Journal of General Virology (2000), **81**, 2573–2604. Printed in Great Britain

REVIEW ARTICLE

Update on adenovirus and its vectors

W. C. Russell

Biomolecular Sciences Building, School of Biology, University of St Andrews, North Haugh, St Andrews, Fife KY16 9ST, UK

Introduction

Adenoviruses have been characterized extensively since their initial description in the early 1950s (Hillemann & Werner, 1954; Rowe *et al.*, 1953) and there is now a panoply of observations on the properties of many of the virus gene products. Nevertheless, there is still a lack of understanding of a number of the molecular mechanisms that operate in the infected cell, particularly in respect of how the virus gene products interact with cellular components and of the nature of the responses mounted by the host in response to infection.

In this regard, it is significant that, although there were almost 4000 references to adenoviruses in the 3 years from 1997 to 1999, most of these have been concerned with the results of investigations using adenoviruses as vectors and relatively few have dealt with the basic virology and immunology of virus infection. Indeed, it is now accepted that the initial enthusiasm for utilizing adenovirus gene vectors in therapy was rather prematurely optimistic and was perhaps over-hyped. In its place, there is a realization that targetting the vector effectively is not so straightforward and that, more importantly, the efficacy of host defences has not been fully appreciated and must be adequately addressed.

This paper reviews the current knowledge in the field of adenovirus vectors as well as advances in our understanding of the properties of the adenovirus gene products. Particular emphasis has been made on developments over the last two to three years. There have been a number of reviews examining different aspects of the vector field, and the reader is referred to these (Benihoud *et al.*, 1999; Hitt *et al.*, 1997; Zhang, 1999) for more comprehensive coverage. The expectation remains that a better understanding of the total spectrum of the virus–cell and virus–host interactions will lead to the design of vectors that provide more efficient delivery along with minimal deleterious host reactions.

General properties of adenoviruses

Adenoviruses have a characteristic morphology (Stewart *et al.*, 1993), with an icosahedral capsid consisting of three major proteins, hexon (II), penton base (III) and a knobbed fibre (IV), along with a number of other minor proteins, VI, VIII, IX, IIIa and IVa2 (Fig. 1). The virus genome is a linear, double-stranded DNA with a terminal protein (TP) attached covalently to the 5′ termini (Rekosh *et al.*, 1977), which have inverted terminal repeats (ITRs). The virus DNA is intimately associated with the highly basic protein VII and a small peptide termed *mu* (Anderson *et al.*, 1989). Another protein, V, is packaged with this DNA–protein complex and appears to provide a structural link to the capsid via protein VI (Matthews & Russell, 1995). The virus also contains a virus-encoded protease (Pr) (Weber, 1976; Webster *et al.*, 1989), which is necessary for processing of some of the structural proteins to produce mature infectious virus.

Members of the adenovirus family (*Adenoviridae*) infect a great variety of post-mitotic cells, even those associated with highly differentiated tissues such as skeletal muscle, lung, brain and heart. Since they deliver their genome to the nucleus and can replicate with high efficiency, they are prime candidates for the expression and delivery of therapeutic genes. They have a wide host-range and are currently divided into three genera with further subdivision into species (also termed subgenera or subgroups) A to F. Division of human serotypes, based mainly on immunological criteria, has historically been the basis of classification (Benkő *et al.*, 1999; Lukashok & Horwitz, 1998; Mautner, 1989). Some adenoviruses produce tumours in animals and can transform cells *in vitro*, but no reference to these characteristics will be made here.

Early events in adenovirus infection

The adenovirus infectious cycle can be clearly defined into two phases. The first or 'early' phase covers the entry of the virus into the host cell and the passage of the virus genome to the nucleus, followed by the selective transcription and translation of the early genes. These early events modulate the functions of the cell so as to facilitate the replication of the virus DNA and the resultant transcription and translation of the late genes. This leads to the assembly in the nucleus of the structural proteins and the maturation of infectious virus. The early phase in a permissive cell can take about 6–8 h (depending on a number of extraneous factors), while the late phase is normally much more rapid, yielding virus in another 4–6 h.

The adsorption of virus to target cell receptors involves high-affinity binding to cell receptors via the knob portion of

Author for correspondence: Willie Russell.
Fax +44 1334 462595. e-mail wcr@st-andrews.ac.uk

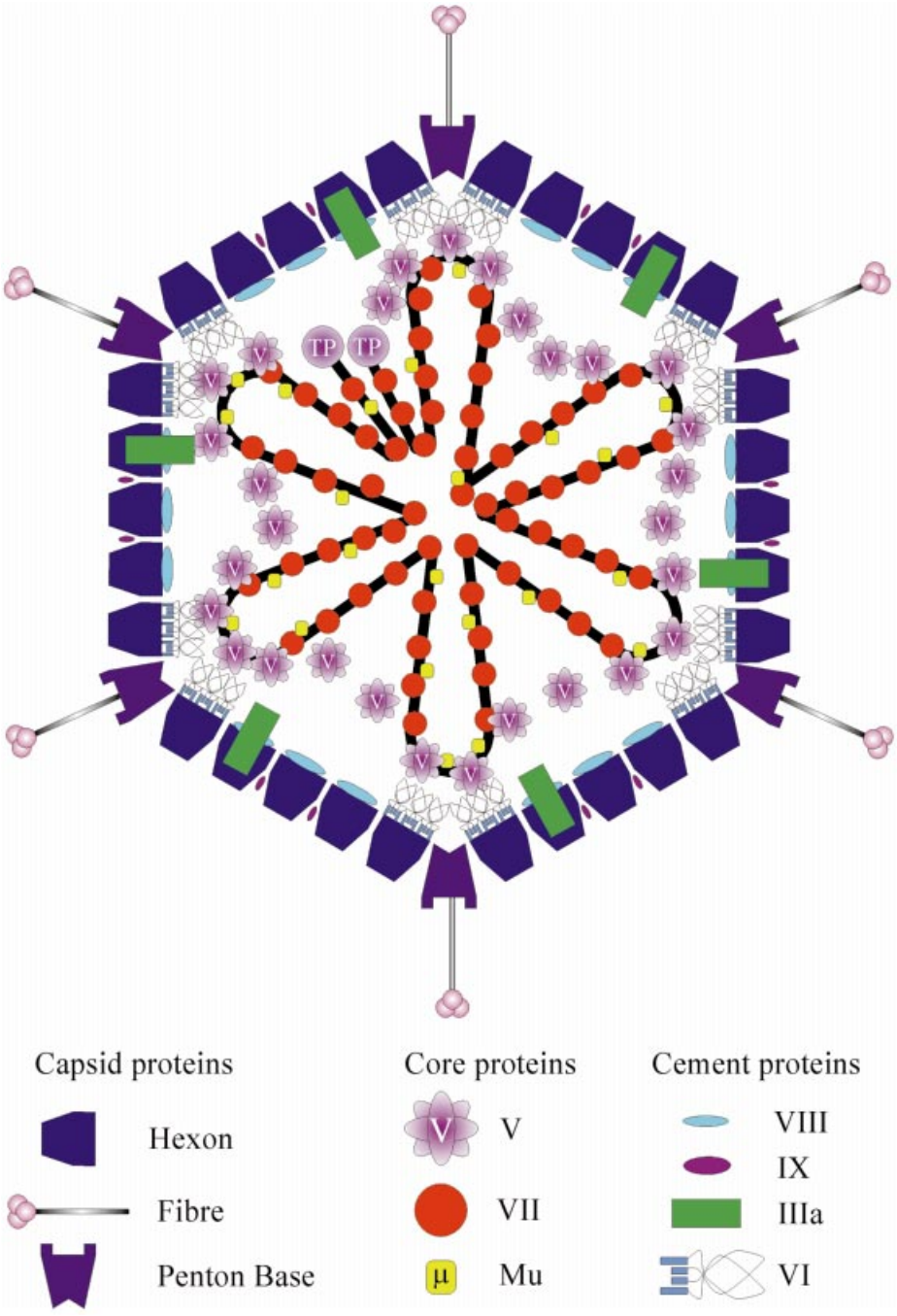

Fig. 1. Structure of adenovirus. The locations of the capsid and cement components are reasonably well defined. In contrast, the disposition of the core components and the virus DNA is largely conjectural.

the fibre; for a review see Chroboczek *et al.* (1995). The prime receptor for the human subgroup C adenoviruses was shown to be identical to that for coxsackie B virus (Bergelson *et al.*, 1997) and has therefore been termed the coxsackie/adenovirus receptor (CAR). This has subsequently been shown to be a plasma membrane protein of 46 kDa belonging to the immunoglobulin superfamily and to contain extracellular, transmembrane and cytoplasmic domains (Tomko *et al.*, 1997), with the extracellular domain being sufficient for attachment (Wang & Bergelson, 1999). A more recent study has indicated that the adenovirus CAR does not completely overlap the coxsackievirus receptor (Tomko *et al.*, 2000). A comprehensive survey of representative members of all the human adenovirus species A to F (Roelvink *et al.*, 1998) suggested that they all

bound to CAR with the exception of members of the subgroup B, which appear to recognize a different receptor (Stevenson *et al.*, 1995). In the same study, it was also noted that adenovirus serotype 41 (Ad41) (of subgroup F) has two fibres of different lengths, and only one of them binds to CAR. Since Ad41 readily infects cells of the gastrointestinal tract, it seems likely that the other fibre will adsorb to a different cell receptor, perhaps displayed on enterocytes. Some cells, such as those of haemopoietic origin, appear to be largely refractory to productive infection by human adenoviruses 2 and 5 and do not display CAR molecules on their plasma membranes (Mentel *et al.*, 1997). This suggests that receptor recognition could be one of the key factors involved in cell tropism. In an attempt to modify cell tropism, fibreless adenoviruses have been constructed. Not surprisingly, these particles showed drastically reduced infectivities and were extremely unstable. The low level of infectivity that could be detected possibly operated by integrin-dependent pathways, which have been demonstrated to operate in some cell systems (Huang *et al.*, 1996; see below). Recent investigations have succeeded in defining the receptor-binding motif on the three-dimensional structure of the fibre head (Kirby *et al.*, 1999; Santis *et al.*, 1999) as well as on the CAR (Kirby *et al.*, 2000). It has also been shown that receptor recognition can be altered by switching fibre heads from other subgroups (Miyazawa *et al.*, 1999). Experiments have also been carried out that have substituted or added other receptor-binding motifs (Hidaka *et al.*, 1999) in the fibre knob; see below. It is interesting to note that some adenovirus serotypes seem to have additional specificities of binding, suggesting that the CAR receptor may be part of a family of receptors (Segerman *et al.*, 2000). Indeed, another receptor, the histocompatibility class I molecule, also a member of the immunoglobulin superfamily, has been shown (Hong *et al.*, 1997) to be available for the subgroup C viruses. Moreover, a recent observation suggests that Ad37, a member of subgroup D, appears to bind to sialoglycoprotein receptors (Arnberg *et al.*, 2000), indicating that receptor specificities are wider than was at first thought.

After the initial interaction of the virus with the fibre receptor, entry of the virus proceeds via clathrin-mediated endocytosis. The critical recognition mechanism for this process is an RGD motif that is exposed on the penton base (Stewart *et al.*, 1997) and interacts with cellular αv integrins (Wickham *et al.*, 1993). There appears to be direct binding of the virus penton base to the integrins in the presence of divalent cations (Mathias *et al.*, 1998). Integrins normally react with the extracellular matrix to facilitate adhesion, differentiation and other cell–cell phenomena (Meredith *et al.*, 1996). They form a large family of heterodimeric receptors and it appears that integrins $\alpha v\beta 3$ and $\alpha v\beta 5$ both support adenovirus internalization. It is noteworthy that integrin $\alpha v\beta 5$ is expressed on human bronchial epithelial cells, a major site of primary adenovirus infection *in vivo* (Mette *et al.*, 1993). Integrins may also play an important part in defining tropism in some situations such as the intestinal epithelium (Croyle *et al.*, 1998*c*) while in others, such as hepatocytes, they have a minimal role (Hautala *et al.*, 1998).

Interaction of the virus with the plasma membrane can induce a number of signalling pathways and there is good evidence for the activation of the phosphoinositide-3-OH kinase (PI-3K) pathway, which in turn triggers the Rho family of GTPases and the polymerization and reorganization of actin to facilitate endocytosis (Li *et al.*, 1998; Rauma *et al.*, 1999). As early as 20 min post-infection, activation of the Raf/mitogen-activated protein kinase (MAPK) pathway and consequential production of IL-8 have been observed (Bruder & Kovesdi, 1997). Since the activation of Raf/MAPK is insensitive to the addition of cycloheximide and is sensitive to prior heating of the virus inoculum at 56 °C, it seems plausible that the initial events at the cell membrane are triggered by a structural component, and this could be via the penton base, since it is heat sensitive (Russell *et al.*, 1967). Triggering these pathways may act as an 'early-warning system' for the induction of defence mechanisms induced in the host (see below).

As noted above, progress of the virus through the endosomes and into the cell cytoplasm is normally mediated by clathrin and the coated pit pathway (Wang *et al.*, 1998). Thereafter, the virus-encoded protease appears to assist in the further disruption of the virus capsid by the proteolysis of the structural protein VI (Greber *et al.*, 1996), which functions as a linker between the capsid and the core components (Matthews & Russell, 1994, 1995). The partially disrupted virus is then transported to the nuclear membrane and the genome is passaged through the nuclear pore and into the nucleus, where the primary transcription events are initiated. The passage through the cytoplasm to the nucleus has been postulated to be mediated by the association of the virus core (the virus DNA and the covalently attached TP, together with the basic proteins VII and V and mu peptide) with a cellular protein, p32 (Matthews & Russell, 1998*b*). The p32 protein is primarily located in the mitochondria but can also be detected in the nucleus, and it has been suggested that it is a component of a cellular transport system that shuttles between the mitochondria and the nucleus and that the virus can hijack this system to gain access to the nucleus. This passage to the nucleus is relatively rapid and also involves the participation of dynein and microtubules (Leopold *et al.*, 2000; Suomalainen *et al.*, 1999). Virus-like particles can be detected at the nuclear membrane by electron microscopy within 1 h of infection (Dales & Chardonnet, 1973) and virus DNA and proteins V and VII can be detected within the nucleus between 1 and 2 h (Greber *et al.*, 1997; Matthews & Russell, 1998*a*). Once inside the nucleus, the genome is targetted to the nuclear matrix (NM), where the TP forms a tight complex with the cellular CAD pyrimidine synthesis enzyme and possibly other NM components (Angeletti & Engler, 1998; Fredman & Engler, 1993). It is interesting that nuclear lamin B, which is a component of the NM, readily binds to p32 (Simos &

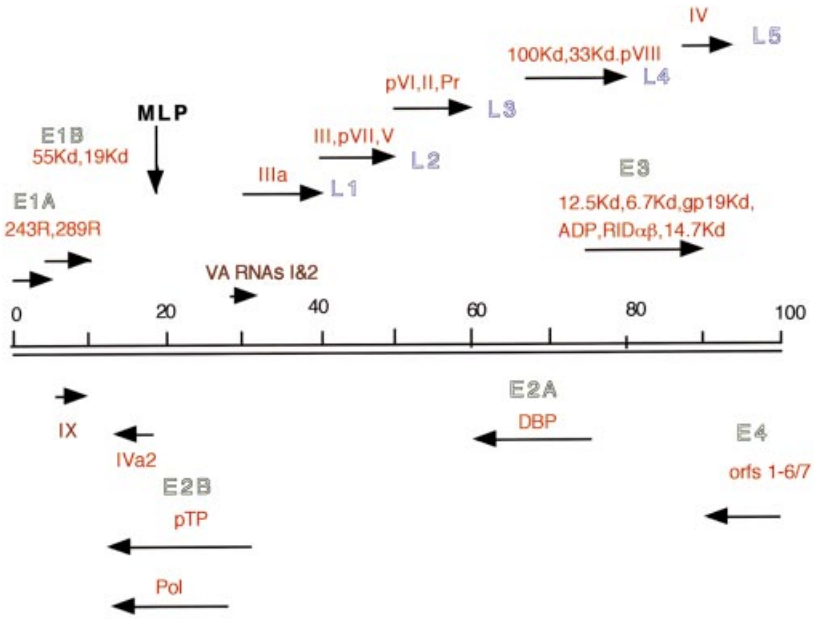

Fig. 2. Transcription of the adenovirus genome. The early transcripts are outlined in green, the late in blue. Arrows indicate the direction of transcription. The gene locations of the VA RNAs are denoted in brown. MLP, Major late promoter.

