Membrane-associated il-10 on natural killer cells

Regulatory subsets of natural killer (NK) cells producing IL-10 have been recently described. We have shown that IL-10 secretion by NK cells was significantly higher in healthy donors compared to cancer patients. We also detected the NK cell secretory function using the alternative method by flow cytometry in IL-10-Detection Assay. In this case, the difference between the IL-10 production of healthy individuals and cancer patients was negligible. We hypothesized that IL-10 Detection Assay detects the presence of membrane-associated form of IL-10 on the surface of NK cells.

Introduction

NK cell-mediated killing activity is known to play a major role in tumor rejection. However, a recent report has shown that NK cells infiltrating human nonsmall-cell lung cancer produce cytokines rather than directly kill tumor cells [1]. Now there is growing evidence that NK cells exhibit regulatory (suppressive) functions [2-6]. *In vitro* generation of non-cytolytic regulatory NK cells secreting IL-10 has been reported recently [7]. The ability of NK cells to secrete constitutively IL-10 has been shown in freshly isolated NK cells from HCV patients [8]. Suppressive influence of IL-10-secreting NK cell subset on Ag-specific T cell proliferation has also been demonstrated [9]. The role of cytokine producing NK cells in tumor development remains to be elucidated.

Moreover the *in vitro* generated subset of non-cytolytic NK cells showed expression of IL-10 like a membrane-associated form, similarly to what reported in human monocytes [7].

Materials and Methods

Purification of NK cells

Peripheral blood samples were collected from 8 healthy donors and 9 patients with different types of cancer (lung cancer, colorectal cancer, cervical carcinoma, esophageal carcinoma and uterine carcinoma). Peripheral blood mononuclear cells (PBMC) were isolated from peripheral blood by Hystopaque-1077 (Sigma-Aldrich, USA) density gradient centrifugation. NK cells were negatively selected using the NK isolation kit (Miltenyi Biotech) by immunomagnetic cell separation (Mini MACS, Vario MACS; Miltenyi Biotech, Germany). The purity of the cell subsets was confirmed by flow cytometry (FACSCalibur, BD Biosciences, Germany).

IL-10 Detection assay

IL-10 Detection assay was performed using the IL-10 Secretion Assay detection Kit (Miltenyi Biotech, Germany) according to the manufacturer's instruction. Purified unstimulated NK cells were labeled with IL-10 catch reagent for 5 min on ice, and subsequently incubated for 45 min at 370C. IL-10 secretion was detected by flow cytometry using IL-10 specific antibody conjugated to phycoerythrin (PE).

Cell fixation was performed with paraformaldehyde according to the manufacturer's instruction (BD Biosciences, Germany).

Results and discussion

Earlier we have reported that IL-10 secretion by NK cells as revealed in ELISPOT-analysis in cancer patients was significantly decreased compared to healthy individuals [10].

To confirm our results we detected the NK cell secretory function in IL-10 Detection Assay by flow cytometry. Freshly purified NK cells from healthy individuals and cancer patients were incubated with capture antibody (catch reagent) and secreted IL-10 was captured on their surface. The caught cytokine on NK cell surface was stained with PE-labeled anti-IL-10 antibodies and analyzed by flow cytometry. But in this case, the difference between the IL-10 positive NK cells of healthy individuals and cancer patients was negligible (Fig.1).

Therefore, using two different methods, we received contradictory results. Other researchers have also noted the discrepancy between the two methods [9]. We hypothesized that the IL-10 Secretion Assay detection Kit detected the presence of membrane-associated form of IL-10 on the surface of NK cells. To confirm our suggestion, we fixed NK cell membrane with paraformaldehyde. Fixation of membrane leads to blocking IL-10 secretion but allows detecting membrane-associated IL-10 after addition of anti-IL-10-PE in absence of the catch reagent. To except intersection with receptors to IL-10 on NK cells, freshly purified NK cells were treated consequently with paraformaldehyde, recombinant IL-10, anti-IL-10-PE and analyzed by flow cytometry.

