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Therapeutic vaccines for non-Hodgkin B-cell lymphoma

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Abstract Despite current therapeutic strategies for B-cell lymphoma, including chemotherapy and transplantation, the majority of patients are not cured. The characterisation of several tumour antigens has made immunotherapy an interesting approach to the treatment of patients with lymphoma. The idiotype region in the immunoglobulin expressed by the tumour B cells is not only a clonal marker but also a tumour-specific antigen. For this reason, the idiotype is an ideal target for immunotherapy. Extensive studies of idiotype vaccination have been done in murine lymphoma models and some of these strategies are now being tested in clinical trials. In the last few years, new strategies to improve the immune response against lymphoma cells have been studied, including the use of DNA or recombinant viruses encoding tumour-antigens, genetically modified tumour cells and a number of immune adjuvants targeting dendritic cells, T cells or NK cells.

Keywords Lymphoma · Idiotype · Cancer vaccines · T cells · Dendritic cells

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Introduction

Cancer vaccines have been the subject of intensive research in recent years. A better understanding of the mechanisms involved in antigen presentation, B- and T-cell activation, and tumour-induced immunosuppression has given researchers tools to manipulate the immune system to elicit a specific antitumour immune response. Manipulating the immune system has taken two forms: passive immunotherapy consists of adoptive transfer of antibodies or effector cells specific to tumour cells; in contrast, active immunotherapy, by means of cancer vaccines, promotes a specific immune response against the tumour by stimulating the tumour-bearing host (Table 1).

In this review, we describe the status of therapeutic cancer vaccines in B-cell non-Hodgkin lymphoma (NHL). We will focus on current clinical trials and will review further approaches by means of cellular and gene manipulation to increase the potential of this approach.

Idiotype protein vaccines

An ideal tumour antigen is one that it is absent in all normal tissues but expressed in all tumour cells. Although this represents an ideal situation for active immunotherapy, it rarely occurs in solid or haematological tumours. In the vast majority of cases, tumour antigens used for active vaccination are expressed in tumour cells but also in some normal cells or embryonic tissues, the so-called tumour-associated antigens.

Each B cell expresses an immunoglobulin molecule on its surface that recognises a unique antigen. Each immunoglobulin has a heavy and a light chain with variable regions that are the ones that bind to the antigen. These variable regions are known as the idiotype, and the entire

Table 1 Cancer vaccines strategies against lymphoma

Vaccine	Antigen/Adjuvant
Peptides or protein	
	Idiotype
Plasmid DNA	
	Encoding idiotype gene
	Immunostimulatory genes:
	Tetanus toxin fragment C
	Cytokines (IL-2, IL-12, GM-CSF)
Recombinant virus	
Adenovirus, poxvirus	
	Encoding idiotype
	Fusion genes: idiotype +
	Cytokines (GM-CSF, IL-2)
	Costimulatory molecules
	CD40L
	OX40
	B7/ICAM-1/LFA-3
Dendritic cells	
	Pulsed with idiotype
	Pulsed with tumour lysates
	Transduced with viral vectors encoding:
	Idiotype
	Costimulatory molecules
	Cytokines, chemokines (MIP1)
	Fused with tumour cells (hybrids)
Whole tumour cells	
Autologous, allogeneic	Transduced with viral vectors encoding:
	Costimulatory molecules
	Cytokines

immunoglobulin with its unique variable region is referred as the idiotype protein. When a normal B cell undergoes malignant transformation (i.e., lymphoma), all tumour cells express an immunoglobulin with the same idiotype. Thus the idiotype can be used as a tumour-specific antigen, which can be recognised by antibodies and some T cells with the appropriate T-cell receptor.

Thirty years ago, studies pioneered by Ron Levy at Stanford University focused on the development of passive immunotherapy with anti-idiotype antibodies for patients with B-cell non-Hodgkin lymphoma [1]. For this purpose, the patient-specific idiotype was injected into mice to produce mouse anti-idiotype monoclonal antibodies that could specifically bind to the patient's tumour cells. With this approach, a number of patients with NHL were treated over a period of 12 years with an overall response rate of 66% and 18% complete response rate, some of them lasting more than 10 years [2]. However, this procedure was very expensive and cumbersome, which precluded its general use in clinical practice. Nevertheless, these findings set the scenario for the development of an active immunotherapy, where the patient's own immune system is stimulated to generate an immune response against the idiotype expressed specifically in the tumour cell. To this end, the idiotype was isolated from the patient's lymphoma cells by using the hybridoma technology and further linked to an immunogenic carrier protein, keyhole limpet haemocyanin (KLH), to elicit a strong immune response. This compound, the idiotype protein-KLH, is used in most of the clinical studies of vaccination in patients with B-cell NHL. However, the production of this protein with the hybridoma technology usually requires more than 6 months, and fails to yield the specific product in at least 20% of cases. In recent years, recombinant DNA technologies have been introduced to generate the idiotype protein more effectively. Generally, these methods use a PCR amplification of the genes encoding the tumour-specific immunoglobulin variable regions, and subsequent cloning into a variety of plasmids for *in vitro* protein expression using either mammalian, bacterial or insect cells. The recombinant technology has several advantages over the hybridoma method: it is much more efficient (95% success rate), requires less tumour tissue, does not require viable cells (frozen tissue can be used) and generates a product in a significantly shorter time.