Georgatos, 1994) and this may allow for the disassociation of p32 from the incoming genome.

Transcription and replication

As noted above, adenovirus transcription can be defined largely as a two-phase event, early and late, respectively occurring before and after virus DNA replication (Fig. 2). Transcription is accompanied by a complex series of splicing events, with four early 'cassettes' of gene transcription termed E1, E2, E3 and E4 (Fig. 2).

The E1 gene products can be subdivided further into E1A and E1B. E1A itself has two major components sharing substantial stretches of sequence that are termed 289R (or 13S) and 243R (or 12S), based on the number of amino acid residues. These E1A proteins are primarily concerned with modulating cellular metabolism to make the cell more susceptible to virus replication. At the risk of being oversimplistic, it is convenient to define cellular metabolism as being devoted principally to promoting accurate cell division while retaining specific cellular functions. In so doing, the cell has devised mechanisms to defend this process from external interference and to remove any defective cells. The former operates by invoking the innate and adaptive immune systems (see below), and some of these pathways appear to be regulated via the transcription factor NF-κB, while the latter is mainly carried out by the induction of apoptosis via a number of routes, one being the transcription factor p53 (for a review of p53 pathways see Prives & Hall, 1999).

NF-κB is a nuclear transactivator that is released by proteolysis of an associated inhibitory factor, IκB, in the cytoplasm (Hay *et al*., 1999*b*), thus leading to its migration to the nucleus and the activation of NF-κB-responsive genes, among the latter being the E3 gene promoter (Deryckere & Burgert, 1996) (see below). Phosphorylation of IκB by a kinase complex, IKK, appears to be crucial for the proteolysis of IκB.

The protein p53 is a tumour suppressor that regulates the transcription of a variety of genes involved in cell cycle arrest and apoptosis. In normal cells, p53 is present in small amounts, but levels increase in response to genotoxic and other stresses. The regulation of p53 seems to mainly at the protein level, utilizing the cellular protein mdm2, which binds to p53 and acts as a ubiquitin ligase, targetting p53 to the proteosome for degradation. Another cellular protein, p19arf, also contributes to this system by binding to mdm2, blocking its ligase activity and thereby stabilizing p53 (de Stanchina *et al*., 1998; Honda & Yasuda, 1999; Tao & Levine, 1999; Weber *et al*., 1999). An additional factor in this regulation has also been uncovered by the finding that p53 can be modified by the small ubiquitin-like modifier (SUMO), leading to activation of p53 (Rodriguez *et al*., 1999).

E1A proteins interfere with the processes of cell division and with the regulation of NF-κB and p53, and do this by a great variety of strategies involving both direct and indirect interaction with cellular proteins. They can also modulate transcription patterns in favour of virus transcription. A summary of the characteristics of E1A is found in Table 1 and Fig. 3. It should be pointed out that many of the properties ascribed to E1A in Table 1 are based on *in vitro* studies, whereas the availability of the relevant cellular components *in vivo* will depend on the nature of the infected cell and its metabolic state. Moreover, other virus gene products can modulate these cellular interactions significantly. For instance,

Table 1. Properties of E1A proteins

Many of the E1A-interacting proteins listed have been mapped to the E1 protein sequences and the locations of those in bold are shown in Fig. 3. It should be noted that no attempt has been made to distinguish between the 243 and 289R formats; they share substantial sequence and differences are more important in the transformation processes. CDK, Cyclin-dependent kinase.

Property	Reference(s)
Bind to p21 and related CDK inhibitors, thereby stimulating cell division and growth	Keblusek *et al.* (1999)
Bind to cyclins A and E–CDK complexes, which regulate passage to cell DNA synthesis	Faha *et al.* (1993)
Bind to the **p300**/CBP family of transactivators, which play a key role in regulating the transcription of many components of the cell cycle. Cellular supply of CBP appears to be self-limiting and sequestration by E1A probably displaces other factors, thereby modulating normal cellular pathways	Arany *et al.* (1995), Jones (1995), Dorsman *et al.* (1997), Lipinski *et al.* (1999)
Binding of E1A also modulates the acetyltransferase activities associated with p300/CBP (**pCAF**), probably by altering nucleosome structure and thereby transcriptional patterns	Reid *et al.* (1998), Chakravarti *et al.* (1999), Hamamori *et al.* (1999), Perissi *et al.* (1999)
Binding of E1A to the p300/CBP family inhibits the activity of the transactivator **STAT-1**, which is required for induction of responses to interferon (see text) and also inhibits the activation of the caspase pathway to apoptosis independent of p53	Look *et al.* (1998), McDonald & Reich (1999), Paulson *et al.* (1999), Putzer *et al.* (2000)
Bind to the **Rb**/p130 family of 'pocket proteins', some of which can function as oncogenes, and in so doing both interfere and promote the activation of a wide range of transcripts	Zantema & van der Eb (2000)
One important factor that is released from Rb on E1A binding is E2F; this is critical for the synthesis of a range of S-phase components as well as the activation of the adenovirus E2 gene cassette. Binding of E1A may also affect the acetyltransferase activities of Rb	Brehm *et al.* (1998)
E1A interacts via a PLDLS motif at its C-terminal region with a protein (**CtBP**) which also binds to Rb at the same motif and then functions as a transcriptional co-repressor	Meloni *et al.* (1999)
E1A activates the promoter of the PCNA gene in HeLa cells. but represses it in rat cells, possibly a factor in the transformation process. This effect appears to be mediated via Rb and involves p53	Kannabiran *et al.* (1999)
Interact with a multiprotein complex **Sur-2**, thereby stimulating the transcription of virus genes	Boyer *et al.* (1999*b*)
Binding to the TATA-box-binding protein (**TBP**) and TBP-associated protein (**TAF**) provides a route to transcriptional regulation	Mazzarelli *et al.* (1997)
E1A proteins can stimulate the induction of apoptosis by a number of routes. An important one is by promoting the synthesis of p53 and maintaining its integrity. Following binding to pRb, release of the transcription factors E2F and ETF can lead directly to transcription of p53 and to the transcription of the p19arf gene	Hale & Braithwaite (1999)
The product of the latter can interact with mdm2 and thus prevent its interaction with p53 and consequential proteolysis via the ubiquitin/SUMO pathway	Lin *et al.* (1994), Honda & Yasuda (1999), Tao & Levine (1999); Weber *et al.* (1999), Rodriguez *et al.* (1999)
The stability of p53 can also be maintained by the interaction of E1A with **Sug1**, a subunit of the proteosome complex that is required for the proteolysis of p53	Grand *et al.* (1999 *a*)
In contrast, it has also been shown that transcriptional activation of p53 can be achieved by binding of CBP to a TRAM motif, which, at the same time, prevents the binding of mdm2 and therefore stabilizes p53. The sequestration of CBP by E1A eliminates this interaction	Somasundaram & El-Deiry (1997), O'Connor *et al.* (1999)
Another route to activation of apoptosis is by sensitization of cells to stimuli such as ionizing radiation, DNA damage, TNF and Fas ligand. This is mediated by inhibiting the IκB kinases, which are critical for the release of NF-κB to the nucleus, and requires the binding of E1A to p300/CBP	Shisler *et al.* (1996), Shao *et al.* (1997, 1999)
E1A can also bind to **UBC9**, a protein involved in the SUMO enzymatic pathway. Binding to E1A may interfere with the SUMO modification (and stabilization) of cellular proteins such as p53	Desterro *et al.* (1999), Hateboer *et al.* (1996), Rodriguez *et al.* (1999)

the E4 gene products can co-operate with E1A in a variety of ways (Goodrum & Ornelles, 1999; Hall *et al.*, 1998; Yun *et al.*, 1999). The E1B gene product 19K also seems to function co-operatively with E1A and p53 in promoting oncogenesis and transformation (Kannabiran *et al.*, 1999), mainly by ensuring that the downstream consequences of cell cycle release do not induce apoptosis.

The E1B 19K gene product is analogous to that from the cellular Bcl-2 gene. This gene product is concerned with prolonging cell survival by interacting and ablating members of the *Bax* family (whose transcription can be promoted by p53), which induce apoptosis and necrosis (Han *et al.*, 1996) (see below). A number of studies have examined the interaction of the other E1B gene product, 55K, with p53. This interaction

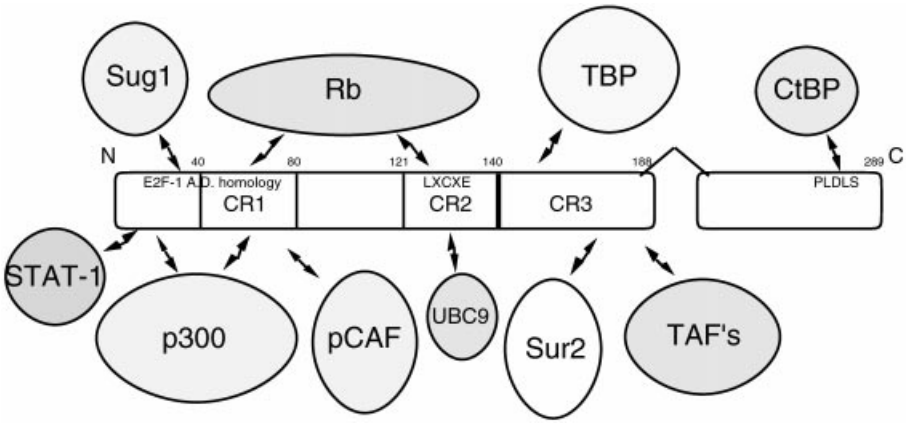

Fig. 3. Binding map of proteins to E1A. The locations have been determined on the basis of *in vitro* studies using deletion and mutational analysis. The abbreviations are explained in Table 1. CR1, CR2 and CR3 are constant regions present in a wide range of adenoviruses. PLDLS and LXCXE etc. are recognition motifs. E2F-1 A.D. homology refers to sequences that can displace E2F-1 from Rb and p300.

appears to be direct and involves the co-localization of the complex to the cytoplasm (Grand *et al*., 1999*b*). Interest in this interaction was stimulated by the development of an E1B-deleted vector that was claimed to act as an oncolytic virus to target tumours with defective p53 genes (Bischoff *et al*., 1996). Further investigations have shown that, while the interaction reduces the transcription of p53, it also seems to be necessary for virus replication (Ridgway *et al*., 1997) and for transport of viral RNAs (Horridge & Leppard, 1998). However, the dependence on intact or wild-type p53 is not essential (Harada & Berk, 1999; Vollmer *et al*., 1999) and, more significantly, the selectivity for replication in defective p53 cells was not apparent when a wide range of cells was tested (Hay *et al*., 1999*a*). The involvement of an E4 gene product, E4orf6 (see below), in the interaction of E1B 55K with p53 also influences the stability of p53. In addition, E1B 55k has an effect on late virus mRNA transcription (Harada & Berk, 1999) and functions as an important effector of inflammation *in vivo* (Ginsberg *et al*., 1999).

The E2 gene products are subdivided into E2A (DBP) and E2B (pTP and Pol). These provide the machinery for replication of virus DNA (Hay *et al*., 1995) and the ensuing transcription of late genes, and this is mediated by interaction with a number of cellular factors.

The E3 genes, which are dispensable for the replication of virus in tissue culture, provide a compendium of proteins that subverts the host defence mechanisms (see below) and their properties are summarized in Fig. 4(A). One of these E3 gene products has been termed the adenovirus death protein (ADP), since it facilitates late cytolysis of the infected cell and thereby releases progeny virus more efficiently (Tollefson *et al*., 1996). The E3 gp19K is localized in the ER membrane and binds the MHC class I heavy chain and prevents transport to the cell surface, where it would be recognized by CTLs. This gene product, in addition, delays the expression of MHC I (Bennett *et al*., 1999). The E3 proteins RIDα&β and 14.7k inhibit proapoptotic pathways (see below). A recent review on these proteins can be consulted for further information on the molecular mechanisms involved (Wold & Chinnadurai, 2000).

The gene products derived from the E4 cassette (termed orfs 1–6/7) mainly facilitate virus messenger RNA metabolism (sometimes in association with E1B gene products) (Goodrum & Ornelles, 1999; Weigel & Dobbelstein, 2000) and provide functions to promote virus DNA replication and shut-off of host protein synthesis (Halbert *et al*., 1985) (see Fig. 4B). They are also associated with resistance to lysis by CTLs (Kaplan *et al*., 1999). Recently, some of the E4 products have been shown to bind to DNA-activated protein kinase, thereby preventing viral DNA concatenation. Since the same kinase is needed for activation of the p53 gene following DNA damage, it has been suggested that such a characteristic could block one of the pathways to apoptosis (Boyer *et al*., 1999*a*; Weiden & Ginsberg, 1994). It is significant that many of the characteristics of both the E1B and E4 gene products are related to counteracting the activities of those encoded by E1A, thus E4 orf4 seems to inhibit the E1A activation of the E2F promoter (Mannervik *et al*., 1999). E4 orf6 has a direct effect on the transactivation of p53 (Boivin *et al*., 1999) by interacting with E1B 55k when it binds to p53, thereby releasing it to be degraded (Boyer & Ketner, 2000). E4 orf3 also appears to relieve the E1B inactivation of p53 independently (Konig *et al*., 1999) and to play a role in relocating sites of replication and transcription in the nucleus (pods) (Doucas *et al*., 1996; Leppard & Everett, 1999). Recent investigations have also shown that p53 can interfere with the binding of the E1A-regulated transcription factor p120E4F, thereby repressing the activities of the E4 promoter (Sandy *et al*., 2000). A review of the E4 gene products can be consulted for further details (Leppard, 1997).

