The CD56 adhesion molecule is the major determinant for detecting non-major histocompatibility complex-restricted cytotoxic mononuclear cells from the intestinal lamina propria

In this study, specific antibodies against natural killer (NK) cell surface markers identify these cells to be commonly present in normal intestinal mucosa of inflammatory bowel disease (IBD) and carcinoma patients. Cells expressing the CD56 adhesion molecule were found to be far more abundant than CD16+ cells. Functional studies revealed that cells mediating non-major histocompatibility complex-restricted cytotoxicity (NK activity) in the lamina propria express the CD56 surface antigen, whereas only a minority of this activity resides in the population with CD16 expression. This is in contrast with peripheral blood NK cells, which were found to be almost exclusively both CD16+ and CD56+. Moreover, in the lamina propria of the intestine we found CD3+ T lymphocytes not to be involved in spontaneous cell-mediated killing of tumor cells.

Considerably higher numbers of cells with the CD16 or CD56 surface markers were found to be present in normal mucosa of IBD patients compared with normal mucosa of carcinoma patients, which was also reflected in higher levels of cytotoxicity detected in lamina propria mononuclear cell preparations from normal IBD mucosa. Because of the disease-related localization of the mucosa studied from both patient groups, i.e., ileum vs. colon, the observed differences may be related to tissue characteristics. Within the IBD group, relatively high levels of cytotoxicity were found in cell preparations from normal mucosa of Crohn’s disease patients compared with ulcerative colitis patients, which might support the current concept that Crohn’s disease affects the whole of the gastrointestinal tract.

1 Introduction

It had been accepted for several years that cells exerting NK activity were absent or sparsely present in the intestinal lamina propria and were therefore, hardly detected in assays determining non-MHC-restricted cytotoxicity [1–3]. This conception was mainly based on earlier reports describing unsuccessful immunohistological detection [4] and failure of measuring NK activity in mucosal mononuclear cell preparations obtained by enzymatic isolation from tissue sections [1, 2, 5, 6]. However, identification of these cells with more specific antibodies against NK cell surface markers [7–10] greatly improved the assessment of these cells in the lamina propria of the gut. Nowadays, there seems to be agreement on the presence of NK cells in the gut lamina propria though they are found in very small numbers.

The low distribution of these cells in the gut does not mean that they are unable to kill virus-infected cells and opsonized bacteria or exert cytolytic activity against malignant cells [11, 12]. Moreover, although these NK cells represent a minor population in the intestinal mucosa, they can play an important role in the regulation of the local immune response [13]. In most studies reported so far, NK activity of isolated lamina propria mononuclear cells (LPMC) was assessed by introducing preculturing conditions [14], high effector-to-target ratios [15], and through enrichment of NK cells by density gradient centrifugation [16], elutriation centrifugation [17] or panning techniques [18]. Potential inhibitory cells were occasionally depleted by adherence but the influence of, for instance, Mφ on cytotoxicity of lymphocytes could not be demonstrated [19]. Primarily because of the sparse distribution of NK cells and the former use of only partly specific antibodies, their phenotypical characterization remained underexposed [6, 20, 21].

Cells in peripheral blood exerting non-MHC-restricted cytotoxicity have been found almost exclusively to express both CD16 and CD56 surface markers and there also seems to be a small population of CD3+, CD16-, CD56+ T lymphocytes involved in this type of spontaneous cell-mediated cytotoxicity [22]. The low presence of cells with NK markers in the lamina propria and their relation to functional activity in both diseased and normal gut mucosa deserves further study with more specific mAb. In the present study we evaluated the functional role of cells with NK activity from the intestinal lamina propria by depletion studies and surface marker expression in relation to localization and disease.
2 Materials and methods

