OncoImmunology 5:1, e1052930; January 2016; Published with license by Taylor & Francis Group, LLC

Activation of cytomegalovirus-specific $CD8^+$ T-cell response by antibody-mediated peptide-major histocompatibility class I complexes

Martina Schmittnaegel[1], Christian Klein[2], Victor Levitsky[2], and Hendrik Knoetgen[1,*]

[1]ROCHE Pharma Research and Early Development: Large Molecule Research; ROCHE Innovation Center; Penzberg, Germany; [2]ROCHE Pharma Research and Early Development: Discovery Oncology; ROCHE Innovation Center; Zurich, Switzerland

Keywords: antibody fusion, CMV-pp65-specific CD8+ T cells, major histocompatibility class I, MHCI restricted T-cell activation, targeted T-cell recruiter, viral mimicry on cancer cells

Imposing antigenicity on tumor cells is a key step toward successful cancer-immunotherapy. A cytomegalovirus-derived peptide recombinantly fused to a major histocompatibility class I complex and a monoclonal antibody can be targeted to tumor cells by antibody-mediated delivery and activate a strong and specific $CD8^+$ T cell response.

Introduction

Cancer-immunotherapy holds promise for becoming a breakthrough treatment of advanced tumors. T lymphocytes are the key mediators of tumor rejection but can be subjugated by an immunosuppressive environment. Furthermore, lack or loss of antigenicity often prevents elimination of cancer by specific T lymphocytes. To address these problems, bispecific T-cell engagers (BiTEs), adoptive T-cell therapy and immunomodulators were recently developed for clinical use.

BiTEs, which indiscriminately activate T cells through binding to the CD3 component of the T-cell receptor (TCR) complex and a tumor-specific antigen, have been approved for EpCAM (catumaxomab)[1] and CD19 (blinatumomab).[2] Treatment with BiTEs causes considerable toxicity such as neutropenia, anemia, neurologic effects and in some cases cytokine release syndrome,[3] necessitating premedication with glucocorticoids along with a stepwise dose increase or even interruption of treatment in a proportion of patients with neurologic side effects. Due to the progress in antibody engineering, the field is moving from non-Fc-based T-cell bispecifics such as BiTE or Tandab formats to more complex, Fc-based, IgG-derived T-cell bispecifics with improved antigen binding and increased half-life.[4] However, to date all T-cell engagers are directed against the CD3ε TCR subunit and therefore trigger polyclonal activation of T cells irrespective of their subtype and specificity.

Adoptive T–cell transfer therapy has made considerable progress in the past ten years both for naturally occurring and genetically engineered lymphocytes.[5] It has been tested only in small clinical trials, yet, but appears to have the potential of becoming a curative treatment for some advanced-stage cancers. The therapy regimen is complex as it requires *ex vivo* expansion and reinfusion of the tumor-specific T cells and may also involve their genetic modification as well as non-myeloablative chemotherapy as preconditioning (lymphodepletion). In addition, it may be accompanied by severe adverse effects such as cytokine-release syndrome, neurological dysfunction requiring hospitalization and even intensive care support[6] which may limit a broader clinical application.

Immunomodulators such as antibodies recognizing checkpoint inhibitory molecules like cytotoxic T-lymphocyte antigen-4 (CTLA-4) and programmed cell death 1 (PD-1) or its ligand PD-L1 have demonstrated clinical successes with favorable safety profiles in treatment of some solid malignancies such as melanoma, non-small cell lung carcinoma and renal cell carcinoma.[7] However, the observed response rates remain below 30% for anti-PD-1 therapy in unselected cancer patients.[8] Interestingly, cancers with higher somatic mutation rates appear to respond best to immune checkpoint blockage.[9] Most likely, a high mutational load produces neoantigens thus increasing the antigenicity of tumor cells which otherwise express only a limited number of poorly immunogenic self-antigens. Some of these neoepitopes obviously share homology with viral and bacterial antigens which may indicate that not only the number but also the nature of the mutations in a tumor triggers their recognition by T cells.[9]