Adenoviruses also transcribe a set of RNAs (see Fig. 2) that are not translated, termed the VA RNAs, and these play a role in combating cellular defence mechanisms (see below).

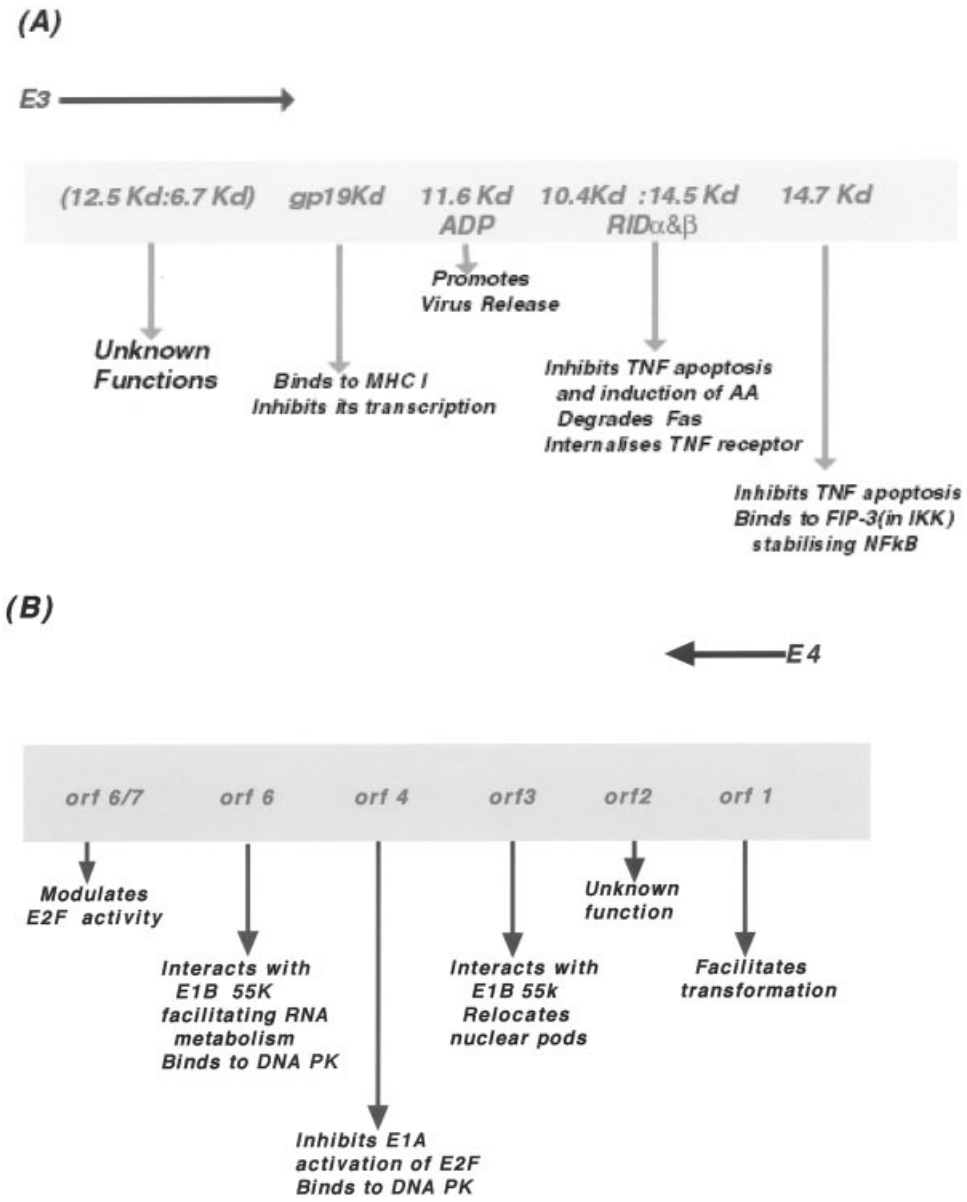

Fig. 4. Characteristics of E3 and E4 gene products. Horizontal arrows indicate the relative directions of transcription.

Other characteristics of these early gene products are described below, and a cartoon depicting the effects of some of them on a few cellular pathways is provided in Fig. 5. DNA replication begins from both DNA termini and requires sequences within the ITRs as origins of replication (Hay *et al.*, 1995). Thereafter, late transcription ensues, with five cassettes of transcripts (termed L1 to L5) resulting from a complex series of splicing events. These lead to the production of the virus structural components and the encapsidation and maturation of virus particles in the nucleus. A key player in the control of transcription is the major late promoter (MLP), which is attenuated during transcription of the early genes. However, it should be noted that there is a low basal level of late transcription occurring early in infection, even before the MLP comes into play. After the onset of virus DNA replication, the IVa2 and IX genes are expressed at high levels (see Fig. 2) and transcription via the MLP is fully functional by specific activation. This is accomplished via the IX and IVa2 gene products (Lutz & Kedinger, 1996; Lutz *et al.*, 1997) and is also influenced by effective competition for the limiting transcription factors (Fessler & Young, 1998). The encapsidation process is governed by the presence in the virus DNA of a packaging signal at the conventional left end, which consists of a series of AT-rich sequences (Hearing *et al.*, 1987). These events are accompanied by major changes in the nuclear infrastructure and the permeabilization of the nuclear membrane (Rao *et al.*, 1996; Tollefson *et al.*, 1996). This facilitates the egress of the virus into the cytoplasm and is followed by

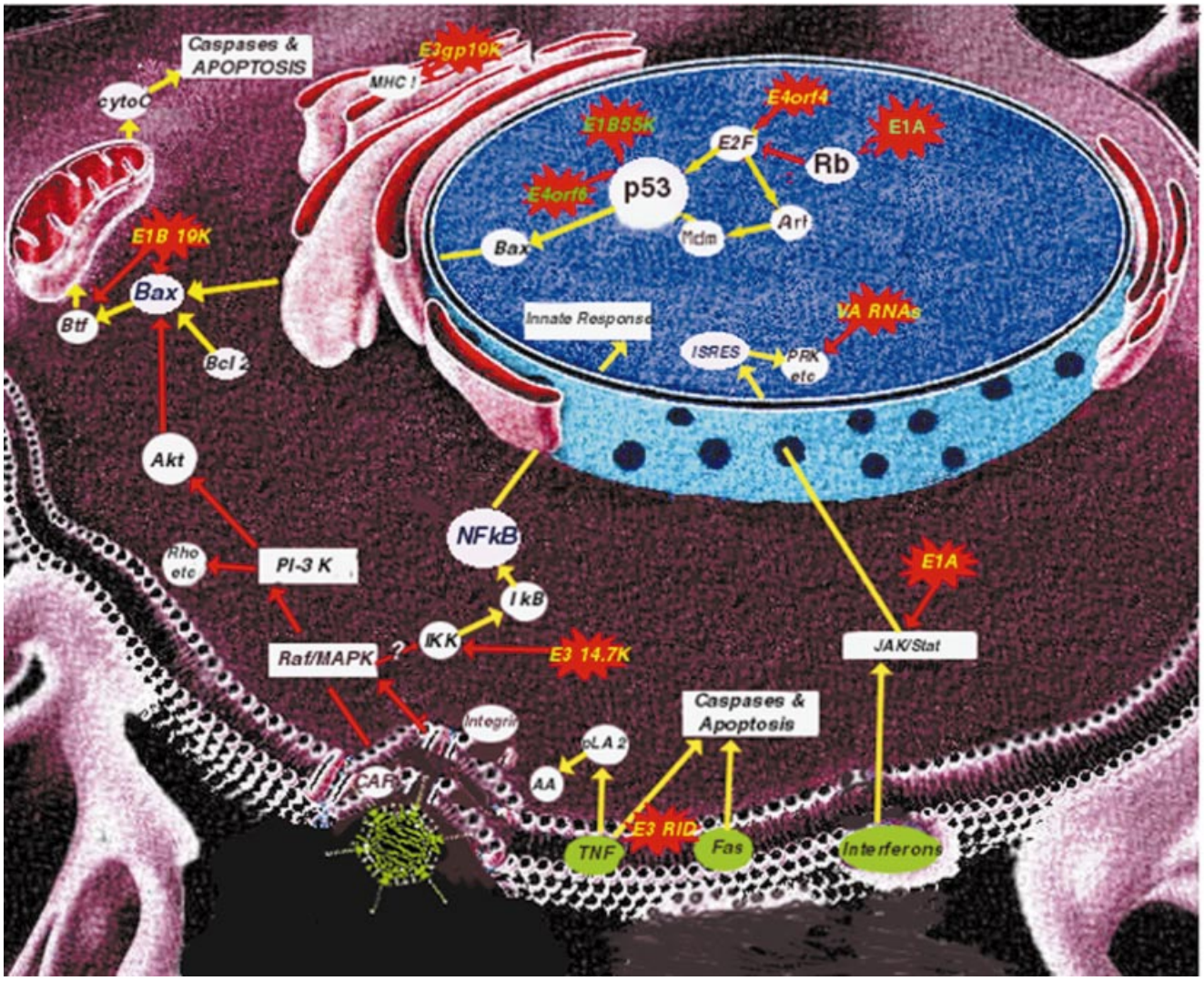

Fig. 5. A cartoon (not to scale) illustrating some of the sites of action of the virus and virus gene products (in red) on a few of the cellular pathways (in yellow). A virus particle at the receptor site is in green.

the disintegration of the plasma membrane and the release of virus from the cell.

Defence mechanisms

Adenoviruses induce only very low levels of morbidity in general and this characteristic initially seemed to make them attractive as gene vectors. However, on closer scrutiny, it becomes evident that a well-orchestrated host defence is the key factor in the suppression of virus spread during infection and, since this could be just as effective against a vector, it becomes important to unravel the complex molecular operations involved in mounting the host defences.

Host cells have a range of strategies to combat any incursion by an intruder; these can be considered as innate and adaptive. With respect to the former, it has recently been established that some epithelial cells release 3–4 kDa antimicrobial peptides termed defensins (Ganz & Lehrer, 1998) and it has been shown that these compounds can provide significant protection from adenovirus infection (Gropp *et al.*, 1999). Indeed, an adenovirus vector expressing a defensin has been utilized to supplement innate defences (Bals *et al.*, 1999). Some tissues, on receiving the appropriate signal (perhaps via the Raf/MAPK pathway; see above), will release multiple chemokines that, in turn, recruit neutrophils and invoke an inflammatory response (Charles *et al.*, 1999; Muruve *et al.*, 1999). Innate defence mechanisms such as recruitment of macrophages, activation of complement and natural killer (NK) cells have been shown to play a significant role in clearing an adenovirus infection *in vivo* (Worgall *et al.*, 1997*a*, 1999). The transcription factor NF-κB appears to be a key regulator of the innate antiviral response (Ferreira *et al.*, 1999), since it can activate the transcription of cytokines and adhesion molecules,

leading to the production of a range of proinflammatory cytokines and the orchestration of other signalling pathways. It has been claimed that adenovirus infection, especially at high multiplicities, can lead to the activation of NF-κB at early stages of infection (Clesham *et al*., 1998; Lieber *et al*., 1998). One mechanism for achieving this could be by the binding of E1A to the p65 subunit of NF-κB, although it is also apparent that this activation can be suppressed by E1B 19K (Pahl *et al*., 1996; Schmitz *et al*., 1996). Reference has been made above to the very early induction, possibly by the interaction of the penton base in the virus with cellular integrins, of the Raf/MAPK and other pathways. These may play a role in the activation of NF-κB (Ghoda *et al*., 1997) as well as in the early release of chemokines (Kuhnel *et al*., 2000; Muruve *et al*., 1999) and interferons, which are important components of the innate response to infection. However, the induction of these endogenous genes following infection appears to be quite cell dependent, with human endothelial cells displaying a range of signalling molecules at 24 h, a scenario not seen in human dermal fibroblasts or alveolar macrophages (Ramalingam *et al*., 1999).

Interferons

The interferons are subdivided into two main classes, type I (containing interferons α and β) and type II (interferon γ).They are cellular proteins ranging in size from 15 to 35 kDa and are released from cells very early after infection by viruses and as a result of other insults to the cell and display a fair degree of cell specificity. In the case of adenoviruses, induction seems to be by interaction with a structural component, since they can be produced by virus particles in the absence of protein synthesis (Reich *et al*., 1988). The interferons function by binding to cell receptors, thereby activating the cellular Jak/STAT pathways, which lead to STAT complexes being transferred to the nucleus and binding to interferon-response elements (ISREs) on the cellular DNA. The ISREs regulate the transcription of a range of gene products, such as a dsRNA-induced protein kinase (PRK) and a 2′–5′ oligoadenylate synthetase as well as a variety of immunomodulators. These form an impressive array of weapons to combat the intracellular activities of the invading virus; for a recent review see Goodbourn *et al*. (2000). Adenoviruses are generally refractive to interferons, since they have provided themselves with a number of strategies to overcome this assault on their activities. Thus, gene products from E1A downregulate the STAT activators (Look *et al*., 1998; McDonald & Reich, 1999; Paulson *et al*., 1999; Leonard & Sen, 1996, 1997).

It has also been claimed (Feigenblum *et al*., 1998) that adenoviruses induce an interferon-regulatory factor (IRF) at later stages of infection that plays a role in cytopathogenicity. In addition, the VA RNAs (Mathews & Shenk, 1991) bind to and inactivate PRK.

