Targeting and exploiting cytomegalovirus for vaccine development
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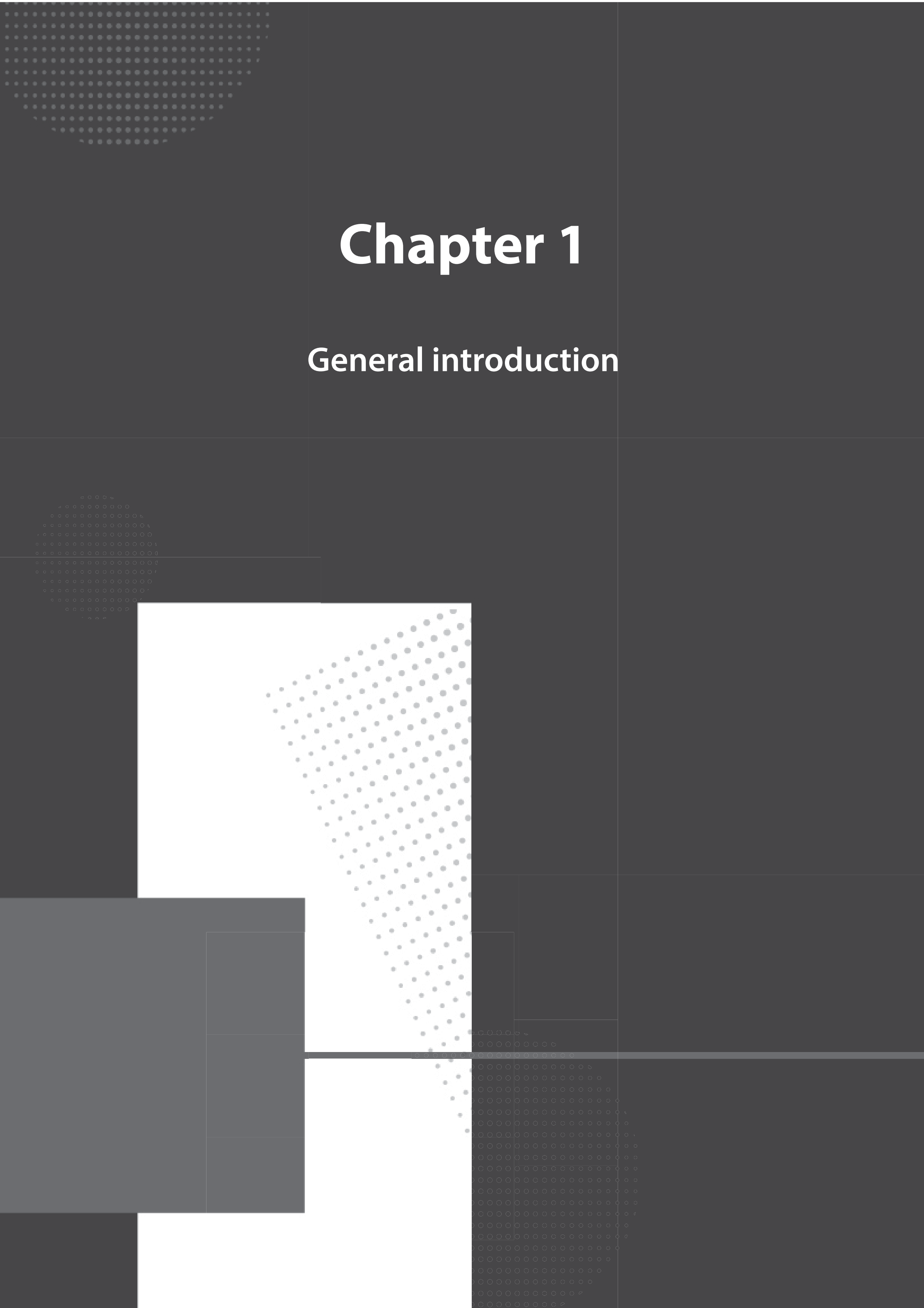

Chapter 1

General introduction

GENERAL INTRODUCTION

Upon pathogen invasion, the innate and adaptive immune systems function in concert to evoke a pathogen specific immune response. Initially, pattern recognition receptors (PRRs) recognise pathogen-associated molecular patterns (PAMPs) and activate innate immune responses such as professional antigen presenting cells (APCs). Consequently, APCs (i.e. dendritic cells (DC), macrophages, B cells) instigate the induction of adaptive immune responses (cytotoxic T cells, helper T cells and antibodies) leading to pathogen clearance [1]. After pathogen eradication, immunological memory is formed and a rapid and more efficient adaptive immune response arises when encountering the same pathogen in the future. Immunological memory can be short- or long– lived, depending on the type and persistence of the pathogen.