Most clinical trials with the idiotype vaccine have been performed in patients with follicular NHL. There are several reasons for this choice. First, it is an indolent lymphoma with a rather slow clinical course. Second, despite new drugs it is considered to be an incurable disease, which makes it ideal to test novel therapies. Third, this type of lymphoma is regarded to be immunogenic based on the fact that spontaneous remissions have been observed and recent gene expression studies have shown an association of survival with the presence of immune cells in the tumour environment [3].

Phase II clinical trials

The first idiotype vaccine clinical trial was done in 1992 by Levy's group at Stanford University [4, 5]. In this trial, patients with follicular NHL in response after a course of chemotherapy were vaccinated with the idiotype protein-KLH. Humoral anti-idiotype immune responses were detected in 41% of the 41 patients treated. More clinically relevant, those patients who mounted an anti-idiotype response had a longer progression-free survival (PFS) than those who did not (7.9 years vs. 1.3 years, respectively; $p<0.001$). This study established the concept that promoting an immune response against a tumour antigen may be clinically relevant, thus stimulating further preclinical and clinical studies with novel formulations of the idiotype vaccine. One such approach consists of the use of cytokines to activate the innate immune system to enhance the potency of the idiotype protein vaccine.

Preclinical studies in murine lymphoma models did show that GM-CSF was able to enhance the anti-idiotype immune response after vaccination with the idiotype protein, presumably by recruiting dendritic cells (DCs) to the site of vaccination and stimulation of a tumour-specific T-cell response [6]. This concept was translated into the clinic and Kwak's group, at the National Cancer Institute, con-

ducted a phase II clinical trial where 20 untreated follicular lymphoma patients were treated with chemotherapy followed by monthly administrations of the idiotype protein-KLH vaccine plus subcutaneous GM-CSF [7]. Following vaccination, 15 out of 20 patients (75%) had an anti-idiotype humoral response and tumour-specific T-cell responses were detected in 95% of the patients. From 11 patients with the translocation t(14;18), a marker of follicular NHL, detected in peripheral blood by means of PCR, 8 converted to PCR negativity after completing all the vaccines. Disease-free survival in this group of patients was 8 years (median), which appears to be superior to that of a historical control treated with chemotherapy alone (about two years). Although these studies suggest a benefit of the idiotype protein vaccination, definitive conclusions cannot be drawn since they were not randomised studies.

In order to improve the immunogenicity and clinical applicability of the idiotype protein vaccine, a number of studies were done which are based upon recombinant techniques to generate the idiotype protein. Veelken's group tested the use of the Fab protein of the immunoglobulin purified from *Escherichia coli* [8]. Eighteen patients with different types of B-cell lymphomas (including follicular, mantle, diffuse large-cell lymphoma and chronic lymphocytic leukaemia), and previously treated with a number of chemotherapy regimens, received four vaccinations with the idiotype Fab protein plus GM-CSF. Despite the fact that the majority of the patients were in progressive disease at the time of the vaccination, almost 50% of the patients developed a specific T-cell idiotype response, but no significant clinical responses were seen.

Another approach consists of the use of the recombinant tobacco mosaic virus with the variable-region immunoglobulin genes cloned into it to produce the idiotype proteins in tobacco plants [9]. Preclinical studies with this approach have shown comparable results to that obtained with the idiotype protein-KLH vaccine, with a significantly shorter manufacturing time (only 2 months). However, early clinical studies done in patients with follicular NHL were disappointing, and plans for future trials were abandoned.

