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Innovative Cancer Vaccine Strategies Based on the Identification of Tumour-Associated Antigens

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Abstract

The identification of tumour-associated antigens has opened up new approaches to cancer immunotherapy. While past research focused on CD8+ cytotoxic T-cell responses, accumulating evidence suggests that CD4+ T cells also play an important role in orchestrating the host immune response against cancer. In this article, we summarise new strategies for the identification of major histocompatibility complex (MHC) class II-associated tumour antigens and discuss the importance of engaging both CD4+ and CD8+ T cells in cancer immunotherapy. The cloning of MHC class I- or class II-associated antigens has made it possible to develop synthetic and recombinant cancer vaccines that express specific tumour antigens. There are three major types of synthetic and recombinant cancer vaccines: recombinant viral and bacterial vaccines; naked DNA or RNA vaccines; and recombinant protein and peptide vaccines. In this article, we also discuss a new generation of recombinant cancer vaccines, 'self-replicating' DNA and RNA vaccines. Studies on the mechanisms of 'self-replicating' nucleic acid vaccines

revealed that the enhanced immunogenicity was not due to an enhanced antigen expression, suggesting that the quantitative difference may not be as important as the qualitative difference in antigen presentation. The presence of the RNA replicase in the 'self-replicating' nucleic acid vaccines mimics alphavirus infection, which triggers the innate antiviral pathways of the host cells. Studies on how viral and cellular modulators of the innate antiviral pathways affect vaccine function should provide molecular insights crucial to future vaccine design.

1. Cytotoxic T Cells in Cancer Immunotherapy

1.1 The Role of Cytotoxic T Cells in Antitumour Immune Responses

Classical vaccines consist of antigens derived from bacteria or viruses that are used to induce an immune response against infectious diseases. Unlike classical vaccines, cancer vaccines can be either prophylactic or therapeutic, and the current forms of cancer vaccines attempt to elicit an immune response specific to a pre-existing tumour and seek to eradicate the tumour in the patient.

Cancer cells are notoriously poor immunogens, indeed, cells that become cancerous may have survived immune surveillance. For many years cancer immunotherapy was just a concept until a major breakthrough in the mid 1980s by a group of scientists led by Steven Rosenberg. These investigators discovered that lymphocytes that infiltrate solid tumours, such as melanoma and renal cell carcinoma, could be grown from single-cell suspensions of tumour by incubation in media containing interleukin (IL)-2, a T lymphocyte growth hormone. The tumour-infiltrating lymphocyte (TIL) clones could specifically lyse autologous tumour cells *in vitro*. In addition, adoptive transfer of *in vitro* expanded TIL plus high-dose IL-2 into patients with melanoma and renal cell carcinoma showed objective clinical effects. In one of the largest reported studies involving TIL therapy, 29 of the 86 patients (34%) had objective tumour regression, almost double the response rate observed in patients receiving IL-2 alone. In addition, many of the patients responding to TIL/IL-2 therapy were patients who had failed to respond to previous treatment with IL-2 alone.[1,2]

1.2 Identification of Major Histocompatibility Complex (MHC) Class I-Restricted Tumour Antigens

The above clinical results demonstrated that TIL specifically recognise tumour cells *in vitro* and have the capacity to directly or indirectly mediate tumour regression *in vivo*. These TIL populations contained mainly T lymphocytes, including both CD4+ and CD8+ T cells. Since CD8+ cytotoxic T lymphocytes (CTLs) are the major effectors for cell killing in animal models, initial interest was focused on the identification of tumour antigens recognised by the CD8+ subset of TIL. The method for cloning tumour antigens recognised by TIL involves transfection of genomic DNA or cDNA libraries into cells expressing the appropriate MHC molecule, followed by the identification of transfectants using cytokine release or lysis by human T cells with specific antitumour reactivity. MHC class I-restricted melanoma antigens have been identified using T-cell clones from TIL as well as from T-cell cultures *in vitro* sensitised with tumour lines.[3,4] Biochemical approaches have had limited success in identifying human cancer antigens. Attempts have been made to elute peptides from tumour cells or from MHC molecules purified from tumour cells and to detect fractions capable of stimulating antitumour T cells after pulsing purified fractions onto antigen-presenting cells (APCs). Mass spectrometric techniques have then been used to sequence the minute quantities of peptides obtained.[5] This approach has been limited by the need for custom-made, highly specialised equip-

