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Replicating Vectors for Gene Therapy of Cancer: Risks, Limitations and Prospects

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INTRODUCTION

MOST COMMON malignancies remain incurable. With chemotherapy, radiotherapy and surgery, it is often possible to reduce the tumour burden dramatically, but regrowth from residual cells is depressingly common, and the disease eventually becomes resistant to all standard forms of therapy. New, effective treatment modalities are therefore required and the spotlight has recently moved to gene therapy.

However, the gene therapy approach is unlikely to be of major value until it is possible to deliver genes efficiently to multiple tumour deposits in the patient, after the disease has disseminated. The efficiency of gene delivery could certainly be increased by the use of replicating viral vectors but, to date, there has been little research in this area. The aim of this article, therefore, is to emphasise the therapeutic potential of replicating vectors, to consider the issues of efficacy and safety relating to their design and use, and hopefully to stimulate further research in this challenging area.

THERAPEUTIC POTENTIAL OF *IN VIVO* GENE TRANSFER TO TUMOUR CELLS

There are three mechanisms by which direct genetic modification of tumour cells *in vivo* might lead to therapeutic benefit:

1. Gene transfer may be used to enhance the immunogenicity of tumour cells, thereby boosting local and systemic immunity against the genetically modified cells and their unmodified counterparts. In this case, it might be sufficient to deliver the 'therapeutic' genes to a relatively small percentage of the tumour cells, although it is expected that the immunostimulatory effects would be directly proportional to the number of tumour cells successfully transduced. However, the approach requires that the tumour cells are inherently antigenic and that tolerance can be broken, preconditions which appear to be met in only a few malignancies (melanoma, renal cell carcinoma and non-Hodgkin's lymphoma).
2. A second possible approach is to correct a genetic defect which contributes to the malignant phenotype of the tumour cells (for example, replace a deleted tumour suppressor gene or deliver an antisense construct to reduce expression of an oncogene). In this case, the therapeutic gene would probably have to be delivered to most, if not all, of the tumour cells in the patient.
3. The third approach is to deliver a gene whose product is directly toxic to the tumour cells in which it is expressed or which renders them susceptible to an otherwise non-toxic prodrug. Again, this requires gene delivery to most or all of the tumour cells. However, there is also the possibility of delivering genes encoding secreted toxins which mediate local killing of bystander tumour cells, or prodrug-activating enzymes which generate drugs with similar bystander effects. In this case, a useful therapeutic effect should be possible with considerably less than 100% efficient gene delivery to the tumour cells.

Candidate genes for each of these approaches have been identified and their potential demonstrated in tissue culture and in animal models. This has led, in some cases, to phase 1 clinical trials in human subjects [1, 2]. The immunostimulatory properties of tumour cells transduced with genes encoding viral antigens, immunomodulatory cytokines or foreign MHC proteins have been adequately demonstrated [3–8]. Transfer of the Rb gene to Rb-negative retinoblastoma cell lines has been shown to reverse their malignant phenotype and antitumour effects of anti-*ras* antisense constructs have been demonstrated in an *in vivo* Lewis lung carcinoma therapy model [9, 10]. Ganciclovir-mediated killing of herpes simplex virus thymidine kinase (HSVtk) gene-modified tumour cells has been demonstrated with significant toxicity to unmodified bystander tumour cells [11, 12], and similar effects have been observed following transfer of genes encoding other prodrug-activating enzymes, such as cytosine deaminase [13].

Armed with a growing number of genes with therapeutic potential, the pressing question is how to deliver them efficiently, accurately and safely to a large number of tumour cells *in vivo* [14].

DELIVERY OF GENES TO TUMOUR CELLS *IN VIVO*

Whether the gene delivery vehicle is a plasmid or a recombinant virus, it should first be capable of gaining access to a sufficient number of tumour cells in the patient to bring about a desired therapeutic outcome.

Reasonably accurate gene delivery can be achieved by direct inoculation of plasmids or recombinant viruses using a needle positioned in a tumour deposit [15]. This strategy achieves a relatively low efficiency of gene delivery, which is confined to tumour cells immediately adjacent to the needle track. Plasmids or viral particles delivered in this way do not permeate freely through the interstitial fluid bathing the tumour. Presumably, adjacent tumour cells are connected by tight intercellular junctions, forming impenetrable sheets of cells which wall off the deposited gene delivery vehicles [16].