Apoptosis

As another means of combating virus infection, the cell can redirect its metabolism to switch on its apoptosis circuits. Cells have complex mechanisms for ensuring that their integrity is not compromised and they have devised a fall-back strategy to switch on proapoptotic proteins when specific alarm pathways are activated (see Fig. 5). Chief among these is the tumour suppressor p53, which regulates the transcription of genes involved in cell cycle arrest and apoptosis. Among the latter are members of the *Bax* family (Pearson *et al*., 2000), which interact with mitochondria and are involved in the induction of caspases, leading to apoptosis. There are other members of this family, such as Bcl-2, which function to inhibit apoptosis and they carry this out by binding to *Btf*, an important transcriptional repressor. *Btf* promotes cell death (Kasof *et al*., 1999) by inducing the permeabilization of mitochondrial membranes (Imazu *et al*., 1999) and releasing cytochrome *c*, thereby initiating the caspase cascade. Adenoviruses can subvert the operation of this pathway by utilizing virus gene products from its E1 cassette; thus, E1B 19K can inactivate *Bax* (Han *et al*., 1996; Ohi *et al*., 1999) and has a similar function to Bcl-2 in binding to *Btf*, thus counteracting the proapoptotic response of the E1A gene product in activating p53. A parallel mechanism of apoptosis is mediated by TNFα, which is secreted by monocytes and lymphocytes following activation as part of the innate response. This cytokine appears to play a significant part in the elimination of adenovirus vectors (Elkon *et al*., 1997) and functions by activating cytosolic phospholipase A2 (pL A2), which permeabilizes cell membranes, releasing arachidonic acid (Wolf & Laster, 1999) and initiating the production of prostaglandins and leukotrienes, which also play a role in inflammation (Krajcsi *et al*., 1996). This pathway is normally modulated by Bcl-2 and its virus analogue, E1B 19K (see above), via downregulation of IκB transcription, thus releasing NF-κB to the nucleus (de Moissac *et al*., 1999). In this regard, it is intriguing to note that the ability of p53 to induce apoptosis requires the participation of NF-κB (Ryan *et al*., 2000), implying a degree of co-operative 'cross-talk'. It is important, however, to note that many of these effects seem to be cell specific: thus, in endothelial cells, Bcl-2 serves to protect the cells from both apoptosis and proinflammatory responses (Badrichani *et al*., 1999). Another route of TNFα action is by the direct induction of caspases (Kimura & Gelmann, 2000). In contrast, in oligodendrocytes, apoptosis by TNFα appears to be mediated by p53 and involves initiating the JNK signalling pathway (Ladiwala *et al*., 1999). TNF-induced apoptosis can be ablated by E3 gene products (Lukashok *et al*., 2000) (see Fig. 5). Other key players in apoptosis are Fas and Fas ligand interactions, and these have been shown to be the major mediators of the elimination of adenovirus vectors from the liver (Chirmule *et al*., 1999). In this case, the E3 gene products RIDα and RIDβ cause Fas to be removed from the cell surface and degraded (Tollefson *et al*., 1998). A cellular protein termed

FIP-3 (Li *et al.*, 1999*b*) has also been implicated in these proapoptotic events. FIP-3 appears to be a scaffolding component of the IKK complex (Ye *et al.*, 2000) and blocks the release of NF-κB by inhibiting the kinase activity of IKK (Fig. 5) (Li *et al.*, 1999*b*). Adenoviruses modulate these events via the E3 gene product 14.7K, which binds to FIP-3 (Li *et al.*, 1999 *b*) and effectively restores NF-κB transcription and thereby cell survival. Many of these apoptotic mechanisms involve the activation of a range of proteases, such as caspases, and it has been demonstrated that inhibition of the related ICE-like proteases can boost adenovirus yields (Chiou & White, 1998). A recent investigation has also shown that E1A can induce apoptosis by activation of caspase-8 and is independent of the status of p53 (Putzer *et al.*, 2000). Interferons can also act proapoptotically by inducing caspase-8 (Balachandran *et al.*, 2000), and this can be amplified in infected cells via the dsRNA route (Tanaka *et al.*, 1998). In this case, however, the inhibition of interferon induction by E1A suppresses this apoptotic response. From the above discussion it will be seen that there is a complex interplay of cellular and virus components seeking to control cell survival and promotion of virus replication and spread. Thus, in utilizing adenoviruses as vectors, it is critical to take these factors into account in devising the optimum conditions for delivery and effective expression of the transgene.

Cellular immune responses

T cells provide an effective defence via both $CD8^+$ cytotoxic cells (CTLs) and $CD4^+$ helper cells. CTLs function by recognizing a virus antigen in a complex with class I proteins of the MHC on the cell surface. This event releases perforin, resulting in cell lysis, thereby eliminating the infected cells even at an early stage before any virus is assembled and released. The recognition mechanism depends on a virus antigen being available to complex successfully within the ER membrane with an MHC component and then being transported to the plasma membrane. The complex formation is a function of the nature of the cell being infected, as well as the MHC status of the host. It also appears that different virus gene products can provide the target depending on their ability to interact with a particular MHC. It is significant, however, that there appears to be some cross-reactivity of human CTLs in recognizing different adenovirus subgroups (Smith *et al.*, 1998).

Adenoviruses can combat this cellular strategy as described above by utilizing E3 gp19K to retain the MHC antigens in the ER and hence disrupt the recognition process (Kvist *et al.*, 1978). E4 gene products have also been demonstrated to function in the inhibition of T cell cytolysis (Kaplan *et al.*, 1999).

The CD4 helper cells are important in mounting a proliferative response to infection. This is mediated in a similar fashion by recognition of a virus target antigen in association with class II MHC. These helper T cells can thereby stimulate proliferation of B cells to provide immunoglobulins for the humoral response (see below). Very few attempts have been made to examine the adenovirus antigens involved in the initiation of the proliferative response. A study on the lymphoid cells from one individual suggested that either the fibre or IIIa structural polypeptides could be targets (Souberbielle & Russell, 1995). However, a more general investigation noted that proliferative responses to the uncommon Ad35 occurred in individuals without any serological evidence of previous Ad35 infection (Flomenberg *et al.*, 1995), implying that $CD4^+$ T cells recognized a conserved antigen. This suggests that this arm of the immune system may play a role in modulating infection with a wide range of serotypes.

The humoral response

The humoral response is a major component of the defence strategy of the host and depends on the ability of B cells, elaborating surface immunoglobulins, to recognize a specific epitope on a foreign antigen. This recognition initiates a massive proliferation via T helper cells and thus the release of specific immunoglobulins of various classes into plasma to interact directly with these antigens. Where these are important in the initial interaction with the host cell, virus infection can be neutralized very efficiently. Given the importance of the fibre and penton base in the recognition of the receptors (see above), it is not surprising that adenovirus-neutralizing antibodies are directed against epitopes on these capsid components (Gahery-Segard *et al.*, 1997, 1998; Willcox & Mautner, 1976). However, there are also antigens on the hexon that induce neutralizing antibodies, and these seem to function by aggregating virus particles and thereby inhibiting adsorption. The efficacy of the humoral response in the case of adenovirus gene therapy is very important and depends on the nature of pre-existing immunity as well as the route and target of infection (Harvey *et al.*, 1999).

For humans, there are 51 different adenovirus serotypes, classified on the basis of their specific neutralizing abilities, and protection by humoral antibodies is therefore tightly restricted to a given serotype. Further subdivision into species or subgenera A to F has also been made, using a variety of criteria (Benkő *et al.*, 1999). Type-specific antigens have been described in the fibre that are associated with the trimeric knob and the proximal regions of the stem (Watson *et al.*, 1988; and W. C. Russell, unpublished data). In the case of the hexon, the type-specific epitopes reside, not surprisingly, on the hexon surface, whereas the internal antigens are conserved, being critical in the formation of the capsid structure, and therefore have a very much wider 'group' specificity. Group-specific hexon antibodies have been used extensively as general adenovirus diagnostic reagents.

Adenoviruses as vectors

Adenoviruses can infect a wide variety of cell types and

tissues in both dividing and non-dividing cells. This characteristic, together with their relative ease of preparation and purification, has led to their extensive use as gene vectors.

The virus can incorporate only about 2 kb of foreign DNA without significant affects on its stability or its infectivity, and the introduction of longer sequences therefore requires the removal of some or all of the virus genes. There are a range of techniques for constructing recombinant adenoviruses, and these are described in detail elsewhere (Hitt *et al.*, 1997; Tashiro *et al.*, 1999; Zhang, 1999).

Vectors can be utilized for: (i) cancer therapy to deliver genes that will lead to tumour suppression and elimination; (ii) gene therapy, i.e. to deliver genes to tissues to augment defective genes; (iii) supplementary therapy to deliver genes, expression of which will combat disease processes.

First-generation vectors

In the first generation of vectors, the E1 and/or E3 gene cassettes were removed, allowing the introduction of up to 6·5 kb of foreign DNA, often under the control of a heterologous promoter. In the case of the E1 deletions, care was taken to ensure the retention of the ITR and the packaging sequences. Removal of the E1 region had the additional apparent advantage of impairing the transcription of the E2 genes (which are E1 dependent) and consequently the replication of virus DNA and the production of the virus capsid proteins. However, it will be evident from the description of the E1 genes given above that there is also the disadvantage of the cellular environment being much less conducive to vector transcription. The defective E1 viruses could be propagated by infection of 293 cells (Graham *et al.*, 1977), which provide the E1 gene products *in trans*. Although many of the initial studies *in vitro* provided much promise, it soon became evident that the expression of the transgene *in vivo* was only transient and was depressed because of the overwhelming immune response, mounted mainly against the virus capsid antigens as well as the expressed transgene. One of the reasons for this was the observation that many cells harboured E1-like proteins that allowed the E2 genes to function, albeit at reduced levels. In turn, this facilitated virus DNA replication and the synthesis of the late structural antigens and the production of replication-competent adenovirus (RCA). It also became evident that, at higher m.o.i., the E1 dependence of E2 gene transcription could be ablated. Bearing in mind these problems, a number of strategies have been adopted in an attempt to minimize the production of RCA (Hehir *et al.*, 1996; Gao *et al.*, 2000). Furthermore, as described above, removal of the E1B products also effectively disarmed one of the mechanisms for combating proapoptotic defences. In the case of the E3-deleted vectors, there were similar sequelae as a result of the elimination of the E3 gene-mediated defences against host responses (Poller *et al.*, 1996).

Second- and third-generation vectors

The next approach was to construct vectors (using suitable complementing cell lines) with some or all of the E2 genes excised (Lusky *et al.*, 1998; Moorhead *et al.*, 1999) and hence with the capacity to replicate virus DNA and to produce RCAs removed. Generation of RCAs could also be prevented by constructing cell lines that do not contain adenovirus sequences that overlap those in the vector (Fallaux *et al.*, 1998, 1999). Nevertheless, the host immune response was still a major impediment to achieving persistent transgene expression and was particularly evident when repeated infections were attempted. A number of studies confirmed that the infecting recombinant virus itself was sufficient to induce the immune response, perhaps not surprising in view of the early activation of signalling cascades noted above and the potent antigenicity of the capsid components.

Other, rather more sophisticated vectors (third generation) have been constructed by deleting other virus genes (Amalfitano *et al.*, 1998) and the latest of these have all or nearly all of the virus genes removed. These so-called 'gutless' vectors (Hardy *et al.*, 1997; Kumar-Singh & Chamberlain, 1996; Lieber *et al.*, 1999; Morsy *et al.*, 1998; Steinwaerder *et al.*, 1999) originally retained only the ITR and packaging sequences and required helper virus and appropriate complementing cells for propagation, followed by careful purification. Nevertheless, there were problems associated with these techniques, mainly due to contaminating helper virus and vector instability. A further development, which prevented the packaging of the helper virus, involved the use of the *Cre-lox* helper-dependent system (Chen *et al.*, 1996; Hartigan-O'Connor *et al.*, 1999; Ng *et al.*, 1999; Parks *et al.*, 1996; Tashiro *et al.*, 1999).

Other methods to simplify and improve the construction of vectors have been described (He *et al.*, 1998; Mizuguchi & Kay, 1998). A more comprehensive review (Hitt *et al.*, 1997) provides details of most of the different techniques available for construction of vectors. One factor in fabricating these vectors is the need to maintain the vector size for efficient DNA packaging (Parks & Graham, 1997). This has been achieved by using 'stuffer' DNA, although the nature of this stuffer segment has been shown to influence transgene expression (Parks *et al.*, 1999*b*). These latest vectors have increased expression dramatically *in vivo* (Morral *et al.*, 1999; Morsy *et al.*, 1998; Ji *et al.*, 1999). However, it has become clear that the retention of some of the E4 genes is important in combating the T cell response (Kaplan *et al.*, 1999; Lusky *et al.*, 1999; Yew *et al.*, 1999) and more recent vectors have been modified accordingly (Gorziglia *et al.*, 1999).

An extension of this approach involves the formation of hybrid vectors with adeno-associated virus (AAV) ITRs, which facilitate transgene integration (Lieber *et al.*, 1999; Recchia *et al.*, 1999). A similar strategy has been developed recently by using the long terminal repeats of Maloney leukaemia virus

(Zheng *et al.*, 2000) and has shown promise both *in vitro* and *in vivo* in a model system in facilitating transgene persistence. Hybrids with other viruses such as Epstein–Barr virus and retroviruses have also been developed (Caplen *et al.*, 1999; Tan *et al.*, 1999). Adenoviruses derived from other species (avian, ovine, bovine, canine) have been investigated as vectors for human gene therapy, since they do not normally invoke endemic humoral immunity (Hofmann *et al.*, 1999; Kremer *et al.*, 2000; Michou *et al.*, 1999; Reddy *et al.*, 1999; Zakhartchouk *et al.*, 1998). Animal adenovirus vectors have also been used for animal vaccination (Hammond *et al.*, 2000; Rasmussen *et al.*, 1999).

Strategies for ensuring effective vectors

The effectiveness of gene therapy is governed in the main by the ability of the vector to be delivered to the relevant tissue and, once there, to express the gene product in appropriate quantities. This, of course, is exactly what the virus has sought to achieve in the course of evolution, and has been demonstrably successful in that adenoviruses are very prevalent without causing excessive morbidity. This seems to have been accomplished by utilizing virus gene products to delay the early innate and immune host defences, thus ensuring that the primary infection produces large amounts of virus. Propagation to other host cells can then be accomplished before the infected host's full immunological armoury is deployed successfully. Whether adenoviruses are able to adopt other strategies to ensure their continuance, such as persistence or latency in the presence of an immune response, has never been adequately demonstrated, although adenovirus sequences can be detected in a proportion of the lungs from healthy individuals (Eissa *et al.*, 1994; Elliott *et al.*, 1995) as well as patients with pulmonary disease (Keicho *et al.*, 1999) (for reviews see Lukashok *et al.*, 2000; Mahr & Gooding, 1999). However, the facts that adenovirus immunity appears to be long lasting and that antibodies to the common serotypes 2 and 5 can be detected in almost 90% of individuals do suggest that persistence may be a factor in virus survival. This latter characteristic is obviously a desirable feature of an effective vector in some applications, but to achieve such an outcome in the tissue of choice will require a much greater understanding of the total spectrum of molecular mechanisms that operate in infection and of the resulting immunity.

Minimizing immune and apoptotic responses. In view of the importance of the immune response in relation to transgene persistence, a number of studies have been carried out to unravel the role of the different arms of the immunological repertoire. Most of them have been carried out in model rodent systems, but a few have involved human subjects.