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ment, and the requirement that peptides be present in sufficient quantity to enable their identification by these techniques. Because tumour specific CD8+ T cells and tumour lines are relatively easy to establish from patients with melanoma using currently available technologies, most MHC class I tumour antigens identified so far are mainly melanoma antigens. Only rarely have tumour antigens from non-melanoma cancers, such as renal cell carcinoma and lung carcinoma, been identified using direct CD8+ T-cell cloning approaches.[6,7] Nevertheless, several melanoma antigens (especially the so-called cancer/testis antigens) are present in various other cancer types. For example, the NY-ESO-1 antigen (identified in melanoma cells) is also present in cancers of the breast, prostate, ovary, and lung cancer. Thus, these antigens are also potential targets for immunotherapies against cancers other than melanoma.

Another technique for the identification of tumour antigens is based on the presence of tumour reactive antibodies in cancer patients. This approach, called serological analysis of recombinant cDNA expression libraries (SEREX), uses diluted serum from cancer patients to screen cDNA libraries prepared from tumours.[8,9] These strategies have identified tumour antigens that are the targets of antibody and/or T-cell immune responses in cancer patients.[10]

The tumour antigens identified thus far fall into four major categories. The first group of tumour antigens is expressed in melanoma and normal melanocytes. These so-called melanocyte differentiation antigens, including Mart-1, gp100, tyrosinase, TRP-1 and TRP-2, are enzymes involved in melanin synthesis pathways.[11,12] The second group of tumour antigens is expressed in a wide array of tumour types. In normal tissues, they are expressed only in the germ cells of the testis. These antigens include members of the MAGE, GAGE, NY-ESO-1 and BAGE families.[13,14] Tumour antigens belonging to the third category are overexpressed antigens including p53, p15, Her-2/neu, PSA, PSMA, CEA, SART-1 and PRAME, which are normally expressed at low levels in a wide range of tissues. However, they are expressed at a much higher level in cancer cells.[15-21] The last category represents unique tumour antigens expressed in tumour cells. These antigens include idiotype antigens in B cell and T-cell lymphomas, viral gene products in virally induced malignancies, and mutated cellular gene products, such as p53, β-catenin and CDK4-R24C.[11,12,22,23]

2. Helper T Cells in Cancer Immunotherapy

2.1 The Role of CD4+ T Cells in Antitumour Immune Responses

The T cells used for cloning most of the above antigens are CD8+ T cells. Although the important role of CD8+ CTLs in antitumour immune response has made human leucocyte antigen (HLA) class I-associated antigens the primary interest of researchers, there is increasing recognition that CD4+ T cells also play a crucial role in initiating and sustaining antitumour immune responses. CD4+ T cells are mainly ‘helper’ cells, although a small percentage of them have a direct cytotoxic effect. In the CTL priming phase, CD4+ T cells may help to ‘condition’ professional APCs by providing critical interactions including, but not limited to, CD40L-CD40 with the APC.[24,25] Such an interactive network of CD4+ T cells, APCs and CD8+ T cells may be responsible for the phenomenon known as ‘cross-priming’ *in vivo*. CD4+ T cells also control the growth and persistence of antigen-specific CD8+ T cells *in vivo* by providing essential cytokines, such as IL-2, in proximity to CTLs.[26] In addition to the direct involvement in priming CTLs, CD4+ T cells also provide cytokines required by other effectors such as natural killer (NK) cells and macrophages.[27] The critical roles of CD4+ T cells in controlling tumour growth have also been documented in MHC class II-negative tumours. In one of these studies, adoptive transfer of CD4+ T cells [specific for Friend murine leukaemia virus (MuLV)-induced tumour (FBL-3)] controlled tumour growth in a CTL-independent fashion.[28] In this model, CD4+ T cells

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were required for tumour protection, in which a macrophage-derived cell type played the role of effector.

