

INTERVIEW about**Bispecific antibody-mediated immunotherapy: cross-linking tumor cells via CD55**

with

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What is the effect of membrane-bound complement regulatory proteins in mAb mediated immunotherapy of cancer?

A. Gorter and K.A. Gelderman: Treatment of cancer patients using monoclonal antibody (mAb)-mediated immunotherapy of cancer has become increasingly successful over the last decade. Immunotherapy is based on the expression of antigens on the tumor cell surface that are absent or expressed at a much lower level on normal cells. Upon binding of (immunotherapeutic) mAb to the tumor cell membrane, activation of the complement system can occur. The complement system is composed of about 30 (mostly) serum proteins. Activation can occur via three different pathways. Antigen-antibody complexes on the tumor cell surface activate the classical pathway initiating an enzymatic cascade that results in the release of chemoattractants (C3a, C5a), membrane deposition of activated complement proteins (e.g. C3b), and pore formation in the (tumor) cell membrane. A split product of C3b, C3bi, is a ligand for complement receptor 3 (CR3) expressed by neutrophils, macrophages and NK cells, thus enhancing or triggering these cells to kill tumor cells via several mechanisms including complement-

dependent cellular cytotoxicity (CDCC) (1). The complement system is a non-discriminatory defense system and therefore host cells need protection from complement-mediated injury by soluble and membrane-bound complement regulatory proteins (mCRP; CD46, CD55 and CD59). CD46 and CD55 act early in the enzymatic cascade. CD55, the major classical pathway mCRP, inhibits the generation of C3b, the generation of the inflammatory mediators C3a and C5a, and subsequent steps such as pore formation, whereas CD59 acts late in the enzymatic cascade and only inhibits pore formation. Tumor cells often overexpress mCRP. This suggests that overexpression of mCRP is advantageous to prevent elimination of the tumor cells by the immune system. Numerous studies have shown that overexpression of especially CD55 and CD59 hampers the efficacy of mAb-mediated lysis of tumor cells (1,2). Furthermore, a number of mAb-mediated immunotherapy studies have reported a positive association between the expression of mCRP on tumor cells and resistance to immunotherapeutic treatment.

How can therapeutic bispecific antibodies target tumor antigens and simultaneously block mCRP?

A. Gorter and K.A. Gelderman: To increase the efficacy of mAb-mediated immunotherapy, mCRP activity on tumor cells should be inhibited. This should be achieved in a tumor-specific fashion; since the majority of

cells in the body express mCRP uncontrolled complement activation must be prevented. To achieve this, bispecific antibodies that recognize both a tumor antigen and a major mCRP were developed. The anti-tumor anti-

gen arm directs the bispecific mAb to the tumor cells, whereas the other arm blocks the most important mCRP at the tumor cells surface. Preferential homing of the bispecific mAb can be further accomplished by using a high affinity anti-tumor arm and a medium/low affinity anti-mCRP arm, thus minimizing binding of the anti-mCRP arm to normal cells. Are these bispecific antibodies superior to their

parental mAb? We have shown that bispecific mAb directed against human CD55 and a colorectal cancer tumor associate antigen (Ep-CAM) or bispecific mAb directed against human CD55 and a renal cell carcinoma antigen (G250) are able to increase C3b deposition, CDCC and complement-mediated lysis compared to the parental mAb (3,4). When studying prophylactic treatment in a rat colorectal metastasis model, we demonstrated that bispecific mAb recognizing rat colorectal cancer cells and the most important rat complement regulatory protein were able to prevent outgrowth of tumor cells. In addition, decreased outgrowth of metastases was shown when this bispecific mAb was administered in a therapeutic setting (5).

What is the advantage if bispecific mAb target effector cell CD55 as well?

A. Gorter and K.A. Gelderman: It has been shown that one of the CDCC mechanisms; CR3-dependent cellular cytotoxicity (CR3-DCC) is much more efficient when CR3 also binds certain sugar group residues that are normally expressed on bacteria or yeast. This sugar, β -glucan, is not expressed on tumor cells, thereby making CDCC less efficient. To incorporate CR3-DCC as an immunotherapeutic mechanism to eradicate tumor cells, tumor cells should be disguised as bacteria, and therefore β -glucan should be administered to the patient (6). When we investigated different conditions involving the complement system to eradicate tumor cells, we observed that bispecific mAb directed against a tumor antigen and CD55 in the presence of β -glucan, induced significant higher level of effector cell-mediated tumor cell lysis than the combination of parental anti-tumor antigen mAb and anti-CD55 mAb (7). To explain this unexpected observation, we hypothesized that not all CD55 binding sites of the bispecific mAb would bind CD55 on the

