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Review

Viral vaccines and vectors – some lessons from cytomegaloviruses

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INTRODUCTION

Vaccination is perhaps the most beneficial public health tool in history. The battle has been won for smallpox, diphtheria, polio, measles, yellow fever and several other diseases *(1)*, saving millions of lives and changing the demographics of the world. However, the war is not over and many challenges such as HIV, influenza, hepatitis C virus (HCV), *Mycobacterium tuberculosis*, Malaria and others remain. Most vaccines have been developed empirically and despite their success we understand little about the ultimate mechanism of their protective immunity. Although the majority of concepts of the new vaccine approaches stem from *in vitro* data, major insights still have to come from *in vivo* analysis. The past decade has witnessed a significant progress in elucidating mechanisms of vaccine-induced immune response *(2, 3)*, providing data necessary for rational development and design of 'smart' vaccines.

The majority of current vaccines rely on protective immunity induced by infection with live, but attenuated pathogens. However, in most of these approaches there is little or no external control over the process of mounting the immune response. Depending on the protective principle needed for controlling certain pathogens, different types of immune response may be desirable, including the production of protective antibodies and various components of specific cellular immune response. In addition, for successful protection against many pathogens, it is essential that immune response mechanisms are operative at the site of infection, e.g. mucosal tissue as the most frequent site of pathogen entry. Novel approaches of vaccine development include delivery of the gene of interest and expression of antigens within a host. These approaches provide much better possibilities to control the process of inducing and maintaining a specific immune response, appropriate to best counteract particular pathogen. The genes encoding the antigens may be delivered to the host by introducing DNA, RNA, modified bacteria or virus vectors. Also, synthetic peptides based vaccines and peptide-loaded dendritic cells (DCs) show promising results as another modality of vaccine approaches.

In this review we briefly discuss why traditional vaccine approaches were not so effective against several present-day epidemic diseases. Thereafter, we highlight some immunological principles that give foundation to viral vector-driven vaccines. We put a special emphasis on how the knowledge gained from cytomegalovirus can be used to design more efficient vaccines and vaccine vectors against multiple human pathogens.

TRADITIONAL VERSUS MODERN VACCINES

Traditional vaccines have shown an outstanding success against several infectious diseases. Because they most closely recapitulate a natural infection, live attenuated vaccines are considered to be the best way to stimulate both humoral and cellular arms of the immune system. Vaccines against smallpox, mumps, rubella, yellow fever and measles can elicit a long-lasting or lifelong protection with even a single dose *(4, 5, 6)*. However, there are many pathogens for which even the immunity acquired after natural infection does not fully protect against re--infection and disease. Therefore, we need to devise vaccines which offer superior protective capacity compared to those obtained by natural infection. For instance, human immunodeficiency virus (HIV) mutates rapidly because its replication includes reverse transcription of the single stranded RNA genome but lacks proofreading mechanisms *(7)*. The result is that each genome of the viral progeny differs from the infecting viral genome in about one nucleotide, which makes it antigenically very heterogeneous *(8, 9)*. Also, live attenuated HIV may regain its virulence through mutations. In the case of influenza virus, live attenuated or inactivated vaccines confer some degree of protection by eliciting neutralizing antibodies to hemagglutinin (HA) and neuraminidase (NA), but frequent mutation of the genes encoding for these major virion surface antigens leads to minor or major antigenic differences between year-to-year strains, resulting in the escape of the virus to antibody-based neutralization *(6, 10, 11)*. Similarly, the existence of multiple serotypes of the dengue virus makes it hard to develop an effective vaccine by traditional methods *(12)*. Live attenuated vaccines have been proven inefficient or of questionable safety in reinfection with respiratory syncytial virus (RSV), malaria, persistent or latent infections, such as hepatitis C virus (HCV), human papilloma virus (HPV) and herpesviruses *(13)*. Immunocompromised patients and immunologically immature infants or children are additional important factors to bear in mind when developing live attenuated vaccines.