Humoral responses can be mounted, as noted above, with a single immunization (Juillard *et al.*, 1995), but this can be modified to some extent by ensuring the retention of the E3 gene cassette in the vector as well as by treatment of the host with anti-CD4 reagents (Poller *et al.*, 1996). This latter protocol reduced the population of T helper cells, which are needed for the activation of B cells and for the production of neutralizing antibodies. Another study implicated factors other than the capsid antigens in modulating the humoral response. It was shown that deletion of the E4 gene cassette diminished Th2 and B cell activities and it was postulated that an E4 gene product facilitated antigen presentation and the production of IL-6 and IL-8, which are important in B cell maturation (Armentano *et al.*, 1997). Not surprisingly, a number of studies demonstrated that the administration of immunosuppressive agents such as cyclosporin, cyclophosphamide (Smith *et al.*, 1996), FK506 (Ilan *et al.*, 1997), deoxyspergualin (Kaplan & Smith, 1997) and CTLa4 Ig (Jooss *et al.*, 1998*b*) enhanced the persistence of the transgene product. Induction of tolerance has also been shown to be successful in some cases, leading to significant transgene persistence (Ilan *et al.*, 1996, 1998; Lee *et al.*, 1999*b*). Another route to immunosuppression was brought into play by the co-administration of an adenovirus vector with another that had a transgene expressing soluble CD8 or CD8 fused to the extracellular regions of a TNFα receptor (Peng *et al.*, 1999). This procedure successfully inhibited the action of TNFα and significantly reduced the humoral antibody responses to both adenovirus and the transgene product. Another, more direct approach to minimizing antibody neutralization was achieved by covalently linking polyethylene glycol (O'Riordan *et al.*, 1999) or a hydrophilic polymer based on *N*-(2-hydroxypropyl)methacrylamide (HPMA) to the capsid components of the virus (Fisher *et al.*, 2000). This latter procedure also allowed retargetting of the vector. It should be noted, however, that the antibody response to the administration of a virus vector is influenced significantly by the pre-existing antibody status and by the route of administration (Harvey *et al.*, 1999). Some improvement in transgene persistence can be achieved by repeated administration with vectors of different serotypes (Parks *et al.*, 1999*a*), although this has its limitations in view of the T cell cross-reactivities described above. Nevertheless, in spite of this plethora of techniques available to minimize the humoral antibody response, there is no doubt that the inability to ablate the response effectively remains a major impediment to exploitation of vectors (Benihoud *et al.*, 1999).

In terms of T cells, a number of investigations have indicated that adenovirus gene delivery can elicit a complex panoply of cellular immune responses. $CD4^+$ and $CD8^+$ T cells specific for the transgene product as well as the vector can all be elaborated with variations dependent on the route of administration, the target organ and other factors such as the host genotype (van Ginkel *et al.*, 1997) and development status (Kass-Eisler *et al.*, 1994). In addition, for delivery to the lung, innate immune mechanisms involving the migration of alveolar macrophages seem to be very important (Worgall *et al.*, 1997*b*).

Apoptosis can also play an important part in minimizing

transgene expression, and this can be combated to a significant extent by using vectors that express Bcl-2 both with and without NF-κB inhibitors (Bilbao *et al.*, 1999*a*, *b*; de Moissac *et al.*, 1999; Lieber *et al.*, 1998), the expression of the inhibitors encouraging greater transgene persistence in mouse livers. The role of the E3 14.7K protein in attenuating inflammation was shown neatly by constructing transgenic mice in which this gene was expressed selectively by using a human SP-C promoter. There was a significant difference in lung inflammation and prolonged transgene expression when an E1/E3-deleted vector was administered (Harrod *et al.*, 1998). A systematic investigation of the target proteins for CTLs and their histocompatibility restriction was undertaken in a murine model of liver gene therapy and revealed that the levels of CTL responses to adenovirus antigens and to the transgene product were varied and very dependent on the MHC haplotype of the host. A range of adenovirus antigens were examined in this system (pTP, Pol, DBP, hexon, penton and fibre) and the structural proteins, especially hexon, appeared to be the major targets (Jooss *et al.*, 1998*a*). Another survey examined both apoptosis and antibody formation in different strains of mice as a result of infection with an adenovirus vector and also concluded that there were differing responses depending on the mouse strain (Schowalter *et al.*, 1999). An interesting study with nude mice, where the immune system was ablated, also concluded that the persistence of a transgene in mouse lung depended on the nature of the vector backbone and on the host background (Kaplan *et al.*, 1997). These results suggest that the efficacy of therapy with adenovirus vectors will exhibit considerable heterogeneity in human populations.

Factors that affect delivery of transgenes. The primary cellular receptors for adenoviruses appear to be distributed so widely in cells that effective and specific delivery to target cells would normally be precluded. On the other hand, a number of tissues and cells express very little, if any, of these receptors (Leon *et al.*, 1998). Thus, the apical surfaces of ciliated airway epithelia, so important in dealing with treatment of cystic fibrosis, do not appear to have CAR available (Walters *et al.*, 1999; Zabner *et al.*, 1997), and the same is true of some primary tumours (Li *et al.*, 1999*c*; Miller *et al.*, 1998). To permit targetted gene delivery, therefore, novel strategies need to be developed and a number of vectors have been constructed in an attempt to do this. Some of these have bispecific conjugates that can ablate the normal receptor binding and introduce novel tropisms, e.g. by using growth factor receptor (Miller *et al.*, 1998), CD3 (Wickham *et al.*, 1997), fibroblast growth factor (Printz *et al.*, 2000), heparin (Wickham *et al.*, 1996) or gastric releasing peptide (Hong *et al.*, 1999*a*). In this way, inflammatory vascular endothelial cells exhibiting E selectin can be targetted by complexing an anti-selectin E MAb with an anti-FLAG MAb and then attaching this dual antibody to a vector expressing the FLAG epitope (Harari *et al.*, 1999). Another, more direct approach has been to incorporate binding motifs into the C-terminal domain of the fibre protein. This procedure facilitated binding to other cells without altering the endogenous binding, but this technique showed that specific delivery could be obtained in cells where the normal CAR was not expressed (Hidaka *et al.*, 1999). A similar strategy using a variety of ligands proved promising in a model system for mouse gliomas (Staba *et al.*, 2000).

One problem in attempting to produce vectors with novel receptors is the need for simultaneous development of culture cells that would allow good propagation of the vector. A neat approach to dealing with this has been demonstrated by incorporating six histidine residues (a His tag) into the H1 loop of the fibre knob (Krasnykh *et al.*, 1998; Michael *et al.*, 1995) and then using the modified virus to infect human glioma cells successfully (which lack normal receptors), which themselves had been modified to display a single-chain antibody against the His tag (Douglas *et al.*, 1999).

A similar method, using a peptide from influenza virus haemagglutinin inserted into either the fibre or the penton base, was used successfully to infect cells expressing the single-chain antibody ligand (Einfeld *et al.*, 1999). This approach could, in principle, be developed to construct vectors that have lost their native tropisms through mutation of the receptor-binding site on the fibre (Bewley *et al.*, 1999; Kirby *et al.*, 1999; Santis *et al.*, 1999) and the RGD motif on the penton base (Chiu *et al.*, 1999; Mathias *et al.*, 1998) and therefore have the capacity to infect cells with other specificities. It has also been shown that delivery can be inhibited by protective extracellular matrices (van Deutekom *et al.*, 1999) and that there are also anatomical barriers to overcome (Fechner *et al.*, 1999).

A survey of a range of human adenovirus serotypes has demonstrated that some of them exhibit different and wider host tropisms, indicating that factors other than CAR must also operate. Thus, a chimeric type 2 adenovirus with a type 17 fibre can enhance gene transfer to airway epithelia (Zabner *et al.*, 1999), in contrast with type 2 on its own. Similar use could be made of the properties of adenoviruses of subgenus H; thus, Ad41 binds selectively to differentiated gut enterocytes (Croyle *et al.*, 1998*b*). Viruses of subgroup D infect primary central nervous system cells more efficiently than do subgroup C (Chillon *et al.*, 1999). One other strategy to ablate the binding properties of the fibre is to use fibreless virus. In this case, infectivity is reduced drastically but entry to cells can still be achieved at low levels via the RGD motif in the penton base (Legrand *et al.*, 1999; Von Seggern *et al.*, 1999). There is additional evidence that virus uptake can be mediated via the penton base alone and that the interaction with integrins can lead to a different route to the nucleus (Hong *et al.*, 1999*b*).

Other, more non-specific ways of bypassing normal receptor-mediated entry are by transfection with the aid of cationic lipids and polymers and by using calcium phosphate (Alton *et al.*, 1999; Campain *et al.*, 1998; Croyle *et al.*, 1998*a*; Dodds *et al.*, 1999; Fasbender *et al.*, 1997, 1998; Lee *et al.*,

1999*a*; Qiu *et al.*, 1998). Although not strictly a vectorial procedure, the ability of adenovirus to enter cells efficiently has been exploited by condensing a plasmid with polyethylenimine and then complexing with psoralen-inactivated adenovirus (Baker *et al.*, 1997; Bischof *et al.*, 1999; Edgell *et al.*, 1998). A variation, with a simpler technique involving the ability of the virus mu peptide to package and deliver DNA to the nucleus with the aid of liposomes, has also been developed recently (Murray *et al.*, 2000). A peptide derived from adenovirus fibre has recently been shown to target to the nucleolus and may provide a vehicle for gene delivery (Zhang *et al.*, 1999). A combinatorial approach, using adenovirus transduction and plasmid transfection as well as lipofection, can also lead to enhancement of expression (Dunphy *et al.*, 1999).

Ensuring expression of the transgene. Assuming that there is effective delivery of the transgene to the host cell, the next step in successful expression depends greatly on the efficiency of promoter and enhancer elements. While the backbone E1A promoter may be sufficient in some cases, heterologous promoters have often been inserted to provide better expression. These promoters may be specific to tissues or cells such as prostate (Rodriguez *et al.*, 1997; Yu *et al.*, 1999), muscle (Acsadi *et al.*, 1998; Amalfitano *et al.*, 1999), lung (Harrod *et al.*, 1998), liver (Sandig *et al.*, 1996) or oligodendrocyte (Horwitz *et al.*, 1997). More ubiquitous promoters, such as those derived from the immediate-early human cytomegalovirus (HCMV) promoter, Rous sarcoma virus (RSV) or the phosphoglycerate kinase gene (Millecamps *et al.*, 1999), have also been inserted. The HCMV promoter has been utilized extensively, since it is relatively powerful and requires the insertion of only a small number of bases (380 bp). An interesting comparison (Sallenave *et al.*, 1998) was made of the effectiveness of a series of adenovirus vectors with three different promoters, the adenovirus MLP, HCMV and MCMV (mouse cytomegalovirus) promoters. *In vitro* studies using human and rat alveolar and pulmonary cells showed the MCMV promoter to be very much more effective than MLP, with the HCMV promoter being intermediate. A similar result was obtained *in vivo* using rat lung. It was also noted that, although the HCMV promoter was much better than the endogenous E1A promoter in inducing persistent expression, this was also influenced by the nature of the vector backbone (Armentano *et al.*, 1997). Careful optimization of the CMV promoter/enhancer sequences can also lead to significant improvements in expression (Massie *et al.*, 1998). The RSV promoter seems to function well in liver (Gorziglia *et al.*, 1999) and has been used in a vector in phase I clinical trials on mesothelioma patients (Sterman *et al.*, 1998) and effectively in ovine vectors (Hofmann *et al.*, 1999). The incorporation of endogenous promoters and enhancers into the vector construct can also improve the transgene expression and persistence dramatically, as demonstrated for apolipoprotein A (De Geest *et al.*, 2000). A similar result was demonstrated for endogenous liver promoters (Pastore *et al.*, 1999). Another strategy, involving the incorporation into the transgene of tissue 'silencer' elements in conjunction with ubiquitous promoters to drive tissue-specific expression in neuronal cells, has been implemented successfully (Millecamps *et al.*, 1999).

From the above discussion, it will be evident that, by modification of receptor-binding characteristics coupled with a judicious use of promoters/enhancers, much more effective delivery and expression of a transgene can be achieved.

Application of adenovirus vectors

The ability of adenovirus vectors to deliver and express genes at high yields, especially *in vitro*, has been amply demonstrated over the last 15 years and has been well documented. However, the supremacy of the immune response *in vivo* has been a limiting factor in the practical development of vectors. Thus, it is critical to control or to suppress the immune response to the vector and the transgene where persistent expression is needed in the case of supplementing the activity of a faulty gene. In contrast, cancer therapy may benefit from the induction of a vigorous immune response. With a better understanding of the molecular and immunological factors that operate *in vivo*, great strides have been made over the last few years in the construction of more effective vectors, and some of these will be described here.

Gene therapy in the treatment of cancer

A variety of techniques have been adopted to suppress or eliminate tumour cells, the approach in each case depending largely on the type and location of the tumour. Most of the vectors have been developed by using *in vitro* models of the tumours and then by testing in the appropriate animal, sometimes using tumour-transplantation methodologies. A number of these approaches have now moved on to clinical trials. The therapies in use can be divided into three groups: (i) tumour suppressors, (ii) oncolytic and sensitizing drug therapy and (iii) vaccines.

(i) Tumour suppressors. Mutations in the p53 gene that lead to loss of function have been implicated in the development of a wide variety of human tumours (Wills *et al.*, 1994). To remedy this defect and to induce apoptosis in the tumour cells, a number of vectors incorporating wild-type p53 have been constructed. Initial studies, using appropriate tumour cell lines and then animal model systems, demonstrated the efficacy of these approaches with anaplastic thyroid cancer (Blagosklonny *et al.*, 1998), human malignant gliomas (Cirielli *et al.*, 1999; Li *et al.*, 1999*a*) and breast cancer (Putzer *et al.*, 1998). In some cases, combination with an immunomodulatory gene such as IL-2 (Putzer *et al.*, 1998) or with a cytotoxic drug such as adriamycin (Blagosklonny *et al.*, 1998) proved more effective. Clinical trials testing the efficacies of these vectors in the treatment of lung, head, neck and liver cancers are under way. However, one of the problems inherent in this approach is the

desirability of efficient targetting of the vector, and direct inoculation via the hepatic artery for treatment of liver cancer has been explored in a mouse model (Anderson *et al.*, 1998). More recent investigations have illustrated dramatically the importance of the ARF–mdm2–p53 interactions in regulating p53 expression, and the discovery of a range of mutations in ARF and in related transcription factors such as *Twist* (Maestro *et al.*, 1999) in a variety of tumours (Eischen *et al.*, 1999; Sanchez-Cespedes *et al.*, 1999; Taniguchi *et al.*, 1999) have suggested that vectors expressing other components of the p53 pathway might be equally productive. Other approaches to induce apoptosis have been explored, and these have involved inhibiting cyclin-dependent kinases, which are critical to the cell cycle. Indeed, one of these, p16, has been shown to be defective in many human tumour cell lines, and vectors expressing p21, p15 and p16 have shown promise in model tumour systems (Tsao *et al.*, 1999). The use of vectors expressing proapoptotic proteins such as Fas ligand and caspase-8 has been limited by the difficulty of production of the vectors. However, the construction of complementing cell lines expressing adenovirus E3 14.7k or the poxvirus serpin gene CrmA has recently provided a route for good production and exploration of their properties (Bruder *et al.*, 2000). Another intriguing strategy to encourage apoptosis has been the use of ribozymes such as anti-H-*ras* for bladder cancer (Irie *et al.*, 1999), anti-Bcl-2 for prostate cancer (Dorai *et al.*, 1999) and anti-HER2 for breast cancer (Suzuki *et al.*, 2000).

