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Evaluating the contribution of anti-myeloma immunity for the efficacy of oncolytic reovirus therapy

Müller LME[1], Parrish C[1,2], Migneco G[1], Scott GB[1], Holmes M[1], Melcher AA[3], Lawson MA[4], Cook G[1,2], Errington-Mais F[1].

1) Leeds Institute of Cancer and Pathology, University of Leeds, UK
2) St. James's Institute of Oncology, St. James' University Hospital, Leeds, UK
3) The Institute of Cancer Research, London, UK
4) Sheffield Myeloma Research Team, University of Sheffield Medical School, UK

Introduction

The aim of this study was to examine the contribution of anti-tumor immunity for the efficacy of oncolytic reovirus therapy against multiple myeloma (MM). Oncolytic viruses (OV) have two main mechanisms of action; direct lytic killing and potentiation of anti-tumor immunity. The direct oncolytic potential of reovirus in MM has previously been demonstrated both *in vitro* and *in vivo*, but the importance of an enhanced immunological anti-tumor response remains under-investigated. Reovirus-induced anti-tumor immunity has been demonstrated in other types of cancer such as melanoma and chronic leukemia, and is of major importance for the efficacy of OV treatment. Thus, it was hypothesized that reovirus-induced anti-myeloma immunity would contribute significantly to the efficacy of reovirus treatment for MM.

Experimental procedures

C57BL/KaLwRij mice were used in the 5TGM1 model system to establish MM *in vivo*. This model closely resembles human MM with induction of osteolytic bone disease and secretion of paraprotein. C57BL/KaLwRij mice have a fully functional immune system, comparable to C57BL/6 mice, and to our knowledge, this is the first immunocompetent model of MM for the study of reovirus efficacy. After establishment of MM in the bone by intravenous injection of bone-homing 5TGM1 cells, mice were treated with repeated injections of reovirus or PBS. Upon sacrifice, direct cytotoxicity and immune activation was examined using flow cytometry. All animal experiments were performed under an appropriate project license following approval by a local ethical review committee. *In vivo* findings were translated into human *in vitro* studies, using MM cell lines, healthy donor (HD) blood and MM patient samples.

Reovirus-induced Natural Killer (NK) cell activation and degranulation was examined using flow cytometry and priming of myeloma-specific T cells was performed using long-term priming cultures.

Results

Tumor burden was reduced by reovirus treatment both in the bone marrow (BM) and spleen of tumor-bearing mice. The immune cell populations, including NK cells, $CD4^+$ T cells and $CD8^+$ T cells were restored to levels of tumor naïve mice in the BM. NK cells were activated in the BM following reovirus treatment, which indicates the onset of an innate immune response. In the spleen, an increase in $CD4^+$ T cells, in combination with $CD8^+$ activation was indicative of an early adaptive immune response. These results translate into human *in vitro* findings, using both HD and MM patient samples, with activation of NK cells in response to reovirus treatment and subsequent enhancement of NK cell degranulation and killing of MM target cells. Encouragingly, reovirus-activated NK cells were able to kill OPM2 cells, which are resistant to direct lytic killing. Preliminary human *in vitro* studies suggest that reovirus treatment can prime anti-myeloma $CD8^+$ T cells for the induction of a long-term protective response.

Conclusions

Introducing a viral agent into the body requires a delicate immunological balance to avoid neutralizing the virus by an antiviral response and simultaneously allowing the enhancement of anti-tumor immunity. The importance of enhanced anti-tumor immunity for OV therapy efficacy is becoming more widely recognized and antiviral immunity can in some circumstances contribute to tumor eradication. The findings in this study indicate that the anti-tumor immune response is also important in the MM setting, with activation of both innate and adaptive immune responses resulting in enhanced killing of MM cells, in particular those resistant to direct lytic killing. This suggests that both arms of OV therapy could play a role for MM eradication, including any minimal residual disease. Future work will explore whether the anti-myeloma response can be further enhanced by combinatorial treatments, including current standard of care treatments.