The second group of traditional vaccines includes inactivated pathogens and subunit vaccines. The discovery that inactivated pathogens may retain immunogenicity was the basis for the development of typhoid, cholera, hepatitis A, whole-cell pertussis and whole virus influenza vaccines. Subsequently, only the extracts of pathogens were used to develop anthrax and pertussis vaccine *(14)*, or pathogen products were used to develop toxoid vaccines against tetanus and diphtheria. Advance of technology enabled the synthesis of polysaccharide alone or protein conjugated polysaccharide vaccines against meningococcus, pneumococcus and *Haemophilus influenzae* type b and recombinant protein based subunit vaccine against hepatitis B virus *(14)* and HPV *(15)*. This group of vaccines may be more safe and stable, as compared to live attenuated vaccines, but their stimulation of the immune system is weaker, raising the need for several booster doses to maintain the appropriate level of immune protection over the time. Further, these vaccines are oriented more towards the production of antibodies, leaving the cellular immune response inadequate *(16)* and often requiring adjuvants to modulate the quality of immune response. Pulsing synthetic peptides into DCs *in vitro* resulted in production of several experimental dendritic cell-based vaccines *(17),* since DCs are major antigen-presenting cells and potent initiators of primary immune responses when pulsed with antigens *(18)*. Several more vaccine approaches, including peptide-based vaccines for cancer, are in different stages of research.

IMMUNE RESPONSE TO VACCINES

The fact that the most currently used vaccines rely on the induction of neutralizing antibodies has, for many years, put aside the development of vaccines based on protective cellular immune response. Indeed, antibodies are extremely powerful in the protection against many pathogens or their products; however, vaccines, or even natural infection with some viral pathogens, often fail to induce a sufficiently protective neutralizing antibody response. This is particularly notable for HIV, since most vaccines based on the induction of antiviral antibodies have failed so far *(19, 20, 21, 22)*. Therefore, the need for developing vaccines based on the induction of protective cellular immunity, which rely on T-cells, particularly cytotoxic $CD8^+$ T-cells, became imminent *(23, 24)*. Moreover, research on vaccines that induce T-cell immunity has dominated a great deal of recent development efforts. Although T-cells are well known for their capacity to contain viral infection, it turns out that the development of T-cell based vaccine is not so straightforward. The reasons for this may be several-fold. Many pathogens, particularly viruses, possess powerful mechanisms to interfere with immune response mechanisms, including T-cells. Furthermore, unlike antibodies, memory T-cells need some time after encountering their cognate antigen to express effector function and it may already be too late for some pathogens. This means that we need vaccines which are able to limit viral infection at a very early stage. It is likely that neither the approach based solely on the induction of neutralizing antibody, nor the one based on $CD8^+$ T-cell response will be successful – what we need are vaccine platforms that could induce both. Moreover, modern vaccine and vaccine vectors aimed at protecting against immunosubversive viruses, such as HIV, need to induce a more powerful cellular immune response of different quality than the one induced by natural infection *(25)*.

$CD8^+$ T-CELL RESPONSE TO VACCINES

It is current belief that a better understanding of the biology of memory T-cell subsets and their homeostasis is crucial for designing efficient vaccines based on cellular immunity. Priming of antigen-specific T-cells takes place in secondary lymphoid tissues triggered by dendritic cells, which present foreign peptides in context of either MHC