2.1 Specimens studied

Intestinal specimens were obtained from patients with a variety of diagnoses including colorectal carcinoma, inflammatory bowel disease, diverticulitis and volvulus, who underwent therapeutic bowel resection in our hospital. The tissue specimens used in our studies were always taken distant from the tumor or inflamed region. Immediately after resection and macroscopic investigation by the pathologist the tissue was transported to our laboratory in cold HBSS (Gibco, Paisley, Scotland) supplemented with antibiotics, pH 7.4. There the tissue specimen was either processed immediately for isolation of mononuclear cells or the mucosal layer was separated and stored overnight on ice (0 °C) in culture medium RPMI 1640 (Gibco) supplemented with 10% FCS (Gibco) and antibiotics. All intestinal tissue samples were considered to be normal as confirmed by macroscopical and histological evaluation by the pathologist. The group of patients included 20 females and 15 males ranging from 21 to 89 years of age with a mean of 57 ± 3 years. Most of the specimens were used for both phenotypical and functional studies of LPMC as indicated.

2.2 Isolation of effector cells

Heparinized venous blood samples were obtained from healthy volunteers. PBMC were isolated by density gradient centrifugation on Ficoll-Isopaque (Pharmacia, Uppsala, Sweden). The cells were recovered from the interface, washed and resuspended in culture medium RPMI 1640 with 10% FCS, supplemented with L-glutamine (2 mM, Flow Laboratories, Irvine, Scotland), penicillin (100 U/ml, Gibco), streptomycin (100 µg/ml, Gibco), gentamicin (50 µg/ml), Flow Laboratories) and amphotericin B (2.5 µg/ml, Flow Laboratories). Viability of these cells, as determined by trypan blue exclusion, was consistently > 98%. LPMC were isolated from intestinal mucosal tissue according to the method previously described by Bull and Bookman [23] and Fiocchi et al. [24] with slight modifications. After the specimen had been washed in Ca2+- and Mg2+-free HBSS (CMF-HBSS) the mucosa was carefully dissected from the underlying submucosal tissue. Small pieces of mucosal tissue of 0.5–1 cm² were incubated in 1 mM DTT (Sigma, St. Louis, MO) in CMF-HBSS to remove the mucus layer. The tissue was collected, washed extensively in CMF-HBSS and rapidly cut in smaller fragments with a pair of scissors. Four or five sequential incubations with 0.75 mM EDTA in CMF-HBSS (Merck, Darmstadt, FRG) were performed at 37 °C for 1-h periods in a Wheaton chamber to remove epithelial cells. The remaining lamina propria tissue fragments were washed twice for 15 min in culture medium, followed by overnight incubation at 37 °C in culture medium supplemented with 20 U/ml collagenase (Worthington, Freehold, MA) and 278 U/ml DNase (Worthington). The next morning the cell suspension was filtered through a nylon mesh (50 µm), washed several times, counted and analyzed for viability. This heterogenous population was further separated by density gradient centrifugation on Ficoll-Isopaque for 20 min at room temperature. The interface was harvested, washed thoroughly and the cells were counted. The average yield (mean ± SD) was 23 ± 13 x 10⁶ cells/g wet mucosal tissue with a mean viability of 92 ± 7%. When comparing the freshly isolated cells with those obtained from tissue which had been stored overnight on ice, no differences in mean viability were found.

2.3 Microscopy

From the PBMC and LPMC preparations 0.2 ml was taken, washed and resuspended in PBS with 0.2% BSA and adjusted to a concentration of 1 x 10⁴ - 2 x 10⁵ cells/ml. Cytospin preparations were made from the PBMC and LPMC suspensions, air-dried, and stained with May GrUNwald Giemsa or used for immunofluorescence staining procedures as described below.