It is yet to be determined whether tumour-specific helper T cells are more effective than non-tumour-specific helper T cells. One can argue that all of the above functions of CD4+ T cells can be provided by non–tumour-specific CD4+ T cells. It is conceivable, however, that the presence of the helper T cells in the vicinity of tumour-specific CD8+ T cells is vital to the priming and sustaining of an antitumour immune response. In other words, both tumour-specific CD8+ and CD4+ T cells need to be physically present in the microenvironment of a tumour-specific immune response. In addition, the role of self-reactive CD4+ T cells could be fundamentally different from that of CD4+ T cells specific to a foreign antigen. Our study showed that adoptive transfer of tumour antigen-specific, self-reactive CD4+ T cells was therapeutically effective in an animal model.[29] Another study showed that there is an absolute requirement for CD4+ T cells to be present in the induction of vitiligo and in tumour destruction in mice vaccinated with a recombinant vaccinia virus encoding TRP-1.[30] In human studies, a recent report indicated that the antibody response against tumour antigen NY-ESO-1 is strongly associated with HLA-DP4 (Fisher's test $p < 0.009$). In addition, the study found that CD4+ T cells specific to one HLA-DP4 epitope of NY-ESO-1 can be efficiently isolated from peripheral blood mononuclear cells (PBMCs) of melanoma patients with NY-ESO-1 antibodies. This study suggests that CD4+ T cells may be involved in gene-specific antibody responses against tumour antigens.[31,32] On the basis of these studies, it is logical to predict that the efficacy of cancer vaccines can be enhanced by the engagement of both CD4+ and CD8+ T cells.

2.2 Identification of Major Histocompatibility Complex (MHC) Class II-Restricted Tumour Antigens

Unlike the cloning of MHC class I-restricted tumour antigens, identification of MHC class II-restricted tumour antigens has been hampered by the lack of a reliable and efficient molecular cloning method. For MHC class I tumour-associated antigens (TAA), which are endogenously expressed gene products, standard methods have been developed to identify such antigens through transient or stable introduction of tumour cDNA libraries into highly transfectable cell lines.[11] Nevertheless, these approaches do not apply to cloning MHC class II-restricted antigens because the processing and presentation of MHC class II-restricted antigens are more complex and require specialised peptide loading compartment and accessory molecules. Recently, a method based on biochemical protein purification in conjunction with mass spectrometry sequencing was successfully used in identifying the unique tumour antigen expressed in 1558 melanoma line, which was recognised by autologous CD4+ TIL.[33] Proteins from 1558 melanoma lysate were purified based on their recognition by 1558 TIL when pulsed onto APC. A search of the databank according to amino acid sequence (revealed by mass spectrum analysis) identified a peptide from triophosphate isomerase (TPI), an important enzyme involved in the glycolytic pathway. Subsequent cDNA cloning identified a mutated version of TPI expressed in the tumour cells as the antigen recognised by 1558 CD4+ TIL in the context of HLA-DR1. The epitope was generated from an amino acid substitution of Thr with Ile due to a point mutation of ACT to ATT in the coding region. The epitope bearing the substituted amino acid residue, which was involved in T-cell receptor (TCR)-peptide-HLA interaction, resulted in enhanced T-cell stimulatory activity by 5 logs. This approach may also be useful for other highly expressed protein antigens that can be efficiently taken up from the exogenous environment by APC, and then presented to CD4+ T cells.[33]

Some tumour cells express MHC class II molecules naturally or upon interferon-γ treatment or CIITA transduction. CD4+ T cells can recognise these tumour cells. Their tumour lysates, however, are usually not recognised when pulsed onto APC bearing the correct MHC class II molecules.[34] To

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facilitate the presentation of exogenous peptides through MHC class II pathways, additional components of the class II pathways are required. A novel gene-based approach has been developed that allows the screening of an Ii-cDNA fusion library in an engineered APC expressing the invariant chain Ii, DMA, DMB and other essential components of the MHC class II processing and presentation pathway (figure 1).[35] This approach has been successfully used in identifying two new MHC class II-restricted tumour antigens that could not be recognised by T cells when tumour lysates were pulsed onto APC exogenously. In addition, this genetic approach has been successfully used to identify TPI,[35] whose gene product could be recognised when pulsed from an exogenous environment. Using a similar gene-based approach, Chiari et al.[36] also reported a new MHC class II-restricted tumour antigen from a melanoma patient.

