Natural Killer Cell Line YT Exerts Cytotoxicity Against CD86+ Myeloma Cells

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**Abstract.** Background: Human NK cell lines providing an unlimited source of effector cells might be suitable for use in adoptive immunotherapy. This study determined the cytolytic activity of the human NK-like cell line YT against myeloma cell lines and primary myeloma cells. Materials and Methods: Lysis of the myeloma cell lines MM1S and U266 and of primary human myeloma cells by YT was measured using a flow-cytometric cytotoxicity assay. Furthermore, it was investigated whether the cytotoxicity correlates with the expression of CD86 on myeloma cells and the effect of different doses of IL-2 on cytolysis was tested. Results: YT showed killing of myeloma cell lines and primary myeloma cells. The extent of cytolysis correlated with the expression of CD86 on myeloma cells and was not augmented by pre-incubation of YT with high dose of IL-2. Conclusion: The human NK-like cell line YT could be useful in immunotherapy of patients with CD86+ multiple myeloma.

Multiple myeloma (MM) is a B-cell neoplasia in which malignant plasma cells accumulate in the bone marrow and secrete large amounts of a monoclonal antibody. Despite significant progress in the treatment of MM due to improved efficacy of autologous and allogeneic stem cell transplantation and introduction of the proteasome inhibitor bortezomib and the immunomodulatory drugs thalidomide, lenalidomide and dexamethasone, a large percentage of MM patients unfortunately experience relapse (1-3). Thus, treatment of MM awaits the development of additional therapeutic options including immunotherapeutic strategies.

The susceptibility of myeloma cells to natural killer (NK) cell-mediated lysis has been shown *in vitro* and *in vivo* (4-6). However, autologous or allogeneic NK cells are difficult to maintain and to expand *ex vivo* on the large scale necessary for adoptive immunotherapy (AIT) (7). Furthermore, the high doses of systemically administered interleukin (IL)-2 that are required for the activation and expansion of NK cells *ex vivo* and after transfer *in vivo* expand also regulatory T (Treg) cells which are able to suppress NK cell activity (8-10). Instead, human NK cell lines, which provide an unlimited source of NK effector cells with stable phenotype, may be suitable for use in AIT (11-15). The human NK-like leukaemia cell line YT was established from cells in the pericardial fluid of a patient with acute lymphoblastic lymphoma (ALL) and thymoma (16). YT cells were described as having their T-cell receptor (TCR) genes in germline configuration being also CD3-, CD16-, CD56+ CD57- and to exert cytotoxicity against CD80/CD86-expressing T and B lymphoblastic cell lines via a CD28-mediated mechanism (16-19). Myeloma cells and plasma cells from healthy donors lack expression of CD86 (20). However, malignant plasma cells show strong variation in the expression of CD86, which is associated with poor prognosis and is absent from or weakly expressed on non-malignant plasma cells (20-22). Malignant and healthy plasma cells of the bone marrow express syndecan-1 (CD138) on their surface but typically differ by the absence or presence of CD19, respectively (23). This study tested the cytotoxic activity of the NK-like killer cell line YT against human primary myeloma cells and myeloma cell lines *in vitro* and determined whether cytolysis correlated with the expression of CD86 on the tumor cells.

**Materials and Methods**

*Primary myeloma cells and cell lines.* Primary myeloma cells were isolated from the bone marrow of a patient with MM after informed consent was given. The myeloma cell lines MM1S and U266 were kindly provided by Dr. Sezer, Charité-Universitätsmedizin Berlin, Germany and the cell line YT was a kind gift from Junji Yodoi, Kyoto University, Kyoto, Japan. All cell lines and primary cells

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were maintained in RPMI-1640 medium supplemented with 10% fetal bovine serum (both from Biochrom AG, Berlin, Germany) and 100 U/ml penicillin and 100 μg/ml streptomycin (Biochrom AG). Maintenance of YT cells required addition of 5-10 U/ml human recombinant IL-2 (Proleukin, Chiron) to the medium.

Isolation of primary myeloma cells. Mononuclear cells were isolated from freshly prepared bone marrow of a patient with MM by ficoll density gradient centrifugation followed by enrichment of CD138⁺ cells using antibody-conjugated magnetic microbeads in accordance to the manufacturer's instructions (Miltenyi, Bergisch-Gladbach, Germany). The purity of primary myeloma cells was determined by flow cytometry using a PE-labeled anti-CD138 antibody (Miltenyi Biotec) and a FITC-labeled anti-CD19-antibody (Jackson ImmunoResearch, Baltimore, USA).

Phenotypical analysis of myeloma cells. The expression of B7-molecules on myeloma cells and on YT cells was determined by flow cytometry using a FACScan (Becton-Dickinson, Heidelberg, Germany). FITC-labeled anti-human CD80 (clone 37711.111) and CD86 (37301.111) antibodies were obtained from R&D Systems (Frankfurt, Germany). YT cells were confirmed to express CD28 as described by others (18) using a biotin-conjugated anti-human CD28 (CD28-2) antibody from eBioscience (data not shown). The biotinylated antibody was counterstained with DTAF-labeled streptavidin (Jackson ImmunoResearch, Baltimore, USA).