2.4 Immunofluorescence

To determine the proportion of cells with the NK cell surface markers CD16 (FcyR) and CD56 (NCAM) in both LPMC and PBMC populations, indirect immunofluorescence studies were performed with mAb Leu-11b (anti-CD16, Becton and Dickinson, Mountain View, CA) and NKH-1A (anti-CD56, Coulter Immunology, Hialeah, FL). Direct immunofluorescence staining with PE-conjugated NKH-1A or FITC-conjugated Leu-11 in our hands appeared to be an insensitive method for detecting the number of NK cells present in LPMC cytopsin preparations. The cells were fixed with methanol for 10 min, which was found to be superior to formaldehyde, glutaraldehyde or acetone fixation when using these mAb, washed in PBS and incubated with Leu-11b (1:10) or NKH-1A (1:10) or control PBS for 30 min at room temperature, washed again in PBS followed by incubation with FITC-goat anti-mouse Ig (1:50, Nordic Immunological Laboratories, Tilburg, The Netherlands) for 45 min at room temperature. Then the slides were washed extensively for 10 min in PBS using a magnetic stirrer device, covered and counted using a Zeiss, Oberkochen, FRG, immunofluorescence microscope. From each preparation approximately 1000 mononuclear cells were counted to determine the percentage of CD16+ or CD56+ cells.

2.5 Depletion experiments

PBMC and LPMC were incubated with mAb against NK cell surface markers CD16 and CD56 or the common T cell marker CD3. Effector cells were incubated with Leu-11b, NKH-1A, OKT3 (anti-CD3, Ortho Diagnostic Systems, Raritan, NJ) or T1 (anti-CD5, Coulter Immunology) at a concentration of 2 µl mAb/10⁶ cells or with medium alone at room temperature for 45 min, followed by a 1-h incubation period at 37 °C with low-toxicity baby rabbit C (Pelfreez Laboratories, Roger, AR), final dilution 1:20. All samples were frequently agitated to improve C-mediated lysis. The cells were subsequently washed three times in medium without additives to ensure removal of C and resuspended in the same volume as control treated effector cells which had been brought to a concentration of 5 x 10⁶ cells/ml for use in the microcytotoxicity assay.
2.6 Target cells

K-562 erythroleukemia cells and Caco-2 colon carcinoma cells were used as targets in the cytotoxicity assay. The cells were cultured in 25-cm² tissue culture flasks at 37 °C in a 5% CO₂/95% air incubator. They were kept in growing phase by transferring approximately 1:5 twice a week. Caco-2 target cells were used in the cytotoxicity assay only when they had reached confluency in culture. The target cells were brought to a final concentration of 2 × 10⁴ cells/ml in their culture medium for use in the assay.

2.7 Cytotoxicity assay

Target cells were incubated with Na₂¹⁴CrO₄ (100 μCi = 3.7 MBq per 5 × 10⁶ cells, Amersham Int., GB) for 1 h at 37 °C and repeatedly gently shaken to improve labeling efficiency. PBMC were tested at an E/T ratio of 50:1, whereas LPMC were tested at a 500:1 ratio, thus correcting for the relatively low number of cells with NK activity in the gut mucosa as reported by others [3]. The high E/T ratio is frequently used in these kinds of experiments and allows studies on the contribution of different cell subsets to non-MHC-restricted cytotoxicity in the lamina propria to be accurately performed. The 4- and 18-h assays were performed essentially as described earlier [25] with both spontaneous and maximal release measured in sextuplicate and the experimental release measured in triplicate. The percentage of variation in our assay was found to be ≤ 3%. Specific cytotoxicity was calculated by the following formula:

\[
\text{Cytotoxicity (\%)} = \frac{\text{Experimental release - spontaneous release}}{\text{Maximal release - spontaneous release}} \times 100
\]

2.8 Analysis of data

Statistical analyses were performed using SPSS/PC+ (Microsoft Corp., Redmond, WA). All data are expressed as the mean ± SEM unless stated otherwise. The Student's t-test or separate variance analysis was used to determine the significance of the differences between groups.