Clinical trials in the rituximab era

Rituximab, a chimeric anti-CD20 monoclonal antibody, induces a high response rate in follicular NHL and is considered a mainstay of the treatment for this lymphoma [10]. Since this antibody produces a profound B-cell depletion, there is a concern that patients treated with this antibody may have very limited capability to develop a humoral immune response. For this reason it has been considered of great interest to test the clinical impact of the idiotype vaccines in patients with rituximab-treated B-cell NHL.

This concept was tested in a clinical trial of patients with follicular NHL [11]. Patients received the standard treatment with rituximab (weekly dose for 4 weeks) and those without progressive disease later received six-monthly idiotype protein-KLH vaccine plus GM-CSF. Although clinical responses attributed to the vaccine itself are difficult to assess, the most important observation of this study was the fact that a very high proportion of the vaccinated patients developed a specific immune response.

In another trial, patients with mantle NHL treated with a chemotherapy-containing rituximab were vaccinated with the idiotype protein-KLH plus GM-CSF [12]. Surprisingly, despite the use of rituximab, 30% of the patients had antibody responses against the idiotype. These responses were delayed and correlated with the B-cell recovery after rituximab. However, $CD4^+$ and $CD8^+$ T-cell responses were also detected in almost 100%, despite the absence of B cells.

These preliminary studies suggest that vaccination in patients after receiving rituximab is feasible, and both humoral and cellular immune responses can be obtained.

Phase III clinical trials

The encouraging results after phase II trials of vaccination with the idiotype protein of patients with indolent NHL led to the initiation of three randomised clinical trials, to definitively answer the question of clinical benefit of the idiotype vaccines.

Currently, three phase III clinical trials are ongoing to test the efficacy of idiotype protein vaccination in patients with previously untreated follicular NHL. The first one is a multicentre trial being conducted by Genitope Corporation, under the leadership of Ron Levy at Stanford University. A second trial, sponsored by the National Cancer Institute, had a target enrolment of 563 patients. In this study, untreated follicular lymphoma patients received chemotherapy with cyclophosphamide, doxorubicin, etoposide, vincristine and prednisone, and those in complete remission were randomised to receive (6 months after chemo) idiotype protein-KLH plus GM-CSF compared with KLH alone plus GM-CSF. Clinical results from this study are not known as yet.

A third study was conducted by the company Favrille Inc. Either untreated or relapsed follicular NHL patients were treated with 4 weekly doses of rituximab and those with response or stable disease randomised to receive idiotype protein-KLH plus GM-CSF or KLH plus GM-CSF. Enrolment was completed in January 2006 with 349 patients randomised. In May 2008, early analysis of the primary end point of the trial, time to progression, failed to show any significant advantage in the treatment arm compared to the control and the trial was discontinued.

The trial conducted by Genitope and Stanford University is the largest and most advanced of all trials, and has just announced its first data analysis [13]. The target enrolment of 676 previously untreated patients was reached in April 2004. Patients received CVP chemotherapy (cyclophosphamide, vincristine and prednisone) and those in response were randomised (n=287) to idiotype protein-KLH plus

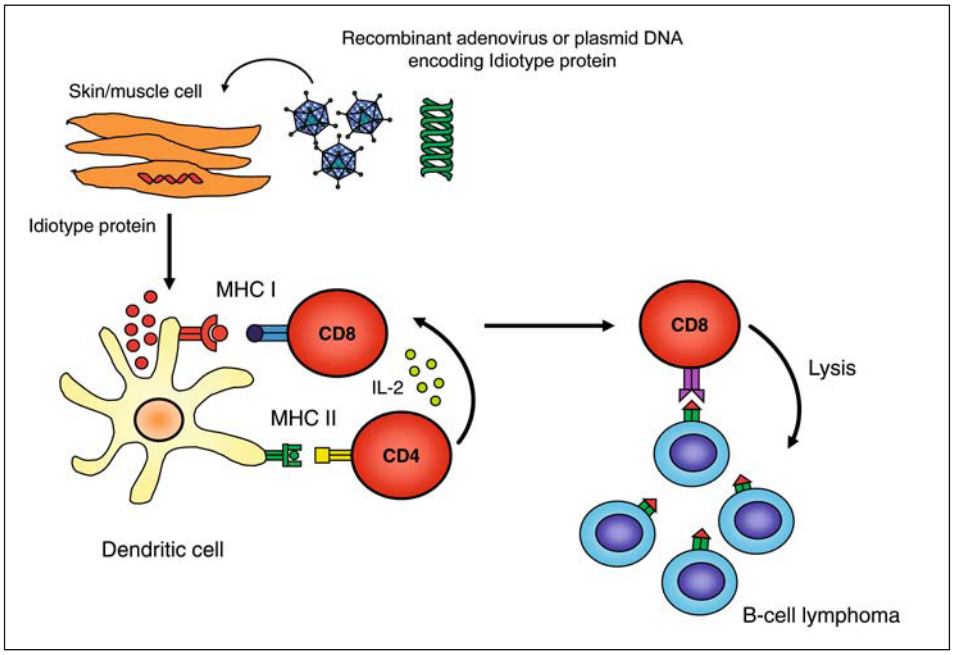

Fig. 1 Costimulatory molecules expressed on antigen presenting cells and their counterreceptors on T cells

