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Therapeutic vaccines: realities of today and hopes for the future

Michael Sela, Ruth Arnon and Bilha Schechter

Vaccines are by definition prophylactic, but in recent years an interest has developed in therapeutic vaccines for infectious diseases such as AIDS and tuberculosis, as well as gastric ulcers, cancer (with different approaches to combat various types of malignancy) and autoimmune diseases (a definite success was the development of a vaccine against multiple sclerosis) and there are potential vaccines in development for myasthenia gravis, lupus and diabetes. Therapeutic vaccines are also being developed against cognitive diseases such as Alzheimer's disease, prion diseases and Huntington's disease. All of these efforts are based on the therapeutic vaccine being closely related chemically to the etiological agent that causes the disease.

***Michael Sela, Ruth Arnon and Bilha Schechter**
Dept of Immunology
Weizmann Institute of Science
Rehovot 76100
Israel
*tel: +972 8 934 4021
fax: +972 8 946 9713
e-mail: michael.sela@weizmann.ac.il

▼ Vaccines are prophylactic in the sense that they are administered to healthy individuals to prevent a disease. Nevertheless, there is a growing trend to use vaccines to alleviate the suffering of those already with a disease. Great effort is being devoted to develop vaccines against tumors, AIDS, hepatitis, tuberculosis, and possibly against the bacteria that cause gastric ulcers. Copolymer 1, used today as a vaccine against multiple sclerosis (MS), is a good example of a beneficial treatment for this autoimmune disease, based on its similarity to the myelin basic protein (MBP), one of the putative causes of MS [1]. This finding could lead to therapeutic vaccines against other autoimmune diseases such as myasthenia gravis, systemic lupus erythematosus (SLE) and rheumatoid arthritis. Furthermore, antibodies prepared against prions raise hopes for a vaccine against bovine spongiform encephalitis (BSE) and Creutzfeldt–Jakob disease (CJD), and antibodies to a peptide derived from β-amyloid plaques could degrade plaques and be used as a therapeutic vaccine against Alzheimer's disease (AD).

By its definition, a preventive vaccine is sufficiently similar in its chemistry to the molecule that provokes the disease so that the immune response directed against it can act against the causative agent. This situation is analogous in the case of therapeutic vaccines.

Infectious diseases

Conventional preventive vaccines against infectious diseases have been highly effective at drastically reducing the incidence and morbidity of many life threatening plagues such as smallpox and polio. Prophylactic vaccines are essential for most infectious diseases caused by viruses or bacteria, because of the short duration of the disease. (An exception is the rabies vaccine, developed by Pasteur >100 years ago, which is administered only after exposure to the virus.) Therapeutic vaccines constitute a relatively recent development, which is applicable and effective only for infectious diseases of a chronic nature, where the duration from exposure to full manifestation of the disease is extended. We will focus on the most prevalent ones.

HIV

Although a vaccine against HIV has been high on the agenda for more than a decade, no candidate vaccine has yet been proven effective. Reduced morbidity, afforded by the introduction of drug treatment, opened the way to the development of therapeutic vaccines, for which several approaches were undertaken (as indicated in a recent interview [2] and summarized in Table 1). Whole, killed HIV seemed to augment immune responses to HIV but with no evidence of significant efficacy, either alone or with the anti-retroviral drug treatment, HAART (highly active anti-retroviral therapy). Defined HIV recombinant proteins such as gp160 proved safe in Phase I trials, but failed to show efficacy in slowing disease progression. A product denoted p24

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Table 1. Vaccination against HIV

Vaccination approach	Viral protein in vaccine	Efficacy of vaccine	Efficacy with drug treatment (HAART)
Whole, killed HIV (Remune)	HIV-1 depleted of gp120	–	– Phase III study
Defined HIV protein (VaxSyn)	Recombinant gp160 of HIV-1 IIIB	–	n.d.
Defined HIV core protein (p24 VLP)	p24 and p17	–	n.d.
DNA vaccine	HIV-1 Env and Rev	+	+ (Rhesus monkeys and human trials)
Live virus vectors: ALVAC1452 (canary pox virus) or NYVAC (vaccinia virus)	HIV-1 Gag, Pol and Nef, and gp120	ALVAC1452: safe NYVAC: effective in macaques	+
Heat shock protein (HSP)	HSP70 and HSP72 alone or linked to gp120 or p27	n.d.	n.d.
Combination with immune-based therapies	Inactivated HIV and GM-CSF and interleukin-2	+	n.d.