3 Results

3.1 Cytotoxic activity of LPMC

All cell populations that were isolated showed significant levels of cytotoxicity against K-562 target cells in both 4-h and 18-h assays. No differences were found in the mean cytotoxic activity of freshly isolated LPMC (n = 20, 4 h: 14 ± 3%, 18 h: 31 ± 7%) compared with the cells obtained from mucosal tissues that were stored overnight in culture medium on ice (n = 14, 4 h: 19 ± 3%, 18 h: 36 ± 5%). The mean cytotoxic activity of LPMC suspensions against K-562 targets was 15.8 ± 2.2% in the 4-h assay and 33.3 ± 3.8% in the 18-h assay (Table 1). Furthermore, in a few cases cytotoxic activity of LPMC against Caco-2 target cells was measured which showed similar differences as obtained by using K-562 targets. The considerably lower levels of cytotoxicity against Caco-2 are in accordance with experiments using peripheral blood mononuclear effector cells where these colon carcinoma cells were found to be less susceptible to lysis. Referring to the cytotoxic activity of LPMC within the miscellaneous and carcinoma groups it is evident that there was increased cytotoxic activity in inflammatory bowel disease (IBD) which, however, was not significant. Similar differences with respect to cytotoxicity between different patient groups were observed when the Caco-2 colon tumor cells were used as targets. Table 1 also shows differences in LPMC cytotoxic activity between colon and ileum specimens in both 4-h and 18-h (p < 0.01) assays. There also tended to be a difference in the cytotoxic activity against Caco-2 cells in both 4-h (p = 0.055) and 18-h (p = 0.064) assays reflecting differences in the activity of the effector arm of the response. When the IBD group is further analyzed, the cytotoxic activity of LPMC from patients with Crohn's disease was found to be much higher in both 4-h (p < 0.01) and 18-h (p < 0.001) assays compared with those from the ulcerative colitis patients group (Table 2). Moreover, within this IBD patients group LPMC from colonic mucosa showed lower cytotoxic activity than their ileal counterparts in both 4-h (p < 0.05) and 18-h (p < 0.02) assays.

3.2 Immunofluorescence quantification of cells with NK cell markers

Only 0.9% of the total LPMC population was found to express the CD16 surface marker, whereas CD56⁺ cells showed to be less sparsely distributed and comprised 3.6% of total LPMC (Table 3). Within the PBMC population the CD16⁺ or CD56⁺ cells were found to be equally distributed, i.e. 10.9% CD16⁺ vs. 12.8% CD56⁺. In the IBD group the relative number of both CD16⁺ and CD56⁺ cells was found to be much higher compared with the carcinoma group. Because both the CD16⁺ and CD56⁺ cells were found to be similarly increased, the calculated ratio remained unchanged, indicating that the proportion of cells with NK cell markers was relatively increased in the lamina propria of IBD patients. Moreover, within the LPMC population isolated from normal ileal mucosa the relative numbers of CD16⁺ and CD56⁺ cells were found to be much higher compared with colonic LPMC.

3.3 Depletion of CD16⁺ and CD56⁺ cells from LPMC and PBMC populations

We next examined the contribution of cells bearing the CD16 or CD56 surface marker in non-MHC-restricted cytotoxicity or NK activity against K-562 target cells. In PBMC the CD16⁺ and CD56⁺ cells were almost exclusively responsible for the NK activity as measured in the Cr-release assay. Depletion of these cells almost completely abrogated NK activity in both 4-h and 18-h assays equally well by using Leu-11b or NKH-1A mAb (Fig. 1A). Mean percentage inhibition of cytotoxicity against K-562 targets by depletion of CD16⁺ or CD56⁺ cells from PBMC was approximately 90% in both assays. In contrast, depletion of CD16⁺ cells from LPMC resulted in only partial inhibition of cytotoxicity against K-562 in both 4-h and 18-h assays, whereas CD56 depletion resulted in total inhibition of activity (Fig. 1B). Mean percentage inhibition by depletion
of CD16+ cells was approximately 40% in both assays (4-h: p < 0.005, 18-h: p < 0.001), whereas depletion of CD56+ cells resulted in a considerably larger reduction of respectively 85% (4-h assay: p < 0.001) and 106% (18-h assay: p < 0.001). The mean percentage inhibition of cytotoxicity by depletion of CD16+ or CD56+ cells was not found to be different when comparing LPMC isolated from normal IBD mucosa with those from the carcinoma patients group (mean inhibition of cytotoxicity in the 4-h assay: CD16; 45% (IBD) vs. 45% (CA), CD56; 97% (IBD) vs. 90% (CA), and in the 18-h assay: CD16; 40% (IBD) vs. 53% (CA), CD56; 113% (IBD) vs. 100% (CA)).