An alternative to direct intratumoural inoculation is the delivery of the plasmid or recombinant virus via the bloodstream. Gene delivery to tumour deposits in the lung was recently demonstrated following intravenous infusion of DNA-liposome

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complexes, although there was no evidence for selective delivery to tumour versus normal host tissues, and the proportion of total tumour cells transduced was low [17]. The selectivity of gene delivery might be increased by introducing the plasmid (or virus) directly into the blood vessels supplying a tumour deposit, but this approach would be unsuitable to reach multiple metastatic deposits. Moreover, given that monoclonal antibodies (which are smaller than gene delivery vehicles) penetrate tumour deposits very poorly after intravenous administration, it is improbable that a high efficiency of gene delivery could be achieved by the same route [18, 19].

The use of non-replicating plasmids and viruses, therefore, imposes severe limitations on the maximum achievable efficiency of gene delivery. One strategy shown to enhance the efficiency of (HSVtk) gene transfer was to inoculate irradiated retrovirus-producing cells into tumour deposits [11]. This improves the efficiency of gene transfer to tumour cells adjacent to the inoculated retrovirus producers, but access to more distant cells in the tumour deposit remains limited.

The use of replicating vectors is a logical strategy whereby genes delivered initially to a small number of tumour cells can be copied and transferred to adjacent cells, thereby increasing the overall efficiency of gene delivery. The following is a review of published data illustrating the therapeutic potential of replicating vectors, their risks and limitations, and a discussion of how safer, more effective versions of these reagents might be designed, constructed and applied in the therapy of cancer.

USE OF REPLICATING VIRUSES FOR THE TREATMENT OF HUMAN CANCER

In 1904, Dock reported the case of a woman who experienced a dramatic remission of her leukaemia following a presumed attack of influenza and suggested that his observation might teach something of therapeutic value [20]. Additional cases were documented subsequently in which the progress of cancer was interrupted temporarily by intercurrent infection [21–23], but the first experimental demonstration of "viral oncolysis" has been attributed to Levaditi [24], who observed in 1922 that vaccinia virus was able to inhibit various tumours of mice and rats. Following this observation, a number of experimental models were established in which solid animal tumours, leukaemias or ascites tumours were inhibited by the administration of viruses, leading to limited and largely unsuccessful clinical experimentation [25, 26].

Interest in the phenomenon of viral oncolysis increased considerably following the demonstration in 1950 that Russian far east encephalitis virus administered by any route could destroy the transplantable mouse sarcoma 180, and other mouse tumours, at a rate dependent on the size of the initial virus inoculum [25, 27, 28]. The virus replicated to higher titre in the tumour than in any other host tissue, but the mice eventually died of viral encephalitis. At the time, in the pre-molecular biology era, there was no viable technique other than serial passage by which to modify the pathogenic and host range properties of a virus. Serial passage of the Russian encephalitis virus in tumour tissue increased its ability to destroy the tumour, whereas similar passage in mouse brain decreased its oncolytic potency. Passage in the Wagner osteogenic sarcoma gave rise to a strain of the virus able to destroy this previously non-susceptible tumour.

Many viruses and many types of malignancy were then surveyed in the hope that it would be possible to identify a single virus with potent activity against all cancers [25]. In the course of these studies, it became clear that many viruses grow in tumours in mice, rats, rabbits, guinea pigs and chickens where they may completely or partially inhibit tumour growth, or have no effect whatsoever. No universally oncolytic viruses were identified and the *in vitro* susceptibility of a tumour cell line did not necessarily correlate with its *in vivo* susceptibility to virus-mediated oncolysis.

The 1950s, 1960s and early 1970s saw a flurry of clinical trials in which patients with advanced cancer were deliberately infected with a variety of viruses. Among those tried in the clinic were adenovirus, bunyamwera, Coxsackie virus, Dengue, feline panleukopenia, hepatitis, Ilheus, mumps, Newcastle disease virus, rabies, vaccinia, varicella, Venezuelan equine encephalomyelitis and West Nile virus. These viruses were adminstered by several different routes (including direct intratumoural inoculation) to hundreds of patients with various treatment-refractory malignancies [29–32]. In general, the results were tantalising, but ultimately disappointing, and it seems that after a long and bitter struggle the pioneers of this research abandoned their crusade.