Abbreviations: GM–CSF, granulocyte macrophage–colony-stimulating factor; gp, glycoprotein; HAART, high-acting anti-retroviral therapy; n.d., not determined; VLP, virus like particles.

VLP, containing the HIV core proteins p24 and p17 encapsulated in liposome particles, was also ineffective. DNA vaccination, however, resulted in increased expression of HIV-1 *env* and *rev*, and led to increased antibody responses in animals and a significant decrease in viral load. Furthermore, DNA vaccination followed by HAART demonstrated high efficacy in rhesus monkeys as well as in human trials.

Live virus vectors such as the recombinant canarypox virus (ALVAC1452, carries HIV-1 *gag*, *pol*, *env* and *nef*), in combination with gp120, was found to be safe in healthy volunteers and produced cytotoxic lymphocyte (CTL) responses. In combination with HAART, volunteers with HIV showed increased efficacy with ALVAC–HIV (recombinant canarypox virus expressing HIV antigens). Another suitable vector, the highly attenuated vaccinia virus (NYVAC) showed efficacy in macaques. Heat shock proteins (HSPs), including HSP70 and HSP72 linked to HIV proteins gp120 or p27, enhanced an antiviral immune response involving natural killer (NK) cells, antibody-dependent cellular cytotoxicity (ADCC), γδ-T-cell and CTL activities against HIV-1 infected cells. Combination with immune-based therapies in Phase II/III studies demonstrated the safety and immunological effects of immune-based therapies, including granulocyte macrophage–colony-stimulating factor (GM–CSF) and interleukin-2 (IL-2) with an inactivated HIV-1 immunogen as adjuvants to anti-retroviral therapy.

Hepatitis B (HBV)

HBV is highly infectious in humans, with a high frequency of persistent chronic infection (~400 million patients worldwide) that can lead to cirrhosis and liver cancer. The best way to control the disease is by prevention using the highly effective vaccine that comprises the recombinant surface antigen (HBsAg) of the virus. However, once infection has occurred, the pathology results from a self-destructive cytotoxic T-cell response modulated mainly by the core antigen (HBcAg). Hence, therapeutic vaccination has been approached recently by introducing DNA-based vaccines. The administration of bacterial plasmids encoding this core antigen [3] induced strong humoral and cell-mediated immunity that conferred protection in some animal models. Therapeutic vaccination was also evaluated in humanized BALB/c mice (trimera mice) subjected to lethal irradiation and transplanted with severe combined immune deficient (SCID) mice cells and human peripheral blood lymphocytes (PBMC). These mice can then be infected with HBV and hepatitis C virus (HCV). Mice reconstituted with PBMC from chronically infected patients simulated the chronic disease. DNA vaccination with plasmid expressing HBcAg led to a marked specific immune response [4].

Another approach that is being considered is the use of peptide-based vaccines, including B-cell- and T-cell-inducing epitopes, delivered either naked or in liposomes or virosomes with or without cytokines. Such peptides, employed in animal models and clinical trials in patients, selectively boost the immune response [5].

Tuberculosis (TB)

Despite the availability of a prophylactic vaccine (BCG) against TB, the incidence of TB is on the rise. Therefore, a

more efficient vaccine for control of the disease is needed, particularly to combat the multidrug-resistant tubercle bacilli (MDRTB). One approach is based on DNA vaccines expressing different antigens of the mycobacterium, which were shown to confer protection in animal models, thus paving the road for human trials [6]. Another interesting approach is based on recent reports that $CD8^+$ T-cells play a crucial role in the control of TB. Dendritic cells (DC) infected with BCG activate these specific $CD8^+$ cells, thus an anti-mycobacterial vaccine based on targeting $CD8^+$ cells via DC is a potential therapeutic strategy [7]. In another study, immunotherapy with heat-killed *Mycobacterium vaccae* in combination with chemotherapy, reduced the rate of drug-resistant TB particularly in patients with a short disease history.

