Macrophages in the liver prevent metastasis by efficiently eliminating circulating tumor cells after monoclonal antibody immunotherapy

Nuray Gül1, Liane Babes2, Paul Kubes2, and Marjolein van Egmond1,3,*

1Department of Molecular Cell Biology and Immunology; VU University Medical Center; Amsterdam, The Netherlands; 2Immunology Research Group; University of Calgary; Calgary, Canada; 3Department of Surgery; VU University Medical Center; Amsterdam, The Netherlands

Keywords: Fc receptors, antibody-dependent cellular phagocytosis, Kupffer cells, liver metastasis, mAbs

Monoclonal antibodies (mAbs) are increasingly being used to treat cancer. In response to mAb therapy, we have identified macrophages in the liver as major effector cells removing circulating tumor cells via antibody-dependent phagocytosis, an immune cell-mediated process that prevented liver metastasis. This discovery extends our understanding of the mechanisms of mAb therapy, and may help to optimize mAb-based anticancer therapeutics.

Introduction

The use of antitumor monoclonal antibodies (mAbs) has increased dramatically in the last decade and is now a mainstream strategy to treat cancer patients. Nonetheless, their mode of action has yet to be completely resolved. mAbs exert direct effects on tumor cells such as growth inhibition or apoptosis induction. Indirect anticancer properties include mAb-mediated activation of the complement pathway leading to complement-dependent cytotoxicity as well as mAb-solicited recruitment of immune effector cells that express high affinity immunoglobulin-Fc receptors. Treatment with antitumor mAbs has been shown to be ineffective in mice lacking 1 or more of the activating Fcγ receptors, supporting the importance of Fc receptor-mediated mechanism(s) of action for in vivo therapeutic efficacy of antibody based immunotherapy.

With live cell imaging and intravital microscopy we recently demonstrated that mAb therapy potently induces phagocytosis of tumor cells by macrophages manifesting in the elimination of circulating tumor cells by Kupffer cells (liver macrophages) and preventing liver metastases. Whereas in the absence of mAbs, Kupffer cells interacted with and sampled portions of tumor cells, antibody-dependent cellular phagocytosis (ADCP) was required for complete tumor cell eradication (Fig. 1). ADCP was found to be dependent upon FcγRI and FcγRIV, consistent with previous studies in which we showed that either FcγRI or FcγRIV was required to prevent outgrowth of liver metastases after mAb therapy.

ADCP was associated with the generation of phagolysosomes within macrophages that were rapidly acidified. However, intracellular degradation of tumor cells was found to be a slower process both in vitro and in vivo. Production of reactive oxygen species (ROS) and nitrogen species were proposed as major cytotoxic mechanisms implemented by macrophages. However, even though ADCP stimulated the generation of ROS, neither ADCP, nor acidification of phagolysosomes and resultant breakdown of tumor cells was found to be dependent on ROS. Thus, intracellular digestion in lysosomes is the most likely mechanism by which macrophages kill tumor cells in the process of mAb-mediated phagocytosis.

Macrophages may play a crucial role in the therapeutic success of anti-CD20 mAb therapy in patients with B cell malignancies. Supporting this premise, macrophage depletion abrogated the ability of anti-CD20 mAbs to eliminate lymphoma cells in an experimental model. Another recent study by Montalvao et al. showed that Kupffer cells trapped circulating normal and malignant B cells in the liver after anti-CD20 mAb therapy, and eliminated them through ADCP, independently confirming our findings. This is most likely due to the convenient localization of Kupffer cells in the vasculature, enabling easy access to both mAbs and circulating tumor cells. Interestingly, clinical responses after treatment with the anti-CD20 mAb rituximab were correlated with polymorphisms in human FcγRIa and FcγRIIia (FcγRIIA-131H/R and FcγRIIia-158V/F) that affect affinity for IgG. Whereas both natural killer cells and macrophages express FcγRIIia, only macrophages express FcγRIIIa, strongly supporting the role for macrophages as effector cells in the depletion of B lymphoma cells after anti-CD20 mAb treatment of cancer patients.

It is currently unclear whether macrophages contribute to tumor cell killing after mAb therapy for the treatment of solid malignancies. Homozygosity for FcγRIIa-131H has been associated with...
stronger antitumor responses and progression-free survival when patients afflicted with metastasized breast cancer were treated with anti-HER2 mAbs (trastuzumab), findings supporting an anticancer role for macrophages. Additionally, macrophages isolated from breast carcinomas in mice have been found to be capable of ADCP. Furthermore, antitumor mAb therapy was reported to be less successful in preventing breast carcinoma outgrowth and metastasis after depletion of macrophages, suggesting that macrophages may be involved as effector cells following mAb therapy of breast cancer.