class I, or class II antigens *(26)*. This leads to the expansion of antigen-specific T-cells and their differentiation and maturation to antigen-specific effector T-cells, which disseminate to non-lymphoid tissues and control infection. Upon clearance of infection, the frequency of these cells contracts, leaving memory T-cells which enable more efficient recall response to cognate antigen and protection against reinfection *(27)*. Keeping in mind that $CD8^+$ T-cells control viral infection by recognition of foreign peptides, presented in context of MHC class I molecules on infected cells, and that $CD8^+$ cells can see several peptides of the same pathogen, or even of the same protein, the chances that the virus will escape $CD8^+$ recognition are smaller than the viral escape from neutralizing antibodies. In addition, unlike antibodies which are neutralizing only when they recognize surface viral proteins, $CD8^+$ T-cells can recognize foreign peptides derived from any viral protein, including non-structural proteins *(28, 29)*. The only way for the viruses to avoid such a powerful recognition mechanism is through interference with antigen processing and presentation, which is indeed the nature of many immunosubversive viruses *(30)*. The above mentioned characteristic of $CD8^+$ T-cells as effector cells can be exploited for designing preventive $CD8^+$ T-cell vaccines. In addition, we now know that $CD8^+$ T-cell response can be modulated by means of innate immune response *(30)*. Therefore, when designing vaccines based on $CD8^+$ cell response, one has to bear in mind not only the viral immunoevasion mechanisms, but also the capacity of innate immune system to modulate $CD8^+$ T-cell response.

Memory $CD8^+$ T-cells are roughly divided into two major subsets, based on the differential expression of homing receptors: central memory $CD8^+$ T-cells (T_{CM}), which express CD62L and CCR7, and effector memory $CD8^+$ T-cells (T_{EM}), lacking those two markers *(31)*. While T_{CM} travel through secondary lymphoid tissues and blood, representing long-lived memory cells with high proliferative capacity upon restimulation, T_{EM} re-circulate through the nonlymphoid tissues where they may be directly involved in virus control *(31)*. We now know that the cellular distribution and frequency of these memory cell subsets may be essential for the efficacy of immune response after challenge infection, but the mechanisms which are involved in the differentiation and maintenance of these subsets are still the subject of intensive research in the field *(25, 32, 33)*. Keeping in mind that $CD8^+$ T_{EM} have a potentially better chance to contain infection at its initial site (e.g. muscosa), one can speculate that successful vaccines for such pathogens need to favour the induction of this type of memory T-cells (see below).

VACCINE VECTORS

Vectors are delivery systems that usually derive from pathogens and deliver foreign genes to express the antigens *in situ (6)*. Plasmid DNA, RNA and modified viruses or bacteria can be used to deliver the antigen coding information to the organism. For instance, DNA, RNA and oligonucleotides are easily delivered into the cells *in vitro* by means of transfection for recombinant protein expression or regulation of gene expression. There have been several methods explored to increase the potency of these vaccines, such as co-transfection with non-coding bacterial plasmid containing CpG motifs, thus stimulating Toll-like receptor 9 (TLR9) and attracting the cells of innate immunity *(34, 35)*, changing the promoters to increase the gene expression *(36)*, or co-expressing of cytokines and other immunomodulatory molecules. The exciting research in this field has already resulted in several vaccines approved for use in animals, and many open clinical trials in phases I to III, exploring this approach in the prevention or therapy of, not only infectious, but also cardiovascular and neurological diseases and cancer [reviewed in *(37)*].

Bacterial vectors can also be used to deliver plasmids or protein antigens expressed within them *in vivo*. Both approaches have been evaluated in different studies and have shown controversial results *(38)*. Amongst the most used bacterial vectors in research are Salmonella, Mycobacteria and Listeria. Invasive bacteria, such as *Listeria monocytogenes* and *Shigella spp*., are able to replicate within the cytoplasm of mammalian cells. Upon lysis, the bacterial content can be released directly into the cytosol of infected cells, providing access to the MHC class I presentation pathway *(39)*. Attenuated and modified for vaccine purposes, bacteria may enter the organism in their natural manner, stimulating diverse immune responses (systemic and mucosal), and, if located intracellularly, deliver the gene or protein to APCs.