Parasitic diseases

No effective vaccine is yet available for parasitic diseases, many of which pose a serious global health issue, inflicting hundreds of millions of people. Because, in most endemic areas, exposure to the parasite occurs at a young age, current efforts are directed at developing therapeutic vaccines that would be effective in patients who are already infected, as exemplified with malaria. Malaria caused by *Plasmodium falciparum* is the world's major parasitic disease (300 million people infected), and effective control measures are urgently needed. DNA- and peptide-based vaccines have thus been employed. DNA vaccines encoding several *Plasmodium* antigens of the infective stage induced $CD8^+$ CTL and interferon-γ (IFN-γ) responses in mice, monkeys and humans. By applying prime or boost immunization strategies, a multi-stage malaria vaccine was developed and is now in clinical trials. Combination therapy with GM-CSF augments the efficacy of the vaccine. Such a vaccine should also be effective against the blood-stage of the parasite, which persists in the chronic infection.

For peptide-based vaccines, the focus was initially on the infective stage (surface) circumsporozoite protein (CSP), but effort has since been concentrated on the blood-stage protein pf55/RESA and six peptides representing T- and B-cell epitopes. Vaccination of women in a malaria endemic area led to a positive cellular response in 77% of the women. The biggest, large-scale clinical trial of a vaccine for malaria, to date, was performed with Spf66, a 35-residue peptide that comprises a synthetic, hybrid molecule containing the protective epitopes of three blood-stage antigens, combined with the NANP epitope (amino acid one letter code) of the CSP. This vaccine was shown to be safe and could confer significant protection when tested in clinical trials in South America, but was less effective in a subsequent trial in Africa.

Gastric ulcers

Recent progress towards a therapeutic vaccine for gastric ulcers caused by *Helicobacter pylori* has mainly focused on the identification of a protective antigen. *H. pylori* urease in live recombinant *Salmonella* [8,9] was administered orally to mice infected with *H. pylori* and led to long-lasting protection. Initially it was assumed that this protective immunity was antibody-mediated. However, it was recently shown that $CD4^+$ helper cells are essential, at least in mouse models, indicating the role played by Th1 cytokines. Another direction in vaccine development is the isolation of a *H. pylori* strain from an asymptomatic individual, called the Baylor strain, which has protective potential.

Cognitive diseases

Prions

Prions are the transmissible pathogenic agents responsible for diseases such as scrapie and BSE [10]. The 'protein only' hypothesis of disease progression proposes that the causative agent, the prion, is identical to a conformational isoform of the normal cellular form of the prion protein (PrP^c). PrP^c is a normal, soluble host protein present in most organs but most abundant in the brain. During disease progression, a largely protease-resistant, aggregated form of PrP, designated PrP^{Sc}, accumulates in the brain. There is an urgent need for effective diagnosis and therapy for prion diseases, such as variant CJD disease.

Recently, three papers have been published that discuss the role of antibodies for treatment and prevention of prion diseases [11–13]. Exposure to the anti-prion protein mAb 6H4 cures cultures chronically infected with scrapie, as demonstrated by the long-term abrogation of PrP^{Sc} accumulation after cessation of treatment. Passive immunization might provide a therapeutic approach to prion disease [11]. Antibodies that bind cell-surface PrP^c inhibit PrP^{Sc} formation by cultured neuroblastoma cells in a dose-dependent manner [12]. Whether such antibodies are effective in animal models is yet unknown because of the long incubation period of scrapie, but the indications are that vaccines against prion diseases might be possible.

In another study [13], ablation of the *Prnp* gene, which encodes PrP^c, enables the immunization of mice with prions to generate specific anti-PrP antibodies, which prevented neuro-invasive scrapie when prions were introduced to the brain or spleen. The authors warn, however, that because of the broad expression of PrP^c, the induction of anti-PrP responses might induce an autoimmune response and hinder vaccination against prions. Nevertheless, it is certainly worth every effort to assess the value of active and passive immunization, as well as the role of T-cells, in this disease process.