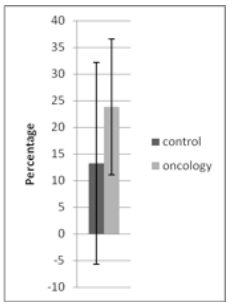

Figure 1. The average number of the CD56/IL-10 positive cells in peripheral blood from 8 healthy donors and 9 cancer patients.

Received results indicated that the IL-10 Secretion Assay detection Kit may reveal membrane-associated forms of IL-10 (Fig.2).

Therefore, our study has demonstrated that both in healthy donors and cancer patients, NK cells have membrane form of IL-10 on their surface. Whether this form of IL-10 is bound to specific membrane receptors or it is a trans-membrane protein remains to be determined. The possible existence of IL-10 receptor-associated form may be consistent

with observation that exogenous IL-10 increase surface staining of NK cells.

Our study raises questions about the role of the IL-10 associated with NK cell membrane in their immune regulatory activity.

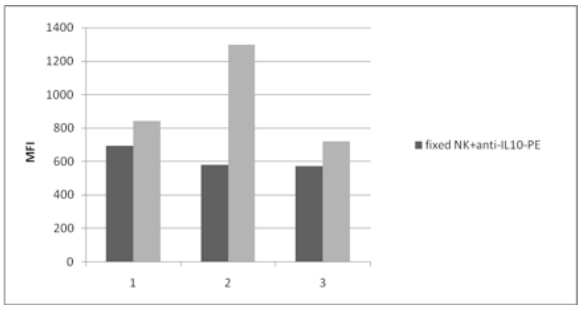

Figure 2. Three representative results of mean fluorescent intensity (MFI) analysis of NK cells from healthy donors are shown. Black bars show the presence of membrane-associated IL-10. Gray bars show summary expression of mem-brane-associated form of IL-10 and IL-10R on the surface of NK cells.

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NK-клеткаларының мембранаға жалғасқан IL-10

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Осы бір қатар жұмыстармен IL-10–ды бөліп шығаратын реттеуші тәбиғи киллерлік клеткалар популяциясының бар екені дәлелденді. Бұдан бұрын онкологиялық аурулардағы NK-клеткалардың IL-10-ды секрециялауы көп мөлшерде төмендегенін көрсеткенбіз. Жұмыстың мақсаты IL-10-ның коммерциялық жинағы secretion Assay detection Kit-ті қолдана отырып ағынды цитофлуориметрия әдісімен алынған бұрынғы нәтижелерді айқындау болды. Біз сау донорлар мен онкологиялық аурулардың NK-клеткалары бөліп шығаратын IL-10 өнімдерінің арасындағы айырмашылықтың көп емес екенін көрсеттік. Біз IL-10 Secretion Assay detection kit NK-клеткаларының бетіндегі мембранаға жалғасқан IL-10-ды анықтай алады деп болжадық.

Мембран-ассоциированный IL-10 на натуральных киллерных клетках

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Рядом работ доказано существование популяции регуляторных натуральных киллерных клеток (NK), продуцирующих IL-10. Ранее нами было показано, что секреция IL-10 NK-клетками значительно снижена у онкологических больных. Целью работы было подтверждение полученных ранее результатов методом проточной цитофлуориметрии с использованием коммерческого набора IL-10 Secretion Assay detection Kit. Однако разница в продукции IL-10 NK-клетками здоровых доноров и онкологических больных была незначительной. Мы предположили, что IL-10 Secretion Assay detection Kit может определять мембран-ассоциированную форму IL-10 на поверхности NK-клеток.

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Теги: membrane-associated form of IL-10, regulatory natural killer cell, мембран-ассоциированная форма IL-10, мембранаға жалғасқан IL-10, регуляторные натуральные киллерные клетки, реттеуші тәбиғи киллерлік клеткалар