Immunisation is the strategy of stimulating the host's defence with i.e. a dead or attenuated pathogen to establish protective or therapeutic immunity [2]. Vaccine development is a complex and multicomponent activity which requires comprehensive understanding of the host-pathogen interactions. Selection of the correct antigenic targets and delivery systems that will shape an effective vaccine-elicited immune response is a crucial step of the vaccine development process. The majority of the peptide- and protein-based vaccine platforms demonstrate low immunogenicity when adjuvants are lacking. Adjuvants act by providing innate and adaptive immune triggers and are currently used in several vaccine models [3-5]. The ultimate goal, is an affordable vaccine that generates strong and long-lasting immunity with the fewest possible side effects.

T CELLS

T cell activation

T cells (thymus-derived) and B cells (bone marrow-derived) are major cellular components of the adaptive immune response. T cell are crucial for mediating cellular immunity and B cells are primary responsible for humoral immunity. T cell activation occurs when a naïve T cell bearing a unique T cell receptor (TCR) encounters a DC presenting cognate antigen in the context of MHC molecules (***Signal 1****)* [6]. In particular, peptides cleaved from endogenously produced proteins are presented by MHC class I molecules and subsequently recognized by T cell receptors of $CD8^+$ T cells leading into the induction of cytotoxic $CD8^+$ T cell responses. Extracellular proteins can be presented by MHC class II molecules to $CD4^+$ T cells leading to helper $CD4^+$ T cell induction [7]. Nevertheless, in some cases antigens from extracellular environment can be presented by APCs on MHC class I molecules and stimulate T- cell immunity, a process widely known as cross-presentation [8]. Priming of T cells requires approximately 16 to 20 hours of antigenic stimulation and activated T cells can be measured within 2 days.

Recruitment of $CD8^+$ T cells ends between 48-96 hours after antigenic challenge due to a feedback mechanisms that limits the duration of effective antigen presentation [9,10]. However, if the antigenic stimulation is less than 4-6 hour the programmed proliferation response is abortive.

Costimulatory signals (***Signal 2***) are considered essential for appropriate T cell activation. Mature DCs express several costimulatory ligands (i.e CD70, OX40L, , ICOSL, and the B7 molecules CD80 and CD86) which upon inflammatory conditions ligate to the equivalent costimulatory molecules (e.g. CD27, OX40, ICOS, CD28) expressed on T cells and strengthen T cell activation [11]. Certain inflammatory cytokines like IL-12 and type I interferons ***(Signal 3)*** play a crucial role in regulating adaptive immune responses and influence both primary and memory T cell development [12]. For generation of fully functional T cells concerted activation of all the three signals described above is required.

T cell expansion, contraction and memory formation

After virus encounter, T cell responses undergo through three phases ***i) expansion, ii) contraction*** and ***iii) memory development*** [13]. During the expansion phase, activated T cells clonally expand and acquire effector cell properties. In mice, the frequency of specific $CD8^+$ T cells can increase in size from 100-200 cells up to 1×10^7 cells during bacterial and viral infections [14,15]. Notably, the expression of interleukin-12 (IL-12) and interleukin-4 (IL-4) pro-inflammatory cytokines by innate immune cells (DCs) stimulates primary T cell activation and induction of Type 1 and Type 2 cytokine producing T cells. Activated $CD4^+$ and $CD8^+$ T cells acquire either a Type 1 or Type 2 cytokine profile. The cytokines produced by Type 1 T cells promote cell-mediated immunity and they include interferon- γ (IFN-γ), tumor necrosis factor (TNF) and interleukin-2 (IL-2) [16,17]. Type 2 T cells, secrete IL-4, IL-5, IL-6, IL-10 and IL-13 cytokines and promote B cell development and antibody responses [18]. Generally, cytokine production by $CD8^+$ T cells occurs in a rapid and transient, tightly regulated by antigenic contact [19]. In addition, $CD8^+$ T cells are also equipped with the cytolysis-inducing molecules perforin and granzyme and the apoptosis-inducing CD95 ligand (FasL/APO-1L) to execute antigen-bearing target cells [20].