Perhaps the three most promising and informative clinical studies were those of Southam and Moore (1954) employing the Egypt 101 strain of West Nile virus [30], of Smith and colleagues (1956) using adenovirus against cervical carcinomas [31], and of Asada (1974) using mumps virus therapy [32].

The Egypt 101 strain of West Nile virus was used in over 150 virus therapy trials in patients suffering from a wide range of different malignancies [30]. Viraemia was documented in 90% of patients, and recovery of viable virus from tumour tissue in 80% of the patients who were tested. Fever was observed in most patients co-incident with viraemia. Not unexpectedly, encephalitis was the most troublesome toxicity associated with administration of this neurotropic virus. Of 8 patients with leukaemia or lymphoma, 5 experienced severe encephalitis, compared with only 1 patient in the much larger group with non-haematological malignancies. Less than 10% of patients overall showed convincing evidence of tumour regression and half of those had lymphoma. Thus, the most dramatic tumour regressions and the most serious virus-mediated toxicities occurred in the same group of patients, namely those with haematological malignancies. There are two possible explanations for this observation. The first is that transformed haemopoietic tissues are more permissive for the replication of West Nile virus than other malignancies, shedding more virus progeny into the bloodstream and thereby increasing the risk of encephalitis. The second explanation is that virus replication proceeds relatively unchecked in these patients (in tumour and brain) because of the global immuno-suppression associated with this group of malignancies. Either way, the therapeutic ratio might have been improved by modifying the tropism of the virus to selectively reduce its ability to propogate in neural tissue.

The use of adenovirus as an anti-neoplastic agent was reported by Smith and colleagues in 1956 [31]. Based on the luxuriant growth of adenoviruses which was observed in HeLa (human cervical carcinoma) cell cultures, the virus was administered as experimental therapy to 30 patients with advanced epidermoid carcinoma of the cervix. Varying volumes of virus-containing tissue culture supernatant were given by direct intratumoural inoculation or by arterial perfusion, and many of the patients were receiving immunosuppressive steroid therapy. Several adenovirus serotypes were employed and the patients were pre-screened for the prescence of anti-adenovirus neutralising

antibodies so that they could be treated with a serotype to which they were not immune. Twenty-six (65%) of the 40 virus inoculations were followed by formation of an area of necrosis and cavity formation in the central portion of the pelvic tumour without apparent damage to normal pelvic tissues. The period of necrosis lasted up to 30 days with production of copious semi-liquid necrotic cancer tissue in the most extreme cases. However, total destruction of the pelvic tumour was not accomplished in any patient and regrowth of the tumour inevitably occurred. 3 patients on corticosteroid therapy developed a febrile toxicity syndrome 2 to 3 days after virus inoculation with photophobia, general malaise and prostration, but all recovered in 7–9 days. Responses were less impressive in patients with pre-existing anti-adenovirus antibodies than in patients with no immunity to the virus, underlining the point that the spread of any replicating vector is likely to be limited by the host immune response.

Mumps virus was selected for cancer virotherapy trials based on its propensity to flourish in actively multiplying germ cells, causing post-pubertal mumps orchitis. Since the virus is otherwise of relatively low pathogenicity (mumps is not fatal) and most adults have antibodies against mumps virus, it was considered reasonable to test its therapeutic potential in human subjects with advanced cancer. Asada administered a tissue culture suspension of the virus to 90 patients with various terminal malignancies [32]. The virus was given by either local application to the tumour surface, intratumoural inoculation, oral, rectal or intravenous routes, or by inhalation. Other than the occasional febrile reaction, there were minimal toxic side-effects. In 37 of the 90 patients, the tumour disappeared or decreased to less than half of its initial size with more minor responses observed in a further 42 patients. Maximal tumour destruction was observed several days after virus administration and was often followed by long-term suppression of tumour growth. It was proposed that the early oncolytic effect is caused by mumps virus multiplication in the tumour, ceasing when spread of the virus is halted by the strong anamnestic antiviral immune response. In keeping with this proposal, anti-mumps antibody titres rose within 1 week after intravenous virus administration or 2 weeks after local injection. The longer-term carcinostatic effect was plausibly attributed to stimulation of antitumour immunity.

To summarise, these studies illustrate that certain viruses can replicate and spread selectively in tumour deposits without causing serious damage to normal host tissues. They also show that, in the context of cancer virotherapy, the host immune response to the virus has desirable and undesirable consequences: it limits collateral damage to normal host tissues and may indirectly enhance the host anti-tumour immune response, but it also serves to limit the spread of virus in tumour deposits. Virotherapy was eventually abandoned as an experimental approach to the treatment of cancer because its effects were unpredictable and, at best palliative rather than curative. At that time, serial passage was the only available method by which to derive a modified form of a virus with improved therapeutic potential but the outcome of such studies was generally disappointing.