*Correspondence to: Hendrik Knoetgen; Email: hendrik.knoetgen@roche.com
Submitted: 05/11/2015; Revised: 08/27/2015; Accepted: 05/14/2015
http://dx.doi.org/10.1080/2162402X.2015.1052930

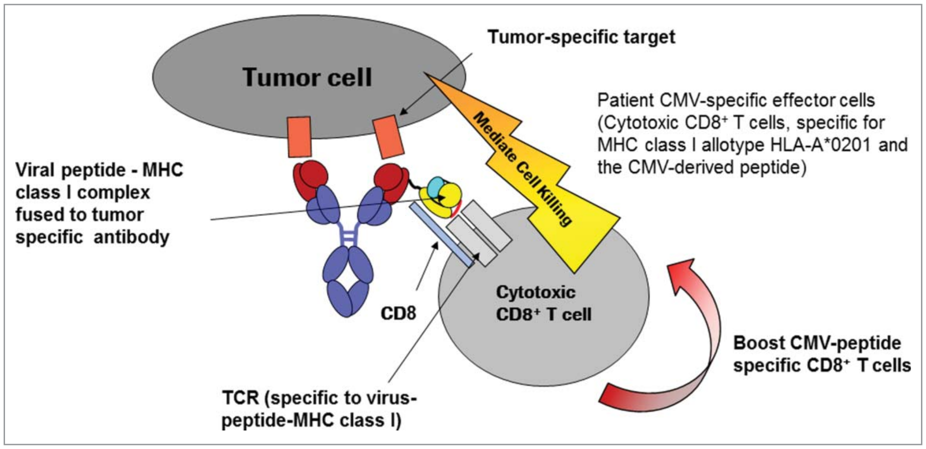

Figure 1. Mechanism of action for tumor-targeted CMV-peptide: MHC class I-antibody fusion proteins (pMHCI-IgG). CMV-pMHCI-IgG selectively recruit CMV-specific $CD8^+$ T cells. Upon crosslinking of the antibody on antigen-specific tumor cells virus-specific T cells mediate cell lysis.

Imposing CMV-antigenicity to Tumor Cells

We believe that in order to increase the success rate of cancer immunotherapy novel approaches allowing controlled manipulation of tumor cell antigenicity need to be developed. Pursuing this goal, we have recently introduced a new technology for selective delivery of a cytomegalovirus (CMV)-derived peptide-major histocompatibility class I complex (pMHCI) to tumor cells.[10] Chronic CMV infection affects the vast majority of humans and results in generation of constantly renewing, antigen-specific and differentiated cytotoxic effector T lymphocytes persisting both in the blood and various organs at high frequencies. CMV-specific $CD8^+$ T-cell responses are mainly focused on a few immunodominant peptides and a single recombinant pMHCI-IgG fusion is sufficient to redirect a large proportion of CMV-specific T lymphocytes against CMV-negative tumor cells expressing the chosen cell surface target (**Fig. 1**). Following exposure to pMHCI of relevant specificity, tumor antigen–expressing cancer cells are decorated with fusion proteins composed of a complete tumor antigen–specific antibody connected to a single MHC class I:peptide complex bearing a covalently linked CMV-derived peptide (pMHCI–IgG). The tumor cells can be specifically eliminated *in vitro* through engagement of antigen-specific $CD8^+$ T cells from peripheral blood mononuclear cell preparations of CMV-infected humans independently of the level of endogenous MHC class I expression on the target. Thus, the paradigm of immune-mediated tumor eradication can be extended even to tumor variants characterized by total loss of MHC expression, which is frequently observed in a sizable proportion of different tumors. Activation of CMV-specific T cells requires surprisingly low pMHCI–IgG concentrations without additional expansion, pre-activation, or provision of T-cell co-stimulatory signals. Our favored molecular format possesses a number of advantageous features related to protein production, stability, IgG-like pharmacokinetics and antigen-binding properties. Due to a single pMHCI complex per molecule and low pMHC-I:TCR binding affinity, target-independent activation of T cells and peripheral sink should not interfere with efficient *in vivo* tumor targeting. In contrast to pan-T-cell recruiters, application of pMHCI-IgGs is HLA-allotype restricted that limits the patient cohort to 30–40% of the population in the case of HLA A*0201. However, it remains to be seen how pMHCI-IgGs compare to conventional T-cell engagers when it comes to safety and the type of activation/death programs induced in T cells *in vivo*. In a side by side comparison with BiTEs, we found that pMHCI-IgGs induce reduced secretion of cytokines despite comparable tumor cell killing *in vitro*. We believe that dressing up tumor cells with CMV-peptide MHCI complexes and subsequent engagement of virus-specific $CD8^+$ T cell subpopulation will be advantageous in clinical settings.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References

