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Selective triggering of the APO-1/CD95 death receptor with bispecific antibodies

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Like many other cell surface receptors the CD95 (APO-1/Fas)-molecule needs to be crosslinked by its physiological ligand or by immobilized or multimeric antibodies to mediate biological activity, that is induction of apoptotic cell death. Monomeric CD95-antibodies of the IgG2a or IgG1 subtype block rather than induce apoptosis. Such antibodies hybridized to a second antibody directed against a different target antigen on the same cell effectively induces apoptosis of the cells if the expression of the target antigen exceeds a certain threshold level. It appears that this effect is due to „bicellular binding" of bispecific antibodies resulting in „mutual crosslinking" of the CD95 death receptor and of the target antigen. Using bispecific reagents it may therefore be possible to restrict the activation of death receptors to a given target site, e.g. a tumor. In general terms, these findings illustrate a principle according to which the triggering of a cell surface receptor (CellSR) may be confined to a given target cell using bispecific reagents with Target X CellSR-specificity.

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Therapeutic Bispecific Antibodies.

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Antibody-based therapy of human cancers has led to several remarkable outcomes, particularly in the therapy of breast cancer and lymphoma. Many solid tumors have proven less responsive, due in part to difficulties in the tumor-selective delivery of antibodies and potential cytolytic effectors. Our work has focused on initiating anti-tumor immune responses using bispecific antibodies and other engineered antibody-based proteins. However, this has not led consistently to inflammation at tumor sites. We have approached the problem in several different ways. The first approach involved the use of antibody-bacterial superantigen fusion proteins; we found that fusion proteins containing native staphylococcal enterotoxin A were unacceptably toxic due to the extraordinary potency of this superantigen. More recently, we have created and tested an immunochemotaxin fusion protein containing an antibody single-chain Fv fragment targeting HER2/*neu* and the human complement fragment, C5a, to provide a chemotactic signal to induce tumor-directed inflammation by neutrophils and mononuclear phagocytes. This inflammation can then be redirected to lyse or phagocytose tumors through other immunological reagents such as bispecific antibodies. The molecule retains both anti-HER2/*neu* binding capacity and promotes in vitro neutrophil migration and degranulation. Animal studies are testing the ability of the fusion protein to induce the migration of inflammatory effector cells to tumor sites in immunodeficient mice. The ability to readily induce tumor inflammation through antibody targeting should facilitate diverse immunotherapy strategies.

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Induction of a long-lasting anti-tumor immunity by a trifunctional bispecific antibody

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Bispecific antibodies (bsAb) can efficiently mediate tumor cell killing by redirecting pre-activated or costimulated T-cells to disseminated tumor cells especially in a minimal residual disease situation. Here, we demonstrate that the trifunctional bispecific antibody BiLu (anti-CD3Xanti-EpCAM) is able to kill tumor cells very efficiently without any additional costimulation of effector cells *in vitro* and *in vivo*. Remarkably, this bsAb also induces a long-lasting protective immunity against the targeted syngeneic mouse tumors (B16 melanoma and A20 B-cell lymphoma, respectively). We observed a strong correlation between the induction of a humoral immune response with tumor-reactive antibodies and the survival of mice. This humoral response was at least in part tumor-specific as shown in the A20 model by the detection of induced anti-idiotype antibodies. Notably, the detected anti-Id response demonstrate that trifunctional bsAb can induce potent anti-tumor responses against antigens not bound by the bsAb directly. Both, the survival of mice and anti-tumor titers were significantly diminished when $F(ab')_2$-fragments of the same bsAb were applied, demonstrating the importance of the Fc region in this process. Using T-cell depletion, we could also demonstrate a contribution of a cellular anti-tumor response. These results reveal the necessity of the Fc region of the bsAb with its potent Ig subclass combination mouse IgG2a and rat IgG2b. The antigen presenting system seems to be crucial for achieving an efficient tumor cell killing and induction of long-lasting anti-tumor immunity. Hereby, the recruitment and activation of accessory cells by the intact bsAb is essential.