3.4 Depletion of CD3+ cells in the lamina propria

To assess further the phenotypical nature of the cells involved in non-MHC-restricted cytotoxicity in the lamina

Table 1. Non-MHC restricted cytotoxicity of LPMC. a)

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Carcinomas</th>
<th>IBD</th>
<th>Miscellaneous</th>
<th>Colon</th>
<th>Ileum</th>
</tr>
</thead>
<tbody>
<tr>
<td>K-562 4 h</td>
<td>n = 35</td>
<td>n = 16</td>
<td>n = 12</td>
<td>n = 7</td>
<td>n = 27</td>
<td>n = 8</td>
</tr>
<tr>
<td></td>
<td>15.8 ± 2.2</td>
<td>14.4 ± 3.3</td>
<td>18.0 ± 3.3</td>
<td>15.4 ± 3.5</td>
<td>13.9 ± 2.5</td>
<td>22.4 ± 3.6</td>
</tr>
<tr>
<td>K-562 18 h</td>
<td>n = 9</td>
<td>n = 5</td>
<td>n = 3</td>
<td>n = 1</td>
<td>n = 6</td>
<td>n = 3</td>
</tr>
<tr>
<td></td>
<td>33.3 ± 3.8</td>
<td>29.8 ± 5.7</td>
<td>39.7 ± 6.7</td>
<td>30.4 ± 8.7</td>
<td>28.0 ± 4.2</td>
<td>51.3 ± 5.7</td>
</tr>
<tr>
<td>Caco-2 4 h</td>
<td>n = 7</td>
<td>n = 5</td>
<td>n = 3</td>
<td>n = 1</td>
<td>n = 6</td>
<td>n = 3</td>
</tr>
<tr>
<td></td>
<td>5.3 ± 1.7</td>
<td>2.8 ± 1.8</td>
<td>9.7 ± 2.6</td>
<td>5.0</td>
<td>3.2 ± 1.5</td>
<td>9.7 ± 2.6</td>
</tr>
<tr>
<td>Caco-2 18 h</td>
<td>n = 9</td>
<td>n = 5</td>
<td>n = 3</td>
<td>n = 1</td>
<td>n = 6</td>
<td>n = 3</td>
</tr>
<tr>
<td></td>
<td>24.4 ± 6.5</td>
<td>14.0 ± 4.9</td>
<td>41.0 ± 14.0</td>
<td>27.0</td>
<td>16.2 ± 4.5</td>
<td>41.0 ± 14.0</td>
</tr>
</tbody>
</table>

a) Mean cytotoxic activity of LPMC against K-562 erythroleukemia and Caco-2 colon carcinoma target cells in both 4- and 18-h 51Cr-release assays. Seven of the IBD patients had undergone surgery for Crohn's disease and the other five for ulcerative colitis. Patients from the miscellaneous group had undergone surgery because of diverticulitis (3), volvulus (1), familial adenomatous polyposis coli (1), colon interposition (1), and rectopexy (1).

b) Significant difference between colon and ileum specimens in the 18-h assay: p < 0.01.

Table 2. Non-MHC-restricted cytotoxicity of LPMC isolated from normal mucosal tissue of IBD patients a)

<table>
<thead>
<tr>
<th></th>
<th>Crohn's disease</th>
<th>Ulcerative colitis</th>
<th>Colon</th>
<th>Ileum</th>
</tr>
</thead>
<tbody>
<tr>
<td>K-562 4 h</td>
<td>n = 7</td>
<td>n = 5</td>
<td>n = 5</td>
<td>n = 7</td>
</tr>
<tr>
<td></td>
<td>24.9 ± 3.7**</td>
<td>8.4 ± 2.2</td>
<td>10.0 ± 3.6***</td>
<td>23.7 ± 3.9</td>
</tr>
<tr>
<td>K-562 18 h</td>
<td>55.7 ± 5.1**</td>
<td>17.2 ± 5.4</td>
<td>21.4 ± 8.4****</td>
<td>52.7 ± 6.4</td>
</tr>
</tbody>
</table>

