New developments in antigen-specific immunotherapies

Novel therapeutic vaccines offer hope in the fight against chronic infectious disease and cancer

Vaccination has been used effectively for more than 200 years to protect individuals from a range of infectious diseases such as polio and smallpox. Many academic and commercial groups are now looking beyond prevention to the therapeutic use of this approach. New insights into the role of the immune system in areas of great unmet medical need such as cancer and chronic infectious diseases are enabling the development of a rational approach to designing novel antigen-specific immunotherapies, therapeutic vaccines, which use the body's own defence system. Far from being a stale approach, technological advances continue to drive vaccination to the forefront of drug discovery. A view supported by forecasts that the 'vaccine' market, including therapeutic vaccines, is predicted to grow from $6 billion in 2000 to $20 billion by 2009 (www.recap.com). This article will examine how increased knowledge of the cell-mediated immune response is making therapeutic vaccines a genuine clinical and commercial possibility.

By Dr Jörg Schneider and Professor Andrew J. McMichael

An improved understanding of the immune system has led to the development of a number of novel treatment approaches for cancer and chronic infectious diseases. These approaches are aimed at inducing or boosting immune effector mechanisms that control or contain tumours and persistent pathogens. There are two major categories of the human adaptive immune response; the most broadly recognised is antibody-mediated immunity, also called humoral immunity. Antibodies are carried in body fluids such as blood or lymph and tackle pathogens found outside the body's cells. However, intracellular pathogens such as all viruses, some parasites and some bacteria are able to avoid exposure to antibodies. Antibodies cannot destroy an infected cell. Similarly, antibodies are not effective at killing tumour cells. Faced with these situations, the body employs cell-mediated immunity to identify and destroy diseased cells.