$CD4^+$ T cell help is critical for proper priming of $CD8^+$ CTL cells. $CD4^+$ T cell help works by stimulating APCs through the CD40-CD40L pathway to prime $CD8^+$ T cell proliferation [21]. Although primary $CD8^+$ T cell responses to several infectious pathogens such as viruses and bacteria are generally unaffected by the absence of $CD4^+$ T cells, the memory and recall $CD8^+$ T cell response to these agents requires $CD4^+$ T cell help to initiate a second round of clonal expansion [22-24]. Moreover, a growing body of evidence indicates a capacity of $CD4^+$ T cells for potent cytolytic killing of virus infected cells [25-28].

After the expansion phase and termination of disease, more than 90% of the effector antigen-specific T cell pool is eliminated by programmed cell death (PCD) [29]. Several

factors are implicated in modulating this phase and retard or accelerate T cell contraction. For instance, IL-2 administration has been clearly shown to augment the magnitude and survival of the antigen-specific T cells, indicating that IL-2 signals are important for T cell expansion and maintenance [30,31]. However, expression of IFN-γ and cytolytic effector molecules such as perforin and granzyme have downsizing influence [32]. The role of TNF and CD95 on cells in the apoptosis of mature T cells is controversial [33-35]. Whether other factors also have contraction-modulating properties remains to be investigated. Down-regulation of activated T cells is of great importance for preventing immunopathology since excessive expansion or defective contraction of T cells (particularly CD8) results in severe or even fatal outcome [36].

After contraction, a small portion of antigen-specific T cells, termed memory T cells, remains. Memory T cell are endowed with the ability to generate a rapid and powerful immune response in case of re-infection with the same pathogen. In particular, extensive literature from animal models describes the superiority of memory T cells precursor frequency (>1000 fold) compared to naïve T cells which fosters induction of stronger and more plethoric immune responses during a secondary antigenic encounter. Memory T cells frequencies are stable over a long time [37]. Memory $CD8^+$ T cell homeostatic proliferation and long term survival is MHC interaction independent and is driven by the cytokines IL-7 and IL-15 [38]. Memory $CD4^+$ T cell replenishment is similar to $CD8^+$ T cells, whilst the need for IL-15 is less marked [39]. Remarkably, memory T cells show delayed secondary contraction and resistance to apoptosis through the upregulation of various anti-apoptotic molecules (e.g. BCL-2, BCL-XL) [40,41]. Memory T cells exhibit also better differentiation capacities and produce IFN-γ, RANTES, and the cytotoxic molecules perforin and granzyme B in larger quantities and more rapidly than naïve T cells [42-44].

Memory T cells can be categorized into effector-memory (T_{EM}), central memory (T_{CM}) or tissue-resident memory (T_{RM}) T cells based on the expression of certain homing and differentiation molecules [45]. T_{EM} can be distinguished based on the expression or lack of $CD62L^+CCR7^-$ surface molecules and the T_{CM} cells express $CD62L^+CCR7^+$ [46]. An important discrete difference between these two subsets is that T_{EM} cells predominantly circulate in non-lymphoid organs whereas T_{CM} cells preferentially localize in lymphoid tissues [47,48]. T_{RM} cells do not circulate but permanently exist in tissues.

CYTOMEGALOVIRUS INFECTION

Cytomegalovirus

Cytomegalovirus (CMV) belongs to the family of the β herpesviruses and it is also known as herpesvirus type 5. CMV has a long life cycle and has a genome size of over 230kb. The virus contains a double-stranded linear DNA enveloped by a proteinaceous matrix, which is packed into a lipid bilayer that contains viral glycoproteins. Transmission of the virus cannot occur through casual contact but via exposure to infected body fluids

(i.e blood, urine, saliva, breast milk, tears). As a result of productive virus replication immediate early, early and late viral proteins are synthesized [49]. After primary infection, the virus establishes lifelong latency within myeloid, endothelial and epithelial cell lineages. Cytomegalovirus replication is cell-type specific and depends on the stage of differentiation of the infected cells. Specifically, while monocytes repress transcription of the major immediate-early promoter and thus impede the production of new virions, macrophages and immature dendritic cells sustain productive infection [50-52]. During the lytic cycle of the infection, the virus replicates in diverse tissues (i.e spleen, liver and lungs) whereas the salivary glands are the primary site where viral replication is detected during latency [53,54].