The identification of these new MHC class II-restricted tumour antigens suggests one surprising notion. Before tumour antigens were identified on the molecular level, one hypothesis was that most tumour antigens recognised by T cells were probably the product of mutated or tumour-specific

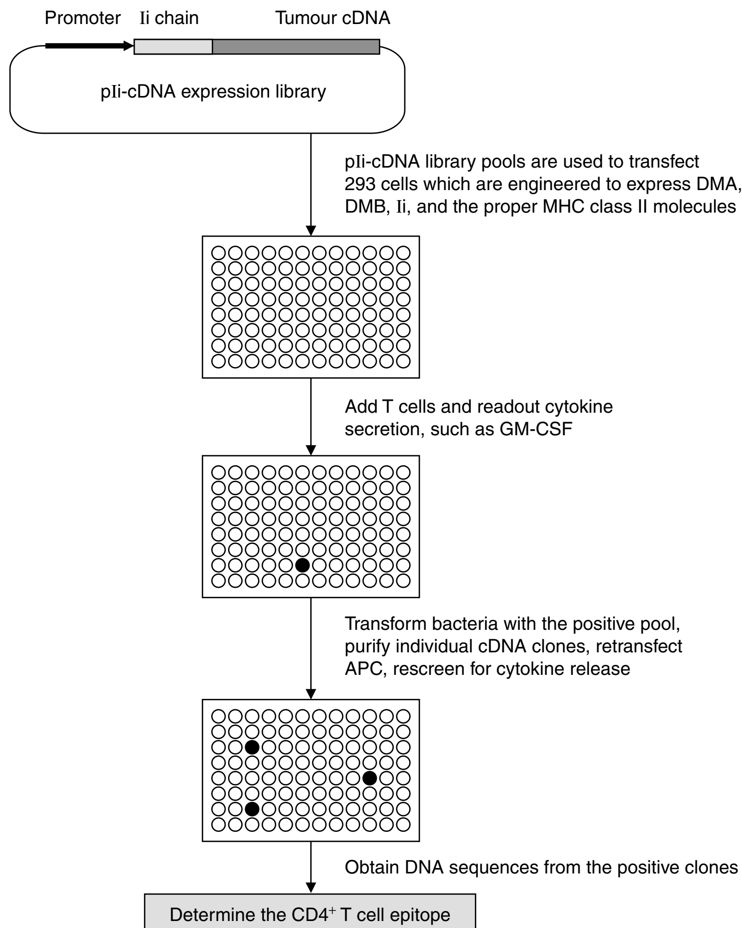

Fig. 1. Cloning of class II tumour antigens by screening of Ii-cDNA fusion library in an engineered antigen-presenting cell (APC). **GM-CSF** = granulocyte-macrophage colony-stimulating factor; **MHC** = major histocompatibility complex.

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genes. However, most of the CD8+ CTL-defined tumour antigens identified so far are products of nonmutated and shared genes. By contrast, many CD4+ T-cell clones derived from TIL, established in our laboratory, recognised unique (and possibly mutated), but not shared, tumour antigens in addition to the above-described mutated unique TAA recognised by CD4+ T cells. Most tumour-specific CD4+ T cells studied in an animal tumour model also suggested that they recognise unique, rather than shared, tumour antigens.[11]

2.3 Identification of Shared MHC Class II Epitopes

Although these pioneering studies of tumour antigens recognised by CD4+ T cells revealed some important molecular details of tumorigenesis, most of the MHC class II-restricted tumour antigens identified by these approaches are not suitable for clinical applications because the antigens are unique to a certain tumour. The critical need for clinical applications is to identify shared tumour antigens recognised by CD4+ T cells. Early studies of TIL-derived tumour-specific CD4+ T cells found that TIL1088 recognised a number of melanoma lines as well as melanocytes in an HLA-DR4-restricted fashion, indicating that the antigen is shared between melanoma and normal melanocytes.[37] Cos cells were transfected with candidate tumour antigens previously identified by CD8+ CTL, and the transfected cos cell lysates were pulsed onto HLA-DR4-EBVB lines for recognition by CD4+ TIL1088. Two nonmutated epitopes from the tyrosinase protein were identified, which represented the first tumour-associated MHC class II epitopes recognised by CD4+ T cells. This finding also indicated the presence of MHC class II epitopes within the proteins previously defined as tumour antigens recognised by CD8+ T cells. Similar to the approach used for identifying peptides bound with MHC class I molecules, endogenously expressed peptides were also eluted from HLA-DR-peptide complex on the surface of a melanoma cell line. In conjunction with mass spectrum analysis, this approach identified a 16-mer peptide derived from gp100. A slightly different version of the peptide was also independently identified by immunising HLA-DR4 transgenic mice with the recombinant GP100 protein emulsified in complete Freund's adjuvant (CFA), and then tested for recognition of the candidate peptides.[38]

Dendritic cells pulsed with the candidate peptides have been successfully used in generating specific CD8+ T cells. This approach was also used to raise specific CD4+ T cells, including HLA-DR1-restricted bcr-abl-specific CD4+ T cells.[39] The peptide was derived from a novel junctional sequence resulting from the fusion of c-abl oncogene on chromosome 9 to break point cluster region (bcr) on chromosome 22, which are characteristics shared in chronic myeloid leukaemia (CML). CD4+ T cells thus generated recognised peptides as well as CML lysates pulsed onto dendritic cells.

