK cells is markedly decreased in intestinal mucosa [9, 18] NK cell activity at the intestinal level can be studied when optimal methods are applied. Thus, to be able to compare NK cell activity from blood with mucosa, other effector:target cell ratios should be used to obtain the same proportions of cytotoxic cells [10]. For these reasons, previous reports of generally minimal or absent NK cell activity cannot be validly interpreted [4, 7]. In this study freshly isolated mononuclear cells of the intestinal mucosa from all patients showed significant cytotoxicity against both cell lines, CaCo-2 and K-562. Using these assay conditions, which overcome most of the methodological difficulties, by culturing cells for 24 h before testing and using a high E:T ratio [10a, b] a defect of NK cell activity at the tumor level, has been demonstrated.

The depressed NK cell activity found in the colorectal tumor appears to be a general finding for tumors of different localizations [16, 24]. Mononuclear cells infiltrating tumors have been shown to have a higher proportion of HNK-1 (Leu-7) and/or CD8 (OKT-8) cells compared to the normal intestinal mucosa [6, 16]. The suppression of the activity of the NK cells may thus be related to the environment of the tumor itself. For example, it is known that tumors contain more macrophages and high prostaglandin E2 production may be a possible mechanism for the depression of the NK cell activity at the tumor level [3a].

IFN-γ, a potent immunomodulator, has been shown to stimulate monocytes and NK cells [20, 21]. In this study mononuclear cells of the tumor were hyporesponders whereas those of blood and mucosa showed a normal response with IFN-γ. Other studies, using other types of interferons, have also shown stimulation of the NK cell activity [8, 9, 13].

Phytohaemagglutinin has been shown to increase cellular cytotoxicity by inducing interleukin-2 production, receptor expression and lymphocyte activation [1, 14]. In this study it increased the cytotoxicity of mononuclear cells of peripheral blood, tumor, and normal adjacent intestinal mucosa in the same fashion. The mononuclear cells of the tumor are capable of becoming fully activated.

We conclude that patients with colorectal cancer have a defect in the NK cell activity at tumor level. The mononuclear cells of the tumor are hyporesponsive to IFN-γ but have high potential lytic capabilities. It seems, therefore, possible that within the tumor, lymphocytes capable of being stimulated to an optimal killing are present but are

Table 1. NK cell activity, as percentage cytotoxicity, of mononuclear cells from normal intestinal mucosa and tumor divided according to Duke's stage

<table>
<thead>
<tr>
<th>Duke's stage</th>
<th>Targets</th>
<th>Mucosa</th>
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<tbody>
<tr>
<td>A and B, n = 5</td>
<td>K-562</td>
<td>32 ± 10</td>
<td>15 ± 4</td>
<td>31 ± 7</td>
<td>12 ± 1</td>
</tr>
<tr>
<td>C and D, n = 6</td>
<td>CaCo-2</td>
<td>33 ± 12</td>
<td>12 ± 3</td>
<td>36 ± 9</td>
<td>13 ± 3</td>
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suppressed by the tumor cells or products produced in the tumor environment. Development of approaches to overcome this local malfunction of the NK cells might provide better local control of tumor growth.

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