GM-CSF or control immunotherapy (KLH plus GM-CSF). Humoral anti-idiotype responses were detected in 40% of the patients receiving the idiotype protein. PFS at 5 years was the same in both arms of the study. However, when comparing patients who mounted an immune response vs. those who do not, PFS was significantly improved in those patients with an anti-idiotype response (39.7 vs. 18.1 months; p=0.0017). This large, randomised study is the first to confirm that follicular NHL patients mounting a humoral immune response to the idiotype have a significantly improved clinical outcome after idiotype protein vaccination. However, the fact that the differences in the clinical outcome were only seen in those patients who developed an immune response to the vaccine raises the question of whether there is a causality relationship between the vaccine and clinical outcome or, on the contrary, the response to the vaccine just reflects a biomarker of the disease status.

Nevertheless, these definitive clinical studies open new avenues to improve the potency and clinical impact of the vaccines with new approaches.

DNA/recombinant virus vaccines

DNA vaccines are currently being explored as an alternative to the idiotype protein vaccination. Since they represent a delivery system that does not require *in vitro* protein expression, a DNA vaccine can be rapidly produced with PCR technology in a matter of 2–4 weeks, which represents a substantial advantage over the vaccines based upon *in vitro* protein production. The concept consists in simply taking a tumour-associated gene sequence and delivering it directly to the patient, so the gene is translated *in vivo* and presented to the immune system.

To generate a DNA vaccine for stimulating anti-lymphoma immune responses the genes encoding the variable regions of the light and heavy chain of the immunoglobulin (the idiotype) are amplified by PCR from a tumour sample and cloned into a bacterial plasmid. In this construction, gene expression is usually driven by the cytomegalovirus (CMV) immediate early promoter to ensure high transcription efficiency [14]. Studies done in murine lymphoma models have shown that vaccination with a plasmid DNA encoding the idiotype genes induces protection against subsequent lymphoma challenge, comparable with that after vaccination with the idiotype-KLH protein [15].

Studies in the last few years have contributed to understanding how these vaccines stimulate the immune system [16, 17] (Fig. 1). DNA vaccines are generally injected into muscle or skin. The encoded antigen (i.e., idiotype protein) is expressed in myocytes or keratinocytes. However, these cells are not able to directly stimulate T cells and, thus, to efficiently stimulate the immune system other mechanisms must exist. DCs are the main antigen-presenting cells (APCs) in the immune system. In that scenario, the antigen protein produced in the muscle or skin cells are picked up by the DCs, and then presentation of appropriate peptides to specific $CD4^+$ and $CD8^+$ T cells occurs, a process known as "cross-presentation". In some cases, depending on the DNA delivery method used (i.e., "gene gun"), DCs can be directly transfected *in vivo* with the plasmid DNA. An important fact that contributes to the stimulation of the innate immune system is the existence in the bacterial

DNA of unmethylated cytidine-phosphate-guanosine (CpG) oligonucleotide-containing motif sequences [18]. These sequences play an important role in the activation and maturation of DCs, and contribute to the stimulation of T and NK cells through the interaction with Toll-like receptors (TLR). CpGs and other TLR agonists are being extensively studied as vaccine adjuvants in different cancer models [19].

DNA vaccination studies in murine lymphoma models have revealed that, in order to generate protection against lymphoma, foreign xenogeneic (human) sequences have to be linked to the murine lymphoma immunoglobulin variable regions [16]. This provides an adjuvant function similar to that provided by the KLH in the case of protein vaccination. Further studies in murine lymphoma models have shown that fusion of cytokine (i.e., GM-CSF) [15] or chemokine genes (i.e., MIP-1α, IP-10) [20] to the idiotype gene can improve the anti-idiotype immune response and protection against lymphoma, this being comparable or even superior to that afforded by the idiotype protein-KLH. Stevenson's group has shown that the fragment C of the tetanus toxin fused to the idiotype gene contributes to an enhanced humoral response against the idiotype in murine lymphoma models [21], due in part to stimulation of specific CD4+ T cells, an approach that has been recently taken into the clinic.