(ii) Oncolytic and sensitizing drug therapy. Direct application of wild-type adenovirus to tumours was attempted soon after their discovery in the 1950s, but only local effects were demonstrated, and it was not until 1996 that it was claimed that an adenovirus that had a mutation in E1B 55k would replicate selectively in p53-defective tumour cells (Bischoff *et al.*, 1996) and therefore could function as an oncolytic virus. This led to the commercial development of the mutant (Onyx 015) and, although a number of publications have shown convincingly that the original premises did not hold up (Hay *et al.*, 1999*a*; Ridgway *et al.*, 1997; Vollmer *et al.*, 1999), it is still claimed that the virus is effective by intravenous administration in treatment of some tumours (Heise *et al.*, 1999). Combination with standard chemotherapy also looks promising (Heise *et al.*, 1997), and phase 3 clinical trials for head and neck tumours using Onyx are under way. Moreover, a recent study using the E1B-deleted virus in parallel with an adenovirus vector expressing IL-2 has shown complete regression of a p53-deficient pancreatic tumour in a mouse model (Motoi *et al.*, 2000).

Another strategy that has been developed for the selective elimination of tumour cells is to deliver a prodrug enzyme via a vector into target cells and then to administer a non-toxic drug that can be converted into a cytotoxic agent *in situ* (Crystal, 1999). Herpes simplex virus thymidine kinase (HSV-tk) has been used extensively as a so-called suicide gene, since administration of ganciclovir will be followed by its phosphorylation by HSV-tk to yield a chain terminator for DNA synthesis. This technique has been used *in situ* for head and neck tumours (Goebel *et al.*, 1998) and phase 1 trials have been completed for malignant mesothelioma (Sterman *et al.*, 1998) and for prostate carcinoma (Herman *et al.*, 1999). A vector with both E1 and E4 deleted has been constructed recently for delivery of the HSV-tk gene (Lanuti *et al.*, 1999), apparently with better results. Cytosine deaminase (CD) has also been used in colon carcinoma as a suicide gene in an adenovirus vector, with co-administration of 5-fluorocytosine (Hirschowitz *et al.*, 1995). A fusion gene of HSV-tk and CD has also been inserted into vectors to treat prostate carcinoma (Blackburn *et al.*, 1999). A trimodal therapy involving a double suicide vector of HSV-tk and CD combined with radiotherapy has provided very effective tumour reduction in a cervical carcinoma xenograft model (Rogulski *et al.*, 2000). Another interesting approach has been to co-administer with the CD vector another containing the gene for uracil phosphoribosyltransferase. In a rat tumour model, this increased the sensitivity of the system very significantly (Adachi *et al.*, 2000). A so-called 'bystander effect' appears to amplify the cytotoxicity with these therapies (Zhang & DeGroot, 2000).

Vectors containing suicide genes have been complemented with vectors delivering a variety of cytokines in an attempt to boost the *in situ* cytotoxicity (Cao *et al.*, 1998).

In all these systems, it was evident that the efficacy of treatment could be enhanced considerably if targetting could be made more specific, and tissue-specific promoters have been incorporated into vectors to facilitate this (Hart, 1996). Applications to breast cancer (Manome *et al.*, 1994), liver cancer (Kaneko *et al.*, 1995) and melanoma (Siders *et al.*, 1998*a*) have been described. However, although specificity was obtained *in vivo*, results were quite often disappointing because of relatively low promoter activity. In an attempt to improve this, an ingenious approach has been developed using the *Cre-lox* system and a tumour-specific antigen in a model system, with promising results (Kijima *et al.*, 1999).

Reference should also be made to the importance of retaining the E1A genes in vectors in these cases because of their ability to promote p53 transcription and to enhance the sensitivity of tumour cells to cytotoxic agents (Brader *et al.*, 1997; Cook *et al.*, 1999; Wildner *et al.*, 1999) and to radiation therapies (Martin-Duque *et al.*, 1999).

(iii) Vaccines. Strategies to invoke anti-tumour cell immunity have been explored using vectors by introducing a variety of immunomodulatory genes and/or tumour-specific antigens. Many cytokines can be effective in this way: thus, IL-2 can induce CTLs, enhance NK cell activity and promote tumour-infiltrating lymphocytes, and high doses of recombinant IL-2 and IL-2-expressing vector have been successful in reducing tumour load in animal models. Nevertheless, problems of toxicity have become apparent (Toloza *et al.*, 1996) and later

developments concentrated on the more-directed delivery of other cytokines such as IL-12 (Bramson *et al*., 1996; Gambotto *et al*., 1999; Mazzolini *et al*., 2000; Siders *et al*., 1998*b*), sometimes in combination with IL-2 (Addison *et al*., 1998) and tumour antigen (Hirschowitz & Crystal, 1999). Intratumoural injection of vectors expressing IL-2 or IL-12 in combination with a vector expressing lymphotactin have been successful in a murine breast cancer model system (Emtage *et al*., 1999). The recognition that many tumours exhibit tumour-specific antigens encouraged the use of vectors expressing these antigens as a means of boosting anti-tumour immunity and led to a trial on metastatic melanomas using adenovirus vectors expressing either MART 1 or gp100 melanoma antigens (Rosenberg *et al*., 1998). The results demonstrated that high doses of the vector could be administered safely, but that the immune response to the vector ablated any longer-term anti-tumour response. In the case of a model system of colorectal cancer (Li *et al*., 1997), expression of a tumour antigen via an adenovirus vector resulted in significant tumour regression and induction of immunity to further tumour challenge. Another promising approach to boosting anti-tumour immunity was modelled on the ability of dendritic cells to present antigens effectively *in vivo*. It has been proposed that one useful strategy would be to isolate dendritic cells from a patient and then to modify them by infection with an adenovirus vector expressing the appropriate tumour antigen, followed by readministration to the patient in combination with standard therapies (Crystal, 1999). Studies with human dendritic cells have shown that dendritic cells modified with adenovirus vectors are not perturbed in terms of their maturation and function (Rea *et al*., 1999; Zhong *et al*., 1999) and significant reduction of lung metastases was achieved with murine dendritic cells (Wan *et al*., 1999). A murine dendritic cell model of melanoma also showed a significant boost to anti-melanoma immunity using an adenovirus vector (Tuting *et al*., 1999). Considerable improvement in the efficiency of targetting of the vector to dendritic cells can be obtained by using a bispecific antibody to redirect the virus to CD40 receptors on the cells (Tillman *et al*., 1999). This technique also facilitated the maturation of the cells and thereby enhanced their immuno-stimulatory characteristics. Vectors expressing CD40 ligand have also been introduced directly into tumour cells and facilitate antigen presentation (Crystal, 1999). Other strategies to do this involved the vector expression of granulocyte–macrophage colony-stimulating factors (Ozawa *et al*., 1999) and increasing the tolerogenicity of the dendritic cells with a vector encoding TGF β1 (Lee *et al*., 1999*b*). A recent study has shown that dendritic cells can be infected with a vector independent of their CAR status and that the cells can elicit the appropriate CTL responses (Linette *et al*., 2000).

Gene therapy for genetic diseases

Cystic fibrosis is a relatively common, inherited, recessive disease caused by mutations in the CFTR gene that result in poor chloride ion conductance and increased sodium ion uptake. Since the defect is manifested primarily in the lung, adenoviruses, with their apparent propensity to infect this organ, would appear to be the vector of choice to deliver the therapeutic gene. However, a multiplicity of investigations have demonstrated that there are many barriers to successful transgene expression. Among these is the inability of the standard Ad2/5 vectors to infect the well-differentiated airway epithelial cells and alveolar macrophages, mainly due to the lack of CARs (Kaner *et al*., 1999; Pickles *et al*., 1998; Walters *et al*., 1999). Attempts have been made to improve uptake by using cationic lipids and calcium phosphate co-precipitates (Alton *et al*., 1999; Fasbender *et al*., 1997, 1998; Lee *et al*., 1999*a*). A more promising approach has been the construction of a chimeric adenovirus vector with serotype 17 fibre, which displays an increased binding to airway epithelial cells (Zabner *et al*., 1999). Another barrier to successful transgene expression was the specific pulmonary-associated T helper cell response (van Ginkel *et al*., 1997), and later studies have shown the importance of retaining E4 genes in the vector to counteract this assault (Armentano *et al*., 1997; Chirmule *et al*., 1998; Lusky *et al*., 1999; Yew *et al*., 1999). A further, natural inhibitor is derived from the ability of airway epithelia to release antimicrobial peptides with anti-adenovirus properties (Gropp *et al*., 1999), and a study of bronchoalveolar lavage fluid has indicated the presence of adenovirus inhibitors in addition to neutralizing antibodies (Bastian & Bewig, 1999). Many of these factors probably account for the results from human trials with earlier vectors in which, although efficacy of transfer was demonstrated, the overall efficiency was low and expression of the transgene was of relatively short duration (Zuckerman *et al*., 1999). It remains to be seen whether the later vectors can improve the efficiency and persistence of the transgene.

Considerable effort using adenovirus vectors has been devoted to trying to repair the gene defects responsible for muscular dystrophy. In a mouse model system, vectors encoding dystrophin can be delivered to muscle fibres with some improvement of function, but this is only transient, because of a potent immune response against the vector and transgene (Yang *et al*., 1998; Yuasa *et al*., 1998). Attempts to circumvent this by using improved vectors (Kumar-Singh & Chamberlain, 1996) and utrophin (a homologue of dystrophin) (Gilbert *et al*., 1999) showed better transgene persistence. One of the barriers to effective gene transfer relates to the lack of receptors for the vectors being used, and a vector with a polylysine-modified fibre improved uptake into muscle cells significantly (Bouri *et al*., 1999). It has been shown that the immune response in the muscle fibre cells appears to be mediated via dendritic cells, and it has been suggested that AAV vectors may be better vehicles for delivery of the transgene (Jooss *et al*., 1998*c*). A canine model system has also demonstrated the significance of the immune response, since treatment with cyclosporin after adenovirus delivery of

dystrophin gives a more sustained improvement of muscle function (Howell *et al.*, 1998).

A number of vectors have been constructed to alleviate gene defects in other tissues, and similar problems in terms of delivery have been encountered.

Supplementary therapy

With an increasingly ageing population, therapy for human neurodegenerative diseases such as Parkinson's offers a major challenge. Adenoviruses, with their ability to infect post-mitotic cells coupled with a potentially high transduction efficiency and low pathogenicity in the immunologically privileged site of the central nervous system, should provide effective vectors for neuronal gene therapy. Two major strategies have been examined for delivering therapeutic genes. One involves direct intracerebral injection of the vector and the other uses *ex vivo* gene therapy, where cells can be modified *in vitro* by vector infection and then transplanted into the relevant areas of the brain (Barkats *et al.*, 1998). Neuro-progenitor cells (Fisher, 1997) and human astrocytes (Ridet *et al.*, 1999) can apparently provide autologous cellular vehicles for *ex vivo* modification and expansion. A tetracycline-regulated adenovirus vector expressing tyrosine hydroxylase, a rate-limiting enzyme in the synthesis of dopamine, has shown considerable promise in model systems using the *ex vivo* technique (Corti *et al.*, 1999*a*, *b*). In an attempt to utilize adenovirus vectors to alleviate Huntington's disease, a construct expressing brain-derived neurotrophic factor was found to give promising results in a rat model system (Bemelmans *et al.*, 1999). Reference has also been made above to the use of neuronal silencer elements in adenovirus constructs in restricting expression to neuronal cells, with the expectation that such a strategy would avoid the side-effects due to ectopic expression of transgenes (Millecamps *et al.*, 1999).

Over the last few years, there has been considerable progress in our understanding of arthritic diseases and the role that cytokines play in invoking the inflammatory processes that occur in joints and synovial fluids. A major discovery has been the role that TNFα plays in the induction of rheumatoid arthritis, amply demonstrated by the success of clinical trials with TNF antibody (Maini *et al.*, 1999) and TNF receptor (Franklin, 1999). Adenovirus vectors have been extremely useful in unravelling the significance of some of the cytokines and their pathways in this disease process. Thus, direct delivery of TNFα receptor and cytokine IL-1 using adenovirus vectors has shown synergistic benefits, both direct and distal, in a rat model system (Ghivizzani *et al.*, 1998). Similarly, TNFα receptor showed significant synergy with an Epstein–Barr virus homologue of IL-10 in an animal model (Kim *et al.*, 2000; Lechman *et al.*, 1999). Use of a vector expressing IL-4 has demonstrated that this cytokine can provide considerable protection for cartilage from inflammatory processes (Lubberts *et al.*, 1999). In contrast, vector delivery of IL-12 accelerated the disease process (Parks *et al.*, 1998). A significant advance was made by the observation that vector expression of an inhibitor of NF-κB inhibited the production of TNFα in macrophages (Foxwell *et al.*, 1998), and later investigations showed that inhibition of NF-κB was accompanied by the inhibition of proinflammatory cytokines, but not the main inflammatory mediators like IL-10 (Bondeson *et al.*, 1999*a*, *b*). These results therefore pinpointed the important role of NF-κB in inflammation and as a therapeutic target. Nevertheless, it should be pointed out that TNFα production did not appear to be NF-κB-dependent in some other cells, such as monocytes (Hayes *et al.*, 1999).

Other applications

Adenoviruses have been useful vectors for the production of a number of proteins for more-detailed molecular analysis, and the reader is referred to the very comprehensive review by Hitt *et al.* (1997). Adenovirus vaccines have been tested thoroughly for safety, as a result of problems with adenovirus respiratory disease in the American military (Chanock *et al.*, 1966), and this has facilitated the development of recombinant adenovirus vaccines for human immunodeficiency virus (Bruce *et al.*, 1999) and rabies virus (Matthews *et al.*, 1999; Yarosh *et al.*, 1996). A comprehensive review of earlier vaccine developments is available (Graham & Prevec, 1992).