a) Mean cytotoxic activity of LPMC from IBD patients against K-562 target cells in the 4- and 18-h 51Cr-release assays. The Crohn's disease group consisted of five ileum specimens and two ileocecal specimens. The ulcerative colitis group were all colon resection specimens except for one ileoccesal specimen. Colon mucosa was obtained from four ulcerative colitis patients and one patient with Crohn's disease, ileum mucosal specimens were obtained from Crohn's disease patients except for one specimen from a patient with ulcerative colitis.

Significant differences between the Crohn's disease and the ulcerative colitis group in the 4-h assay: * p < 0.01, and in the 18-h assay: ** p < 0.001.

Significant differences between cytotoxicity of LPMC from colon and ileum specimens within the IBD group in the 4-h assay: *** p < 0.05, and in the 18-h assay: **** p < 0.02.

Table 3. Detection of CD16+ or CD56 cells in LPMC and PBMC preparations by indirect immunofluorescence staining

<table>
<thead>
<tr>
<th></th>
<th>LPMC total</th>
<th>LPMC carcinomas</th>
<th>LPMC IBD</th>
<th>LPMC colon</th>
<th>LPMC ileum</th>
<th>PBMC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leu-11b (anti-CD16)</td>
<td>n = 14</td>
<td>n = 7</td>
<td>n = 6</td>
<td>n = 9</td>
<td>n = 5</td>
<td>n = 5</td>
</tr>
<tr>
<td></td>
<td>0.9 ± 0.2a)</td>
<td>0.6 ± 0.1</td>
<td>1.4 ± 0.3</td>
<td>0.6 ± 0.1</td>
<td>1.5 ± 0.4</td>
<td>10.9 ± 0.5b)</td>
</tr>
<tr>
<td></td>
<td>(0.2-2.3)</td>
<td>(0.2-1.1)</td>
<td>(0.4-2.3)</td>
<td>(0.2-1.1)</td>
<td>(0.4-2.3)</td>
<td>(9.0-12.0)</td>
</tr>
<tr>
<td>NKH-1A (anti-CD56)</td>
<td>n = 17</td>
<td>n = 8</td>
<td>n = 7</td>
<td>n = 11</td>
<td>n = 6</td>
<td>n = 8</td>
</tr>
<tr>
<td></td>
<td>3.6 ± 0.6</td>
<td>2.9 ± 1.0</td>
<td>4.5 ± 0.7</td>
<td>2.9 ± 0.8</td>
<td>4.8 ± 0.8</td>
<td>12.8 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>(0.7-9.3)</td>
<td>(0.7-9.3)</td>
<td>(2.0-6.8)</td>
<td>(0.7-9.3)</td>
<td>(2.0-6.8)</td>
<td>(7.3-15.3)</td>
</tr>
<tr>
<td>Ratio CD16+/CD56+</td>
<td>n = 36</td>
<td>n = 30</td>
<td>n = 36</td>
<td>n = 34</td>
<td>n = 41</td>
<td>n = 98</td>
</tr>
<tr>
<td></td>
<td>0.36 ± 0.11c)</td>
<td>0.39 ± 0.20</td>
<td>0.36 ± 0.11</td>
<td>0.34 ± 0.16</td>
<td>0.41 ± 0.13</td>
<td>0.98 ± 0.13</td>
</tr>
</tbody>
</table>