The incidence of the CMV infection varies and its true sero-prevalence rate is difficult to determine due to virus latency and difficulty to be detected. Recent epidemiologic evidence suggests that approximately 60-80% of the human population is infected by the virus and 90% or more of the infected individuals acquire the virus till aged 40 years old age [55]. CMV infection usually is benign, with no clinical disease manifestations in immunocompetent individuals but it can reactivate and lead to severe complications in immunosuppressed and immunocompromised individuals, including allograft recipients and HIV patients. Moreover, CMV is currently the leading cause of congenital infections, and can cause long term neurological consequences such as hearing loss, visual impairment, and cognitive delays. Antiviral therapy for CMV exists, but it is accompanied with significant toxicity and prolonged treatment periods. The most commonly used antivirals for CMV disease include ganciclovir, valganciclovir, foscanet and cidofovir, all of which target to inhibit viral DNA amplification. At present there are no licensed vaccine-based countermeasures against CMV [56].

Vaccines against CMV seek to recapitulate or to improve the degree of protection bestowed by natural infection. In the last decades, many prophylactic and therapeutic vaccine strategies have been explored such as live attenuated viruses, human dense bodies, recombinant viral vectors, DNA, and subunit/adjuvant vaccines. The majority of those vaccines focused on the induction of broad neutralizing antibodies against the major envelope protein B (gB) or, more recently, against the gH pentamer complex [57]. Although some highly potent humoral-inducing vaccines have been developed, their main limitation is that they fail to provide long term protection. A series of studies on mice, non-human primates and humans provide evidence that vaccines against CMV should engage cellular T cell immunity. T-cell based vaccines designed to induce $CD4^+$ and/or $CD8^+$ T cell responses of sufficient magnitude, particular phenotype and functional traits that directly contribute to pathogen clearance, although not yet providing sterilizing immunity via cell mediated effector mechanisms is a rapidly expanding vaccination strategy that gains increasing attention [58-62]. Clinical phase I and II trials, incorporating humoral immune targets in combination with cellular immune targets, predominantly focus on the immediate early 1 protein (IE1) and protein 65 (pp65) as immunogens for cytotoxic T cells and gB as immunogen for neutralizing

antibodies. In these trials promising immune responses have been elicited. To this end, numerous novel vaccines modalities including replication-defective viral vectors and genetically disabled CMV are currently tested in experimental studies and preliminary results engender optimism that a successful vaccine will be developed [56,63].

T cell responses to cytomegalovirus

CMV-specific T cell responses exhibit unique features and follow an atypical kinetic profile. T cell responses to CMV dominate the memory T cell pool more than other (known) viruses. It is estimated that about 10% of the total circulating $CD4^+$ and $CD8^+$ memory T cell responses are CMV specific and these percentages may rise throughout the course of life [64]. The unique nature of CMV-specific T cell responses has been studied in depth using mouse CMV (MCMV) [54]. The majority of the MCMV-specific $CD8^+$ T cell responses that predominate during the acute infection phase follow the typical course, which is characterised by massive proliferation after antigen encounter, rapid contraction and long term maintenance at low levels [65]. These $CD8^+$ T cells are characterised by a conventional central memory phenotype ($CD127^+$, $CD62L^+$, $CD27^+$, $CD28^+$, KLRG-1^-, $CD44^-$ and IL-2^+) during the persistent phase of infection.

However, $CD8^+$ T cell responses to certain proteins do not contract but continue to intensify gradually over time and display an effector-memory phenotype ($CD127^-$, $CD62L^-$, $CD27^-$, $CD28^-$, KLRG-1^+, $CD44^+$ and IL-2^-). These T cells were termed inflationary to denote an ongoing rise in the response rate [66]. Inflation of memory CMV-specific $CD4^+$ T cells has also been observed [67]. Inflationary CMV-specific $CD8^+$ T cells with similar phenotypic features have been detected in humans and in primate models of CMV. Inflationary T cells often show restricted T cell receptor (TCR) usage, with many carrying T cell receptors specific for immunodominant CMV antigens. Despite their ongoing proliferation capacity, inflationary T cells retain their functionality and do not exhibit features of exhaustion as observed with certain other persistent viral infections.