This review has only touched on a relatively small number of the applications of vector technology and is certainly not comprehensive, but is illustrative of the considerable progress that has been made; Table 2 provides further examples of applications.

Safety considerations

The protocols for developing a virus vector for clinical use invariably employ a series of steps that begin with a study of infection of appropriate cells *in vitro*, followed by application to an animal model system of some kind. This latter step can bring to light some of the problems inherent in delivery to the site of interest and in the expression and persistence (where that is required) of the transgene. Very often, the efficiency of delivery has been found to be poor and the expression of the transgene to be only transient, although some of these effects may be a function of a different distribution of receptors in the model systems. With a greater understanding of the nature of the responses *in vivo*, many of these problems can be alleviated to some extent, as described above, by constructing vectors that improve targetting and minimize immune responses to both the vector and the transgene (Miller & Whelan, 1997). Nevertheless, in the course of *in vivo* studies, it has become apparent that there can be acute injury and inflammation of infected tissues quite independent of the possibility of helper virus replication. This was particularly apparent in the liver (Lieber *et al.*, 1997), where it was shown that a vector can induce production of chemokines within 1 h, even with psoralen-inactivated virus (Muruve *et al.*, 1999). This was followed by an influx of neutrophils into mouse liver and, with

Table 2. Some recent vector applications

APC, Adenomatous polyposis coli; GM–CSF, granulocyte–macrophage colony-stimulating factor; PKC, protein kinase C.

Site/disease	Transgene(s)	Reference
Tumours		
Prostate	p16	Steiner *et al.* (2000)
	PKC	Fujii *et al.* (2000)
	Cytolytic virus	Rodriguez *et al.* (1997)
	Fas ligand	Hedlund *et al.* (1999)
Colon	GM–CSF/IL-2	Diaz *et al.* (1998)
	CD suicide gene	Topf *et al.* (1998)
	APC	Shih *et al.* (2000)
	Lymphotactin + suicide	Ju *et al.* (2000)
Cervix	p21	Tsao *et al.* (1999)
	Papillomavirus p21	He *et al.* (2000)
Ovary	Stomatostatin receptor	Rogers *et al.* (1999)
	Cytolytic virus/IL-6	Rancourt *et al.* (1999)
Endometrium	p53/p21	Ramondetta *et al.* (2000)
Gliomas	Caspase-3/Fas ligand	Shinoura *et al.* (2000)
Carcinoembryonic antigen-producing cancer	HSV-tk	Kijima *et al.* (1999)
Other conditions		
Autoimmune diabetes	Adenovirus E3	von Herrath *et al.* (1997)
Glycogen storage disease II	Glucosidase	Amalfitano *et al.* (1999)
CNS conditions	(via astrocytes)	Ridet *et al.* (1999)
Factor VIII deficiency	Factor VIII	Balagu *et al.* (2000)
OTC deficiency	OTC	Batshaw *et al.* (1999)
Liver graft	Bcl-2	Bilbao *et al.* (1999*a*)
Leishmania infection	IL-12	Gabaglia *et al.* (1999)
Tay–Sachs disease	Hexosaminidases	Guidotti *et al.* (1999)
Motor neurone disease	Neurotrophic factor	Haase *et al.* (1999)

high virus inputs, to hepatic necrosis and apoptosis. It seems evident that the innate system, possibly via NF-κB mediation, is a major player that must be taken into account even with so-called gutless vectors, and consideration could therefore be given, in appropriate cases, to concomitant introduction of a vector that expresses inhibitors of this pathway. This problem has been highlighted by the recent tragic death of a patient undergoing adenovirus vector therapy for a defect in the ornithine transcarbamylase (OTC) pathway. The preliminary enquiries appear to point to a massive activation of innate immunity followed by systemic inflammation (Marshall, 1999). It may be significant that a large dose of virus was given in an effort to get enough functioning OTC genes into the liver. Apparently, this dramatic reaction had not been noted before, and it was suggested that this response was an unusual one related to this particular individual's genetic repertoire. However, it has now also come to light that a substantial number of adverse effects had not been reported adequately in some clinical trials. These events highlight the necessity to carry out trials using monitoring procedures that have the confidence of the public as well as all of the parties that are directly involved. As indicated above, use of animal model systems had indicated previously that host responses to vector were very dependent on host genotype, and this unfortunate incident emphasizes the need to understand the basic factors that control host–vector responses.

It should be noted that there have been a number of reports of inflammatory responses in animals, not only following vector administration to the liver. Thus, airway neurogenic inflammation was apparent in a rat cystic fibrosis model system, but this could be alleviated by pharmacological methods (Piedimonte *et al.*, 1997).

A significant improvement was seen by utilizing gutless vectors in a rat model system for leptin delivery, where liver toxicity, inflammation and cellular infiltration were reduced significantly compared with an E1-deleted vector (Morsy *et al.*, 1998). However, the problems and considerable costs of scaling up, purification and production of these vectors are not insignificant. The need for careful evaluation of long-term inflammatory responses in human therapy has been underlined further by the report of chronic brain inflammation following the successful inhibition of a glioma using cytotoxic gene therapy (Dewey *et al.*, 1999). In this report, persistence and expression of the HSV-tk gene could be found in the brain

some 3 months later and co-incident with the presence of a variety of inflammatory markers. In contrast, a study examining the toxicity associated with suicide gene therapy in prostate cancer found that toxicity was not a major factor, even with multiple applications (Shalev *et al*., 2000). The importance of the route of vector delivery has been demonstrated by the finding that vascular inflammation occurred in a rabbit vascular model when delivery was by the intraluminal route, but was greatly reduced when the adventitial route was used (Schneider *et al*., 1999). Another, perhaps surprising finding in a porcine model was that direct myocardial administration of a vector proved successful without any apparent inflammatory responses, although the experiment was continued for only 28 days and therefore longer-term chronic toxicity was not assessed (Patel *et al*., 1999). A study in sheep, in contrast, led to the conclusion that administration of adenovirus vectors *in utero* was accompanied by inflammatory and fibrotic responses (Iwamoto *et al*., 1999).

Future developments

Adenovirus vectors have been in vogue for over 15 years and the early enthusiasm in their therapeutic application has now been tempered by the realization that there is, as yet, no evidence of significant clinical success. As noted above, the protective responses of the host had been underestimated and it is only in the last few years has there been some understanding of the complexities of virus expression and the host responses. In this connection, it should be noted that most of the clinical trials presently under way have utilized the earlier first- and second-generation vectors. This rather negative scenario has been compounded by the news of the death of the patient undergoing vector therapy. Nevertheless, great progress has been made and lessons should have been learned. Principal among these is the necessity to explore the many factors involved in vector administration in depth before venturing into clinical application. Thus, as well as trying to understand the molecular nature of both the innate and active immune responses in the context of genetic background, the target organ, the route of administration etc., the possibility of pharmacological and vectorial supplements will need to be examined. This, of course, implies that much longer development protocols will be required and much greater patience will need to be exercised by the institutions and companies that invest in this technology. Nevertheless, adenovirus vectors offer great promise and should not be abandoned in the light of these early mishaps in clinical trials.

The author is indebted to his colleagues, who supplied advice on both the text and the compilation of the figures: viz. Rick Randall, Ron Hay, Martin Ryan, Bernie Precious and Alex Houston. Thanks also to David Matthews, who supplied the basis of Fig. 1, and Phil Gallimore and Andy Turnell for Fig. 3.

References

Acsadi, G., O'Hagan, D., Lochmuller, H., Prescott, S., Larochelle, N., Nalbantoglu, J., Jani, A. & Karpati, G. (1998). Interferons impair early transgene expression by adenovirus-mediated gene transfer in muscle cells. *Journal of Molecular Medicine* **76**, 442–450.

Adachi, Y., Tamiya, T., Ichikawa, T., Terada, K., Ono, Y., Matsumoto, K., Furuta, T., Hamada, H. & Ohmoto, T. (2000). Experimental gene therapy for brain tumors using adenovirus-mediated transfer of cytosine deaminase gene and uracil phosphoribosyltransferase gene with 5-fluorocytosine. *Human Gene Therapy* **11**, 77–89.

Addison, C. L., Bramson, J. L., Hitt, M. M., Muller, W. J., Gauldie, J. & Graham, F. L. (1998). Intratumoral coinjection of adenoviral vectors expressing IL-2 and IL-12 results in enhanced frequency of regression of injected and untreated distal tumors. *Gene Therapy* **5**, 1400–1409.

Alton, E. W., Stern, M., Farley, R., Jaffe, A., Chadwick, S. L., Phillips, J., Davies, J., Smith, S. N., Browning, J., Davies, M. G., Hodson, M. E., Durham, S. R., Li, D., Jeffery, P. K., Scallan, M., Balfour, R., Eastman, S. J., Cheng, S. H., Smith, A. E., Meeker, D. & Geddes, D. M. (1999). Cationic lipid-mediated CFTR gene transfer to the lungs and nose of patients with cystic fibrosis: a double-blind placebo-controlled trial. *Lancet* **353**, 947–954.

Amalfitano, A., Hauser, M. A., Hu, H., Serra, D., Begy, C. R. & Chamberlain, J. S. (1998). Production and characterization of improved adenovirus vectors with the E1, E2b, and E3 genes deleted. *Journal of Virology* **72**, 926–933.

Amalfitano, A., McVie-Wylie, A. J., Hu, H., Dawson, T. L., Raben, N., Plotz, P. & Chen, Y. T. (1999). Systemic correction of the muscle disorder glycogen storage disease type II after hepatic targeting of a modified adenovirus vector encoding human acid-alpha-glucosidase. *Proceedings of the National Academy of Sciences, USA* **96**, 8861–8866.

Anderson, C. W., Young, M. E. & Flint, S. J. (1989). Characterization of the adenovirus 2 virion protein, mu. *Virology* **172**, 506–512.

Anderson, S. C., Johnson, D. E., Harris, M. P., Engler, H., Hancock, W., Huang, W. M., Wills, K. N., Gregory, R. J., Sutjipto, S., Wen, S. F., Lofgren, S., Shepard, H. M. & Maneval, D. C. (1998). p53 gene therapy in a rat model of hepatocellular carcinoma: intra-arterial delivery of a recombinant adenovirus. *Clinical Cancer Research* **4**, 1649–1659.

Angeletti, P. C. & Engler, J. A. (1998). Adenovirus preterminal protein binds to the CAD enzyme at active sites of viral DNA replication on the nuclear matrix. *Journal of Virology* **72**, 2896–2904.

Arany, Z., Newsome, D., Oldread, E., Livingston, D. M. & Eckner, R. (1995). A family of transcriptional adaptor proteins targeted by the E1A oncoprotein. *Nature* **374**, 81–84.

Armentano, D., Zabner, J., Sacks, C., Sookdeo, C. C., Smith, M. P., St George, J. A., Wadsworth, S. C., Smith, A. E. & Gregory, R. J. (1997). Effect of the E4 region on the persistence of transgene expression from adenovirus vectors. *Journal of Virology* **71**, 2408–2416.

Arnberg, N., Edlund, K., Kidd, A. H. & Wadell, G. (2000). Adenovirus type 37 uses sialic acid as a cellular receptor. *Journal of Virology* **74**, 42–48.

Badrichani, A. Z., Stroka, D. M., Bilbao, G., Curiel, D. T., Bach, F. H. & Ferran, C. (1999). Bcl-2 and Bcl-XL serve an anti-inflammatory function in endothelial cells through inhibition of NF-kappaB. *Journal of Clinical Investigation* **103**, 543–553.

Baker, A., Saltik, M., Lehrmann, H., Killisch, I., Mautner, V., Lamm, G., Christofori, G. & Cotten, M. (1997). Polyethylenimine (PEI) is a simple, inexpensive and effective reagent for condensing and linking plasmid DNA to adenovirus for gene delivery. *Gene Therapy* **4**, 773–782.

Balachandran, S., Roberts, P. C., Kipperman, T., Bhalla, K. N., Compans, R. W., Archer, D. R. & Barber, G. N. (2000). Alpha/beta

interferons potentiate virus-induced apoptosis through activation of the FADD/caspase-8 death signaling pathway. *Journal of Virology* **74**, 1513–1523.

Balagu, C., Zhou, J., Dai, Y., Alemany, R., Josephs, S. F., Andreason, G., Hariharan, M., Sethi, E., Prokopenko, E., Jan, H., Lou, Y. C., Hubert-Leslie, D., Ruiz, L. & Zhang, W. W. (2000). Sustained high-level expression of full-length human factor VIII and restoration of clotting activity in hemophilic mice using a minimal adenovirus vector. *Blood* **95**, 820–828.

Bals, R., Weiner, D. J., Moscioni, A. D., Meegalla, R. L. & Wilson, J. M. (1999). Augmentation of innate host defense by expression of a cathelicidin antimicrobial peptide. *Infection and Immunity* **67**, 6084–6089.

Barkats, M., Bilang-Bleuel, A., Buc-Caron, M. H., Castel-Barthe, M. N., Corti, O., Finiels, F., Horellou, P., Revah, F., Sabate, O. & Mallet, J. (1998). Adenovirus in the brain: recent advances of gene therapy for neurodegenerative diseases. *Progress in Neurobiology* **55**, 333–341.

Bastian, A. & Bewig, B. (1999). Inhibition of adenovirus-mediated gene transfer by bronchoalveolar lavage fluid. *Gene Therapy* **6**, 637–642.

Batshaw, M. L., Wilson, J. M., Raper, S., Yudkoff, M. & Robinson, M. B. (1999). Recombinant adenovirus gene transfer in adults with partial ornithine transcarbamylase deficiency (OTCD). *Human Gene Therapy* **10**, 2419–2437.

Bemelmans, A. P., Horellou, P., Pradier, L., Brunet, I., Colin, P. & Mallet, J. (1999). Brain-derived neurotrophic factor-mediated protection of striatal neurons in an excitotoxic rat model of Huntington's disease, as demonstrated by adenoviral gene transfer. *Human Gene Therapy* **10**, 2987–2997.

Benihoud, K., Yeh, P. & Perricaudet, M. (1999). Adenovirus vectors for gene delivery. *Current Opinions in Biotechnology* **10**, 440–447.

Benkő, M., Harrach, B. & Russell, W. C. (1999). *Adenoviridae*. In *Virus Taxonomy. Seventh Report of the International Committee on Taxonomy of Viruses*, pp. 227–238. Edited by M. H. V. van Regenmortel, C. M. Fauquet & D. H. L. Bishop. San Diego: Academic Press.

Bennett, E. M., Bennink, J. R., Yewdell, J. W. & Brodsky, F. M. (1999). Cutting edge: adenovirus E19 has two mechanisms for affecting class I MHC expression. *Journal of Immunology* **162**, 5049–5052.