VIRAL VECTORS

The characteristic of viral vectors, such as their cell tropism, capacity for carrying heterologous genetic material, quantity of expression of heterologous genes, ability to induce diverse immune response and persistence in host, are some of the factors influencing the selection of a particular viral vector to its specific application. Viruses have evolved highly effective mechanisms for entering the cells and using their machinery for the expression of viral proteins *(40, 41)*. This property has made them very attractive as transporters of the genes of interest into the cell. Advances in molecular biology have made possible the modification of the viral genome, rendering viruses highly attenuated or replication-deficient (i.e. safe) and, at the same time, capable of infecting the cell and delivering the genetic material. Viral vectors offer many advantages compared to traditional vaccines, including robust antibody response, but, more importantly, they activate both $CD8^+$ and $CD4^+$ T-cell response that is essential for control of intracellular pathogens and cancer *(42)*. Additionally, by using viral vectors, immune response can be focused on a particular protein, or even epitope, conserved between different strains of pathogens, making them useful for creating universal heterosubtypic vaccines (e.g. for Influenza) *(43, 44)*. However, viral vectors may have several weaknesses. First of all, attenuated viruses may gain virulence *in vivo*,

making the vaccine vector unsafe. Secondly, these vectors may also recombine with endogenous viruses and gain their virulence. Under the selective pressure imposed by the immune system, viruses may mutate or delete the inserted foreign genes and lose antigenicity. Pre-existing immunity against viruses that commonly infect the human population can be a major problem. This is particularly the case for adenovirus vectors, and also for herpesvirus vectors. Many efforts to overcome the problem of pre-existing immunity are underway, including the development of different serotypes of viral vectors as well as the better design of prime-boost approaches.

The list of recombinant virus vectors that have been tested for vaccines is extensive and includes adenoviruses, adeno-associated viruses, vesicular stomatitis virus, polio virus, etc. One should not forget pox viruses, which have the longest use in human history, since the vaccination with vaccinia virus resulted in the eradication of smallpox. In addition to adenovirus vectors, several members of the pox virus family are currently under evaluation as vaccine vectors *(41)*.

Despite many disappointing outcomes in attempts to generate an efficient HIV vaccine *(45, 46),* a recent trial in Thailand, using prime-boost regimen consisting of Canary pox vector (Alvac-HIV, Sanofi Pasteur), followed by GP120 in Alum (AIDSVAX B/E Global Solutions for Infectious Diseases) clearly demonstrated the positive effect with regard to preventing HIV infection in the population at risk *(47)*. Curiously, and in contrast to what was expected, the data failed to provide any evidence that protection was mediated through $CD8^+$ response, although the pox virus as a vector is clearly a good inducer of these cells.

HERPESVIRUSES – VACCINES AND VECTORS

Herpesviruses are a large family of DNA viruses, among which eight can cause diseases in humans, particularly in children and in immunocompromised individuals. Herpesviruses can be subdivided into the α-, β- or γ- subfamilies based on their biological and sequence characteristics, but they share common features such as the establishment of persistence and latency in specific cell types, and the ability to cause lytic infection in permissive cells *(48)*.

Persistence in host and intermittent reactivation are key features of the herpesvirus infections. These features, however, in the context of viral vectors, may translate to periodic antigenic restimulation, which is highly desired for the induction and maintenance of effector T-cell response. The development of vaccines against herpesviruses has major public health significance. A range of vaccine platforms, has been used thanks to the major advances in molecular biology and genetic engineering *(49)*. Varicella-zoster virus (VZV), and herpes- simplex virus (HSV) have also been extensively researched as vectors for vaccines against HIV *(50, 51, 52, 53, 54)*, and several other pathogens *(55, 56, 57)*.

In spite of the outstanding properties of herpesviruses to induce potent immune response, including the response to vectored antigens, one should keep in mind that the use of live persistent viruses bears the risk of recombination of attenuated herpesvirus vaccine or vaccine vectors and the restoration of virulence, as recently demonstrated for laryngotracheitis herpesvirus *(58)*. Yet, a large proportion of herpesvirus genomes are occupied by non-essential genes encoding viral immunoevasins and other genes involved in interaction with host cells. Deletion of these genes usually does not compromise virus growth *in vitro*, but, alternatively, results in strong attenuation *in vivo (59, 60)*. Thus, it is unlikely that such deletion mutant viruses could easily recombine to regain the virulence. In addition, as will be discussed below, further manipulation with herpesviral genome, such as insertion of genes encoding ligands for various immune receptors, should make such a vector extremely sensitive to immune control *(61)*. We assume that, in spite of the obvious resistance of regulatory agencies to approve live recombinant persistent viruses as vaccine vectors, accumulated data point out that many of them may reach the highest level of safety standards. The use of recombinant viruses, such as a CMV vaccine and CMV-based vaccine vector, will be discussed further in the text.

