INTRODUCTION

Colorectal adenocarcinoma is a neoplasm in which prognosis is mainly determined by the histological stage. However, prognosis of patient groups, especially of intermediate stages, remains vague\textsuperscript{1}. The main weakness of the currently used prognostic markers in colorectal adenocarcinoma, is their inability to point out patients without metastases, whose clinical course will progress unfavorably and patients with metastatic disease who will have a relatively mild prognosis\textsuperscript{1}. Therefore, development of new prognostic markers is essential, as they might help in the planning of more effective treatment modalities. Markers of the host’s immune response could potentially be helpful in this field.

Natural killer (NK) cells play a pivotal role in innate immunity and immunological surveillance. Their cytotoxic effects may be initiated without prior immunization and thus NK cells have been considered a first line of defence of the host against a developing cancer\textsuperscript{2,3}. In spite of their small presence in tissue samples from colorectal adenocarcinomas, NK cells manifest a potent cytotoxic anti-tumor effect\textsuperscript{3,4,5}. Expression of various protein surface markers is essential for these cells to perform their activities\textsuperscript{2}. Among these markers, CD16 is used to identify active NK cells in immunohistochemical studies, while CD57 is expressed on NK cell-like elements.

The aim of this study was to evaluate tissue presence of NK cells in a series of colorectal adenocarcinomas and to attempt to relate their presence with clinical and pathological variables and prognostic markers of colorectal cancer.
MATERIALS AND METHODS

From June 1997 to May 2000, 82 patients from our Department underwent colectomy, due to a diagnosis of colorectal adenocarcinoma of conventional histologic type. NK cell presence was examined via standard 3-step immunohistochemical analysis (ABComplex), which was performed on formalin-fixed, paraffin-embedded tissue sections. Briefly, two appropriate monoclonal antibodies were used (anti-Fc Gamma Receptor II, CD16 and an equivalent to Leu-7, specific for CD-57, Dako, Glostrup, Denmark) at dilutions of 1/200 with overnight incubation. Antigen retrieval was necessary and was performed by usual microwave treatment. In the examined samples, tissue identification of NK cells was based on strong CD16 immunostaining, which was frequently accompanied by CD57 immunoreactivity. Tissue sections from hypertrophic tonsils were used as positive markers. Diaminobenzidine tetrahydrochloride 0.06% in phosphate-buffered saline buffer containing 0.03% hydrogen peroxide was used as a chromogen. Images were acquired using a Zeiss Axiolab microscope (Carl Zeiss GmbH, Jena, Germany) with a mechanical stage, fitted in a Sony-iris CCD video camera (Sony, Tokyo, Japan). The video camera was connected to a Pentium II PC, loaded with the appropriate image analysis software (Sigma Scan Pro, Science, Erkrath, Germany). Slides were examined at a x200 magnification. The ratio, expressed in percentile proportion (%), between the number of immunohistochemically positive-stained cells and the total number (stained and unstained) of lymphocytes was calculated. All tumors had been characterized according to the following classical clinical and pathological variables: patient gender (male 52, female 30, 63.4% and 36.6%, respectively) and age (mean: 75.9, median: 76, ranging between 35-95 years), tumor location (rectum 27, 32.9%,...
sigmoid 26, 31.7%, descending colon 4, 4.8%, transverse colon 5, 6%, ascending colon 10, 12.1%, cecum 10, 12.1%), size (mean diameter: 4.4cm, median diameter: 4cm, ranging between 1.2-10cm), grade (I: 24 cases, 29.3%, II: 48 cases, 58.5%, III: 10 cases, 12.2%), bowel wall invasion (present in 66 cases, 80.5%), lymph node metastases (present in 33 cases, 40.2%) and Dukes’ stage (A: 5, 6.1%, B: 44, 53.7%, C: 29, 35.4%, “D”: 4, 4.9%). All cases of “D” Dukes’ stage cases involved hepatic metastases. The tumors were categorized according to their location into two larger groups: those involving the rectum as well as the left colon (57 cases, 69.5%) and those involving the right colon (25 cases, 30.5%). This was performed for reasons of statistical analysis.