TIME FOR A REVIVAL?

Significant advances have been made recently. The structures and life cycles of many viruses are understood in intricate molecular detail which logically relates back to their genome sequence. Many viral genomes are now available as infectious molecular plasmid clones which generate fully infectious virus upon transfection into mammalian cells [33, 34]. Such clones are amenable to precise experimental sequence manipulation. Techniques for virus culture, purification and quantitation have also advanced greatly from the days in which arbitrary volumes of untitred culture supernatant were used in human trials. Excellent *in vivo* models of human malignancy have been developed in SCID mice which can even be reconstituted with a functional human immune system [35].

In the light of these advances, especially the ability to engineer the nucleotide sequences of viral genomes, there is now a strong case for reviving research into the use of replicating viruses as vehicles for the delivery of therapeutic genes to tumour deposits *in vivo*. The remainder of this paper, is therefore, devoted to a discussion of some of the ways in which replicating vectors might be designed, constructed and used as safe, effective, non-toxic alternatives to conventional cancer treatments. For clarity, it is assumed that the treatment will be administered intravenously to patients with multiple metastases, although it should not be forgotten that local therapy may be beneficial in many clinical situations.

REPLICATING VECTORS FOR MORE EFFICIENT GENE DELIVERY

The first challenge is to maximise the efficiency of gene delivery to tumour cells. For a replicating vector, initial delivery is followed by replication and spread to adjacent cells. Within limits, it is logical to expect that the larger the dose of the vector, the greater should be the efficiency of initial delivery, but there is a need for experimental definition of the exact relationship between dose and efficient delivery.

There is an option to deliver the vector as preformed virus or as infectious nucleic acid (for example, plasmid DNA) and the relative merits of the two approaches have yet to be directly compared in an experimental model. Preformed virus may be rapidly inactivated by complement or circulating antibody and the particles may be too large to escape efficiently from small blood vessels into the tumour parenchyma. This is in contrast to infectious nucleic acid which is not immunogenic [36, 37], resists complement, is smaller than preformed virus and can be administered in vast quantities (10^{16} copies of the vector genome per dose is quite realistic). Its smaller size should give more uniform uptake by tumour cells distributed throughout a deposit and its lack of immunogenicity should allow repeated dosing. Ease of production and physical stability are also important advantages compared with infectious viruses. The main disadvantage of infectious nucleic acid is that it is not readily taken up by living cells in the body unless complexed to cationic lipid or a suitable protein carrier.

The potential gain in gene transfer efficiency that derives from use of a replicating vector is a function of its ability to spread locally from the initially transduced cells. The kinetics of this process vary, depending on the nature of the virus and the details of its replication cycle. For example, lytic viruses release their progeny in a single large burst (up to 10^4 per cell for adenovirus) coincident with cell death, whereas non-lytic virus progeny are continuously released from viable cells (retrovirus, for example). Transfer of the replicating vector genome to neighbouring cells may, therefore, occur suddenly after a time lag of a few days (lytic cycle) or more slowly but continuously from day one (non-lytic cycle). Further rounds of virus replication and radial spread to adjacent cells would, if unchecked, lead to an exponential rise in the number of cells harbouring the vector genome. The rate of spread would reflect the rate of virus release by infected cells and, perhaps more importantly, their ability to diffuse locally

from the site of production to more distant 'neighbouring' cells. The time-honoured method of serial passage in a representative tumour cell line is probably the best way to adapt a virus for more rapid replication and release from its target cells, although due consideration should be given to the cellular and microenvironmental heterogeneity that exists within and between tumour deposits [38–40]. The viral factors influencing virus diffusibility in the interstitial compartment would be an interesting area for further study since they may be amenable to manipulation.

In vivo, the local spread of virus is held in check and eventually contained by host defences. These include non-specific effector mechanisms (such as interferon or complement) and a specific antiviral immune response comprising humoral and cell-mediated effector arms. Therefore, replicating vectors should be chosen with these effector mechanisms in mind and, where possible, should be adapted accordingly.