For establishment of memory T cell inflation, repetitive antigen exposure is required, whilst systemic viral production does not seem to be a prerequisite to drive memory inflation. Also differences in the functional avidity for peptide-MHC complexes were not predictive whether epitopes elicit inflationary or non-inflationary T cell responses. Importantly, $CD4^+$ T cells, costimulatory molecules such as CD27 and OX40 and IL-2, IL-7, and IL-15 cytokines provide important signals for the maintenance of the inflationary T cell population but their precise mechanisms are under investigated [66,68,69] .

Animal models to study CMV pathogenesis and therapies

Treatments for CMV infections have been studied in murine, guinea pig, non-primate and primate models before being tested in humans. All models of CMV infection have significantly contributed to CMV research and they all contain a plethora of advantages and disadvantages.

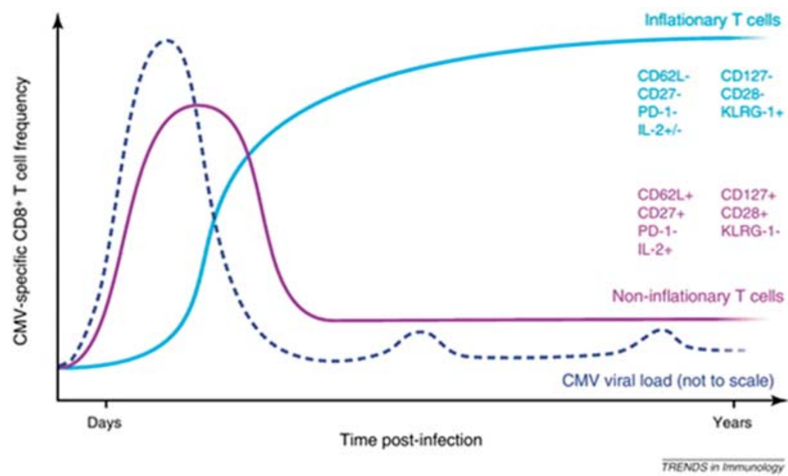

Figure 1. Longitudinal analysis of the CMV-specific T cell subsets. During CMV infection two types of T cells arise i) Conventional (non-inflationary) T cells which undergo expansion, contraction and development of stable memory pools and ii) Inflationary T cell pools which do not contract but gradually continue to accumulate in frequency throughout time. Phenotypic characteristics of inflationary and non-inflationary T cells are displayed. (Figure adapted from O'Hara, Weltens, Klenerman and Arens, Trends in Immunology, 2012; 33:84-90.)

Mice are widely used to study CMV immune functions and pathogenesis. MCMV infection resembles at a great extend HCMV biology. C57BL/6 mice alike humans are not considered particularly sensitive to CMV infection. Thus, C57BL/6 mice are a suitable model to study CMV immune responses and to investigate virus immune evasion mechanisms and new therapies in the context of immunocompetent settings. On the other hand, the BALB/c mouse strain lacks Ly49-associated NK cell functions and it is considerably susceptible to CMV infection [54]. Importantly, BALB/c mice infected with high dose MCMV (10^6 plaque-forming units) develop viremia rapidly (day 4-7 post infection) in several organs (e.g. liver, lungs, spleen, kidney) and direct disease manifestation that can even be lethal. Based on the aforementioned characteristics of MCMV infection in BALB/c mice, this mouse strain is particularly useful for studying CMV disease and testing potential antiviral treatments in the context of immunocompromised individuals. Whereas MCMV animal models recapitulate at most HCMV and there is increased availability of inbred mouse strains, a limitation of this model is that no vertical virus transmission can occur making MCMV a less suitable model to study congenital CMV infection [70,71].

Recently, ***humanised mice models*** have been developed for HCMV research. Humanised mice allow direct investigation of HCMV tropism, latency, reactivation and antiviral treatments. However, the generation of humanized mice requires technical modifications that restricts their use, and the development of immunity to HCMV is crippled [72].