a) Proportion of LPMC positive for the CD16 or CD56 NK cell surface marker, expressed as percentage of a total of approximately 1 x 10^8 mononuclear cells counted.
b) Proportion of PBMC from healthy volunteers positive for the CD16 or CD56 marker. Detection by immunofluorescence staining of cytospin preparations; number of individuals screened as indicated, values are given as mean ± SEM (range).
c) Mean CD16/CD56 ratio of all individuals screened for both markers.
propria we performed depletion of CD3+ lymphocytes, a population of cells known to contribute to this type of cytotoxicity in peripheral blood. Depletion of LPMC expressing the CD3 antigen of the TcR complex did not result in alteration of cytotoxicity (Fig. 2), whereas depletion of CD56+ cells in the same cell suspensions resulted in complete inhibition of cytotoxicity (4-h: \( p < 0.05 \), 18-h: \( p < 0.02 \)). Intriguingly, the extended 18-h assay depletion of CD3+ T lymphocytes did not alter cytotoxic activity of LPMC against the NK-sensitive K-562 target cells. Trypan blue dye exclusion of the OKT3 and C-treated population revealed that 34 ± 3% of total LPMC was killed which corresponds with the proportion of T lymphocytes present in the lamina propria. Similar results were obtained when, alternatively, mAb T1 (anti-CD5) was used to deplete T cells from LPMC preparations (data not shown).

**4 Discussion**

In this study it is demonstrated that considerable NK cell activity against both K-562 and Caco-2 target cells can be measured in LPMC isolated from normal mucosa of IBD and carcinoma patients. Moreover, this study describes the relative contribution of cells with the CD16 or CD56 surface marker to intestinal NK activity by depletion experiments. Interestingly, overnight storage of mucosal tissue did not influence cytotoxic activity and might facilitate the experimental assessment of NK activity in resection specimens. From depletion experiments with anti-CD16 and anti-CD56 mAb it can be concluded that cells involved in non-MHC-restricted cytotoxicity in the lamina propria are of different phenotype, i.e. predominantly CD56+ and only partly CD16+, than their peripheral blood counterparts which are mainly both CD16+ and CD56+. Such an observation on the presence and relative functional contribution of cells from the lamina propria with the CD16 or CD56 marker to non-MHC-restricted cytotoxicity has not been reported earlier. Depletion of CD16+ or CD56+ LPMC in various patient groups revealed that within normal mucosa the relative contribution of these cells to NK activity was not disease dependent because the percentage inhibition of cytotoxicity by depleting cells expressing CD16 or CD56 did not differ between IBD and carcinoma patients. The observed differences in cytotoxic activity of LPMC between IBD and carcinoma patients, therefore, are due to differences in numbers of NK cells rather than to a shift in NK cell subsets. Moreover, CD6 depletion of LPMC resulted in abrogation of cytoxicity in both 4- and 18-h assays, indicating that even in the prolonged assay the lytic activity is mediated by cells expressing the CD56 adhesion molecule. The importance of intestinal CD56+ cells was previously shown by Shanahan et al. [18]. Using panning techniques they had been able to demonstrate the cytotoxic capacity of intestinal CD56+ cells at very low E/T cell ratios. However, they did not unravel the relative contribution of CD16+ or CD56+ cells.
to cytotoxicity and found intestinal CD16+ cells to be absent by phenotyping. Selective depletion of cells with the CD16 surface marker in our study resulted in only partial reduction of cytotoxicity independent from the underlying disease. In similar experiments of CD16 depletion Gibson and Jewell [19], also found reduction of cytotoxicity by depletion of NK cells and although percentual inhibition was found to be higher, approximately 70%, it differed between preparations from different intestinal origin, and was much lower than we observed by using anti-CD56 mAb. The CD16 marker, therefore, seems to be less adequate in detecting NK cells in LPMC preparations compared with the CD56 adhesion molecule. Our observations made on NK cells in the lamina propria contrasted with the characterization of these cells in peripheral blood as shown by Lanier et al. [22]. They found that cells simultaneously expressing CD16 and CD56 almost exclusively mediate non-MHC restricted cytotoxicity. Interestingly, they also found a rather small subset of T cells with CD3+CD16-CD56+ phenotype capable of mediating non-MHC-restricted cytotoxicity in peripheral blood and, according to the differences in these marker expressions, they suggested the presence of three types of cytotoxic lymphocytes [26]. When evaluating the contribution of these non-MHC-restricted T cells to cytotoxicity by LPMC we found these cells not to be important. Depletion of CD3+ cells from LPMC preparations did not result in alteration of cytotoxicity. More importantly, the cytotoxic activity of LPMC as measured in the extended 18-h assay was also found to be independent of the presence of these T cells which was also confirmed by depletion of CD5+ T cells. Verification of the amount of cells killed by C lysis with anti-CD3 revealed that approximately one third of LPMC were lysed which represents the percentage of T cells present in the lamina propria as reported by others [4, 21]. However, lamina propria T cells can be involved in non-MHC-restricted killing by pretreating them with anti-CD3 antibodies as shown by Shanahan et al. [27]. In our view, their study describes another mechanism of cell-mediated cytotoxicity than the spontaneous killing of tumor cells, we report here, since they mimicked the in vivo activation of T cells primed by environmental antigenic stimuli.

