emerging therapeutic vaccines

The excitement over the past 30 years for immunotherapy of cancer and other diseases has not led to the expected clinical successes. Over-enthusiasts predicted a cure for cancer with the initial development of monoclonal antibody technology, and later the 'magic bullets' or toxin-labelled antibodies. Identification of proteins restricted to, or at least overexpressed in tumours has also led to disappointing clinical results. The main barriers have been a lack of immunological understanding of the processes at work, eg immune tolerance. Advances in our understanding of how to induce strong immune responses and how to manipulate the immune system to avoid immunological tolerance have opened the way for emerging therapeutic vaccinations in the treatment of not only cancer, but other diseases as well. This review will focus on immunotherapy for cancer and chronic human diseases characterised by the altered expression of self-proteins.

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Immunological tolerance regulates the immune system such that foreign pathogens are quickly attacked, while immune responses directed against the body are rare. This is achieved by functionally removing cells that recognise self-antigens from the immune system. In most cases, a breakdown of immune tolerance is undesirable, and leads to autoimmunity. However, in some situations it is beneficial to elicit an immune response against self-proteins aberrantly expressed in chronic human diseases and in cancer (eg the overexpression of Her-2 in breast cancer). The immune system is divided into two main responses, the innate immune response and the adaptive immune response. The innate immune response is the body's first line of defence and is mostly nonspecific; while the adaptive immune response is a specific response to the invading pathogens, and is the response targeted by vaccination. The adaptive response is composed of B and T lymphocytes that recognise specific 'epitopes' or structures of the pathogen. B lymphocytes recognise three-dimensional structures on proteins and produce antibodies that bind these structures. T lymphocytes recognise short peptides (8-15 mers), presented in the context of the major histocompatibility complex (MHC) on antigen presenting cells, and become activated. T helper (Th) cells recognise peptides presented in MHC class II complexes and provide signals that help to activate other cells. Cytotoxic T lymphocytes (CTLs) recognise peptides presented in MHC class I complexes and kill target cells that present the same peptide/MHC class I complexes.

Considerable excitement concerning the treatment and diagnosis of disease, mainly cancer, arose with the development of monoclonal antibody technology in 1975 by Kohler and Milstein[1]. Unfortunately, very little clinical progress has been made utilising monoclonal antibodies to treat disease, with several noted exceptions (Rituximab (α-CD20), Remicade (α-TNF) and Herceptin (α-Her-2). Part of the failure of monoclonal antibodies in treating disease may partly be due to the lack of support from the pharmaceutical industry, which pulled its R&D funding after initial endeavours did not live up to expectations. However, there has been a resurgence of interest and success with monoclonal

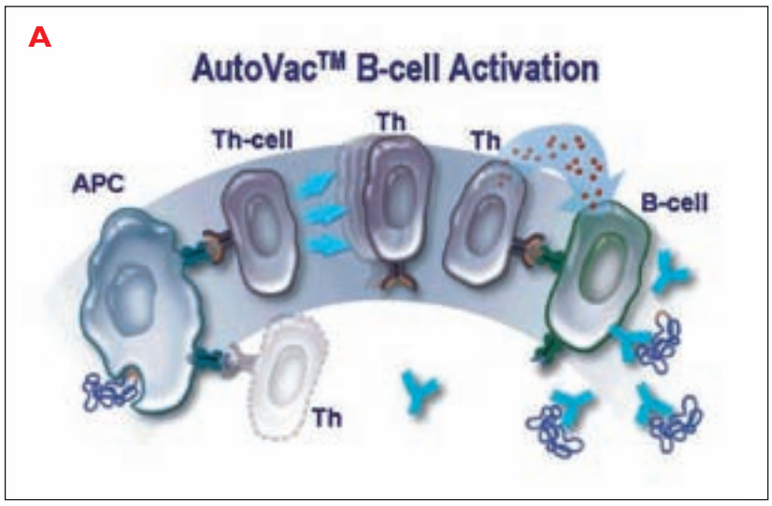
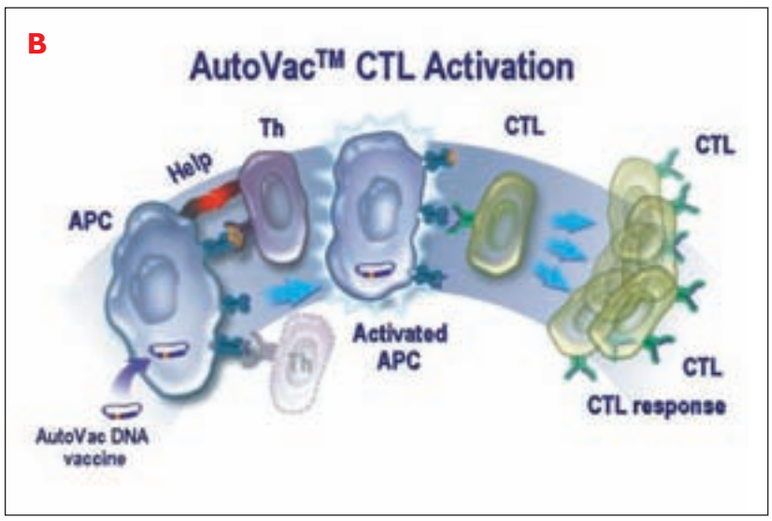