MAGE-3 is a cancer/testis antigen widely expressed in a number of tumour types. Monocyte-derived dendritic cells pulsed with recombinant MAGE-3 proteins were used to generate CD4+ T cells from the PBMC of normal donors. CD4+ T cells recognising MAGE-3 in the context of HLA-DR13 and HLA-DR11 were established.[40,41]

NY-ESO-1 is another tumour antigen of the cancer/testis category and possibly the most immunogenic tumour antigen of this category. In addition to CTLs recognising epitopes from the normal as well as the alternative open reading frame (ORF), antibodies were present in approximately 50% of patients with tumours expressing NY-ESO-1.[42] The targeting of NY-ESO-1 by both the cellular and humoral arms of the immune system may suggest the presence of CD4+ T cells specific for this antigen. Recently, an HLA-DR4-restricted and an HLA-DP4-restricted CD4+ T-cell epitope from NY-ESO-1 were identified[43] using peptides to stimulate PBMCs from patients with antibodies against NY-ESO-1.[31,43] This strategy was based on a hypothesis that such patients might have higher frequencies of specific helper T cells. Thus, this strategy may be generally applicable in identi-

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fying CD4+ T-cell epitopes from other candidate antigens previously identified by antibodies.

3. Synthetic Cancer Vaccines Based on Identification of Tumour-Associated Antigens

3.1 Classification of Cancer Vaccines Targeting Tumour-Associated Antigens

Based on the methods used for identification of tumour antigens, it is clear that immune response against tumour antigens is present in individual cancer patients. The next challenge is to induce a more powerful and more specific immune response in cancer patients to eradicate tumour cells. The cloning and characterisation of tumour antigens has made it possible to develop synthetic and recombinant cancer vaccines expressing specific tumour antigens. The development of synthetic and recombinant cancer vaccines falls into three major areas:

- recombinant protein and peptide vaccines
- recombinant viral and bacterial vaccines
- naked DNA or RNA vaccines.

3.2 Peptide-Based Cancer Vaccines

Peptides are cheap, stable and easy to make and have demonstrated their preliminary efficacy in clinical trials when used in conjunction with adjuvants. One study involved the use of an HLA class I epitope from the melanoma-associated antigen gp100. The parental peptide gp100:209-217 was modified by replacing T with the M residue at position 210, which enhanced the binding to HLA A2 as well as the immunogenicity *in vitro*. A strong antitumour CD8+ CTL was generated in the PBMCs of 91% of patients receiving the modified peptide in conjunction with incomplete Freund's adjuvant (IFA). However, objective clinical responses were not seen in these patients. In subsequent studies, high dose IL-2 was administered immediately after the immunisation of patients with the modified gp100 peptide in IFA. The new regimen showed an increased clinical response rate of 35 to 42% in comparison to the 15 to 20% response rate in patients receiving IL-2 alone.[44] Nevertheless, systemic immunisation with the gp100 peptide did not correspond with the clinical responses observed in this study, which was similar to conclusions drawn from other clinical trials involving MHC class I peptide vaccines.[45] One possible reason why these experimental approaches lack clinical effectiveness is that immunisation with MHC class I peptides gives rise only to CD8+ T cells. CD4+ helper T-cell activity may also be required to induce tumour regression. Therefore, peptides from helper T-cell epitopes may also be required for future clinical studies. We envisage a new generation of cancer vaccines based on the recent MHC class II-restricted tumour antigen discoveries that may involve a combination of both class I and class II peptide epitopes. These peptides would be directly injected into patients in combination with IFA. Alternatively, dendritic cells could be loaded with the combined MHC class I and class II peptides and used to immunise patients. Efficacy of peptide-based immunotherapies may be enhanced by the engagement of both CD4+ and CD8+ T cells against cancer.