Huntington's disease

Even in the case of Huntington's disease (HD), the possibility of vaccination has reached a hopeful stage. The expansion of glutamine repeats in proteins is responsible for several pathologies including HD. The mechanism that results in tissue damage is poorly understood, but the formation of protein aggregates rich in β-sheet structures is clearly important [14]. The abnormally elongated polyglutamine near the N-terminus of the huntingtin protein induces pathological protein–protein interactions and aggregate formation by huntingtin or its exon-1-containing fragments. Selection from a large human-phage display library yielded a single-chain Fv (sFv) antibody specific for the 17 N-terminal residues of huntingtin adjacent to the polyglutamine in HD exon 1. This anti-huntingtin sFv intrabody was tested in a cellular model of the disease in which huntingtin exon 1 had been fused to green fluorescent protein (GFP). Coexpression of anti-huntingtin sFv intrabodies with the abnormal huntingtin–GFP fusion protein dramatically reduced the number of aggregates, compared with controls lacking the intrabody. Intrabody-mediated modulation of abnormal neuronal proteins might contribute to the treatment of neurodegenerative diseases such as HD [15]. This work is under evaluation in an animal model as a further step in preclinical development.

Alzheimer's disease

Alzheimer's disease (AD), the most widespread and one of the most devastating neurological disorders, remains without an effective cure. One of the main characteristics of the disease is the accumulation of extracellular protein deposits called amyloid or senile plaques. The main constituent of these amyloid plaques is the amyloid-β peptide (Aβ), a fibrilar 40–42 amino acid peptide that accumulates in the brain of AD patients and elicits neuronal cell death. Aβ is generated by the proteolytic processing of amyloid precursor protein (APP). Recently, Schenk *et al.* [16] have shown that immunization with Aβ inhibited the formation of amyloid plaques and associated dystrophic neurites in mice genetically engineered to develop an Alzheimer's-like condition. These results raised the possibility of vaccination with Aβ against human AD, although many questions still have to be answered [17]. The most crucial question is whether the depletion of amyloid plaques is accompanied by an improvement in the behavioral or neurophysiological impairments and a reduction in neuron death. Aβ deposits in the brains of living mice, observed by direct imaging, appeared to clear following immunotherapy, indicating that the humoral response mediated the attenuation of Aβ deposition after immunization with Aβ [18]. Thus, passive immunization might be effective in preventing and clearing Aβ deposits in AD.

Two independent teams reported recently that Aβ vaccination seems to preserve memory and learning ability in plaque-producing mice. In one study [19], the performance of vaccinated and control animals was compared in the Morris water maze (a standard memory test in which animals are supposed to learn the location of a submerged platform in a water bath. The researchers found that even weeks after Alzheimer's-like memory disorders were apparent in non-treated litter-mates, immunized mice performed as well as mice that weren't engineered to develop amyloid plaques. In a similar study using the maze [20], mice were trained to find a hidden platform and the wrong turns of mice searching for the platform were counted after 30 min (to measure short-term memory). Mice engineered to have amyloid plaques were unable to find the platform whereas vaccinated mice were almost flawless in their attempts. The mice appeared to remember the location of the platform, as did control mice lacking the genes that cause amyloid plaque deposition.

A different approach to vaccination against AD was taken by Frenkel *et al.* [21], who developed an immunization procedure for producing effective anti-Aβ-aggregating antibodies based on filamentous phages displaying the EFRH peptide (amino acid one letter code), which correspond to amino acid residues 3–6 within the human Aβ peptide. The past two years have seen an intense effort in several laboratories to produce a peptide vaccine against Alzheimer's disease, or to alleviate it by passive antibody administration. However, recent reports announced the cessation of a clinical trial by Elan (Dublin, Ireland) because of detrimental responses of patients to such treatment.

Autoimmune diseases

For every autoimmune disease for which there is a putative molecule responsible for the disease, it should be possible to produce an analog that could serve as a therapeutic vaccine. Such a vaccine against MS has been developed by the authors of this review, but similar approaches will also be described for other autoimmune diseases such as myasthenia gravis, SLE, juvenile diabetes, optical neuritis and anti-phospholipid syndrome.

MS

Copolymer 1 (Cop 1, glatiramer acetate, Copaxone®) is a synthetic amino acid random copolymer, composed of L-alanine, L-glutamic acid, L-lysine and L-tyrosine [1,22]. Cop 1 was designed to simulate MBP, one of the major myelin-derived autoantigens implicated in the pathogenesis of MS, which induces experimental autoimmune encephalomyelitis (EAE) – an animal model of MS. Cop 1 was shown to suppress EAE induced by MBP in guinea pigs,