CMV VACCINES

The Committee for Vaccine Development at the Institute of Medicine (The National Academies, Washington DC) ranked human cytomegalovirus (HCMV) vaccine, aimed to prevent congenital HCMV infection, among the candidate vaccines of the highest priority *(62)*. In order to design any successful vaccine, vaccines against persistent viruses in particular, it is essential to understand its biology and the immune response mechanisms involved in its control. Human cytomegalovirus (HCMV) is ubiquitous in humans, with seroprevalence rates from 50 to 90%. After primary infection, the immune response effectively terminates virus replication. However, the clearance of the viral genome is not achieved, and HCMV establishes a lifelong latency with periodic reactivation and virus shedding *(63)*. While HCMV infection is readily controlled in an immunocompetent host, the virus displays its severe pathogenic potential when immune control is impaired. Although there is sound basis to believe that vaccination may ameliorate HCMV infection or disease in some high-risk populations, any vaccine approach must take into account different clinical situations.

The components of innate immunity, particularly the natural killer (NK) cells, are considered important for early virus control, but components of specific immune response are needed for termination of productive infection and establishment of latency *(64)*. Among them, $CD8^+$ T-cells play a major role in the control of primary CMV infection, whereas antiviral antibodies are responsible for virus neutralization after the reactivation from latency *(65, 66, 67, 68)*. Notably, $CD8^+$ T-cells specific for mouse CMV (MCMV) have been shown to slowly

accumulate after the primary infection has been resolved *(69, 70)*. The protective capacity was associated with the emergence of epitope-specific $CD8^+$ T_{EM} cells, which provide long-term protection and apparently undergo continuous activation and proliferation. The expansion of the memory pool of $CD8^+$ (and $CD4^+$) T-cells specific to HCMV *(71)* was also described. Altogether, any attempt to prevent or ameliorate the primary CMV infection should bear in mind the key role of both innate and acquired cellular immune control whereas the quality of antiviral antibody response determines the ability of the host to limit virus spread after reactivation and re-infection. Another hallmark of all CMVs is the presence of a large range of so-called 'immune evasion' genes, targeting the host immune response, whose function may influence the success of the vaccine itself. Numerous studies using viral mutants, carrying targeted deletions in regions of CMV containing immunoevasive genes, have demonstrated their importance as virulence factors *in vivo (72)*.

A number of CMV vaccine strategies have been developed, including: protein vaccines, DNA vaccine, peptide vaccines, dense bodies' vaccines, virally vectored vaccines and live attenuated vaccines *(73, 74, 75)*. Unlike subunit vaccines, which induce immunity to selected viral antigens, live virus vaccines elicit an immune response which mimics natural immunity and provides broader protection. The use of live virus vaccines, however, carries the risk of reactivation of the vaccine strain in immunocompromised patients, unless such vaccine virus is susceptible to residual immune control preserved after immunosuppression. For instance, if some components of innate immunity are preserved after immunosuppression (e.g., NK cells) and the vaccine virus is sensitive to them, one would expect that any recurrence of the vaccine virus would be controlled. One such pre-clinical approach to generate immunogenic, yet safe, live vaccine is the deletion of viral genes that subvert host immune response *(59, 76)*. Another approach would be the insertion of ligands, for activating receptors on immune cells, into the CMV genome (Figure 1). We