3.3 Recombinant Virus versus Nucleic Acid Cancer Vaccines

Despite significant advances in using recombinant viral or nucleic acid tumour vaccines in animal tumour models, evidence of clinical efficacy in cancer patients is scarce.[46,47] Immunisation with recombinant viral vaccines has been ineffective because of pre-existing immunity to the vector or because of a lack of immuno-dominance of the transgene due to the antigenic complexity of the vector itself. The DNA or RNA core of the recombinant viral vaccines are covered by a protein 'coat' that both protects the viral genome from degradation and facilitates entry into the host cell. The viral coat is generally subject to neutralising antibody reactions that in many cases eliminates the multiple immunisation potential of recombinant viral vaccines. The lack of a protein coat in nucleic acid vaccines makes them, on the one hand, less efficient for cell entry and vulnerable to degradation,

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and on the other, more suitable for multiple immunisation because of the absence of the neutralising antibodies against the viral coat. Furthermore, the lack of a viral coat eliminates the possibility that immune responses to the coat could potentially overwhelm the immune responses to the desired antigen space.[48,49]

3.4 Strategies to Improve Recombinant Cancer Vaccines

Many methods have been used to improve recombinant viral and nucleic acid vaccines.[6] Antigen expression can be improved by using better promoters, intron and enhancer elements, or polyadenylation sequences.[49-52] The TAAs are notoriously poor immunogens, because most are 'self' antigens. Various strategies have been used to make them better immunogens, such as adding sequences to target antigens to the MHC class I or class II processing pathways.[53,54] Epitopes can also be modified to increase their ability to bind to MHC molecules, thereby increasing their immunogenicity.[55] Because most TAAs are self antigens, T cells that are highly specific for the TAA are either deleted or anergised during T-cell development. Therefore, attempts to directly immunise using the original self antigen was unsuccessful. However, antigens can be modified to stimulate an immune response that not only reacts with the modified epitope, but also 'cross-reacts' with the original TAA. Additionally, the TAA from one species can induce an immune response that cross-reacts with the TAA from another species. It is, therefore, possible to induce immune response against a 'self' epitope by immunising with a 'foreign' relative.[44]

In addition to the improvement of a tumour antigen itself, exogenous immunomodulatory molecules can also be used to enhance nucleic acid vaccination. Cytokines, such as IL-2, IL-6 or IL-12, can augment therapeutic efficacy when coadministered with DNA encoding a tumour model antigen.[56,57] Costimulatory molecules, such as B7-1 or B7-2, provide the second signal required for T-cell activation. Without the second signal, T cells become anergised or undergo apoptosis upon receiving only the first signal through TCR. This may be what happens when T cells contact a tumour cell, because tumour cells do not express costimulatory molecules on their surfaces. It has been demonstrated that coadministration of B7-1 and B7-2 significantly enhances DNA vaccination.[58] It is critical, however, that the costimulatory molecules be on the same plasmid as the antigen. Perhaps the antigen and the costimulatory molecules have to be expressed in the same cell. Alternatively, DNA immunisation can be enhanced by inhibiting CTLA4, which sends a negative signal for T-cell activation.[59,60] Another group of molecules, termed chemokines, have also been explored for their potential in augmenting nucleic acid vaccination. Chemokines are chemoattractants. They can induce the activation and directional migration of a variety of immune cells. Genetic fusion of a model tumour antigen with a chemokine moiety may allow efficient targeting of APCs *in vivo*.[61] Finally, the so-called immunostimulatory sequences (ISS), which are nonmethylated, palindromic DNA sequences containing CpG-oligodinucleotides, can activate B cells, T cells, NK cells and dendritic cells *in vitro* and *in vivo*. The immunostimulatory function of ISS may come from its bacterial origin. Evolution enables the immune system to recognise the foreign DNA as 'dangerous' signals when it enters a cell. ISS can either be incorporated into the backbone of nucleic acid immunogens or coadministered with plasmid DNA in the form of oligonucleotides.[62,63]

3.5 Formation of a 'Self-Replicating' Nucleic Acid Vaccine

The use of alphavirus replicons in nucleic acid vaccines has recently attracted great interest. This new generation of genetic vaccines takes advantage of the self-replication machinery used by members of the alphavirus genus, including Sindbis virus, Semliki Forest virus and Venezuelan equine encephalitis virus. Alphavirus is a positive-stranded RNA virus. Its RNA encodes structural proteins and a polyprotein, called RNA replicase. Upon infecting a cell, the viral RNA first translates