Figure 1
AutoVac™ can bypass immunological tolerance by providing help to B and T lymphocytes. The AutoVac™ recombinant protein, or DNA, is taken up, processed and peptides are presented by MHC class II on antigen presenting cells (APC). Simultaneously, the T helper (Th) cells that recognise the promiscuous Th epitopes presented by MHC class II molecules, and the APC, become activated, upregulate costimulatory molecules and produce cytokines. **A** B-cells recognising the self-antigen also internalise the AutoVac™ protein via the B cell antigen receptor, process and present the peptides to Th cells. Activated Th cells that recognise the peptides presented by B-cells activate the B-cells to differentiate into antibody secreting plasma cells that produce antibody capable of crossreacting with the non-modified self-protein. **B** The activation of APCs by Th cells is required to stimulate CTLs, recognising peptides bound to MHC class I molecules, capable of killing target cells expressing the self-protein.



antibodies, which is mirrored in the number of monoclonal antibodies marketed or in late stage clinical trials for both cancer and immune system disorders such as asthma, allergy and autoimmunity (see **Table 1**).

The treatment efficacy of a monoclonal antibody is usually lower than that of a strong polyclonal response, and the high doses and frequent patient administrations can cause problems. Most notably, endogenous immune responses to the 'foreignness' of the monoclonal antibody in the form of anti-idiotypic antibodies and immune reactions to xenogenic and isotypic aspects of the monoclonal antibody if it is not fully humanised have caused significant problems. The recent generation of mice expressing human immunoglobulin gene segments should facilitate the production of fully humanised monoclonal antibodies[2]. In addition to producing humanised monoclonal antibodies, much improved preclinical data can be obtained using these mice in combination with mice expressing human major histocompatibility complex (MHC) molecules[3-5]. Better immunological understanding of how monoclonal antibodies function *in vivo* has greatly increased their success in therapy; however, therapeutic vaccinations may have overwhelming advantages to monoclonal antibody therapy (see **Table 2**).

Active vaccination has the advantage of recruiting the entire immune response to subdue the targeted disease. Vaccination can induce a polyclonal antibody response that generates antibodies to several epitopes and of different isotypes[6]. The efficacy of clearing a soluble antigen is arguably increased by a polyclonal antibody response compared to the ability of a monoclonal antibody. In addition, the activation of antibody-dependent effector functions, the key to the success of antibodies in cancer immunotherapy, is orders of magnitude better with a polyclonal antibody response than with a monoclonal antibody. In fact, the inability of monoclonal antibodies to activate effector functions can explain some of the poor results obtained in previous cancer trials. In addition to the humoral response, vaccination has the advantage of activating cell-mediated immunity such as CTLs. Activated CTLs can directly kill other cells, such as a tumour, expressing the target antigen and MHC class I. The advantages of inducing both a polyclonal humoral response and a strong cell-mediated response would provide a new generation of therapeutic vaccinations with a greater chance of clinical success.

The first therapeutic vaccines utilised donor tumour cells as immunogens. Irradiated devitalised tumour cells are injected back into patients with the aim of raising an immune response to the tumour. Tolerance may prohibit immune responses to the dominant epitopes, but by providing some T cell help

Table 1
Monoclonal antibodies and therapeutic vaccines in clinical trials[a]