The speed of the primary or anamnestic antiviral immune response, following administration of the replicating vector, is important since it defines a time window during which the virus can spread. This time window might be significantly prolonged by concurrent administration of immunosuppressive drugs, permitting more extensive spread of the vector genome. That tumour cells are often capable of evading or suppressing potentially damaging host antitumour immune responses may be important [41]. By the same token, they might locally suppress the host antiviral immune response, thereby providing a protected haven for the spread of a replicating vector.

MORE SELECTIVE GENE DELIVERY WITH REPLICATING VECTORS

The risk of collateral damage to normal host tissues must be addressed if treatment with replicating vectors is to be contemplated seriously. The ideal solution would be a vector which replicates selectively in the tumour cells, but can neither infect nor damage normal host tissues.

Viruses are variably dependent on host cell factors for successful transition of the various stages in their life cycles (binding, entry, intracellular transport, genome replication, viral gene expression, assembly and release of progeny). Thus, a virus cannot bind to a target cell unless there is a suitable receptor present on the surface of the cell [42]. Subsequently, the viral genes may not be expressed unless the cell provides transcription factors which bind to promoter/enhancer elements in the viral genome. Not uncommonly, viruses are dependent on host cell factors which are expressed in a species-specific or cell lineage-specific manner, or even at a single stage of cell differentiation in a single cell lineage in a single mammalian species. Hence, the host ranges of naturally occurring viruses are often restricted by the binding specificities of their coat proteins (for example, HIV binds to CD4 [43]) or by their transcription factor requirements (B19 parvovirus transcription is active only in cells of the erythroid lineage [44]).

Since viral coat protein genes and viral promoters are readily amenable to manipulation, it may now be possible, by non-random genetic engineering, to generate replicating vectors whose host range is restricted to specific populations of transformed human cells. Targeting might be achieved through selective binding of the vector to tumour cells, selective gene expression (and hence vector replication) in the tumour cells and selective toxicity of the vector-encoded therapeutic gene product for the tumour cells. The combination of two or more different tumour-selective features in a single replicating vector may enhance selectivity to the point at which the vector can be termed tumour-specific.

Viral coat protein engineering

If a replicating vector were to encode a remodelled coat protein binding specifically to a tumour antigen, its progeny might preferentially infect the antigen-positive tumour cells. When monoclonal antibodies were used to crosslink ecotropic retroviral particles to human cells (mouse ecotropic retroviruses neither bind nor infect human cells), gene transfer was observed, although the efficiency was low and not all cell surface antigens could act as surrogate receptors [45]. Moreover, in another study, mouse ecotropic retroviral particles, which had been chemically modified with lactose, were shown to bind specifically to the asialoglycoprotein receptor on human hepG2 cells [46]. Binding was followed by retroviral infection of the human cells as indicated by transfer of a functional β-galactosidase gene. Subsequently, it was shown that functional antibody fragments expressed as a viral envelope-single chain antibody fusion protein could be incorporated into retroviral particles to confer a predictably altered binding specificity [47]. Together, these studies demonstrate the feasibility of manipulating the host range of a replicating vector by modifying its coat protein genes.

Viral promoter engineering

A virus controlled by tumour-selective promoter and enhancer elements should generate progeny solely in infected tumour cells since its genes would not be expressed in other cells. Tissue-specific regulation of gene expression has been well demonstrated from heterologous (non-viral) promoters included in recombinant viral genomes [48–50]. Moreover, interleukin genes delivered by parvoviral vectors based on minute virus of mice (whose early promoter is preferentially active in transformed cells [51]) were well expressed in oncogene-transformed cell lines, but not in the corresponding non-transformed parental cells [52].

To date, there have been few published experiments attempting to modify virus host range by insertion of heterologous tissue-specific non-viral promoters. Cell-specific utilisation of the albumin (liver-specific) and immunoglobulin (B-cell specific) promoters has been demonstrated within non-replicating adenovirus genomes [53], but cell specificity was partially lost after replication of the viral DNA[54]. The albumin promoter remained active and the immunoglobulin and β-globin promoters were inactive when adenoviral DNA was replicated to high copy number in hepatoma cells. However, the cell specificity of all three promoters was lost, giving high levels of gene expression when the viral genomes were replicated in HeLa cells. Nevertheless, these studies strongly suggest that with careful attention to the stoichiometry and kinetics of gene regulation by cellular transcription factors, it should be possible to engineer the promoters of replicating vectors for tissue-specific, transformation-dependent expression.