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its structural proteins as well as the replicase complex, which in turn drives its own RNA replication. Up to 200 000 copies of RNA can be made in 4 hours, and expression of the encoded antigen can be as much as 25% of total cellular protein. By replacing the genes for structural proteins of the virus with an antigen of interest, a 'self-replicating' RNA vaccine can be generated.[64,65]

To increase the stability of the construct, and to facilitate the production and handling of the vaccine, the self-replicating RNA can be encoded by a DNA plasmid where a cytomegalovirus (CMV)-promoter 'jump-starts' the production of the self-replicating RNA.[65-67] The alphavirus replicase functions in a broad range of host cells (mammalian, avian, reptilian, amphibian and insect cells), making it a very attractive delivery vehicle. We have recently demonstrated that both an RNA vaccine and different plasmid DNA replicons encoding a model TAA under the control of alphaviral RNA replicase are effective in the treatment of an experimental tumour.[66] In animal models of infectious disease, these plasmid DNA replicons are 100 to 1000 times more potent in stimulating antigen-specific immune responses, particularly cellular responses, than conventional plasmid DNA expression vectors.[65,66] As little as 10ng of DNA is enough to induce a significant amount of immune response. Using less DNA would make DNA vaccines more economical for clinical applications. An RNA-based vaccine has been a long-ignored strategy because of its low efficiency and instability. However, RNA does not integrate into the host genome, making it a potentially safer vaccine vehicle than a DNA vaccine. The self-replicating capability of the replicon-based RNA vaccine overcomes the efficiency problem of conventional RNA vaccine. Thus, it significantly increases the potential of an RNA vaccine.

3.6 Quantitative versus Qualitative Antigen Presentation

The initial rationale for using the alphaviral RNA replicase was to enhance antigen expression. However, the level of antigen expression of replicase-based constructs *in vitro* is comparable to that obtained using conventional DNA vectors. A fundamental difference between replicase-based DNA vaccines and conventional DNA vaccines is the virus-like RNA replication inside the transfected host, resulting in the production of a large amount of double-stranded RNA (dsRNA), which is the requisite intermediate of RNA replication. Many viruses, especially some RNA-based viruses including alphavirus, mediate the production of dsRNA during viral replication or during the transcription of overlapping RNA species.[68] Higher organisms have evolved a number of redundant and complimentary pathways to recognise foreign dsRNA. dsRNA from viruses is known to trigger two major antiviral mechanisms of the host cell: the protein kinase-RNA activated (PKR) and 2′-5′-oligoadenylate (2-5A) synthetase pathways (figure 2). Activation of the 2-5A system contributes to the antiviral effect of the interferons through the synthesis of 2-5A and its subsequent activation of RNAse L, which degrades both viral and cellular RNA. Induction of the PKR antiviral pathway by dsRNA up-regulates host cell interferon production and also triggers an inhibition of translation. The activation of both of these pathways predisposes the cell to death by apoptosis.[69] Indeed, transfection of cells with self-replicating RNA, as well as with plasmid DNA-replicon, causes apoptotic death, as does the infection with the complete alphavirus (figure 2).[48,70,71]

The above studies suggest that the amount of antigen expressed does not correlate with the amount of immune response generated. The quantitative difference in antigen presentation may not be as important as the qualitative difference in antigen presentation. It may be possible to harness the primitive viral defence pathways to enhance vaccine efficacy, and there are a variety of gene products known to modulate these pathways that may be useful for vaccine design. Questions that remain, however, are how viral and cellular modulators of the antiviral pathways affect vaccine function and how we can incorporate these components into our current vaccine design.