Cells of the immune system constantly monitor

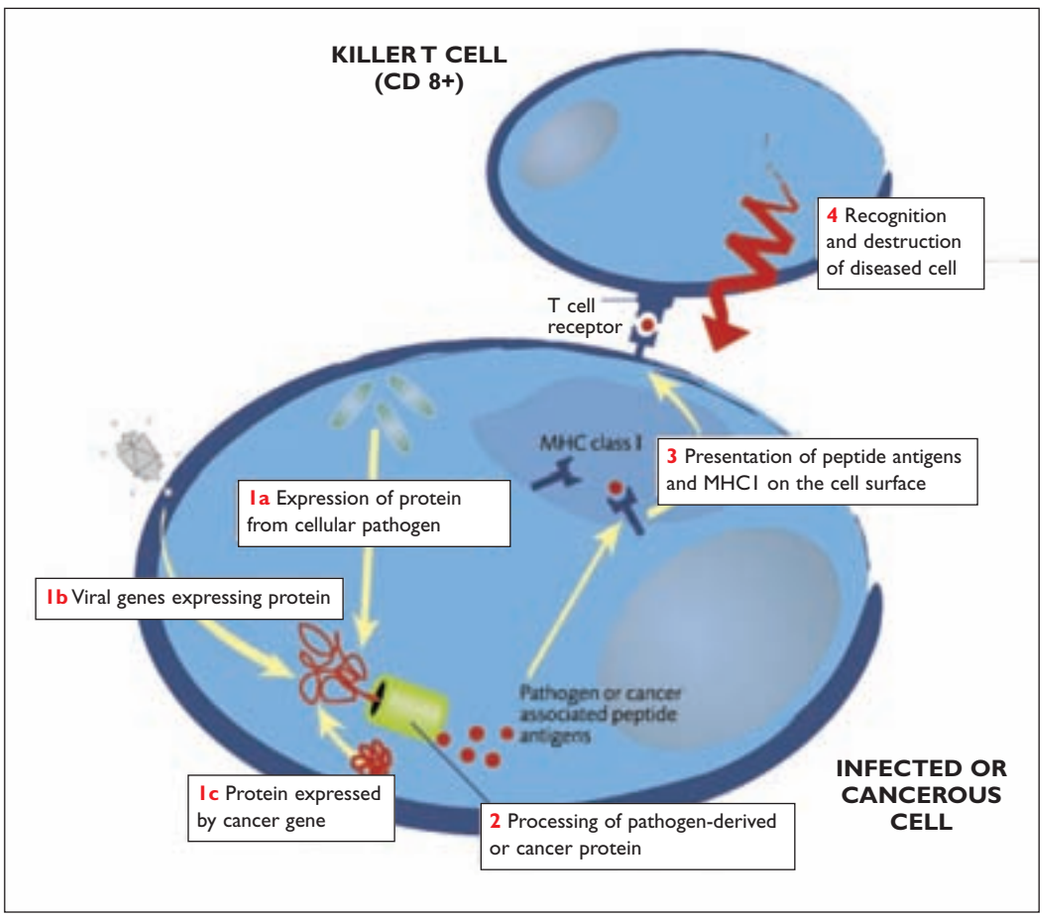

Figure 1
In order to fight intracellular pathogens and tumours, the immune system has evolved specialised effector cells. CD8+ 'killer' T cells have the unique ability to scan tissues for the presence of pathogens or malignant transformation. This scanning system relies on sophisticated machinery within the diseased cells that places pathogen-associated or tumour-associated antigens on the cell surface in a manner that allows the CD8+ T cell to see them. Proteins from cellular pathogens (**1a**) or expressed from viral genes (**1b**) or proteins associated with cancer (**1c**) are processed to form antigens (**2**). The recognition process involves presenting the antigen within a cell-surface protein called MHC class I (**3**). Recognition of the MHC class I/antigen complex is mediated via the highly specific T cell receptor. On encountering and recognising the diseased cell the T cell locks on to it and is activated to release special contents that cause cell death (**4**)

for the presence of malignant or pathogen-infected cells within the body (**Figure 1**). In the case of chronic virus infection or tumour development the patient's immune system has failed to contain the virus or appearance of malignancies. Therapeutic vaccination is based on the concept that the immune system can be effectively stimulated in the presence of such conditions. There are several naturally occurring chronic virus infections caused by pathogens such as cytomegalovirus (CMV) and Epstein-Barr virus (EBV) which, following an initial acute infection, are properly controlled in the majority of immunocompetent individuals. Observations in cancer patients, particularly melanoma and renal cell carcinoma, suggest that strong cellular immune responses can lead to tumour regression[1]. Studies of these diseases revealed the presence of disease antigen specific immune cells called CD8+ cytotoxic T cells that evolved to deal with the intracellular pathogens or tumours. In contrast to disease antigen specific antibodies, CD8+ T cells exert numerous effector functions targeting the antigen expressing cells. In vivo, antigen specific CD8+ T cells can actively reach the site of virus infection or infiltrate tumours rather than passive diffusion of antibody molecules. Antigen expressing target cells are then eliminated by CD8+ T cells through secretion of anti-viral and tumoricidal factors. In addition, in viral infections the localised release of cytokines can prevent virus replication in neighbouring cells, therefore preventing further spread. Many current strategies in therapeutic vaccination aim to induce or increase antigen-specific CD8+ T cells.

Antigen delivery platform technologies for therapeutic vaccination

Historically it has been difficult to reliably induce and monitor CD8+ T cell responses in humans. Traditional antigen delivery technologies such as inactivated pathogens or subunit protein vaccines are potent at inducing antibody responses but fail to elicit significant levels of CD8+ T cells. Therefore a number of clinical trials testing protein-based products as therapeutic vaccines inducing CD8+ T cells were disappointing[2]. Different antigen delivery technologies are being developed aimed at introducing the antigen into antigen presenting cells which in turn stimulate CD8+ T cells. Recombinant protein-based approaches now

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use improved adjuvants, or use dendritic cells to deliver antigens. Nearly all human cells present antigens to the immune system in a non-professional fashion, ie in addition to their defined function. However, certain cells of the immune system, such as dendritic cells, present antigens via a different mechanism that results in potent immune stimulation – these cells are described as professional antigen presenting cells or APCs. Gene-based antigen delivery systems introduce the antigen into both professional and non-professional antigen presenting cells. This method has predominantly used recombinant viruses and DNA vaccines as antigen delivery systems. Initial success in small animal models raised high hopes for the potency of these antigen delivery systems since both antibody and cellular responses were observed[3]. However, assessment of this technology in non-human primates and humans revealed that these antigen delivery systems are far less immunogenic than predicted from small animal studies. Currently several new approches are being tested in clinical trials in order to increase the immunogenicity of gene-based antigen delivery systems[4]. Simply presenting the immune system with the same antigen in the same context (repeated administration) or larger amounts might not be sufficient to induce strong immune responses. The sequential administration of the same antigen in different (heterologous) antigen delivery systems has proved to be particular effective for the induction of high levels of CD8+ T cell responses in rodents and non-human primates[5,6]. This powerful heterologous prime-boost approach is now being tested in clinical trials for a number of different diseases such as HIV and malaria in a preventive setting, and for chronic hepatitis B infection, HIV and melanoma in a therapeutic setting.