In accordance to their functional detection we found that cells with the CD56 surface marker outnumber the CD16+ cells within the lamina propria. CD56+ cells comprise about 3.6% of total LPMC, whereas only 0.9% were found to express the CD16 antigen. In most studies reported so far NK cells in the lamina propria of the gut have been found to be absent or sparsely present when using anti-CD57 (Leu-7) or anti-CD16 antibodies. When a more specific mAb like NKH-1 was used, the number of NK cells present was found to be much higher. However, whereas others could only detect the NK cell CD56 surface marker on lamina propria lymphocytes by FCM [18] or both CD16 and CD56 in LPMC but only at very low percentages [21], we found both markers to be commonly present. The low number of cells with the CD16 marker possibly reflects low surface antigen expression and might indicate the presence of a special NK cell subset in the lamina propria. Different NK cell subsets in the peripheral blood with respect to their CD16 and CD56 expression have been described by Lanier et at. [22]. In addition, this group found low CD16 expression to be associated with low cytotoxic activity but high proliferative capacity of NK cells [28]. Moreover, they showed these cells to be capable of presenting the CD44 adhesion molecule, a structure involved in particular in adherence to vascular endothelium and migration into the extravascular tissue. Thus, there might be selective compartmentalization of NK cell subsets with low CD16 and high CD56 expression in the lamina propria. Such a phenomenon of selective homing of NK cells in the gut mucosa has been proposed earlier by Gibson et al. [29].

NK activity was found to be higher in LPMC isolated from normal mucosa of IBD patients compared with the carcinoma patients group, the latter showing similar levels of activity to those of the miscellaneous group. There is no decisive answer yet whether NK activity in IBD is disease related or depends on the origin of the tissue. A similar observation in this respect was made by Hirata et al. [4] by immunofluorescence detection using the former NK cell marker CD57. In general, functional evaluation of NK activity was done using inflamed IBD mucosa compared with normal mucosa from carcinoma patients which made it inaccurate to discuss differences between normal and diseased mucosa. Becken et al. [30] found high levels of cytotoxicity in both normal and inflamed mucosa in Crohn's disease compared with LPMC isolated from mucosa from carcinoma patients. Our group earlier reported decreased cytotoxicity of LPMC isolated from the tumor lesion compared with adjacent normal mucosa [31].

From our studies presented here, it can be concluded that normal mucosa in IBD reveals higher levels of cytotoxicity which is accompanied by higher numbers of cells positive for the CD16 or CD56 surface marker. Therefore, the increased cytotoxicity we measured could not be attributed to a larger influx of inflammatory cells in IBD tissue [32], because equal numbers of LPMC in the assay were used, but rather to the increased proportion of NK cells, especially in Crohn's disease. Inevitably, normal IBD mucosa more frequently involves small bowel tissue, whereas normal mucosa from carcinoma patients usually originates from the large bowel region. Differences in cytotoxic activity in normal mucosa, therefore, may be caused by distinct distribution of NK cells due to tissue characteristics. Currently, studies are being performed to dissect whether the increased NK cell numbers and activity in IBD mucosa is tissue or inflammation dependent, applying the sensitive and more specific CD56 NK cell marker.

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5 References

CD56+ cells and non-MHC restricted cytotoxicity in the gut mucosa