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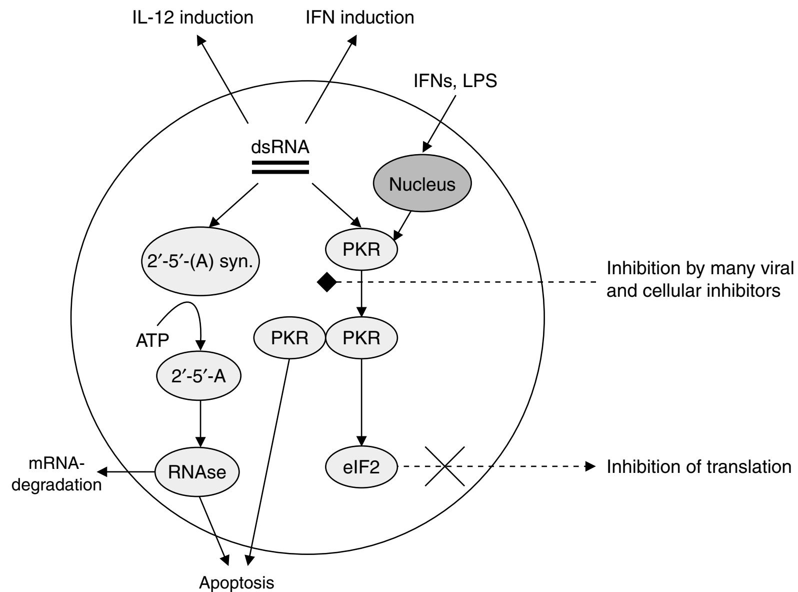

Fig. 2. Cellular responses induced by the presence of double-stranded RNA (dsRNA). **eIF$_2$** = eukaryotic initiating factor 2 ; **IFN** = interferon; **IL** = interleukin; **LPS** = lipopolysaccharide; **PKR** = protein kinase-RNA activated; **2′-5′-A** = 2′-5′-oligoadenylate; **2′-5′-(A) syn.** = 2′-5′-oligoadenylate synthetase.

It needs to be mentioned that there are other strategies that are not discussed in detail in this review, such as whole tumour cell-based strategies, dendritic cell-based approaches, etc. Dendritic cells are the most potent 'professional' APCs and have moved to the centre stage of active immunotherapy. Clinical trials using dendritic cell-based immunotherapy have been published for five cancers, namely lymphoma,[72] melanoma,[73] prostate cancer,[74] renal cancer[75] and malignant brain tumour.[7] Many dendritic cell-based strategies are currently under development, including pulsing dendritic cells with whole tumour lysate,[72] total tumour RNA,[76,77] tumour peptides,[74] plasmid DNA coding for a specific tumour antigen,[78] dendritic cell-tumour hybrids[75] and dendritic cell uptake of apoptotic or necrotic tumour cells.[79] Many of these strategies are being tested in clinical settings.

4. Conclusions and Future Directions

The development of synthetic and recombinant cancer vaccines is still maturing. Which vaccine vector and tumour antigen will be the best for a specific cancer? It is too early to make the call. Nonetheless, we can imagine that the ideal vector for a recombinant vaccine should offer the following: it should be safe, highly immunogenic, non-integrative, easy to manipulate, capable of multiple immunisation and cheap. Recombinant viruses are powerful, but their efficacy is hindered by neutralising antibodies to their envelope proteins. Naked nucleic acid vaccines do not contain a protein 'coat'; therefore there are no neutralising antibodies against the immunogen. However, they are also much less efficient at entering a host cell than recombinant viruses because of the lack of a protein coat. It is conceivable that the future design of recombinant cancer vaccines may focus on the middle ground between recombinant viral vaccines and nucleic acid vaccines.

The molecular identification of tumour antigen has brought us to an exciting new era of cancer vaccine development. Although many established tumours have been successfully treated in animal models, clinical success has happened in only an extremely limited number of patients. The follow-

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ing are some of the issues that require consideration. First, animal tumour models do not sufficiently reflect what occurs in cancer patients. More clinically relevant animal models need to be designed. Second, the immune systems of mice and humans are different. Treatment that works in mice does not necessarily work in humans. Third, studies have shown that expression of specific tumour antigens is lost in cancer cells after the patients are immunised with vaccines encoding the tumour antigens.[80,81] In other words, cancer cells that grow back after vaccination are immune-selected to lose the expression of the target tumour antigen. Fourth, the tumour antigens used as cancer vaccine targets exist in patients prior to immunisation. These tumour antigens are believed to be either actively tolerated or ignored by the immune system. To overcome the absence of specific T cells in cancer patients who fail to respond to active immunotherapy, techniques are developed to generate large numbers of antigen-specific CD8 T cells *in vitro*.[82,83] Up to 1 billion antigen-specific T cells can be adoptively transferred back into cancer patients. It is conceivable that each therapeutic strategy may be suitable for only a certain type of cancer. Finally, advancement of vaccine design is still hindered by our limited understanding of their mechanisms. The convergence of information from studies of tumour biology, immunology and molecular virology will undoubtedly contribute to our burgeoning knowledge of how to design a better cancer vaccine in the years to come.

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