Unlike mice, ***guinea pig*** models enable the study of congenital CMV infection. Specifically, guinea pig's placenta has a single trophoblast layer similar to humans which allows the virus to cross the placenta and infect the foetus. Vertical transmission rate in quinea pigs is approximately 40-60%, similar to what observed in the human settings. Notably, lytic guinea pig CMV (GPCMV) infection of immunocompetent guinea pigs leads to clinical disease symptoms such as splenomegaly, peripheral blood mononucleosis and self-limited viremia, analogous to what it is observed during HCMV infection. As with HCMV, the most important target of humoral immune response following GPCMV infection appears to be the gB homolog. This animal model has numerous advantages in terms of monitoring disease progression (i.e virologic endpoints) and evaluating therapeutic interventions. However, the usage of guinea pigs is less practical when it comes to large-scale vaccine testing, studies of the role of the immune system or experiments with (long term) follow up as compared to mice or rats [70].

Like mice also ***rats*** display human-like physiological responses to CMV infection. Importantly, almost all human genes noted to be associated with disease have known counterparts in the rat genome and extensive literature on rat CMV (RCMV) pathogenesis exists [73]. Furthermore, as with the guinea pig model RCMV can cross the placenta and infect the foetus, although the exact mechanism is still unclear. Recent studies suggest the potential use of the RCMV model to study CMV congenital transmission and vaccine development [74,75]. Hence, the rat model is significantly advanced compared to the guinea pig and mouse model for studying various aspects of CMV pathogenesis, therapeutic interventions and evaluating vaccine candidates for CMV congenital infection in humans. However, a major limitation in the study of RCMV is like for GPCMV the restricted availability of tools (e.g. monoclonal antibodies) to study the role of the immune system [70].

Finally, ***Rhesus macaques* infected with Rhesus** cytomegalovirus (RhCMV) is studied extensively. The RhCMV model showed a strong antigenic relationship with the HCMV and the chimpanzee CMV (CCMV) genome [76]. Some important limitations of the use of Rhesus macaques is the limited availability of inbred animals, the significantly higher cost for breeding and the requirement for specialised facilities and trained personnel compared to the rest available CMV animal models [77,78].

CMV AS A VACCINE VECTOR

Today numerous viral vectors are being exploited for potential clinical application against tumour malignancies and a broad range of infectious pathogens. Viral vaccine vectors are usually harmless, well tolerated viruses which carry an inserted antigen of choice. Viral vectors can be manufactured similarly to conventional vaccines and their safety prolife can be easily assessed, which makes them particularly attractive as vaccine platforms [79,80]. Modified vaccinia Ankara (MVA) and the adenovirus (Ad) vectors

are among the most popular and well investigated vectors which have shown very promising results [81-90]. The success of viral vector vaccines relies on their capacity to express antigens intracellularly, thereby inducing robust cellular immune responses in tissues, and in addition can carry proteins on their surface eliciting humoral (antibody-mediated) immunity. Early-phase trials have shown that targeted delivery via viral vector vaccines is feasible and ample opportunities for further improvement exist.

The majority of the viral vectors tested do not establish sufficient immunological memory, which may be related to their attenuated status [91]. Moreover, T cell responses to most viral vectors follow the conventional course which leads mostly to the formation of T_{CM} cells. Hence, upon antigen re-encounter, T_{CM} cells first undergo a phase of expansion and effector differentiation. In certain instances, this delay in the response may be detrimental for the host. Finally, immunological memory wanes over time and repetitive stimulation with the same viral vector or targeted antigen is required to maintain lifelong immunity [79,92,93]. All these crucial limitations led to the conceptualization of an alternative T cell based vaccine model that induces T_{EM} that are maintained for a long time. Unlike T_{CM} cells, T_{EM} cells are equipped with immediate cytotoxic function and localise in peripheral sites such as the mucus, one of the main portals for pathogens entry [94,95]. Hence, a vaccine with the ability to induce and maintain a large population of T_{EM} cells is expected to provide rapid effector activity and intercept pathogen replication at the most vulnerable phase of infection. T_{EM} -inducing vaccine vectors might overcome conventional viral vector vaccine limitations and establish long term efficacy.