Clinical trials of naked DNA vaccination

The first clinical DNA vaccination study was done in a small group (n=12) of follicular lymphoma patients previously treated with chemotherapy [22]. Patients in remission received three monthly injections (intramuscle or intradermal) of a DNA plasmid encoding a chimeric immunoglobulin molecule containing the variable light- and heavy-chain immunoglobulin sequences derived from each patient's tumour fused to the mouse immunoglobulin constant regions. Since that was a phase I/II study, the plasmid DNA dose varied in different groups of patients. Although the vaccination process was safe, significant anti-idiotype responses were rare and clinical responses were not observed.

Because of the improved immunogenicity of DNA vaccines with fusion genes in animal models, clinical trials have begun in follicular lymphoma patients. Stevenson et al. initiated a phase I/II trial of vaccination with plasmid DNA encoding the patient's tumour idiotype gene fused to the fragment C of the tetanus toxin [14]. Preliminary data from 16 evaluable patients have indicated that 40% of them developed anti-idiotype humoral responses. However, their impact on their clinical outcome has not been reported.

Recombinant virus

The potential use of recombinant viruses as cancer vaccines has been extensively explored in recent years [23]. Recombinant viruses offer several advantages over naked DNA: they are highly immunogenic, and can be produced in high titres in a short period of time; they can efficiently transduce genetic material into a variety of cell types either *in vitro* or *in vivo*, yielding high levels of protein.

Adenoviruses are of special interest for cancer vaccines because they meet all of the above-mentioned criteria. The concept consists of the use of replication-defective adenoviruses with a particular antigen-encoded gene [24, 25]. The most used adenovirus vector is based on the adenovirus type 5. The viral vector was rendered replication-defective by removing the E1 gene, which is required both for adenovirus replication and for viral gene expression. Genes encoding tumour antigens with or without immunostimulatory genes may be inserted in place of the E1 gene with expression driven by the CMV early promoter [26].

After intradermal or intramuscle administration, the protein encoded by the gene is expressed and presented by DCs to stimulate both humoral and cellular immune responses (Fig. 1). In addition, the adenovirus itself functions as a strong adjuvant to enhance cellular immune response against the antigen-encoded gene. In infectious disease animal models, vaccination with adenovirus encoding a model antigen has proved to be efficacious in preventing disease [27], and a similar approach has been taken for cancer.

Studies in murine lymphoma models have shown that vaccination with adenovirus encoding the idiotype genes was able to stimulate a specific immune response against lymphoma [28]. Importantly, immunity against the tumour seems to be superior to that afforded by the classical idiotype protein vaccination and naked DNA. Despite the importance of the preclinical data obtained, no clinical trials with recombinant adenovirus have been done in lymphoma so far.

Recombinant poxviruses are also attractive candidates for cancer vaccination [29]. They are large, DNA viruses that can efficiently express antigen-encoded genes that potentially can be recognised by T cells. In addition, they have a very high tropism for a variety of mammalian cells, but are replication incompetent. Studies in several murine tumour models have shown that administration of antigen-encoded recombinant fowlpox viruses, an avipox virus, elicits a strong T-cell immune response against the tumour antigen [30]. Although clinical trials of vaccination with fowlpox virus have been done in patients with solid tumours [31], no such studies have been conducted in lymphoma patients.

Dendritic cell vaccines

Presentation of an antigen to T cells is one of the crucial steps to stimulate an efficient immune response against that antigen. DCs are the most important APCs and play a crucial role in both induction and regulation of the immune re-

sponses [32]. These bone marrow-derived cells have a great surface expression of class I and II MHC proteins along with costimulatory molecules needed to stimulate naive T cells. In addition, DCs are able to produce a variety of chemokines and immunostimulatory cytokines that contribute to the final activation of the effector cells, including T, B and NK cells.