tumor cell, because of the relatively low expression of CD55 compared to the expression of the tumor antigen, and that therefore unoccupied anti-CD55 arms bind to CD55 on effector cells (Fig. 1). Due to complement activation by bispecific mAb on the tumor cell membrane, C3b, further converted to C3bi, is present on the tumor cell membrane. CR3 primed by β -glucan binds this iC3b and these activated CR3 form complexes with (effector cell) CD55 that are immobilized by bispecific mAb, resulting in increased tumor lysis. Our hypothesis was supported by previous observations that glycosylphosphatidylinositol anchored proteins, such as CD55, can be recruited by CR3 and increase cell activation (8). Furthermore, cross-linking CD55 and CR3 on the effector cells and subsequently offering mAb opsonized-tumor cells indeed increased the levels of cellular cytotoxicity comparable to the situation where tumor cells were opsonized with bispecific mAb and complement in the presence of β -glucan. Additional sup-

port for the proposed mechanism came from experiments where effector cells incubated with either anti-CD55 mAb or anti-CR3 mAb were unable to increase the lysis of anti-Ep-CAM mAb and complement opsonized colorectal tumor cells, suggesting that both CR3 and CD55 were required for the observed effect. In conclusion, co-activation of CD55 and CR3, in the presence of β -glucan, on effector cells allowed lysis of tumor cells that express relatively low levels of tumor antigen and that otherwise would not be eradicated with mAb immunotherapy due to low levels of complement activation. Therefore, bispecific mAb that contain anti-CD55 may, apart from recruiting inflammatory cells and inducing or enhancing CDCC, promote the formation of CR3-CD55 complexes on the effector cells. These cells have the capacity to enhance tumor cell lysis and thus may increase the success rate of immunotherapy in cancer patients.

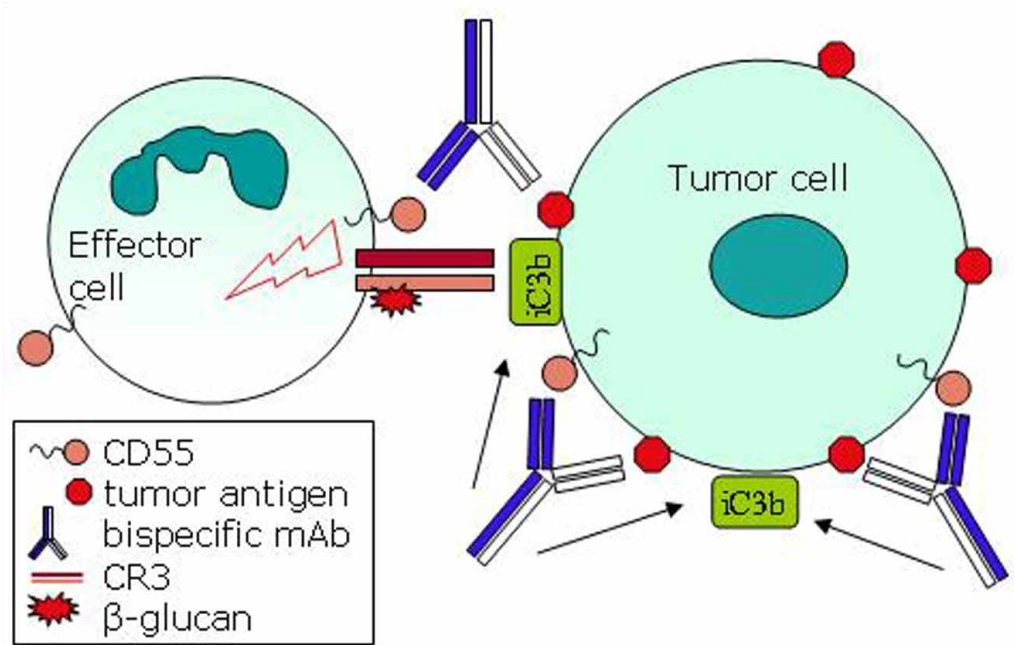


Figure 1: Upon cross-linking of a tumor antigen with effector cell CD55 by bispecific mAb, an interaction occurs between CD55 and CR3 that is bound to iC3b deposited on the tumor cells, resulting in efficient effector cell activation.

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