Promoters and enhancers are composed of multiple genetic elements, or modules, allowing their transcription to be regulated in response to diverse intracellular signals [55]. Oncogenic mutations often lead (directly or indirectly) to changes in the activity of abundance of various transcription factors [56]. In the future, it may therefore prove possible to design and construct patient-specific, tumour-selective promoters whose modular composition is tailored to fit the unique transcription factor profile of the tumour.

PUBLIC SAFETY OF REPLICATING VECTORS

Replicating viruses have been used extensively in human vaccination programmes leading to the eradication of smallpox with significant control of poliomyelitis, measles, mumps, rubella and yellow fever. Vaccination involves the administration of a randomly mutated variant of a pathogenic virus strain, derived by serial passage in cultured non-human cells [57]. There is, therefore, much precedent for the release of genetically altered viruses into the environment.

As with live viral vaccines, replicating gene transfer vectors raise serious safety concerns which relate to the inherent mutability of animal viruses. The accidental emergence of a new virus strain could cause harm to the patient, an unborn child, or worse still, could initiate an epidemic. Important safety questions which will have to be addressed before a replicating vector can be approved for human use are set out below:

1. Could the replicating vector damage normal tissues and cause disease in the treated patient?
2. Could the replicating vector mutate or recombine *in vivo* to generate a more pathogenic strain and, if so, would the new strain be more pathogenic than the wild type virus from which the vector was derived?
3. Could the replicating vector or a more pathogenic mutant spread from the patient to other human subjects and what would be the consequences of such spread?
4. Could the recombinant virus or a more pathogenic mutant be passed transplacentally and cause damage to a developing embryo or fetus?

Serious epidemics arise when pathogenic viruses gain access to susceptible populations under conditions which favour transmission between individual members of the population [58]. In selecting a virus from which to develop a replicating vector for human use, potential pathogenicity should be the primary consideration. Viruses which occur naturally in non-human species and are non-pathogenic for humans, seem at first sight to be attractive candidates. However, it is clear that such viruses are totally unsuitable, since adapting them for replicative spread in tranformed human tissues could render them pathogenic for humans. In the absence of natural immunity, the entire human population would be susceptible to the new pathogen. This argument against the use of relicating vectors derived from veterinary viruses is strongly supported by the recent observation that murine retroviruses can cause serious pathology in non-human primates [59]. Three of 10 monkeys became viraemic and developed aggressive, fatal T cell lymphomas after they had been transplanted with bone marrow that had been infected with murine retroviruses. Moreover, serious viral epidemics and pandemics may be initiated by a sudden change in the species tropism of a naturally occurring virus [60].

Replicating vectors for cancer therapy should therefore be derived from viruses which are naturally endemic to the human population, if only to permit a realistic assessment of the risks they pose to public health. Exotic human viruses should be avoided for similar reasons and serious human pathogens are obviously unsuitable because of the risk of reversion to wild type in the treated patient. The optimal strategy might be to derive the replicating vector from a highly prevalent but weakly pathogenic human virus. Reversion to wild type would then pose no serious risk to the patient or to the population but the possibility of recombination or mutation giving rise to a pathogenic variant of the original virus would have to be addressed. For example, it would be dangerous to extend the

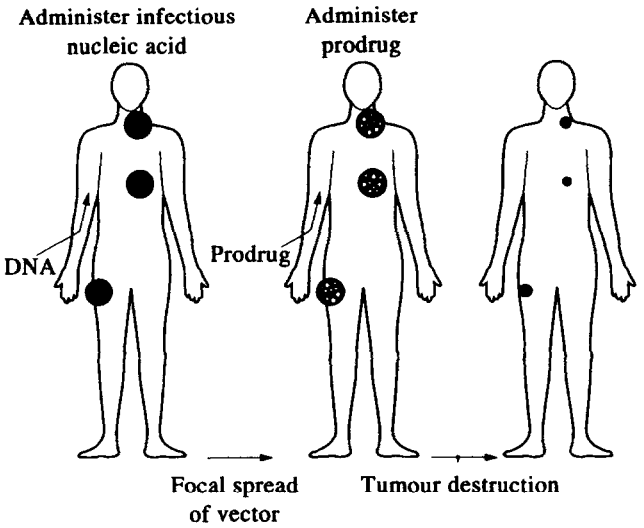

Figure 1. Purative therapeutic strategy employing targeted replicating vector. Replicating vector is administered intravenously as infectious nucleic acid in (targeted) liposomal formulation. Focal spread of vector occurs exclusively in tumour deposits causing direct cytophathic effect, local synthesis of vector-encoded prodrug-activating enzyme and tumour cell killing due to local antiviral immune response. Infused prodrug is converted locally to cytotoxic drug which kills uninfected 'bystander' tumour cells. The cycle is repeated to eradicate residual disease.