In the early phase of the immune response process, DCs are in an "immature" state and express an array of surface receptors specialised in antigen uptake. In this situation, DCs can uptake antigens from bacteria, viruses, death or dying tumour cells, proteins and immunocomplexes, through phagocytosis and endocytosis. The proteins are then processed into peptides, coupled to MHC molecules, and expressed in the membrane for recognition by antigen-specific T cells: peptide class I molecules are recognised by $CD8^+$ T cells and peptide class II molecules by $CD4^+$ T cells. A specific pathway, called "cross-presentation", allows DCs to present antigens derived from virus-infected or tumour cells to stimulate antigen-specific $CD4^+$ and $CD8^+$ T cells. After capturing the antigen, DCs undergo another process called maturation. This process transforms DCs into cells specialised for T-cell stimulation. Maturation is characterised by reduced antigen uptake, enhanced ability of migration to lymphoid tissues and activation of antigen-specific T cells. A number of molecules released by damaged tissues stimulate the maturation process in which several receptor types, such as the TLRs, are involved. Mature DCs express high levels of surface molecules such as CD80, CD86, ICAM-1, CD40 and MHC molecules, all devoted to efficiently activate antigen-specific T cells. Mature DCs secrete chemokines and cytokines that recruit inflammatory cells and specific T-cell subsets, all of which eventually influence the T-cell response.

Because of the pivotal role of DCs in inducing an antigen-specific immune response, these cells constitute an important part of several cancer vaccines strategies. The list of possibilities includes the use of DCs cocultured *in vitro* with tumour-derived peptides or proteins, tumour lysates, or DCs fused to irradiated tumour cells. Alternatively, DCs can be transfected with DNA or RNA, or transduced with recombinant viruses encoding tumour antigens with or without immunostimulatory genes [33].

Studies done in murine lymphoma models showed that vaccination with diotype-pulsed DCs stimulated a specific immune response against a lymphoma challenge [34]. Moreover, in the same experimental setting the DC vaccine was superior to the classical idiotype-protein KLH, suggesting this approach could be of interest for treating patients with lymphoma. To improve the activity of DC vaccines a number of strategies have been tested in murine lymphoma models. One strategy explores the ability to enhance the immunogenicity of DCs by means of the genetic manipulation of DCs [35]. DCs can be transduced with a number of recombinant viruses with tumour-antigen encoded genes. This strategy can result in prolonged presentation of multiple epitopes by various class I and II MHC molecules, with the ability to stimulate antigen-specific T cells. Experimental studies have shown that immunisation with DCs transduced with recombinant adenoviruses encoding a tumour antigen stimulates a potent $CD8^+$ T-cell response able to eradicate established tumours [36–38].

All the approaches previously discussed are based on the knowledge of the tumour antigen. However, this is not the case for the majority of tumours. To circumvent this problem, studies with DCs stimulated with whole tumour cells have been done. Thus, vaccination with DCs cocultured *in vitro* with a tumour lysate improved the survival of lymphoma-bearing mice compared to the idiotype-protein KLH vaccine [39]. Another strategy takes advantage of the ability of DCs to fuse to tumour cells [40, 41]. This approach ensures proper presentation of an array of tumour antigens coupled to appropriate stimulation of antigen-specific T cells. The potential advantage of this method relies on the fact that several relevant tumour antigens, instead of just one, may be presented to the effector cells along with proper T-cell costimulation, thus increasing the immune response against the tumour. Our group has recently explored this strategy in murine lymphoma models. Fused DCs plus tumour cells are not only able to stimulate rejection of the tumour but also to eradicate established lymphoma, showing the strength of this approach. The system can be further improved by using adjuvants such CpG sequences or by genetic modification of the hybrids with recombinant adenoviruses encoding the CD40L immunostimulatory gene [42].

Clinical trials with DCs

Clinical trials with DCs have been increasingly use in recent years in patients with different types of cancer [43]. Based on preclinical studies, a pilot clinical trial of vaccination with idiotype-pulsed DCs was conducted more than 10 years ago at Stanford University [44]. In that small study, cellular immune responses and clinical responses were noted after administration of *ex vivo* idiotype-pulsed DCs in 3 out 4 patients. Encouraged by these results, the study was extended by including 35 patients with follicular NHL, 10 in relapse and 25 in first clinical remission after chemotherapy [45]. Among 10 relapsed patients, 8 developed an idiotype-specific T-cell immune response and 4 had clinical responses; 3 patients achieved a complete response lasting more than 4 years and the remaining one had a partial response. Concerning the 25 patients vaccinated in complete remission after chemotherapy, 65% developed either a humoral or cellular anti-idiotype response. Importantly, 70% of the patients remain progression-free after vaccination, at a median of 43 months after completion of chemotherapy. Interestingly, among 6 patients that progressed after DC vaccination, significant clinical responses were obtained again in 3 of them, after receiving idiotype protein-KLH, which confirms the antitumour effect of this approach.