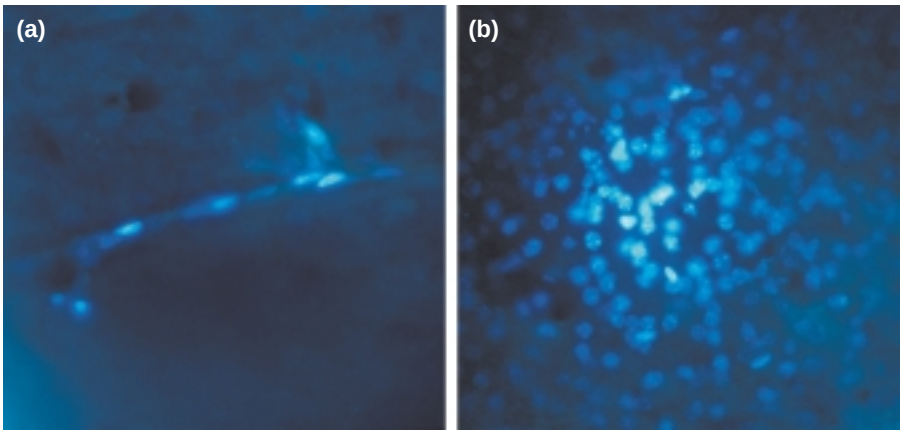

Figure 1. Brain sections of mice taken seven days after injection of fluorescent-labeled Cop1-specific T-cells to the periphery. **(a)** Cells in blood vessels. **(b)** A cluster of cells (perivascular infiltration) in brain tissue (magnification ×400).

rabbits, mice, rhesus monkeys and baboons. The suppressive effect of Cop 1 in EAE is4] not restricted to a certain species, disease type or encephalitogen used for EAE induction. In Phase II [23] and III [2clinical trials, Cop 1 was found to slow the progression of disability and reduce relapse rate in exacerbating-remitting MS patients. As an antigen-specific intervention, treatment by Cop 1 has the advantage of being less likely to cause long-term damage to the patient.

The mechanism of Cop 1 activity in EAE and MS seems to involve, as an initial step, the binding of Cop 1 to major histocompatibility complex (MHC) class II molecules, thus competing with myelin antigens for T-cell activation, both at the MHC and T-cell receptor level. Cop 1 also induces specific suppressor cells of the Th2 type, which probably play a major role in its mechanism of action [25–27]. Th2 cells cross the blood–brain barrier and accumulate in the CNS (Fig. 1), where they can be stimulated *in situ* by MBP and thereby exert their therapeutic effects in the diseased organ [28]. This therapeutic effect was manifested in the brains of EAE-induced mice, by a decrease in inflammatory cytokine IFN-γ and by secretion of the anti-inflammatory Th2 cytokine IL-10 in response to the autoantigen MBP. Another possible mechanism of action for Cop 1 is T-cell receptor antagonism [29]. More recent clinical results show that after six years of treatment, 152 of 208 (73%) of the patients who entered the open-label trial continue to feel that they are getting benefit from continuing treatment [30]. The high patient compliance is backed up by a major decrease in relapse rate (72%) accompanied by the fact that the majority of patients has not deteriorated during the six years.

Two clinical trials have recently been reported for MS, stressing the specific immunotherapy approach but also emphasizing its problematic outcome. In one of these, an altered peptide ligand related to sequence 83–99 of MBP was used [31]. Because the peptide ligand was poorly tolerated at the dose tested, the trial had to be halted. In the other trial, using a similarly altered peptide ligand, a safety board suspended the trial because of hypersensitivity reactions in 9% of the patients [32]. Notwithstanding the problematic results of these two trials, the specific approach of using peptides is certainly worth further investigation. In another interesting study, a peptide of an amino acid sequence designed to bind crucial MHC pockets and to interfere with T-cell activation, was shown to ameliorate EAE in Lewis rats [33].

Cop 1 has been approved for use against relapsing-remitting MS in Northern America and the European Union (altogether in >40 countries).

Myasthenia gravis (MG)

Whereas MS is mainly a T-cell-mediated disease, myasthenia gravis is a T-cell-regulated disease mediated by antibodies against the acetylcholine nicotinic receptor (AChR). Nevertheless, T-cells are of crucial importance for the formation of these antibodies [34]. Of the many studies on the use of peptides in therapy (or for vaccination) of MG, two approaches should be mentioned. In one, the tolerance-inducing and suppressive potential of mucosal administration of recombinant peptides has been successfully investigated [35]. The prevention of experimental autoimmune myasthenia gravis (EAMG) has recently been reported by oral [36] and nasal [37] administration of relevant peptides.

