Monocyte- and neutrophil-mediated lysis of SCCL by a bispecific molecule comprised of Lys^3-BN and mAb22

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Introduction

Small cell carcinoma of the lung (SCCL) grows rapidly, metastasizes early and eventually causes the death of about 30,000 people per year in the U.S.A. Various combinations of chemotherapy have been used with little improvement in the long-term survival rate. For these reasons recent efforts have focused on developing other therapeutic strategies for the treatment of SCCL, such as immunological and hormonal therapy [1], and intensive chemotherapy with autologous bone marrow or peripheral stem cell support [2]. Most human SCCL cell lines produce gastrin releasing peptide (GRP), which is similar in action and sequence to bombesin (BN), a peptide from frog skin. These SCCL cell lines produce GRP and express a single class of high-affinity receptors for BN/GRP [3].

As a result of BN/GRP receptors having limited distribution in the body, these receptors could serve as targets for directing specific immune reactions. We have developed a novel approach of immunotherapy using the BN/GRP receptors expressed on SCCL cells as targets. We have made a bispecific molecule (immunoconjugate, IC) between Lys^3-BN and a monoclonal antibody (mAb22) [4] against the human high-affinity Fc gamma receptor (FcγRI, CD64), which is expressed on the surface of human monocytes (Mo) and IFNγ-activated polymorphonuclear neutrophils (PMN). We hypothesized that this IC should be able to redirect these immune effector cells towards SCCL cells and, thus, elicit specific antibody-dependent cell-mediated cytotoxicity (ADCC) of the carcinoma cells.

Results and Discussion

The IC, mAb22-Lys-BN, was obtained by the reaction of Lys^3-BN with *N*-succinimidyl *S*-acetylthioacetate (SATA). The Lys^3-BN-SATA conjugate was purified by reversed phase HPLC. Deacetylation to the free sulfhydryl, Lys^3-BN-SH, was accomplished with hydroxylamine (NH_2OH) and purification was performed by HPLC. The antibody mAb22, or its F(ab')$_2$ fragment (Medarex, Inc.), was reacted with sulfosuccinimidyl 4-(*N*-maleimidomethyl)-cyclohexane-1-carboxylate (Sulfo-SMCC, Pierce) to produce a maleimide-containing Ab. The final conjugation of Lys^3-BN-SH with the maleimide-containing Ab was effected by mixing equimolar amounts overnight at RT.

The ability of two immunoconjugates, mAb22-Lys-BN and F(ab')$_2$-Lys-BN, to bind to four SCCL cell lines (NCI-H69, H345, SHP-77, and DMS273) was determined by flow cytometric analysis, using the indirect staining method. The binding was directly proportional to the amount of IC used to stain the cells. This was manifested both by an increase in the absolute percentage of cells stained positively and by an augmentation

of the mean fluorescence intensity (MFI) of the entire cell population. In general, the IC prepared between the whole antibody, mAb22, and Lys^3-BN had a higher MFI than that prepared between the $F(ab')_2$ fragment and Lys^3-BN.

ADCC assays used target (T) SCCL cells that were incubated with ^{51}Cr. The effector (E) cells, Mo or PMN, were obtained from different donors and activated by prior incubation with rIFNγ. The cytotoxic potencies varied among donors. In all four SCCL cell lines, more than 80% of the cells were lysed by Mo from different donors. Tumor cell lysis was primarily dependent on the E/T ratio used for each donor. The greatest lysis, with either Mo or PMN, was consistently achieved at an E/T ratio of 100:1. About 80% of SHP-77 cells were lysed by PMN from two different donors, while with the H69 cells the lysis was about 45%. Since PMN do not express FCγRI on their cell surface without rIFNγ stimulation, non-stimulated PMN had much less cytotoxicity on target cells.

Mo-mediated cytotoxicity was studied under different assay conditions in each of the four SCCL cell lines. When the target cells were incubated with activated effector (Mo) cells in the absence of IC, about 15-60% tumor cell lysis was observed, depending upon donor. The addition of mAb22 did not cause any further cell lysis. However, the addition of IC (mAb22-Lys-BN) resulted in an increase in tumor cell lysis to about 50-95%. The IC-induced SCCL cell lysis could be significantly ($p < 0.05$) blocked by adding an excess amount of either mAb22 or Lys^3-BN. Similarly, with two SCCL lines, PMN IC-induced tumor cell lysis could be significantly blocked by adding an excess amount of either mAb22 or Lys^3-BN.

Conclusions

We have produced a novel IC between mAb22 and Lys^3-BN, which binds to BN/GRP receptors on the cell surface of four SCCL cell lines and to FcγRI on both human Mo and PMN. As hypothesized, IC mAb22-Lys-BN can direct immune effector cells towards target tumor cells and elicit a specific ADCC, which leads to the lysis of target SCCL cells. This cytotoxicity is dependent on E/T-cell ratios, can be induced in a wide range of IC concentrations, and can be blocked by the addition of an excess amount of either parental molecule, mAb22 or Lys^3-BN.

References

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