Heterologous prime-boost

The heterologous prime-boost regimen exploits the ability of the immune system to generate large numbers of secondary antigen specific T cells following the initial priming step. The same antigen is delivered in sequence using different vectors. Following a priming immunisation, the antigen specific T cell population expands to modest levels and then contracts. Over time a proportion of these cells transform into memory T cells. This results in an increased number of antigen-specific memory T cells. Memory T cells have the ability to expand rapidly upon encounter with the same antigen a second time round. In an heterologous boost situation, because the priming and boosting vectors are different, T cells specifically targeting the viral vector are not boosted and do not activate cell number control mechanisms, therefore allowing for greater expansion of the disease antigen-specific T cell populations (**Figure 2**)[6].

Several groups have now established that heterologous prime-boost regimens are among the most potent strategies to induce strong cellular immune responses. For example, a primary response can be induced by plasmid DNA vaccines. Boosting of this primary response with a heterologous (viral) vector will result in 4-10 fold higher T cell responses compared to homologous boosts with the same DNA vaccine[7-9]. It is speculated that the cytokine microenvironment created by a local virus infection during boosting is responsible for the effective expansion of effector T cells. Viral vectors such as vaccinia virus or adenovirus proved to be particularly effective at boosting immune responses[10,11].

The superiority of heterologous prime-boost immunisation regimen has been confirmed in many pre-clinical models including small rodents, ruminants and non-human primates. In humans, heterologous prime-boost immunisation regimen are currently being tested for cancer (melanoma, colon carcinoma, prostate carcinoma) and infectious diseases (chronic hepatitis B infection, HIV, malaria and tuberculosis).

Target diseases for therapeutic vaccines

Chronic infectious disease: HIV

Infection with the human immunodeficiency virus results in the initial rapid replication of the virus until initial immune effector mechanisms are activated. This acute phase of primary HIV infection is often associated with flu-like symtoms. Once high levels of HIV-specific CD8+ T cells are generated the virus load decreases and initially the virus is controlled but not eliminated. Eventually after 3-20 years of persistent virus infection the control by CD8+ T cells fails and AIDS develops. The reasons why HIV eventually evades control by the immune system remain unclear. Other persistent virus infections such as EBV and CMV seem to be controlled by the immune system for life. Probable reasons include the gradual decline of CD4+ (helper) T cell numbers and function (HIV invades and infects this important subset of immune cells), damage to antigen presenting cells and repeated escape from the CD8+ T cells by mutation in the virus.

The role of HIV-specific T cells in HIV infection is being exploited for preventive and therapeutic HIV vaccines. In the preventive setting the induction of HIV specific effector T cells provides a build-up defence to combat incoming viruses. Studies in a small minority of commercial sex workers with high frequencies of virus exposures who remain uninfected yielded the first clues on the role of CD8+ T cells in HIV immunity. These individuals have high levels of