CANCER

Monoclonal antibodies			
Anti-EGFR (C225)	ImClone Systems	Phase III	head & neck
Anti-EGFR + cisplatin (C225)	ImClone Systems	Phase II/III	non-small cell lung
Lym-1-I^{131} (Oncolym)	Techniclone International	Phase III	non-Hodgkin's lymphoma
Anti-CD20-I^{131} (Bexxar)	Coulter Pharmaceuticals	FDA action	non-Hodgkin's lymphoma
Anti-CD33 (SMART M195)	Protein Design Labs	Phase III	acute myeloid leukemia
Anti-VEGF	Genentech	Phase III	colon & non-small cell lung
Anti-CD52 (Campath)	Ilex Oncology	PLA/NDA[b]	chronic lymphocytic leukemia
Anti-CD52 (Campath)	LeukoSite	PLA/NDA	chronic lymphocytic leukemia
Anti-CD33 + cytotoxin (CMA676)	Celltech Group	Phase II/III	acute myeloid leukemia
Anti-CD33 (AHP)	Celltech Group	PLA/NDA	acute myeloid leukemia
Anti-CD20 (Mabthera/Rituxan)	IDEC Pharmaceuticals	Marketed	non-Hodgkin's lymphoma
Anti-Her-2 (Herceptin)	Genentech	Marketed	breast & ovarian
Anti-CD20 + yttrium^{90} (Zevalin)	IDEC Pharmaceuticals	Phase III	non-Hodgkin's lymphoma
Vaccines			
hCG vaccine (Avicine)	AVI BioPharma	Phase II/III	pancreatic
hCG vaccine (Avicine)	AVI BioPharma	Phase III	colon
CEA vaccine (CeaVax)	Titan	Phase III	colorectal
GM2 conjugate vaccine (GMK)	Progenics	Phase III	melanoma
T cell vaccine + GM-CSF gene (GVAX)	Cell Genesys	Phase III	melanoma
Melanoma vaccine (Melacine)	RIBI Immunochem	PLA/NDA[c]	melanoma
Melanoma vaccine (Melacine)	RIBI Immunochem	Phase III	breast cancer
OncoVax®	Intracell	PLA/NDA	colon
Gastrin 17 peptides (Gastrimmune)	Aphton	Phase III	pancreatic
Gastrin 17 peptides (Gastrimmune)	Aphton	PLA/NDA	gastrointestinal
Hapten-modified tumour cell (M-vax)	Avax Technologies	Phase III	melanoma

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Monoclonal antibodies			
Anti-TNFα (D2E7)	Cambridge Antibody	Phase III	rheumatoid arthritis
Anti-TNFα (Remicade)	Centocor	Marketed	rheumatoid arthritis & Crohn's disease
Anti-TNFα (Norasept)	CellTech Group	Phase III	Crohn's disease
Anti-CD20 (Zenapax)	Protein Design Labs	Marketed	kidney transplantation
Anti-IgE (E25)	Tanox	Phase III	asthma & allergic rhinitis
Anti-IgE (E25)	Tanox	Phase III	allergy
Anti-IgE	Genentech	Phase III	asthma & allergic rhinitis
Anti-leukointegrin (LeukoArrest)	ICOS	Phase III	ischemic stroke
Anti-T cell (MEDI-500)	Medimmune	Phase III	graft versus host disease
Vaccines			
TCR peptide vaccine ()	Immune Response	Phase III	rheumatoid arthritis
MHC peptide (AnervaX)	Anergen (Corixa)	Phase II	rheumatoid arthritis
Heat-killed M. vaccae extract (PVAX)	Corixa	Phase II	psoriasis

a Clinical trial data taken from www.recap.com, and is not presented as a complete listing
b Product licence application/new drug application
c Approved for sale in Canada

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Table 2
Comparison of monoclonal antibody therapy to other vaccination methods

	MONOCLONAL ANTIBODY	CONJUGATE VACCINE	CELLULAR VACCINES	PEPTIDE VACCINES	VIRAL VECTOR	AUTOVAC™
Dose Required	High	Low-to-moderate	High	High	Low	Low
Duration/Titre	Weeks	Months	Months	Months	Months	Months
Efficacy	Moderate-to-High	Low- Moderate	Low	Moderate	High	High
Antibody Secondary Effector Functions	Low	High	High	Low	High	High
Immune Response to Dominant vs Subdominant Epitopes	Single Epitope	Subdominant	Subdominant	Subdominant	Both	Both
Antibody Fine Specificity	Monospecific	Variable	Variable	Limited	Variable	Polyspecific
Carrier/Viral/Anti-idiotypic Suppression	Yes	Yes	No	Yes	Yes	No
Cell-mediated Immunity	Not Applicable	Low	Low	High	High	High
Hospitalisation	Required	None	Required	None	None	None
Production Costs	High	Low	High	Low	Low	Low
Safety Characterisation	Easily Characterised	Difficult to Characterise	Difficult to Characterise	Easily Characterised	Difficult to Characterise	Easily Characterised

in the form of recombinant cytokines, viral oncolysates or genetically modified tumour cells (eg to express higher levels of MHC and co-stimulatory molecules) limited success can be achieved[7].