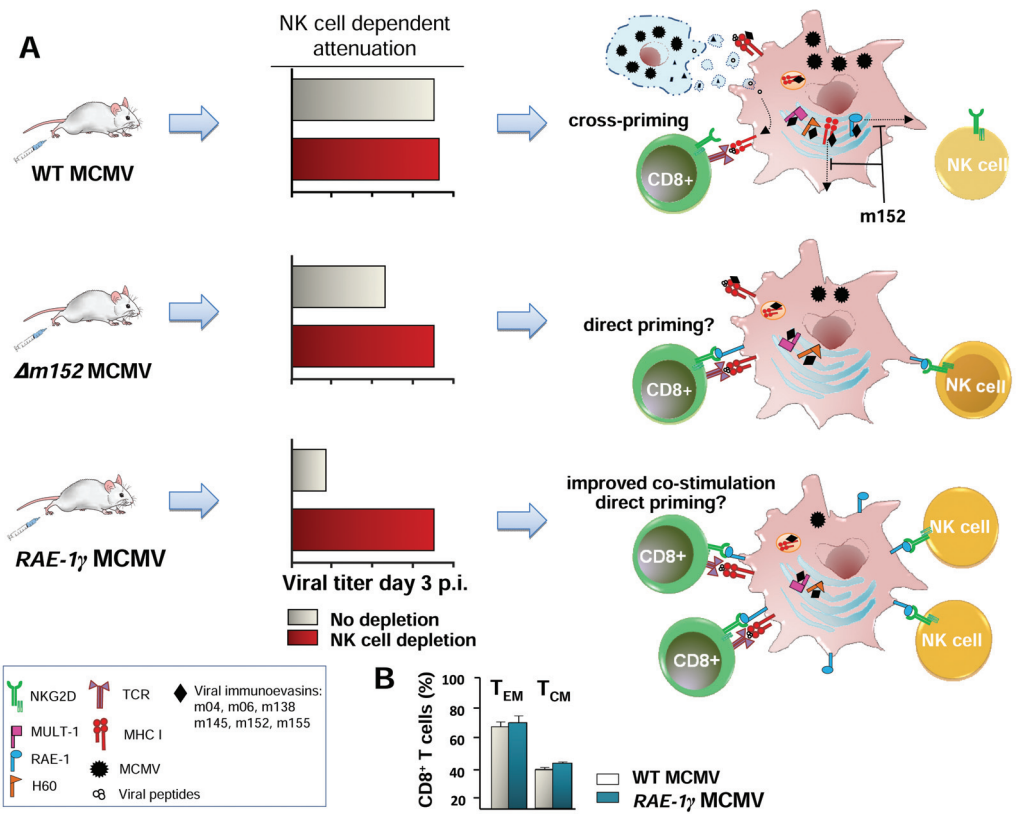

Figure 1. *Despite profoundly attenuated replication in vivo and reduced antigenic load as compared to the WT MCMV, recombinant virus expressing NKG2D ligand RAE-1γ instead of its viral inhibitor m152 efficiently primes and maintains virus-specific $CD8^+$ T cell response. Naive $CD8^+$ T cells require engagement of TCR by MHC-I molecules on antigen presenting cells (APCs) in order to be activated. (A) MCMV has evolved several mechanisms for evading the immune system and thus escaping the virus clearance. Wild-type MCMV (WT MCMV) downregulates MHC class I and co-stimulatory molecules from the surface of infected APCs and reduces their capacity for direct T cell priming. Consequently, the cross-priming becomes the dominant mechanism in generation of specific $CD8^+$ cells following WT MCMV infection. However, infection with MCMV mutants lacking MHC class I evasion genes (e.g., m152) and other immunoevasins or mutants possessing insertion of genes encoding cellular ligands for activating receptors (e.g., RAE-1γ) may improve not only virus control but also T cell/APCs interaction and $CD8^+$ T cell priming. Accordingly, despite profoundly attenuated replication in vivo and reduced antigenic load as compared to the WT MCMV, RAE-1γMCMV efficiently primes and maintains virus-specific $CD8^+$ T cell response (61, 85). (B) Similar to WT MCMV infection, T cell induced by RAE-1γMCMV is of dominantly of T_{EM} phenotype.*