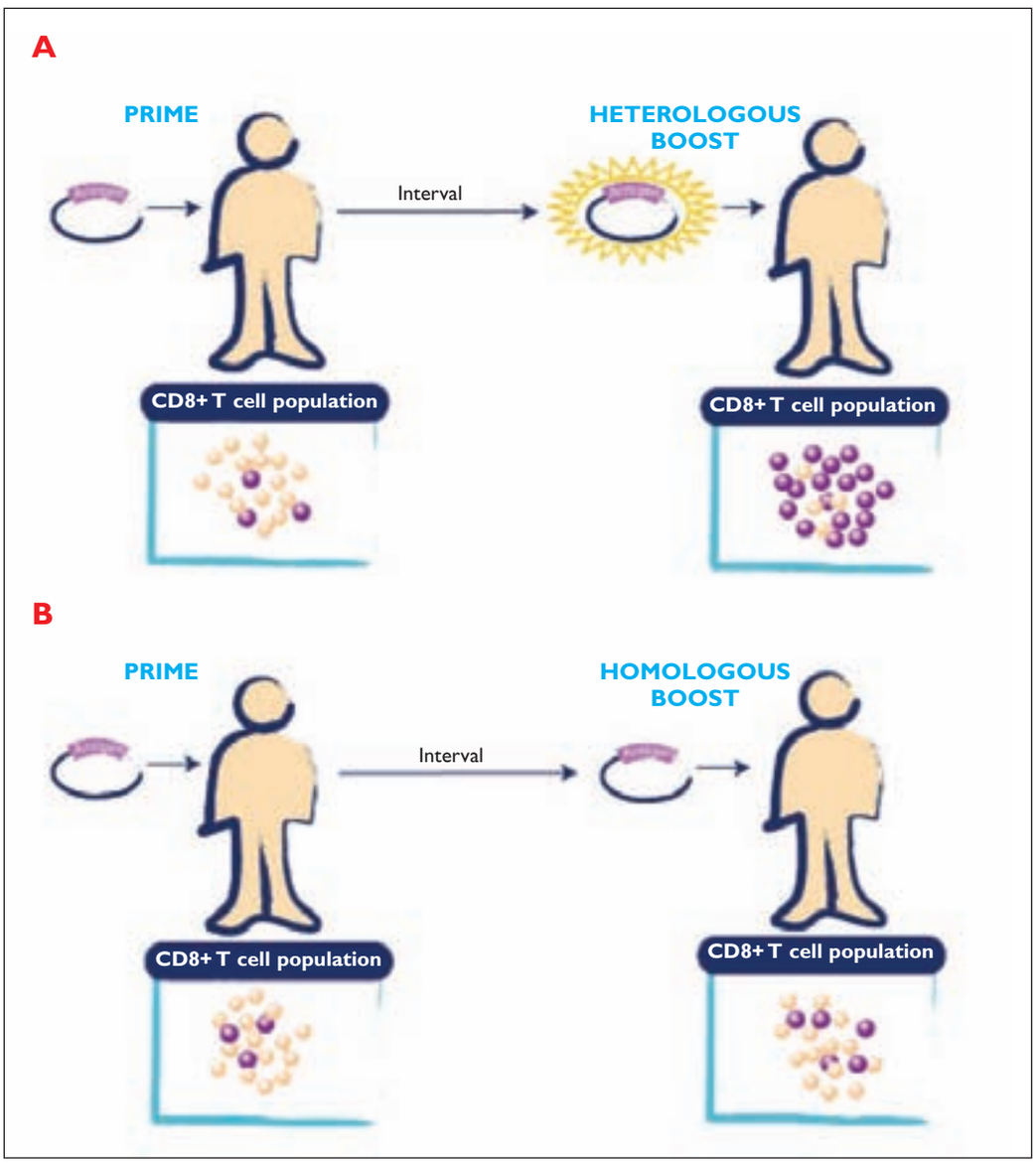

Figure 2
Heterologous PrimeBoost exploits the tendency of the immune system to focus an immune response. The approach aims to bias the preference for CD8+ 'killer' T cell amplification towards the important viral or tumour associated antigens and not vector antigens, resulting in a stronger boost. The prime immunisation acts to focus the immune response towards the important CD8+ T cell antigens, essentially setting the preference for the subsequent boost. Boosting with a different (heterologous) vector (**A**) results in a greater increase in the number of antigen specific CD8+ T cells (purple) within the CD8+ T cell population (purple and cream), when compared to using the same (homologous) vectors (**B**)