Polymorphisms in FcγRIIa-131H/R and FcγRIIIa-158V/F have been further correlated with clinical responses of patients with colorectal cancer after treatment with the anti-epidermal growth factor receptor (EGFR) mAb cetuximab. However, we found that mAb therapy was ineffective in treating existing liver micro-metastases, as Kupffer cells proved stationary and were not recruited into micro-metastases. Thus, these results argue against an important role for Kupffer cells in mAb therapy once liver metastases have been established, a premise supported by current standard clinical practice. Anti-EGFR mAbs are only indicated for treatment of metastatic colorectal cancers with wild type RAS, as this therapy is ineffective when tumors harbor a RAS mutation. This further supports the notion that the direct effects of anti-EGFR mAbs (such as growth inhibition) are more important than Fcy receptor-mediated effector mechanisms in the treatment of established and already metastatic colorectal tumors.

Nevertheless, our results may hold great promise for the treatment of colorectal cancer patients. Approximately 1.2 million patients worldwide are diagnosed with this devastating disease each year, with an estimated annual death rate of 600,000 patients. Surgical removal of the primary tumor is currently the only therapeutic efficacy. This may be particularly important for patients with hematological malignancies, as well as those with circulating tumor cells at the time of elective cancer surgery, in order to prevent the development of post-surgical liver metastases.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

References
1. Scott AM, Wolchok JD, Old LJ. Antibody therapy of cancer. Nat Rev Cancer 2012; 12:278-87; PMID:22437872; http://dx.doi.org/10.1038/nrc3236
2. Clynes RA, Towers TL, Presta LG, Ravetch JV.FcγRIIa mediates antibody-dependent cellular phagocytosis after treatment with antitumor monoclonal antibodies. J Exp Med 2001; 195:1135-49; PMID:11507608; http://dx.doi.org/10.1084/jem.2000220
3. Gul N, Babes L, Siegmund K, Korthouser R, Bogels M, Braester R, Vidarsson G, ten Hagen TL, Kubes P, van Egmond M. Macrophages eliminate circulating tumor cells after monoclonal antibody therapy. J Clin Invest 2014; 124:812-23; PMID:24430180; http://dx.doi.org/10.1172/JCI66776
4. Otten MA, van der Bij GJ, Verbeek SJ, Nimmerjahn F, Ravetch JV, Beelen RH, Van Rooijen N, Bousso P. The mechanism of anti-CD20-mediated effector mechanisms in vivo. J Immunol 2008; 181:6829-36; PMID:18981101
5. Minard-Colin V, Xiou Y, Poe JC, Horikawa M, Magro CM, Hamaguchi Y, Haas KM, Tedder TF. Lymphoma depletion during CD20 immunotherapy in mice is mediated by macrophage FcgammaRII, FcgammaRIIb, and FcgammaIRV. Blood 2008; 112:1205-13; PMID:18495955; http://dx.doi.org/10.1182/blood-2008-01-135160
6. Montalvao F, Garcia Z, Celli S, Breatnacht D, Deguine J, Van Rooijen N, Bousso P. The mechanism of anti-CD20-mediated B cell depletion revealed by intravital imaging. J Clin Invest 2013; 123:5098-103; PMID:234177426; http://dx.doi.org/10.1172/JCI70972
7. Overdijk MB, Verploegen S, Bleeker WK, Parren PW. Role of IgG Fc Receptors in monoclonal antibody therapy of cancer. Chapter 13, Antibody Fc: Linking Adaptive and Innate Immunity. Elsevier. 2014.

8. Grugan KD, McCabe FL, Kinder M, Greenplate AR, Harman BC, Ekert JE, van Roosijen N, Anderson GM, Nemeth JA, Strohl WR, et al. Tumor-associated macrophages promote invasion while retaining Fc-dependent anti-tumor function. J Immunol 2012; 189:5457-66; PMID:23105143; http://dx.doi.org/10.4049/jimmunol.1201889

9. Groot Koerkamp B, Rahbahi NN, Büchler MW, Koch M, Weitz J. Circulating tumor cells and prognosis of patients with resectable colorectal liver metastases or widespread metastatic colorectal cancer: a meta-analysis. Ann Surg Oncol 2013; 20:2156-65; PMID:23456317; http://dx.doi.org/10.1245/s10434-013-2907-8

10. Gül N, Bügels M, Grewal S, van der Meer AJ, Rojas LB, Fluitsma DM, van den Tol MP, Hooberman KA, van Marle J, de Vries HE, et al. Surgery-induced reactive oxygen species enhance colon carcinoma cell binding by disrupting the liver endothelial cell lining. Gut 2011; 60:1076-86; PMID:21278144; http://dx.doi.org/10.1136/gut.2010.224717