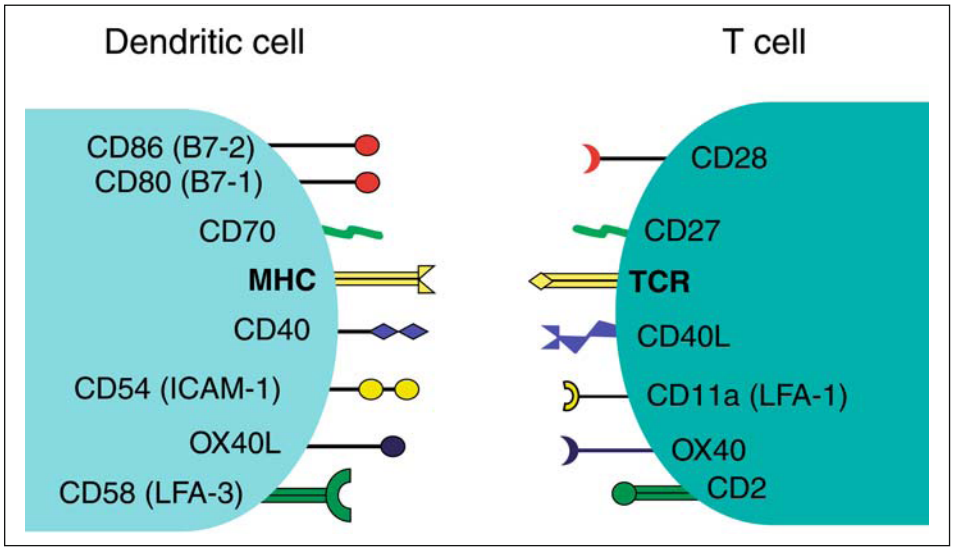

Fig. 2 Antitumour vaccination with recombinant DNA or virus. Lymphoma antigens (i.e., idiotype) encoded by DNA or recombinant virus are expressed on muscle or skin cells and acquired by DCs. DCs process the tumour antigen proteins into peptides that are presented within class I and II MHC molecules to T cells. Antigen-specific T cells are activated and migrate to the tumour to generate a specific anti-lymphoma cytotoxic response

Tumour cell vaccines

A completely different approach to generate a cancer vaccine consists of the use of tumour cells to induce a host immune response. This is based on the assumption that some tumour cells can present their own tumour antigens to DCs or T cells. An advantage of the use of whole tumour cells as a vaccine is that characterisation of the tumour antigen is no longer required since they represent a source of a broad spectrum of tumour antigens. Thus, with this strategy a polyvalent immune response against several tumour antigens could be generated, in contrast to a response against a single antigen when a peptide is used for vaccination.

For appropriate antigen-specific T-cell activation to occur, T cells require cellular interactions with the APCs, recognition of peptide-MHC complexes through the T-cell receptor and costimulation of T cells [46]. In fact, recognition of antigens by T cells in the absence of proper costimulation results in anergy rather than activation. Among the costimulatory molecules, the B7 family of proteins –mainly B7.1 or CD80 and B7.2 or CD86– is of great importance for the stimulation of T cells [47]. The expression of these molecules is tightly controlled and is restricted to APCs, including DCs and B cells. Both B7.1 and B7.2 interact with the CD28 molecule on activated T cells to further enhance its activation. In addition, other molecules expressed by APCs, such as ICAM-1 and LFA-3, interact with different receptors on T cells to promote adhesion and activation of these cells (Fig. 2).

Experimental data have shown that tumour B cells, in contrast to normal B cells, are very inefficient at presenting antigens for the stimulation of a productive T-cell response. However, there are several approaches, based on genetic manipulation of the tumour cells, to increase their immunogenicity.

Tumour cells can be genetically modified to secrete cytokines and chemokines that contribute to activation of APCs and T cells. Studies in animal models with different tumours have shown that vaccination with tumour cells engineered to express cytokines (i.e., IL-2, IL-12, GM-CSF) stimulates T cells that are able to recognise and kill unmanipulated tumour cells, thus providing systemic immunity [48]. GM-CSF has been one of the most extensively studied. In murine models, vaccination with irradiated-tumour cells transduced with a recombinant virus encoding GM-CSF provides a protective systemic immunity that is mediated by both $CD4^+$ and $CD8^+$ T cells [49]. In the clinical scenario, the use of autologous tumour cells for vaccination is hampered by the long, cumbersome and expensive manufacturing process. For this reason, an allogeneic GM-CSF transduced human cell line was used for the trials. This cell line was chosen because it shows a very low expression of class I and II HLA molecules, thus minimising the chance of rejection. Clinical trials with this approach were conducted at the Johns Hopkins in patients with multiple myeloma [50]. In a phase I/II trial, patients were vaccinated with a mixture of irradiated tumour cells plus the allogeneic GM-CSF transduced cell line following autologous stem cell transplantation. However, because of lack of clinical efficacy the trial was prematurely discontinued.