HIV-specific CD8+ T cells and no detectable antibody responses to HIV[12]. Studies in monkeys have also shown that vaccination to stimulate CD8+ T cells can protect against challenge with SIV, the simian equivalent of HIV. Therapeutic HIV vaccines aim at boosting numbers or changing the quality of CD8+ T cells in chronically infected individuals. Given that natural infection stimulates a very good response in the early stages, it is unlikely that vaccination alone will do much good. A more promising idea is to combine vaccination with drug treatment. The new antiretroviral drugs can reduce virus levels in HIV infected people to undetectable levels, but they do not eliminate virus and if treatment is stopped virus rebounds within weeks. When virus levels are reduced by drugs the CD8+ T cell response falls because of reduced antigen stimulation[13]. Therapeutic vaccination could restore this T cell response, so that if drug treatment was stopped the rebounding virus could be controlled. A recent experiment in monkeys infected with SIV shows that this could be effective[14]. Several groups are now developing effector T cell inducing vaccines against HIV using heterologous prime-boost immunisation strategies. One of the most clinically advanced programmes uses plasmid DNA prime followed by recombinant MVA boost to deliver antigens[15].

Cancer: melanoma

Melanoma is one of the more immunogenic human solid tumours. It is well known that cutaneous melanomas can demonstrate partial regression of the primary tumour and that melanoma patients

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can develop both antibodies and CD8+ T cells against tumour antigens[16]. These tumour-reactive CD8+ T cells can produce tumour regression after expansion *in vitro* and re-injection into the same patient[17]. From other clinical studies, it is known that 3-15% of cutaneous melanomas are first diagnosed as lymphatic or visceral metastases with no physical evidence of a primary tumour. The ability to cure a subgroup of these patients with surgical resection suggests that immunological mechanisms are capable of managing residual micrometastatic disease[18]. All of these observations support the idea that melanoma tumours express antigens that can serve as targets for immunotherapy.

Central to the development of a therapeutic cancer vaccine is the molecular identification of the antigens present on the tumour cells that are recognised by the immune system. Many of the melanoma antigens that have been identified are either tissue-specific differentiation antigens (Melan-A/Mart-1[19], tyrosinase[20], gp100[21], etc) or are expressed by a diversity of tumour tissues, but are not expressed by normal tissues, other than the testis. These are the so-called 'cancer-testis' antigens and are encoded by genes with family names such as MAGE, BAGE, RAGE and NY-ESO-1[22]. Antigens or CD8+ T cell epitopes from both antigen groups are currently being tested in a number of clinical trials using different antigen delivery platforms.

Most current melanoma vaccine trials use peptide CD8+ T cell epitopes mixed with adjuvants, cytokines or loaded on to dendritic cells as antigen delivery platforms. The first clinical data from peptide immunisation trials show that peptide immunisation can induce some melanoma-specific CD8+ T cell responses[23-25]. Oxxon Pharmaccines is currently evaluating a DNA vaccine and a recombinant MVA expressing a series of different CD8+ T cell epitopes in a heterologous prime-boost regime. This antigen delivery approach offers the posibility to deliver whole antigens or even multiple antigens to ensure a broad immune responses.

Challenges and outlook

There are numerous challenges associated with the induction of an effector immune response in the presence of viral or tumour antigens, and these differ for chronic infectious diseases and cancer. In the case of chronic infectious diseases, one of the main criticisms of the concept of therapeutic vaccination is the idea that delivery of more viral antigen will not stimulate the immune system in an organism that is already confronted with large quantities of antigen. A possible answer is that despite the fact that antigen is presented by a persistent pathogen, the route of antigen presentation might be important to trigger an immune response. Combining immune modulatory interventions with drugs to lower viral load, or surgery to lower tumour burden, might help lead towards greater clinical efficacy. Many tumour antigens are weakly immunogenic and presenting them in a more immunogenic context may trigger an effective response. Finally, therapeutic vaccination might induce broader, more effective T cell specificities that are different to the specificities induced during the natural history of a disease. Delivery of antigens in a more inflammatory context might be able to overcome low immunogenicity of self/tumour antigens.

Another key issue for therapeutic vaccination will be the maintenance of sufficiently high levels of an immune response with a therapeutic effect. Treatment cycles of heterologous boost-boost regimes offer a realistic chance of maintaining high levels of effector T cells. For example priming with DNA vaccines boosted by a recombinant poxvirus (ie MVA) followed by boosting with a recombinant adenovirus.

The combination of advanced antigen delivery platforms, such as heterologous prime-boost, with enhanced methods of measuring antigen-specific T cells will enable clinical researchers to correlate levels and quality of T cell responses with clinical efficacy. DDW

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