

The role of antibody-mediated cellular cytotoxicity (ADCC) in the mechanism underlying the action of trastuzumab

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Trastuzumab is a recombinant antibody drug, which is widely used for the treatment of breast cancer. Despite encouraging clinical results some cancers are primarily resistant to trastuzumab, and a majority of those initially responding become resistant during prolonged treatment. The mechanisms of trastuzumab resistance have not been fully understood.

It is well documented that trastuzumab acts directly in cancer cell signaling, as well as indirectly via the immune system. We examined the possible mechanisms of trastuzumab resistance in detail using the breast cancer cell line JIMT-1 established from the pleural metastasis of a patient who was clinically resistant to trastuzumab. Despite ErbB2 gene amplification and receptor overexpression, JIMT-1 cells are resistant to trastuzumab *in vitro*, and also *in vivo*, if therapy is initiated 45 days after establishing xenografts.

Our main and unexpected finding was that trastuzumab caused a significant growth inhibition of the outgrowth of macroscopic JIMT-1 xenograft tumors in both nude and SCID mice. The effect was probably mediated via the Fc portion of trastuzumab IgG because the F(ab')₂ fragment of trastuzumab was ineffective in the SCID mouse model system, in spite of inhibiting proliferation of trastuzumab sensitive cells *in vitro* equally well as intact trastuzumab IgG. We attribute the Fc-mediated effects of trastuzumab to ADCC because both nude and SCID mice have functioning macrophages and natural killer cells capable of killing tumor cells by ADCC. These findings reflect the central role of the immune system in mediating the effects of trastuzumab *in vivo*. In a previous study, Clynes et al. inoculated trastuzumab-sensitive BT-474 cells into knock-out mice lacking activating FcRγIII receptors. In this model system, the antitumor activity of trastuzumab was reduced but not ablated: about 25% of the effect was retained. In the same manner, treatment of wild-type mice with the mutated form of 4D5, the parent antibody of trastuzumab, which was made unable to bind to Fc receptors, had a similar partial effect. These results indicate that in the case of BT-474 cells, trastuzumab probably triggers both the intrinsic growth-inhibitory and apoptotic regulatory pathways, as well as evokes ADCC. In the case of the intrinsically resistant JIMT-1 cells, the mechanism of action of trastuzumab seems to be exclusively ADCC.

The mutual independence of the intrinsic and immune-mediated effects was further evidenced by *in vitro* ADCC experiments using human peripheral leukocytes as effector cells. The capacity of these cells to kill JIMT-1 and SKBR-3 cells in the presence of trastuzumab by ADCC was the same despite significant differences in the direct drug sensitivity assays. Moreover, we found that the downmodulation of ErbB2 receptor from the cell surface upon trastuzumab treatment, which has previously been postulated as the central phenomenon for direct growth inhibition, seems to be mechanistically unrelated to the action of trastuzumab *in vivo*.

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The subline JIMT-X+, generated from a trastuzumab treated JIMT-1 xenograft, did not differ from the parental JIMT-1 cells in the *in vitro* ADCC assays. JIMT-1 X+ cells were able to form xenografts in SCID mice and the growth of the xenografts was reduced by trastuzumab administration, suggesting that JIMT-1 X+ cells were initially sensitive to trastuzumab also *in vivo*; although trastuzumab resistance has developed after 3 weeks.

Finally, we showed that trastuzumab therapy was able to reduce the number of both circulating tumor cells (CTC) in the blood and disseminated tumor cells (DTC) in the bone marrow, even when the antibody has already lost its tumor inhibitory effect on the primary tumor. To the best of our knowledge, this is the first study to investigate the effect of trastuzumab on circulating and disseminated tumor cells shed from a trastuzumab resistant primary tumor in a xenograft model. We suggest that ErbB2 expressing CTCs and DTCs may be sensitive to trastuzumab even if trastuzumab resistance of the primary tumor has already developed, especially if the mechanism of resistance is masking of ErbB2. So trastuzumab treatment might have benefit in the case of patients with already trastuzumab-resistant breast cancer.