Another novel approach focuses on the ability of some tumours to present antigens to T cells. As mentioned above, this involves the expression of several costimulatory molecules by tumour cells to activate T cells. B-cell lym-

Table 2 Novel immune adjuvants for cancer vaccines

Toll-like receptor agonists	CpGs
	Flagellin
Regulatory T-cell depletion	Anti CD25
	Anti GITR
	Anti folate receptor 4
T-cell modulation	
Inhibitory	CTLA4 blockade
	PD1 blockade
Stimulatory	Anti 4-1BB
	Anti OX40
NK/NKT cell activation	Anti NKG2D
	α-Galceramide

phomas, despite being able to present tumour antigens to T cells, often lack the expression of costimulatory molecules rendering these cells inefficient for T-cell stimulation [51]. However, a number of methods have been developed to effectively turn lymphoma B cells into powerful APCs. Direct transfer to the tumour of costimulatory molecules (i.e., B7, ICAM-1 and LFA-3) by the use of recombinant viruses may enhance their immunogenicity and this strategy has been proved in lymphoma models [52]. Another approach involves the CD40-CD40 ligand (CD40L) system. CD40 is a molecule expressed by APCs (DCs and B cells, including their tumour counterparts) that interacts with its ligand (CD40L) expressed on activated T cells [53]. In B lymphoma cells, stimulation of CD40 induces upregulation of several adhesion (ICAM-1) and costimulatory molecules (CD80, CD86), which leads to efficient activation of tumour-specific T cells [51]. Studies in lymphoma models have shown that vaccination with tumour cells transduced with a recombinant adenovirus encoding the CD40L gene stimulates a systemic immune response against the lymphoma [54, 55]. This concept has been translated into the clinic. To date, two clinical studies based upon CD40 activation have been reported, both in patients with B-cell chronic lymphocytic leukaemia. In the first study, autologous tumour cells were ex vivo transduced with a recombinant adenovirus encoding CD40L and reinfused to the patients with no other concomitant treatment [56]. There were no significant toxicities, and a reduction in the numbers of tumour B cells and the size of lymph nodes was seen. In other study, patients received subcutaneous injections of autologous tumour cells transduced with a recombinant adenovirus encoding both CD40L and IL-2 genes [57]. Activation of T cells was noted and, more importantly, 30% of the patients achieved a partial response, which suggests that CD40-based immunotherapy may be useful in B-cell lymphoma.

Future strategies to improve cancer vaccines efficacy

Despite all the advances in the development of cancer vaccines, their clinical impact is far from ideal. The understanding of tumours' mechanisms to evade the immune system has led to the development of new strategies to improve antitumour immune responses (Table 2).

Immunosuppressive molecules secreted by tumours (i.e., VEGF, TGFβ) inhibit DCs and T-cell functioning, and this could be potentially reverted using neutralising monoclonal antibodies against these molecules [58].

Blockade of T-cell inhibitory signals may also enhance antitumour responses. CTLA-4 is an inhibitory molecule expressed in activated T cells that contributes to shut down the immune response [59]. Blocking this molecule with anti-CTLA-4 monoclonal antibodies has been shown to improve the immune responses of anticancer vaccines [60].

Regulatory T cells constitute another important pathway that has been the object of much attention in recent years. In humans, these cells have been characterised as $FOXP3^{+}CD4^{+}CD25high$ [61]. These cells suppress immune responses by contact mechanisms and release of inhibitory cytokines such as IL-10 and TGFβ. Depletion of these cells by monoclonal antibodies has been shown to improve the efficacy of anticancer vaccines, both in murine models and in humans [62, 63].

Recent studies have focused on the activation of NK cells since they play a pivotal role in immunosurveillance and tumour killing [64]. Activation of NK cells through the use of antibodies to the NKG2D-activating receptor increases their killing capacity and is being tested in tumour models in the laboratory [65]. Finally, activation of a specific type of NK cell, the NKT cell, by α-galactosylceramide is able to stimulate a systemic immune response that eradicates established tumours in animal models [66, 67].

In summary, understanding the interactions between tumours and the immune system will contribute to the development of more efficacious cancer vaccines that hopefully will have a significant impact on the outcome of patients with lymphoma and other types of cancer.

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