restricted tissue tropism of any virus by coat protein engineering or promoter insertion. Conversely, restricting the tissue tropism of a virus already capable of replicating in a variety of human tissues (tumours included) would pose no serious risk. The choice of therapeutic gene for insertion into the replicating vector genome also has important public safety implications. A weakly pathogenic virus might be converted to a dangerous pathogen by insertion of a gene coding for a potent cellular toxin, whereas a gene coding for a drug-activating enzyme would carry no such risk.

To qualify as a suitable candidate from which to derive a replicating vector for human cancer therapy, a virus must therefore satisfy a large number of stringent preconditions and the choice will vary depending on the malignancy. One promising candidate to emerge from this theoretical selection process is adenovirus type 5, although there are other viruses worthy of consideration. Adenovirus 5 is already prevalent in the human population, is of low pathogenicity, does not persist in the infected host and there is a safe, effective vaccine available. Its life cycle is well studied, its genome is available as an infectious molecular (plasmid) clone and it is potentially suitable for coat protein engineering (the trimeric fibre protein) or promoter/enhancer engineering to restrict its broad host range [61, 62]. It has room for insertion of a therapeutic gene coding for a drug-activating enzyme and recombinants are known to be stable provided they do not exceed 105% of wild type genome size [63]. Moreover, the virus is not cell cycle dependent (i.e. can infect non-dividing tumour cells) and is capable of rapid spread in tumour deposits *in vivo*.

SUMMARY

There are good theoretical arguments for exploring the use of replicating gene-transfer vectors for human cancer therapy. Such vectors should be derived from weakly pathogenic human viruses with intially broad tissue tropism. Coat protein engineer-

ing and promoter engineering might be used successfully to narrow the tropism of the vector, enhancing its ability to target tumour cells. Killing of uninfected 'bystander' tumour cells could be achieved through prodrug activation by a vector-encoded enzyme. Rapid elimination of infused vector particles by circulating antiviral antibody would limit access to tumour deposits after repeated administration, but might be circumvented by the use of infectious nucleic acid which is poorly imunogenic [64]. This putative therapeutic strategy is illustrated in Figure 1.

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Gene Marking After Bone Marrow Transplantation

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INTRODUCTION

BONE MARROW stem cells are desirable targets for gene therapy, since their genetic modification could treat many inherited and acquired diseases. Marrow progenitor cells are also appealing targets for logistic reasons; the cells are easily obtained and handled *ex vivo* and readily returned. Most importantly, genetic modification of just one single stem cell could, in principle, be sufficient to repopulate the haemopoietic and lymphoid system of an individual for their entire life. However, most pre-clinical experiments have suggested that currently available methods for transferring, expressing and regulating new genetic material have too limited an efficiency for clinical use [1, 2]. The problem has arisen because exploitation of the advantages of marrow stem cells for gene therapy requires the use of a vector which will stably integrate in the host cell genome and thereby retain its presence, not only in the original stem cell, but in the multitudinous progeny thereof. At present, retroviruses are the only vectors capable of achieving such integration, but will behave in this way only in dividing cells. Because the great majority of marrow stem cells at any one time are non-cycling, transfer efficiencies have been exceedingly low, and the expression levels obtained would have been wholly inadequate to obtain any clinical benefit. Because gene transfer carries a finite risk to the patient, there can be no justification for opening a protocol in which the risk:benefit ratio was infinitely high [1, 2].

These limitations led us to propose a series of gene marking experiments, using recipients of autologous bone marrow transplantation (ABMT) [3, 4]. In these patients, clinically relevant issues could be addressed, even with the extremely limited efficiency of gene transfer then available. Our initial aim was to use gene marking techniques to discover the source of relapse after ABMT, and to learn more about the biology of normal marrow reconstitution. We have also begun to use gene marking in patients following allogeneic bone marrow transplantation as part of a study aimed at adoptively transferring specific T cell immunity against Epstein–Barr virus (EBV)-infected B-cells, in

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