Flow cytometric cytotoxicity assay. The cytotoxicity of YT cells pre-incubated with a low dose (10 U/ml) or high dose (1000 U/ml) of IL-2 over one week was determined by a flow cytometric based assay. In brief, 1x10⁶ target cells were stained with 0.1 μM carboxyfluorescein diacetate succinimidyl ester (CFDA-SE) in PBS over 5-10 min; 5x10³ labeled target cells were incubated with YT effector cells for four hours in 200 μl complete medium in triplicates and at E:T ratios as indicated. Subsequently, the percentage of dead target cells was determined by flow cytometry using a FACscan after staining the cells with 2 μg/ml propidium iodide (PI).

Statistics. Data are presented as mean±the standard error of the mean (SEM). Analysis of flow cytometric data was performed using the CellQuest software version 4.0.2 (BD Biosciences, Heidelberg, Germany).

Results

YT cell-mediated cytolysis of primary myeloma cells and myeloma cell lines. YT cells treated with a low dose of IL-2 showed moderate cytotoxicity against the myeloma cell line MM1S and exerted less cytolytic activity against U266 (Figure 1A). Pre-treatment of YT cells with a high dose of IL-2 did not increase their cytotoxicity against the myeloma cell lines (Figure 1B). Primary myeloma cells which were enriched from bone marrow cells to a purity of 73% were lysed by YT to a similar degree as U266 (Figure 1 C, D).

Expression of CD80/CD86 on myeloma cells. The bone-marrow-derived CD138⁺ plasma cells of the patient expressed CD86 at very low density and lacked expression of CD80 (Figure 2A, B). MM1S showed moderate expression of CD80, whereas U266 exhibited low expression of this surface molecule. Both myeloma cell lines were negative for expression of CD80 (Figures 2C-F).

Discussion

Recent studies revealed that bone-marrow-derived myeloma cells are sensitive to NK cell-mediated cytolysis (4-6). Therefore this study tested whether the human NK cell-like cell line YT shows cytolytic activity against myeloma cell lines and primary myeloma cells in vitro. Indeed, YT cells exerted moderate killing of MM1S cells and lower cytotoxicity against U266 cells and CD19⁺CD138⁺ plasma cells freshly isolated from the bone marrow of a patient with MM. Myeloma cells lack expression of CD80 but show different amounts of CD86 on their surface as described by others and observed in this study (20-22). Furthermore, the extent of CD86 surface expression was recently shown to be associated with the pathophysiology of myeloma, including enhanced growth rate, induction of immune-suppressive CD4⁺ T-cells and shorter survival time of patients (21, 22). In accordance to the described MHC-non-restricted cytotoxicity via interaction of CD80 with CD86 on YT cells with CD80/86 on T and B lymphoma and leukemia cells, the cytosis correlated with the extent of the expression of CD86 on the surface of the myeloma cells (18, 19). Similarly, the myeloma cell line RPMI-8226, which expresses low levels of CD86, was shown to be almost resistant to YT-mediated lysis (18), whereas the CD80⁺CD86⁺⁺ ARH-77 cell line which was reportedly strongly killed by YT was meanwhile re-classified as a B-lymphoblastoid and not as a myeloma cell line (16, 24-26).

NK cell cytotoxicity is significantly increased after treatment with larger amounts of IL-2 (27, 28). Thus, this study determined the effect of a pre-treatment of YT with a high dose of IL-2 on the lysis of myeloma cell lines. However, YT cell cytotoxicity was not increased by IL-2, which could be due to the CD28-mediated killer mechanism shown to be executed in the absence of IL-2 (18, 19). Moreover, YT cells reportedly express the IL-2 receptor (IL-2R) α-chain (CD25) only weakly or lack its expression and, in contrast to primary NK cells, do not up-regulate CD25 in response to IL-2 which obviates the generation of the trimeric high affinity IL-2R (16, 29, 30). Similarly, the growth rate of YT cells was shown not to be increased after incubation with high doses of IL-2 (30). Nevertheless, the independence of YT cell killer activity from significant amounts of exogenous IL-2 might be advantageous compared with the need for high doses of the cytokine for activation and expansion of autologous or allogeneic NK cells before and after adoptive transfer, which is associated with the expansion of NK cell inhibitory Treg cells and...
induction of a vascular leak syndrome (8-10, 28, 31-34). In fact, YT cells were shown to provide a constitutively activated killer phenotype due to production of significant amounts of cytotoxic molecules, such as perforins and granzymes, and expression of the apoptosis-inducing Fas-ligand (35). Moreover, the absence of surface-expressed killer immunoglobulin-like receptors which regulate recognition and lysis of tumor cells by NK cells on YT cells may enable therapeutic usage of the NK-like cell line independently from the MHC class-I repertoire of the recipient (36-38).

Taken together, this study provides evidence that human myeloma cells are sensitive to YT cell-mediated cytolysis dependent on the expression of CD86 on tumor cells. Furthermore, the cytotoxicity of YT cells is not improved by treatment with a large dose of IL-2.

References


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