DEFINITE SPONTANEOUS CELL-MEDIATED CYTOTOXICITY AND HNK-1 CELLS IN THE HUMAN LARGE INTESTINE

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Summary

Spontaneous cell-mediated cytotoxicity (SCMC) and the marker of natural killer (NK) cells mediating SCMC of the human large intestine were studied. Lamina proprial lymphoid cells (LPL) were isolated by sequential dithiothreitol-EDTA-collagenase treatment of the gut specimen. SCMC was measured by the chromium release method. Target cells included P4788 in monolayer, a cell line derived from colon cancer, Chang cells in monolayer, and K562 in suspension. Target cells in monolayer including colon cancer cell line were chosen because they were thought to be more appropriate to assess SCMC for lymphoid cells in the solid organ. While lower compared to cytotoxicities (CT) by peripheral blood lymphoid cells (PBL), define CT were observed in LPL against all three targets. NK cells marker was studied both on LPL by an indirect fluorescent antibody method and on the gut tissue by indirect immunoperoxidase staining using anti HNK-1 monoclonal antibody which defines virtually all NK cells. HNK-I positive (HNK-1 + ) cells were identified in both methods. HNK-1 + cells were observed in the epithelium, lamina propria, and lymph follicle with or without germinal centers. These results clearly demonstrated the presence of SCMC and HNK-1 + cells in the human large bowel.

Key Words: HNK-1 (Leu-7), Large intestine, Lymphocytes, intestinal (mucosal), Natural killer (NK) cells, Spontaneous cell-mediated cytotoxicity (SCMC).
tion to nonspecific mitogens\cite{1,7,11} and the ability to mediate mitogen-induced cell-mediated cytotoxicity\cite{4,6,8,9} and antibody-dependent cell-mediated cytotoxicity\cite{2,4-6} have been described by several groups. However, the data on the ability of LPL to mediate spontaneous cell-mediated cytotoxicity (SCMC) have been conflicting\cite{4,6,8,10,12,15-17}.

SCMC is a phenomena in which lymphocytes from any healthy donor, in the absence of known sensitization, are spontaneously cytotoxic in vitro for malignant or non-malignant cells. The effector cells responsible for this type of SCMC are functionally defined as natural killer (NK) cells\cite{18}. NK cells are thought to play a role in vivo in the resistance to tumor, viral infections, and in the immunoregulatory network\cite{18-22}. As the large intestine is a major site of neoplastic, viral infection and chronic inflammatory disorders of unknown etiology, the presence or absence of NK cells and their activity (NK activity) is of great interest. Chiba et al.\cite{6} and Targan et al.\cite{10} detected low SCMC and MacDermott et al.\cite{4} and Gibson et al.\cite{12} detected only minimal SCMC, while Falchuk et al.\cite{8} and Fiocchi et al.\cite{15} did not detect any SCMC. As for the study of the phenotype of NK cells using anti-HNK-1 monoclonal antibody\cite{23} which defines virtually all NK cells, Gibson et al.\cite{19} identified anti-HNK-1 positive (HNK-1+) cells in the large bowel while Fiocchi et al.\cite{15} did not. Since there is heterogeneity in target cell structures and these antigenic specificities evoke the specific characteristics of the cytotoxicity\cite{24,25}, it is important to choose an appropriate target cell in the study. For this reason, P4788 colon cancer cell line\cite{26,27} was chosen as one of the targets in this study. In addition, since the effector cells are derived from the solid organ, the large intestine, the form of target cells should be more appropriate in monolayer\cite{27,28} than in suspension form. Therefore P4788 and Chang cells\cite{18} in monolayer as well as K562\cite{18} in suspension were used as targets. HNK-1 phenotype was also studied both for the isolated LPL and for gut tissue. Our results of functional studies were consistent with those of phenotypic studies. We observed a definite SCMC and positivity for HNK-1+ in the human large intestine.

Materials and Methods

1. Study Materials

Sixteen patients with colorectal cancer, excluding adenomatosis coli (7 males, 9 females) ranging from 38 to 78 years old (mean 60) and 2 patients with colon polyp (F. 41 yrs, F. 47 yrs) were studied. 1) LPL and PBL from 7 patients with colorectal cancer were studied in terms of their cytotoxicity and HNK-1+ by an indirect immunofluorescence study. PBL from 15 healthy subjects ranging from 20 to 63 (mean 36) served as controls. 2) 16 specimens of gut tissue (3 rectum specimens, 4 sigmoid, 1 descending, 3 transverse, 3 ascending colon, 2 cecum) from 12 patients (10 colorectal cancers, 2 colon polyps) were studied for HNK-1+ by indirect immunoperoxidase staining. These tissue specimens were obtained either by resection (12 specimens) or by endoscopic biopsy (4).

2. Effector Cells

1) PBL (peripheral blood lymphoid) cells

Venous blood samples were drawn from patients with colorectal cancer and healthy controls. In colorectal cancer, venous blood samples and autologous colon specimens for LPL isolation were obtained during operation. PBL were isolated from venous blood samples by a Ficoll 400 (Pharmacia Fine Chemicals, Uppsala, Sweden)—Conray (Daichi Seiyaku, Japan) gradient as described in a previous paper\cite{29}. Monocyte-macrophage depletion was not done. Appropriate numbers (0.5, 1.0, 2.5, and 5.0 × 10^6) of PBL were suspended in 1 ml of RPMI 1640 (Nissui, Japan) containing 10% V/V heat inactivated fetal bovine serum (Flow