1. Heiss MM, Murawa P, Koralewski P, Kutarska E, Kolesnik OO, Ivanchenko VV, Dudnichenko AS, Aleknaviciene B, Razbadauskas A, Gore M et al. The trifunctional antibody catumaxomab for the treatment of malignant ascites due to epithelial cancer: Results of a prospective randomized phase II/III trial. Int J Cancer 2010; 127:2209-21; PMID:20473913; http://dx.doi.org/10.1002/ijc.25423
2. Bargou R, Leo E, Zugmaier G, Klinger M, Goebeler M, Knop S, Noppeney R, Viardot A, Hess G, Schuler M et al. Tumor regression in cancer patients by very low doses of a T cell-engaging antibody. Science 2008; 321:974-7; PMID:18703743; http://dx.doi.org/10.1126/science.1158545
3. Topp MS, Gokbuget N, Stein AS, Zugmaier G, O'Brien S, Bargou RC, Dombret H, Fielding AK, Heffner L, Larson RA et al. Safety and activity of blinatumomab for adult patients with relapsed or refractory B-precursor acute lymphoblastic leukaemia: a multicentre, single-arm, phase 2 study. Lancet Oncol 2015; 16:57-66; PMID:25524800; http://dx.doi.org/10.1016/S1470-2045(14)71170-2
4. Spiess C, Zhai Q, Carter PJ. Alternative molecular formats and therapeutic applications for bispecific antibodies. Mol Immunol 2015; 67(2 Pt A): 95-106; PMID:25637431; http://dx.doi.org/10.1016/j.molimm.2015.01.003
5. Rosenberg SA. Decade in review-cancer immunotherapy: entering the mainstream of cancer treatment. Nat Rev Clin Oncol 2014; 11:630-2; PMID:25311350; http://dx.doi.org/10.1038/nrclinonc.2014.174
6. Maude SL, Frey N, Shaw PA, Aplenc R, Barrett DM, Bunin NJ, Chew A, Gonzalez VE, Zheng Z, Lacey SF et al. Chimeric antigen receptor T cells for sustained remissions in leukemia. N Engl J Med 2014; 371:1507-17; PMID:25317870; http://dx.doi.org/10.1056/NEJMoa1407222
7. Hodi FS, O'Day SJ, McDermott DF, Weber RW, Sosman JA, Haanen JB, Gonzalez R, Robert C, Schadendorf D, Hassel JC et al. Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med 2010; 363:711-23; PMID:20525992; http://dx.doi.org/10.1056/NEJMoa1003466
8. Tumeh PC, Harview CL, Yearley JH, Shintaku IP, Taylor EJ, Robert L, Chmielowski B, Spasic M, Henry

G, Ciobanu V et al. PD-1 blockade induces responses by inhibiting adaptive immune resistance. Nature 2014; 515:568-71; PMID:25428505; http://dx.doi.org/10.1038/nature13954
9. Snyder A, Makarov V, Merghoub T, Yuan J, Zaretsky JM, Desrichard A, Walsh LA, Postow MA, Wong P, Ho TS et al. Genetic basis for clinical response to CTLA-4 blockade in melanoma. N Engl J Med 2014; 371:2189-99; PMID:25409260; http://dx.doi.org/10.1056/NEJMoa1406498
10. Schmittnaegel M, Levitsky V, Hoffmann E, Georges G, Mundigl O, Klein C, Knoetgen H. Committing cytomegalovirus-specific CD8 T cells to eliminate tumor cells by bifunctional major histocompatibility class I antibody fusion molecules. Cancer Immunol Res 2015; 3(7):764-76; PMID:25691327; http://dx.doi.org/10.1158/2326-6066