In the other study, analogs have been synthesized of two immunodominant myasthenogenic T-cell epitopes (p195–212 and p259–271) derived from an α-subunit of the nicotinic AChR. Ideally, the goal of therapy for MG should be the elimination of autoimmune responses to the AChR specifically, without interfering with immune responses to other antigens. To this end, the dual analog composed of the tandemly, reciprocally arranged two single analogs of p195–212 and p259–271, namely Lys262–Ala207, was prepared and shown to efficiently inhibit the proliferation of T-cell lines specific to the myasthenogenic peptides, and of lymph node cells primed *in vivo* to either of these peptides. The dual analog specifically

inhibited *in vitro* T-cell stimulation to either myasthenogenic peptide in >90% of the responding MG patients. When administered orally, the dual analog could treat EAMG induced in mice by immunization with the multideterminant native Torpedo AChR [38]. Moreover, it had beneficial effects on the clinical manifestations characterizing EAMG.

Thus, the dual analog is an efficient immunomodulator of EAMG in mice and could be of specific therapeutic potential for MG [38]. The dual analog vaccine candidate acts by specifically and actively suppressing myasthenogenic T-cell responses. This active suppression is mediated by the upregulation of transforming growth factor-β (TGF-β) secretion and downregulation of IFN-γ and IL-2 (Th1 type cytokines) [39]. A state of non-responsiveness is induced by the dual analog, which, at least partially, causes the cells to undergo anergy. Thus, the dual analogue can definitely be considered as a candidate for a therapeutic vaccine.

SLE

Peptides that are based on the complementarity determining region (CDR) of autoantibodies have been demonstrated to be novel potential candidates for vaccination against autoimmune diseases. This was exemplified by the use of peptides from anti-DNA autoantibodies for specific treatment of SLE patients. SLE is an autoimmune disease characterized by the increased production of autoantibodies and systemic clinical manifestations.

Experimental SLE can be induced in mice by immunization with the human anti-DNA mAb that bears the common idiotype, 16/6 Id. Autoantibodies isolated from the diseased mice were shown to be highly homologous to anti-DNA mAb of SLE-prone [(NZB × NZW)F1, mrl/lpr/lpr] mice. Peptides based on CDR1 and three of the pathogenic mAb to DNA of both murine and human origins were synthesized and shown to inhibit SLE-associated T- and B-cell responses. The peptides were capable of either preventing SLE or treating an established disease that was either induced or developed spontaneously in (NZB×NZW)F1 mice [40]. Treatment of an established disease correlated with immunomodulation of Th1- and Th2-type cytokines, and a significant upregulation of the immunosuppressive cytokine TGF-β. In addition, levels of both disease-associated matrix metalloproteinases (MMPs), MMP-3 and MMP-9, were diminished in the sera and kidneys of the peptide-treated mice (E. Mozes, pers. commun.).

Furthermore, the CDR-based peptides efficiently inhibited the SLE-associated 16/6 Id-specific proliferation and IL-2 secretion by peripheral blood lymphocytes (PBL) of SLE patients. This inhibition correlated with an upregulated production of the immunosuppressive cytokine TGF-β, suggesting that the peptide-mediated modulation of autoreactive responses of PBL from patients is via a mechanism similar to that observed in the SLE animal models (E. Mozes, pers. commun.).

Type 1 diabetes

Insulin-dependent diabetes mellitus is a chronic autoimmune disease that develops spontaneously in humans and in the experimental murine model of non-obese diabetic (NOD) mice. A peptide of the heat-shock protein 60 (hsp60) molecule, designated peptide p277, is a target of T-cells in autoimmune diabetes in NOD mice. A single administration of p277 peptide can arrest the autoimmune process even after it is far advanced. Successful therapy was associated with downregulation of the autoimmune process and regression of islet inflammation [41]. Cessation of β-cell destruction seemed to result from a p277-induced shift in the cytokine profile of hsp60 autoimmunity from a pro-inflammatory Th1 phenotype to an anti-inflammatory Th2 phenotype. A recently published [42] randomized, double-blind, Phase II study of p277 peptide treatment in patients with newly diagnosed type 1 diabetes showed that endogeneous insulin production was preserved, perhaps through the induction of a shift from Th1 to Th2 cytokines produced by the autoimmune T-cells.