CMV elicits a unique T cell repertoire containing both the conventional T_{CM} phenotype and the unconventional inflationary T_{EM} -like phenotype. The inflationary CMV T cells exhibit no signs of exhaustion and form a promising T cell subset that can be exploited in various vaccination settings [66,68,96,97]. Experimental studies with RhCMV vectors expressing SIV antigens have been conducted with very encouraging outcomes. Specifically, RhCMV-SIV vectors elicited and maintained robust and highly polyfunctional T_{EM} cell responses that were widely distributed at all potential SIV replication sites and recognized a broad range of MHC class I and II restricted epitopes. Remarkably, RhCMV-SIV vaccinated animals manifested an unprecedented long term pattern of protection or elite virus control without need for anamnestic stimulation [98-100].

However, the high seroprevalence rate of CMV among people raises concerns about the potential risk for tolerance to CMV-based vaccines due to pre-existing host immunity to the virus [101]. CMV has however the capacity to re-infect the same hosts [102]. Thus, the ability of CMV to re-infect encourages the use of CMV vector vaccines in CMV seropositive individuals as well as the potential for re-use of CMV vectors encoding the same or different target antigen in the same individuals. In addition, the ability of CMV to arrest and clear overtime residual SIV infection suggests that CMV vector based

vaccines can be used in therapeutic vaccine settings against HIV-1 or other chronic infectious diseases [103].

Finally, CMV-based vector vaccines could synergize with other vaccine platforms and potentially enhance overall vaccine efficacy. For instance, vaccines that are designed to elicit humoral immune responses could be combined and work together in a complementary fashion with CMV vectors designed to elicit robust cytotoxic T_{EM}-based cellular responses. Induction of acquisition blocking antibodies and highly effective long lasting T_{EM} responses could manage early and long term control of infections and cancer [103,104]. Important considerations in the use of wild-type human CMV vectors is the persistence and potential pathogenicity of the virus. These issues need to be actively evaluated in the upcoming first-in-human clinical studies for CMV vectors.

SYNTHETIC LONG PEPTIDE VACCINES

Vaccines consisting of multiple overlapping synthetic long peptides (SLPs) containing T cell epitopes constitute a very popular and promising prophylactic T cell-based immunization strategy. SLPs are chemically stable, can be easily synthesized, lack toxicity and oncogenic potential [105]. SLPs are usually designed as approximately 30-mer peptides overlapping by 10 to 15 amino acids. There is compelling evidence that the length of the peptide vaccine regulates the magnitude of the induced T cell responses [106,107]. Vaccination with exact MHC-binding peptides, usually 8-10 amino acids has been tested in many infectious diseases and following combination with adjuvants (i.e incomplete Freud adjuvant, montanide ISA-51) managed to protect against subsequent pathogen infection. However, a growing body of research evidence suggests that vaccination with short peptides is frequently not able to induce memory $CD8^+$ T cell responses and immunological tolerance to the immunizing antigens may occur [108-111]. Direct comparison of whole protein and short peptide vaccines with SLP vaccines has shown that the latter are cross-processed by intracellular routing leading to superior $CD8^+$ T cell stimulation [106,112]. Specifically, SLPs cannot bind directly to MHC class I molecule but are internalised and processed by specialised APCs (i.e DCs) before being presented to either $CD4^+$ or $CD8^+$ T cells. This process results in enhanced T cell activation and induction of more effective antigen-specific immune responses [107,113]. Hence, vaccination with SLPs is considered more efficient and an optimal approach for priming protective immunity compared to short peptides or protein vaccines.

Another important advantage of SLP vaccines is that they allow epitope selection *in vivo* based on the animal's or patient's own MHC profile. Additionally, multivalent SLP vaccines may facilitate simultaneous priming of T cells against multiple epitopes stimulating a broad immune response. Markedly, professional APCs can handle large pools of long peptides and are capable of properly exercising multiple MHC class I and

II peptide epitopes for presentation at the cell surface. Therefore, injection of pools of long peptides will lead at the induction of both CD4+ and CD8+ T cells, specific to all targeted epitopes, each of which can contribute to pathogen control. However, competition among SLPs may occur leading to lower responses.