With the identification of tumour-associated antigens, peptide-based vaccinations can be developed to target specific antigens aberrantly expressed in cancer. Peptides are either obtained directly by acid-elution from MHC molecules on the cell surface of tumours (that can then be sequenced), or indirectly acquired from tumour-specific heat shock proteins (HSPs) that chaperone a wide variety of peptides[8]. Unfortunately, these approaches have the disadvantage of being expensive and labour intensive. In addition, because a complete set of peptides derived from both normal and tumour-associated proteins are included, development of autoimmunity to normal tissue may occur[9]. Determining the peptide specificity of tumour-specific CTLs *ex vivo* can be used to generate tumour-specific peptide vaccines for the generation of CTL responses. Peptides themselves are poorly immunogenic, and need to be conjugated to a carrier protein. Conjugate vaccines are able to elicit immune responses to poorly immunogenic peptides by providing T cell help in the form of a highly immunogenic carrier protein, such as keyhole limpet hemocyanin (KLH). Unfortunately, the dominating immune response to the carrier protein results in a poor immune response to the target peptide[10]. Additionally, conjugated peptides may have problems entering the MHC class I presentation pathway for CTL activation. The other disadvantage of peptide-based therapies is that the immune response elicited is to that single peptide epitope; whereas a given protein probably contains several MHC class I and II binding peptides.

Other methods of employing peptide-based therapies include pulsing peptides onto dendritic cells that specialise in presenting antigen to B and T lymphocytes. Bone marrow-derived dendritic cells are harvested from the patient, peptide is loaded on to the MHC molecules on the surface of the dendritic cell and the cells are reintroduced into the patient. The advantage of this approach is that the tumour-specif-

ic peptides are efficiently 'presented' to the immune system in a way that optimally activates T cells. Multiple target epitopes can be presented to the dendritic cells with a cocktail containing several disease-associated peptides. This approach is limited by cost and efficacy: having to develop specific vaccines for each patient and bypassing immune tolerance to the peptide epitopes.

Therapeutic vaccines have been hindered by their inability to break immunological tolerance and to generate a robust response composed of both a humoral and a cellular immune component. Several recent advances have addressed these problems, including recombinant viral vaccinations, polynucleotide (RNA and DNA) vaccinations, and autovaccinations (AutoVac^{TM}). All of these techniques can induce immune responses to conserved self-proteins by providing exogenous T cell help to lymphocytes, albeit at varying efficiencies.

Recombinant vaccinia virus can induce strong immune responses, both humoral and cell-mediated, to weakly immunogenic self-proteins. Recombinant viruses can be engineered to co-express multiple peptide epitopes[11], immunostimulatory cytokines (such as IL-12) or costimulatory molecules (such as B7 family members). The extreme immunogenicity of vaccinia virus limits its use to a single vaccination that cannot be boosted[12]. The inability to boost or vaccinate with subsequent vaccinia-based therapies, and other safety concerns, limit the use of recombinant viral-based vaccinations.

Polynucleotide vaccinations are able to generate both a humoral and a cell-mediated immune response, but appear to favour cell-mediated immunity. Recombinant DNA or RNA is introduced into the patient, where it is expressed in either antigen presenting cells that directly activate lymphocytes, or it is expressed and released by some other cell type and the protein is sequestered by the antigen presenting cell. Preclinical data are promising, but more time is needed to generate clinical data in humans and to resolve safety concerns. RNA vaccinations have safety advantages over DNA vaccinations, in that RNA cannot incorporate into the host genome and has a shorter half-life than DNA.

Autovaccination is another approach that can bypass immunological tolerance. Rapid and robust humoral and cellular immune responses can be induced to conserved self-proteins by providing exogenous T cell help to self-reactive lymphocytes. By constructing proteins containing promiscuous foreign T cell epitopes inserted into flexible regions of self-proteins, therapeutic autovaccination can trick the immune system into responding to otherwise immunologically tolerated proteins. The mechanism is shown in **Figure 1**. This therapeutic vaccination approach utilises the basic concepts of B-T lymphocyte collaboration to induce self-reactive antibodies and Th cell activation of antigen presenting cells to stimulate CTLs[13-17]. Self-proteins carrying promiscuous foreign Th epitopes are endocytosed by antigen presenting cells and the epitopes are presented to Th cells by MHC class II molecules expressed on the antigen presenting cell. Activated Th cells then provide help (eg cytokines) to those B cells that have also internalised the modified self-proteins via the B cell antigen receptor; and antigen-specific B cells then secrete antibodies capable of cross-reacting with non-modified self-protein (**Figure 1A**). Using the appropriate delivery system, or DNA vaccination (**Figure 1B**), AutoVac^{TM} molecules can also induce CTLs. As in **Figure 1A**, internalised peptide epitopes induce Th cells that then activate antigen presenting cells via CD40-CD40L interactions, for example. Activated antigen presenting cells can stimulate naïve CTLs capable of killing target cells displaying tumour-specific peptides presented by MHC class I molecules. This approach has the advantages of bypassing immunological tolerance and activating both arms of the adaptive immune system to target self-antigens aberrantly expressed in disease, while avoiding several of the disadvantages of other therapeutic vaccine technologies (eg carrier suppression and peptide therapies, see **Table 2**). DDW

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