Other autoimmune diseases

Antiphospholipid syndrome is characterized by the presence of high titers of autoantibodies against phospholipid β-2-glycoprotein-I, by recurrent fetal loss, repeated thromboembolic phenomena and thrombocytopenia. Three hexa-peptides identified by a phage-display library react specifically with these antibodies, and inhibit their damaging effects [43]. The use of synthetic peptides that focus on neutralization of the pathogenic antibodies represents a possible therapeutic approach to anti-phospholipid syndrome. Vaccination was also considered for experimental autoimmune neuritis in rats, by using an oligomerized T-cell epitope containing 16 repeats of amino acid residues 58–73 within the sequence of myelin P2 protein [44]. The experimental disease in animals could be a model for Guillain–Barré syndrome.

Neurodegenerative diseases

Recent studies revealed an additional benefit of glatiramer acetate activity, which might also be relevant to MS. Similarly to MBP, active immunization with glatiramer acetate, as well as the adoptive transfer of T-cells reactive to glatiramer acetate, inhibits the progression of secondary degeneration after crush injury of the rat optic nerve [45]. Furthermore, vaccination with glatiramer acetate protected

neurons against glutamate cytotoxicity, whereas immunity to MBP or myelin oligodendrocyte glycoprotein (which provides effective neuroprotection after axonal injury) did not protect the neurons from glutamate-induced toxicity [46]. Immunization with glatiramer acetate also protected retinal ganglion cells from death induced by ocular hypertension in rats. This has obvious implications for vaccination against glaucoma. It was further demonstrated that activated glatiramer-acetate-specific T-cells secrete significant amounts of brain-derived neutrotrophic factor (BDNF), a neutrotrophin that plays a major role in neuronal survival.

Autoimmunity is not only relevant to disease but can also be protective [47]. Spinal cord injury results in a massive loss of neurons and loss of function. The passive transfer of autoimmune T-cells directed against myelin-associated antigens provides acutely damaged spinal cords with effective neuroprotection. Post-traumatic T-cell-based active vaccination is also neuroprotective [48]. Thus, prospects for vaccination have been raised because of the beneficial immunity after CNS injury [49]. Post-traumatic vaccination with a Nogo-A-derived peptide elicits a T-cell-mediated response that limits neuronal degeneration after incomplete spinal cord injury [50].

T-cell vaccination against autoimmune diseases

T-cell vaccination (TCV) refers to a form of cell therapy, usually autologous, which is aimed at curing or ameliorating autoimmune diseases by eliminating the aggressor T- cells [51]. TCV, like any other vaccination, activates the subject's immune system to neutralize a pathogenic agent. However, TCV differs from classical vaccination in that the agents to be resisted are populations of the subject's own T-cells; the vaccine is devised using a component of the immune system itself. TCV has already been experimentally used in rats and mice in autoimmune encephalomyelitis, adjuvant arthritis, experimental autoimmune thyroiditis, collagen-induced arthritis, experimental autoimmune uveitis, murine lupus and type 1 diabetes. The lupus and diabetes models are spontaneous diseases arising in genetically susceptible mice, and so TCV in these mice is carried out after the spontaneous onset of the autoimmunity; therapeutic TCV could be demonstrated in these models. TCV using T-cells that are reactive against allo-antigens has also been shown to prolong skin allografts in experimental animals.

Antitumor vaccines

Vaccination against cancer should induce an anti-tumor immune response, to prevent disease in healthy high-risk individuals or effectively eradicate tumor cells during an ongoing disease. Tumor cells must, therefore, carry immunogenic epitopes that will be recognized by the immune system as antigens. In recent years, our knowledge of human tumor antigens recognized by autologous CTLs or antibodies has increased considerably [52–54].

Normal tissue proteins overexpressed on tumor cells fall within the category of tumor-associated antigens. The major candidates for these are cell-surface receptors such as the tyrosine-kinase growth-factor receptors of the ErbB family [55]. Cell-surface receptors are vital for cellular growth and differentiation and interaction with specific growth factors stimulates a cascade of signaling pathways instructing the cell to undergo growth and differentiation, or regulates the transformation of normal cells into malignant tissue. Interaction with specific antibodies can modify or obstruct receptor function and arrest cellular growth, for example, by inhibiting ligand binding or inducing receptor downregulation. mAbs against the extracellular domain of ErbB1 or ErbB2 have been effective in inhibiting tumor growth [56], eventually leading to the antibody-based anticancer agent Herceptin, which is particularly effective in combinations with chemotherapy [57]. These results suggest that immunizing with the receptor, or with structure-based or conformation-mimicking peptides [58], could open the way for active vaccination against ErbB-expressing tumors.