Importantly, SLPs vaccines alone are poorly immunogenic and need to be combined with adjuvants or other immune modulators for sufficient innate immune stimulation and T cell induction. In several preclinical models, agonists of TLR3, TLR4, TLR7 and TLR9 significantly boosted the size and efficacy of virus/and or tumour-specific T cell responses when where combined with SLPs [114-116]. Similarly, in humans, the use of SLP vaccine formulations combined with adjuvants have managed to enhance not only the magnitude but also the activation status of the T cell response and provided promising benefits in preclinical and clinical cancer immunotherapy studies [117-121]. Although the capacity of the SLP vaccines to induce CD8+ T cell responses is not CD4+ T cell ''helper" dependent, the presence of a helper peptide markedly improves the magnitude of the CD8+ T cell response [122]. Furthermore, co-administration of agonistic antibodies (anti-OX40, anti-CD40, anti-4-1BB) *in vivo* to stimulate DC activation significantly improved the efficacy of the SLP vaccines in both mice and patients [116,123].

In conclusion, SLP vaccines has shown promising clinical results in a number of published and ongoing clinical trials. Combination of SLPs with other methods, such as nanomaterials and chemotherapy, or improved delivery systems is investigated and their clinical efficacy remains to be evaluated.

SCOPE AND OUTLINE OF THIS THESIS

The overarching research question studied within this thesis is whether the SLP vaccine modality can form an efficacious vaccination strategy against CMV infection.

In **chapter 2**, the preventive efficacy of a SLP vaccine comprising MHC class I MCMV epitopes that exclusively elicit cytotoxic MHC class I T cell responses against lytic MCMV challenge was examined. The quality of the vaccine induced CD8+ T cell responses was studied in depth and the vaccine efficacy was evaluated in two mouse strains of CMV infection (C57BL/6 and BALB/c mice). This study showed that the breadth of MHC class I SLP based vaccines determines the efficacy of the vaccine to significantly reduce the viral load.

Next in **chapter 3**, an effort was undertaken to optimize the efficacy of the MHC class I SLP based vaccine by the addition of CD4+ T cell ''help" and/or OX40 costimulation. SLPs containing MHC class II epitopes from 5 immunodominant MCMV antigens were tested individually or in combination with the MHC class I SLPs for possible prophylactic effect against lytic MCMV infection in C57BL/6 mice. In addition, OX40 signalling was enhanced to optimise vaccine-induced CD4+ T cell responses and to enforce induction

of vigorous vaccine-specific CD8$^+$ T cell responses. Vaccination with both MHC class I and II SLPs in combination with OX40 costimulation led to superior protection and a strong reduction in viral titers after challenge. Subsequently in the appendix section of chapter 3, the therapeutic efficacy of the combinatorial MHC class I and II SLP vaccines was examined in the C57BL/6 mouse strain.

In **chapter 4**, studies were undertaken to discover novel HCMV T cell epitopes and enlarge the choice of antigens for potential HCMV T cell-based SLP vaccines. *In silico* methods using public immune epitope databases and *in vitro* screening of T cell reactivity against the indispensable for virus replication IE2 HCMV protein was performed in PBMCs obtained from healthy CMV seropositive and seronegative mid age volunteers. Highly immunogenic areas with MHC class I and II T cell epitopes were identified and could be used for vaccination purposes. These results were complemented with *in silico* screening for previously published epitopes to design the most promising MHC class I and II IE2-based CMV vaccine.

In **chapter 5,** the capacity of the CMV-based viral vector vaccines to protect and/ or treat virus inducing cancer was tested. MCMV-based vaccine vectors expressing immunodominant HPV antigens 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 induced was thoroughly examined over time and the vaccine effectiveness was evaluated and showed that the level of pre-existing immunity determines the efficacy of MCMV-based vaccine vectors.

Chapter 6 provides a critical narrative overview of literature focused on the importance of T cell-based eliciting vaccines in counteracting chronic viral infections. The main determinants and mechanistic factors required to shape immunity and maximize prophylactic T cell vaccine efficacy were discussed.

Finally, a general discussion of the core research findings, the main strengths and weaknesses and fruitful future research directions are outlined in **chapter 7**. Important emphasis is also placed on whether the knowledge obtained from this thesis can be clinically utilised and translated into the design of more potent prophylactic T-cell based vaccines.

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