Moreover, positive cases were divided into two groups: those in which immunopositive cells ranged from 0-9% of the total number of lymphocytes (“minimal” tissue presence of NK cells) and those in which they were ≥10% (“sufficient” NK cell presence). This was performed somewhat arbitrarily, as we considered that the cutoff level of 10% is in accordance to what a pathologist would consider to be the cutoff level between “negative” and “positive” cases in a qualitative examination, and in order to perform statistical analysis. Associations of NK cell presence with the patients’ sex, tumor location, grade, and presence of bowel wall invasion, as well as lymph node metastases and Dukes’ stage were examined using chi-square statistics; associations with the patients’ age and tumor size were determined using the Mann-Whintey U test.
RESULTS

NK cells were detectable in the primary tumor site of 79 cases (96.3%) in percentages up to 32% of the primary site lymphocytes (Figure). Of the 33 cases with metastatic lymph nodes, NK cells were found in 27 cases (81.8%); moreover, NK cells were found among liver lymphocytes in 3 of the total 4 cases with hepatic metastases (75% of stage “D” cases).

Forty-nine of the 82 primary tumors (59.8%) had a “minimal” tissue presence of NK cells (0-9%), whereas the remaining 33 primary tumors (40.2%) had “sufficient” NK cell presence (≥10%). Of the 33 cases with metastatic lymph nodes, 18 cases (54.5%) had “minimal” and 15 “sufficient” NK cell presence.

NK cell presence in the primary tumor site was not associated with the patients’ sex (chi-square, p=0.462), tumor size (Mann-Whintey U test, p=0.377), tumor location (chi-square, p=0.262), tumor differentiation (chi-square, p=0.556), wall invasion (chi-square, p=0.999), the presence of metastatic lymph nodes (chi-square, p=0.720), or Dukes’ stage (chi-square, p=0.992). However, a negative association between the presence of NK cells at the primary tumor site with the patients’ age was noticed (Mann-Whitney U test, p=0.031).

Moreover, the presence of NK cells in the metastatic lymph nodes was not associated with the patients’ sex (chi-square, p=0.999), age (Mann-Whintey U test, p= p=0.212), tumor size (Mann-Whintey U test, p=0.729), tumor location (chi-square, p=0.442), tumor differentiation (chi-square, p=0.872), wall invasion (chi-square, p=0.999), or Dukes’ stage (C or D) (chi-square, p=0.308).
DISCUSSION

Although colorectal adenocarcinoma has been characterized as a model neoplasm in which clinical prognosis is mainly determined by the histologic stage at diagnosis, the inability of the classical pathologic markers to predict the prognosis of a great number of patients has necessitated the evaluation of several alternative prognostic markers. Immunohistochemical studies have proved useful in the evaluation of such potential markers; although they are relatively cheap and easy-to-perform, they may be as effective as other advanced molecular techniques in evaluating the role of several molecules in human carcinogenesis.

CD16 and CD57 were chosen in the present study, as they are markers of the presence of NK cells, a cellular line which could reasonably play a significant role in the pathophysiology of colorectal adenocarcinoma. NK cells were considerably present in the primary tumor site, as well as the metastatic lymph nodes of many cases in our series. This observation is in line with data deriving from the literature. Their presence had no association with the clinical or pathological variables of our series, besides a negative association between the presence of NK cells at the primary tumor site with the patients’ age (p=0.031), i.e. less NK cells were found in the stroma of the primary tumor site in the older patients of our study. Reports concerning alterations of the number and/or the cytotoxicity of NK cells in patients of older ages seem to be controversial; this has been attributed to the diversity of the parameters examined in these studies. In most reports, ageing results in an increase of the number of circulating NK cells; however, other studies point out that although total levels of NK cells actually remain steady, some of their biologically active sub-populations actually diminish. The cytotoxicity of NK cells on the other hand, has been
reported to increase\textsuperscript{13}, remain steady\textsuperscript{8,14} or diminish\textsuperscript{12} in patients of older age; some reports connect presence of certain nutrients and hormonal factors with the maintenance of NK cytotoxicity in these patients\textsuperscript{8,14}.

However, what seems to be pivotal for rapid and efficacious migration of NK cells from the circulation to the tumor stroma, is the expression of appropriate adhesion molecules\textsuperscript{15}. As expression of adhesion molecules in lymphocytes, monocytes and the interstitial tissue decreases with age\textsuperscript{16} this might result in decreased adhesion molecule-mediated migration of NK cells to the tumor stroma in the older-aged patients of our series, which could possibly interpret the negative association observed in this study\textsuperscript{5,15}.

In conclusion, our findings suggest that less NK cells are found in the stroma surrounding the primary tumor site in older patients with colorectal adenocarcinoma. This could possibly be attributed to decreased adhesion molecule-mediated migration; however, this hypothesis needs to be further investigated through more studies, planned to evaluate the probability of this proposed mechanism.
REFERENCES


LEGEND TO ILLUSTRATION

Figure: Presence of NK cells within cancerous formations. (ABC, magnification X250).