have recently combined these approaches, designing an experimental CMV vaccine encoding ligand for NKG2D receptor, and demonstrated excellent characteristics of such virus *(61)*. In fact, the idea was to create an experimental CMV vaccine, extremely sensitive to control by NK cells, but at the same time, able to prime and maintain robust virus-specific response. Such an idea has been somewhat against the current dogma, since it is believed that the intensity of virus replication and antigenic load positively influences ongoing immune response *(64, 77)*. To achieve both, strong attenuation and potent antiviral response, we inserted Rae-1γ, the cellular ligand for the NKG2D receptor, in place of its viral inhibitor. Thus, the obtained recombinant virus was able to activate NK cells via NKG2D and circumvent all other immunoevasion mechanisms. At the same time, the priming of the antiviral T-cell response was equal to, or even higher, than in mice infected with the WT MCMV strain.

The powerful immune response was probably a consequence of NKG2D co-stimulation, since this receptor plays a co-stimulatory function on $CD8^+$ cells. Moreover, we have shown that the vaccination of female mice before pregnancy results in the induction of an antiviral antibody response which, after transplacental transfer, can protect newborn mice from lethal MCMV infection. The two other features of such recombinant vaccine are worth mentioning. Firstly, it is attenuated even in mice lacking a receptor for type I interferons (IFNAR k.o. mice), or mice immunosuppresed by sublethal gamma irradiation, which are otherwise extremely susceptible to this virus. Secondly, since such a virus is under stringent selective pressure by NK cells *in vivo*, one would expect the viral escape from NK cell control by mutation or deletion of Rae-1 transgene. Surprisingly, unlike in the case of the m157 gene, which is subject to numerous mutations and deletions when the virus is exposed to NK cells expressing Ly49H receptor *(78),* the gene encoding Rae-1, expressed in MCMV, remained completely intact, even when reactivated several months after infection. Altogether, the work by Slavuljica and colleagues nicely demonstrates that one can design a CMV vaccine or CMV-based vaccine vector which does not cause disease, even in immunologically immature and immunodeficient hosts, but is at the same time able to induce strong antiviral immunity. Based on the results presented above, one can predict that a similar virus could serve as an excellent vaccine vector *(79)*.

CMV-BASED VACCINE VECTORS

As mentioned above, CMVs are excellent inducers of virus-specific $CD8^+$ T-cell response, in spite of the numerous immunoevasion mechanisms aimed to compromise antigen presentation in context of MHC class I molecules *(80)*. Indeed, a recent study demonstrates the ability of MCMV-based vaccine vector expressing CD8 epitope of Ebola virus nucleoprotein to protect against challenge infection with this virus *(81)*. The ability of attenuated recombinant CMVs to induce and maintain robust immune response was most likely the reason for efforts in preclinical studies aimed at developing an HIV vaccine, based on CMV as a vector. Hansen and co-workers used rhesus macaques injected with rhesus CMV (RhCMV) expressing simian immunodeficiency virus (SIV) genes, and tested its capacity to induce SIV-specific $CD8^+$ T-cells (Figure 2). Indeed, they were the first to demonstrate that majority of animals vaccinated with the RhCMV/SIV recombinant virus, expressing multiple SIV proteins, could resist progressive infection following repeated SIV administration via an intrarectal route *(82)*. Next, they showed that this CMV-based vaccination preferentially induces $CD8^+$ T-cells of effector memory phenotype, which should therefore be able to restrict SIV replication on the site of infection. Moreover, they compared the vaccination with RhCMV/SIV recombinant virus to the conventional recombinant adenovirus vaccine that predominantly induces central memory T-cells *(83)*. The obtained results were very encouraging