Significant progress in tumor immunology, mainly because of the pioneering work by Boon and colleagues, accelerated the development of different strategies for antitumor vaccination. A wide range of tumor antigens, presented to T-cells by human leukocyte antigen (HLA) molecules and recognized by autologous CTL, was identified by genetic and biochemical procedures. One group is the tumor-specific antigens encoded by mutated genes [59] that can mediate class-restricted cellular immunity. These are individual antigens that are less applicable for clinical use. Another group comprises the tissue-specific differentiation antigens expressed on both normal and tumor cells, for example, gp100, tyrosinase and, in particular, melan-A/MART-1 [60–62]. Peptides derived from these antigens can elicit specific CTL responses, and some clinical effects or tumor regression were observed with GM-CSF added to a pool of HLA-A2 restricted peptides from melan-A, tyrosinase and gp100 [63].

Another group comprises antigens encoded by genes of the MAGE (melanoma associated antigen) family, expressed in various tumor cells of different histological origins [64]. These antigens are silent in normal tissues except for the male germ-line cells, which are devoid of HLA molecules and, therefore, immunologically inert. The MAGE antigens were first identified in melanomas by screening gene expression libraries using tumor-specific

class-restricted CTL, mostly from the tumor infiltrates of cancer patients [60,65]. Peptides recognized by both class-I-restricted $CD8^+$ and an emerging number of class II $CD4^+$ T-cells, induce tumor-specific cellular immune responses [such as delayed type hypersensitivity (DTH) and CTL] in cancer patients, with some objective clinical responses. These are considered promising candidates for broad-spectrum antitumor vaccination as single agents or as polyepitope vaccines. Many of the antigens identified by CTL also induce antibodies, as shown by serological analysis of recombination libraries (SEREX) using cancer patients' autologous sera [66]. SEREX can thus be used for defining antigens for CTL induction.

A poor correlation was frequently observed between T-cell induction and clinical responses, leading to the assumption that low-level CTL can initiate tumor rejection. Antigenic peptides encoded by MAGE-3 and presented by HLA-A1 induced tumor regression in patients with metastatic melanoma [67]. Peptides derived from NY-ESO-1, a highly immunogenic antigen overexpressed in several tumors, induced a DTH response, disease stabilization and regression of single metastases [68,69]. More effective immunization is expected with dendritic cells (DC), acting as antigen presenting cells to activate MHC-class-restricted CTL. DC can either be loaded *ex vivo* with the antigenic peptides or are capable of processing and presenting multiple antigenic peptide fragments when loaded with apoptotic, killed or lysed tumor cells [70,71]. Vaccination with MAGE-3A1 peptide-pulsed mature, monocyte-derived DC expanded tumor-specific CTL and induced the regression of some metastases in advanced-stage IV melanoma [72]. Combination with cytokines or using allogeneic, genetically modified, IL-2-producing melanoma cell line, appeared to augment CTL, DTH and the regression of subcutaneous metastases [73]. The significance of these results will be resolved by the outcome of several large multicenter trials that are under way.

Conclusions

At least one 'therapeutic vaccine', copolymer 1 (glatiramer acetate) for the relapsing-remitting form of multiple sclerosis, is being used by many thousands of patients. Another vaccine for type I diabetes has recently completed a Phase II trial successfully, and several vaccines against cancer are already being studied, some of which are planned to enter, or have just begun, clinical trials. Therapeutic vaccine preparations against infectious diseases such as HIV, tuberculosis and malaria are in Phase II clinical trials to evaluate their efficacy in patients. In most cases the therapy is based on the resemblance between the etiological agent causing the disease and the therapeutic vaccine. However, in some cases the vaccine is based on stretches of CDRs, on cytokines [74] or on idiotypes. There is no doubt that a review written in ten years time will have many more successful clinical cases, but – like in science generally – this does not mean that the realm of the unknown, in this case the search for new therapeutic vaccines, will diminish.

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