Targeting and exploiting cytomegalovirus for vaccine development

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ENGLISH SUMMARY

The development of novel vaccine strategies for CMV is major research priority. Vaccines based on SLPs may form an efficient vaccination strategy against CMV infection. However, a detailed and systematic examination of the potentially promising prospects of SLP vaccines is lacking. This PhD thesis presents a series of experimental studies undertaking elaborate evaluations of the efficacy of SLP vaccines against MCMV infection. SLP vaccines alone or in combination with immunomodulatory agents of the TNFR superfamily were investigated for prophylactic and therapeutic efficacy in various mouse models of CMV infection. The pivotal knowledge obtained from the MCMV studies led to the investigation of highly immunogenic IE2 HCMV antigenic targets and formed the basis for the design of a HCMV vaccine in this doctoral thesis.

The first experimental study **(chapter 2)** tested the preventive efficacy of SLP vaccines comprising immunodominant MHC class I MCMV restricted epitopes that exclusively elicit cytotoxic MHC class I T cell responses against lytic MCMV infection in C57BL/6 and BALB/c mouse strains. Prime-booster vaccination induced strong and polyfunctional $CD8^+$ T cell responses. Interestingly, the degree of the $CD8^+$ T cell response was determined by the naive $CD8^+$ T cell precursor frequency of each epitope rather than the T cell functional avidity. Vaccination with a combination of SLPs produced the best reduction in viral titers, suggesting that the breadth of the vaccine-induced $CD8^+$ T cell response is critical for preventive vaccine strategies against MCMV.

The second study **(chapter 3)** examined whether the efficacy of the MHC class I SLP based vaccine is boosted with $CD4^+$ T cell "help" and/or OX40 co-stimulation. The prophylactic effects of SLPs containing MHC class II epitopes from 5 immunodominant MCMV antigens were examined alone or in combination with the MHC class I SLPs in C57BL/6 mice. Enforced OX40 signalling during booster SLP vaccination improved vaccine-specific $CD4^+$ T cell and $CD8^+$ T cell reactivity. The combined vaccination of SLPs containing various MHC class I and II SLPs plus OX40 agonistic antibodies produced the best protection and virus dissemination effects. Drawing on these promising findings, the therapeutic efficacy of the combinatorial MHC class I and II SLP vaccines was also tested in the C57BL/6 mouse strain **(appendix section of chapter 3)**. The strong T cell response to MCMV was not boosted following SLP vaccination and no therapeutic effect was observed. These results do not support the therapeutic prospects of the combinatorial MHC class I and II SLP vaccines against MCMV infection.

Chapter 4 presents an endeavour to translate this research in humans. A study to discover novel HCMV T cell epitopes, which would enlarge the choice of antigens for potential HCMV T cell- based SLP vaccines, was undertaken. IE2 HCMV T cell responses were tested in the peripheral blood of 15 HCMV- seropositive and 6 HCMV- seronegative healthy donors using IE2 SLP pools and cytokine flow cytometry. Whereas the IE1 protein is frequently recognized by $CD8^+$ T cells, a limited number of HLA-class I restricted IE2

T cell epitopes were found. Several new HLA class II-restricted IE2 T cell epitopes were detected in addition to previously detected epitopes. Five highly immunogenic IE2 SLPs recognized by polyfunctional Th1 cytokine (IFN-γ^+/ TNF^+/ IL-2^+) producing cells were identified during the functional characterization of the IE2-specific T cell responses. Testing of these 5 highly immunogenic IE2 SLPs as a potential T cell based vaccine for CMV is highlighted as a fruitful future research direction.

Since CMV has a unique ability to re-infect the same host and initiate a second cycle of immune responses, a study **(chapter 5)** was performed in which the protective and therapeutic efficacy of MCMV-based viral vector vaccines against HPV^+ tumours were evaluated in mice with and without pre-existing immunity. MCMV-based vaccine vectors expressing an immunodominant MHC class I-restricted HPV E7 epitope in either inflationary or non-inflationary T cell epitope regions were generated. The diversity of the inflationary versus non-inflationary HPV specific T cell responses was thoroughly examined over time and the vaccine effectiveness was compared. All MCMV vector-based vaccines exhibited long term prophylactic vaccine efficacy. Interestingly, therapeutic evaluation of the MCMV vectored vaccines showed that the level of pre-existing immunity determines the efficacy of MCMV-based vaccine vectors.

The empirical findings presented in the previous 4 chapters are complemented by a narrative review of the literature in **chapter 6,** which critically evaluates the research evidence on the potency of prophylactic T cell eliciting vaccines for chronic viral infections. It outlines the main factors which determine the quality of the $CD4^+$ and $CD8^+$ T cell responses. In parallel, the complexities in understanding the mechanisms underpinning potent T cell responses are discussed as T cell mediated protection is driven by several factors.

The final **chapter 7** provides an insightful discussion of the key knowledge gaps filled in this thesis, the core strengths and limitations of the utilised methodologies and findings, and the most fruitful research ideas to be prioritised by future studies in this area. Importantly also, a balanced debate is presented about the likely direct and indirect clinical implications of this thesis in the design of efficacious prophylactic T cell based vaccines.