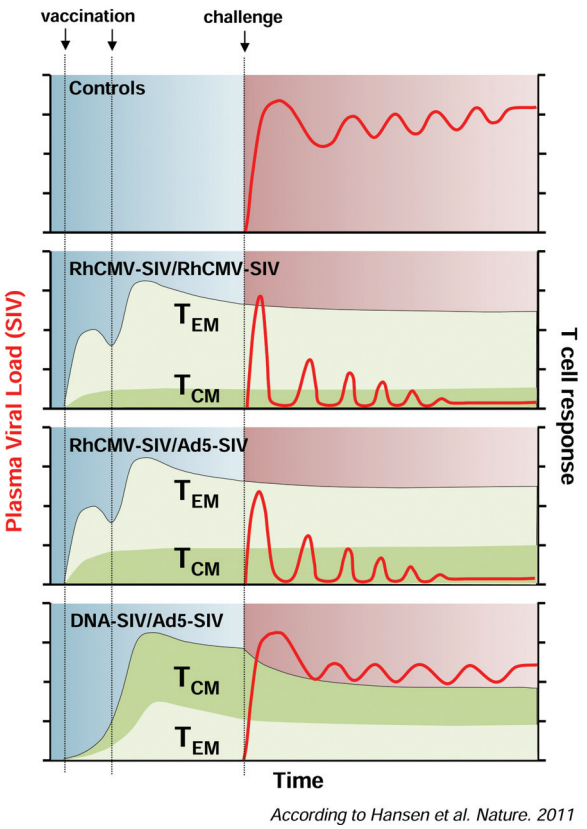

Figure 2. *RhCMV vectors expressing SIV genes induce a strong and protective effector- memory T cell response in rhesus macaques. Three different vaccination protocols which included RhCMV-SIV, Ad5-SIV vectors and plasmids with SIV genes, were recently compared by Hansen* et al. (83). *The first group of animals received RhCMV-SIV vector alone; the second group was primed with RhCMV-SIV vector and boosted with Ad5-SIV vector, while the third group was primed with plasmids containing SIV genes and boosted with Ad5-SIV vector. The effects of various vaccine approaches with respect to T cell response and virus control after challenge infection with highly pathogenic SIV are schematically displayed.*

with regard to the potential of generating HIV vaccine using similar approach and HCMV as a vector. Namely, the repeated rectal challenge infections of primed animals with SIV showed a remarkable protection in majority of animals challenged with transient SIV viremia followed by decrease in virus load. Additionally, periodic SIV reactivation was also less frequent over the time, which is in stark contrast to the course of persistent SIV infection and high level of virus replication in unvaccinated macaques. The efficacy of this vaccine approach as compared to others, which are also able to induce $CD8^+$ T-cells, is likely to be related to the ability of RhCMV/SIV vector to induce effector memory $CD8^+$ T-cells which became resident in mucosal tissue. This close proximity of memory $CD8^+$ cells to the site of SIV infection seems to be essential for early containment of SIV infection, which was not the case with vaccines unable to induce effector memory $CD8^+$ T-cells in mucosal tissue. Altogether, the above mentioned studies demonstrated that CMV-based vaccine vector can be used to induce the protective T-cell response which is able to contain SIV infection. Therefore, these studies indeed shed the new light on the development of preventive HIV vaccine by using persistent herpes viral vectors *(84)*.

CONCLUDING REMARKS

Recent developments in the use of viruses as vaccine vectors have been enhanced by expanding knowledge of immunology and viral biology. It is current dogma that the optimal vaccine platform should induce both cellular and humoral immune response. It is well established that live attenuated virus vaccines and vectors still represent the best inducers not only of cellular immunity, but also of antibody response. As there are a large number of pathogens for which even the immunity acquired after natural infection does not fully protect against re-infection and disease, we need to devise vaccines which offer superior protective capacity compared to those obtained by natural infection. At present, a wide range of viruses, replication competent and attenuated, are under development as vaccine vectors for human use. Several preclinical studies on animal models, including non-human primates, indicate the capacity of these persistent herpesvirus vectors to elicit and maintain potent memory T and B cell response. Several sets of data indicate that, by deleting various herpesviral immunoevasion genes and/or by inserting cellular ligands for activating immune receptors, one can create vectors that can induce robust protective immunity in spite of dramatic attenuation. We believe that similar platforms can be used in the development of vaccination strategies against multiple human pathogens.

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