Bergelson, J. M., Cunningham, J. A., Droguett, G., Kurt-Jones, E. A., Krithivas, A., Hong, J. S., Horwitz, M. S., Crowell, R. L. & Finberg, R. W. (1997). Isolation of a common receptor for coxsackie B viruses and adenoviruses 2 and 5. *Science* **275**, 1320–1323.

Bewley, M. C., Springer, K., Zhang, Y. B., Freimuth, P. & Flanagan, J. M. (1999). Structural analysis of the mechanism of adenovirus binding to its human cellular receptor, CAR. *Science* **286**, 1579–1583.

Bilbao, G., Contreras, J. L., Gomez-Navarro, J., Eckhoff, D. E., Mikheeva, G., Krasnykh, V., Hynes, T., Thomas, F. T., Thomas, J. M. & Curiel, D. T. (1999*a*). Genetic modification of liver grafts with an adenoviral vector encoding the Bcl-2 gene improves organ preservation. *Transplantation* **67**, 775–783.

Bilbao, G., Contreras, J. L., Zhang, H. G., Pike, M. J., Overturf, K., Mikheeva, G., Krasnykh, V. & Curiel, D. T. (1999*b*). Adenovirus-mediated gene expression in vivo is enhanced by the antiapoptotic bcl-2 gene. *Journal of Virology* **73**, 6992–7000.

Bischof, J., Vietor, I., Cotten, M. & Huber, L. A. (1999). Transient transfection of mammary epithelial cells with a PEI/DNA/adenovirus system. *Biological Chemistry* **380**, 269–273.

Bischoff, J. R., Kirn, D. H., Williams, A., Heise, C., Horn, S., Muna, M., Ng, L., Nye, J. A., Sampson-Johannes, A., Fattaey, A. & McCormick, F. (1996). An adenovirus mutant that replicates selectively in p53-deficient human tumor cells. *Science* **274**, 373–376.

Blackburn, R. V., Galoforo, S. S., Corry, P. M. & Lee, Y. J. (1999). Adenoviral transduction of a cytosine deaminase/thymidine kinase fusion gene into prostate carcinoma cells enhances prodrug and radiation sensitivity. *International Journal of Cancer* **82**, 293–297.

Blagosklonny, M. V., Giannakakou, P., Wojtowicz, M., Romanova, L. Y., Ain, K. B., Bates, S. E. & Fojo, T. (1998). Effects of p53-expressing adenovirus on the chemosensitivity and differentiation of anaplastic thyroid cancer cells. *Journal of Endocrine Metabolism* **83**, 2516–2522.

Boivin, D., Morrison, M. R., Marcellus, R. C., Querido, E. & Branton, P. E. (1999). Analysis of synthesis, stability, phosphorylation, and interacting polypeptides of the 34-kilodalton product of open reading frame 6 and of the early region 4 protein of human adenovirus type 5. *Journal of Virology* **73**, 1245–1253.

Bondeson, J., Browne, K. A., Brennan, F. M., Foxwell, B. M. & Feldmann, M. (1999*a*). Selective regulation of cytokine induction by adenoviral gene transfer of IκBα into human macrophages: lipopolysaccharide-induced, but not zymosan-induced, proinflammatory cytokines are inhibited, but IL-10 is nuclear factor-κB independent. *Journal of Immunology* **162**, 2939–2945.

Bondeson, J., Foxwell, B., Brennan, F. & Feldmann, M. (1999*b*). Defining therapeutic targets by using adenovirus: blocking NF-κB inhibits both inflammatory and destructive mechanisms in rheumatoid synovium but spares anti-inflammatory mediators. *Proceedings of the National Academy of Sciences, USA* **96**, 5668–5673.

Bouri, K., Feero, W. G., Myerburg, M. M., Wickham, T. J., Kovesdi, I., Hoffman, E. P. & Clemens, P. R. (1999). Polylysine modification of adenoviral fiber protein enhances muscle cell transduction. *Human Gene Therapy* **10**, 1633–1640.

Boyer, J. L. & Ketner, G. (2000). Genetic analysis of a potential zinc-binding domain of the adenovirus E4 34k protein. *Journal of Biological Chemistry* **275**, 14969–14978.

Boyer, J., Rohleder, K. & Ketner, G. (1999*a*). Adenovirus E4 34k and E4 11k inhibit double strand break repair and are physically associated with the cellular DNA-dependent protein kinase. *Virology* **263**, 307–312.

Boyer, T. G., Martin, M. E., Lees, E., Ricciardi, R. P. & Berk, A. J. (1999*b*). Mammalian Srb/mediator complex is targeted by adenovirus E1A protein. *Nature* **399**, 276–279.

Brader, K. R., Wolf, J. K., Hung, M. C., Yu, D., Crispens, M. A., van Golen, K. L. & Price, J. E. (1997). Adenovirus E1A expression enhances the sensitivity of an ovarian cancer cell line to multiple cytotoxic agents through an apoptotic mechanism. *Clinical Cancer Research* **3**, 2017–2024.

Bramson, J. L., Hitt, M., Addison, C. L., Muller, W. J., Gauldie, J. & Graham, F. L. (1996). Direct intratumoral injection of an adenovirus expressing interleukin-12 induces regression and long-lasting immunity that is associated with highly localized expression of interleukin-12. *Human Gene Therapy* **7**, 1995–2002.

Brehm, A., Miska, E. A., McCance, D. J., Reid, J. L., Bannister, A. J. & Kouzarides, T. (1998). Retinoblastoma protein recruits histone deacetylase to repress transcription. *Nature* **391**, 597–601.

Bruce, C. B., Akrigg, A., Sharpe, S. A., Hanke, T., Wilkinson, G. W. G. & Cranage, M. P. (1999). Replication-deficient recombinant adenoviruses expressing the human immunodeficiency virus Env antigen can induce both humoral and CTL immune responses in mice. *Journal of General Virology* **80**, 2621–2628.

Bruder, J. T. & Kovesdi, I. (1997). Adenovirus infection stimulates the Raf/MAPK signaling pathway and induces interleukin-8 expression. *Journal of Virology* **71**, 398–404.

Bruder, J. T., Appiah, A., Kirkman, W. M., III, Chen, P., Tian, J., Reddy, D., Brough, D. E., Lizonova, A. & Kovesdi, I. (2000). Improved

production of adenovirus vectors expressing apoptotic transgenes. *Human Gene Therapy* **11**, 139–149.

Campain, J. A., Matassa, A. A., Felgner, P. L., Barnhart, K. M., Curiel, D. T. & Harrison, G. S. (1998). Lipid- and adenoviral-mediated gene transfer into AIDS-Kaposi's sarcoma cell lines. *Cancer Gene Therapy* **5**, 131–143.

Cao, X., Ju, D. W., Tao, Q., Wang, J., Wan, T., Wang, B. M., Zhang, W. & Hamada, H. (1998). Adenovirus-mediated GM-CSF gene and cytosine deaminase gene transfer followed by 5-fluorocytosine administration elicit more potent antitumor response in tumor-bearing mice. *Gene Therapy* **5**, 1130–1136.

Caplen, N. J., Higginbotham, J. N., Scheel, J. R., Vahanian, N., Yoshida, Y., Hamada, H., Blaese, R. M. & Ramsey, W. J. (1999). Adeno-retroviral chimeric viruses as in vivo transducing agents. *Gene Therapy* **6**, 454–459.

Chakravarti, D., Ogryzko, V., Kao, H. Y., Nash, A., Chen, H., Nakatani, Y. & Evans, R. M. (1999). A viral mechanism for inhibition of p300 and PCAF acetyltransferase activity. *Cell* **96**, 393–403.

Chanock, R. M., Ludwig, W., Heubner, R. J., Cate, T. R. & Chu, L. W. (1966). Immunization by selective infection with type 4 adenovirus grown in human diploid tissue cultures. I. Safety and lack of oncogenicity and tests for potency in volunteers. *Journal of the American Medical Association* **195**, 445–452.

Charles, P. C., Chen, X., Horwitz, M. S. & Brosnan, C. F. (1999). Differential chemokine induction by the mouse adenovirus type-1 in the central nervous system of susceptible and resistant strains of mice. *Journal of Neurovirology* **5**, 55–64.

Chen, L., Anton, M. & Graham, F. L. (1996). Production and characterization of human 293 cell lines expressing the site-specific recombinase Cre. *Somatic Cell Molecular Genetics* **22**, 477–488.

Chillon, M., Bosch, A., Zabner, J., Law, L., Armentano, D., Welsh, M. J. & Davidson, B. L. (1999). Group D adenoviruses infect primary central nervous system cells more efficiently than those from group C. *Journal of Virology* **73**, 2537–2540.

Chiou, S. K. & White, E. (1998). Inhibition of ICE-like proteases inhibits apoptosis and increases virus production during adenovirus infection. *Virology* **244**, 108–118.

Chirmule, N., Hughes, J. V., Gao, G. P., Raper, S. E. & Wilson, J. M. (1998). Role of E4 in eliciting CD4 T-cell and B-cell responses to adenovirus vectors delivered to murine and nonhuman primate lungs. *Journal of Virology* **72**, 6138–6145.

Chirmule, N., Moscioni, A. D., Qian, Y., Qian, R., Chen, Y. & Wilson, J. M. (1999). Fas–Fas ligand interactions play a major role in effector functions of cytotoxic T lymphocytes after adenovirus vector-mediated gene transfer. *Human Gene Therapy* **10**, 259–269.

Chiu, C. Y., Mathias, P., Nemerow, G. R. & Stewart, P. L. (1999). Structure of adenovirus complexed with its internalization receptor, $\alpha v\beta 5$ integrin. *Journal of Virology* **73**, 6759–6768.

Chroboczek, J., Ruigrok, R. W. H. & Cusack, S. (1995). Adenovirus fiber. In *The Molecular Repertoire of Adenoviruses*, pp. 163–200. Edited by W. Doerfler & P. Bohm. Berlin: Springer.

Cirielli, C., Inyaku, K., Capogrossi, M. C., Yuan, X. & Williams, J. A. (1999). Adenovirus-mediated wild-type p53 expression induces apoptosis and suppresses tumorigenesis of experimental intracranial human malignant glioma. *Journal of Neuroncology* **43**, 99–108.

Clesham, G. J., Adam, P. J., Proudfoot, D., Flynn, P. D., Efstathiou, S. & Weissberg, P. L. (1998). High adenoviral loads stimulate NF kappaB-dependent gene expression in human vascular smooth muscle cells. *Gene Therapy* **5**, 174–180.

Cook, J. L., Routes, B. A., Walker, T. A., Colvin, K. L. & Routes, J. M. (1999). E1A oncogene induction of cellular susceptibility to killing by cytolytic lymphocytes through target cell sensitization to apoptotic injury. *Experimental Cell Research* **251**, 414–423.

Corti, O., Sabate, O., Horellou, P., Colin, P., Dumas, S., Buchet, D., Buc-Caron, M. H. & Mallet, J. (1999*a*). A single adenovirus vector mediates doxycycline-controlled expression of tyrosine hydroxylase in brain grafts of human neural progenitors. *Nature Biotechnology* **17**, 349–354.

Corti, O., Sanchez-Capelo, A., Colin, P., Hanoun, N., Hamon, M. & Mallet, J. (1999*b*). Long-term doxycycline-controlled expression of human tyrosine hydroxylase after direct adenovirus-mediated gene transfer to a rat model of Parkinson's disease. *Proceedings of the National Academy of Sciences, USA* **96**, 12120–12125.

Croyle, M. A., Roessler, B. J., Hsu, C. P., Sun, R. & Amidon, G. L. (1998*a*). Beta cyclodextrins enhance adenoviral-mediated gene delivery to the intestine. *Pharmacy Research* **15**, 1348–1355.

Croyle, M. A., Stone, M., Amidon, G. L. & Roessler, B. J. (1998*b*). In vitro and in vivo assessment of adenovirus 41 as a vector for gene delivery to the intestine. *Gene Therapy* **5**, 645–654.

Croyle, M. A., Walter, E., Janich, S., Roessler, B. J. & Amidon, G. L. (1998*c*). Role of integrin expression in adenovirus-mediated gene delivery to the intestinal epithelium. *Human Gene Therapy* **9**, 561–573.

Crystal, R. G. (1999). In vivo and ex vivo gene therapy strategies to treat tumors using adenovirus gene transfer vectors. *Cancer Chemotherapy and Pharmacology* **43**, S90–S99.

Dales, S. & Chardonnet, Y. (1973). Early events in the interaction of adenoviruses with HeLa cells. IV. Association with microtubules and the nuclear pore complex during vectorial movement of the inoculum. *Virology* **56**, 465–483.

De Geest, B., Van Linthout, S., Lox, M., Collen, D. & Holvoet, P. (2000). Sustained expression of human apolipoprotein A-I after adenoviral gene transfer in C57BL/6 mice: role of apolipoprotein A-I promoter, apolipoprotein A-I introns, and human apolipoprotein E enhancer. *Human Gene Therapy* **11**, 101–112.

de Moissac, D., Zheng, H. & Kirshenbaum, L. A. (1999). Linkage of the BH4 domain of Bcl-2 and the nuclear factor kappaB signaling pathway for suppression of apoptosis. *Journal of Biological Chemistry* **274**, 29505–29509.

Deryckere, F. & Burgert, H. G. (1996). Tumor necrosis factor alpha induces the adenovirus early 3 promoter by activation of NF-kappaB. *Journal of Biological Chemistry* **271**, 30249–30255.

de Stanchina, E., McCurrach, M. E., Zindy, F., Shieh, S. Y., Ferbeyre, G., Samuelson, A. V., Prives, C., Roussel, M. F., Sherr, C. J. & Lowe, S. W. (1998). E1A signaling to p53 involves the p19 (ARF) tumor suppressor. *Genes & Development* **12**, 2434–2442.

Desterro, J. M., Rodriguez, M. S., Kemp, G. D. & Hay, R. T. (1999). Identification of the enzyme required for activation of the small ubiquitin-like protein SUMO-1. *Journal of Biological Chemistry* **274**, 10618–10624.

Dewey, R. A., Morrissey, G., Cowsill, C. M., Stone, D., Bolognani, F., Dodd, N. J., Southgate, T. D., Klatzmann, D., Lassmann, H., Castro, M. G. & Lowenstein, P. R. (1999). Chronic brain inflammation and persistent herpes simplex virus 1 thymidine kinase expression in survivors of syngeneic glioma treated by adenovirus-mediated gene therapy: implications for clinical trials. *Nature Medicine* **5**, 1256–1263.

Diaz, R. M., Todryk, S., Chong, H., Hart, I. R., Sikora, K., Dorudi, S. & Vile, R. G. (1998). Rapid adenoviral transduction of freshly resected tumour explants with therapeutically useful genes provides a rationale for genetic immunotherapy for colorectal cancer. *Gene Therapy* **5**, 869–879.

Dodds, E., Piper, T. A., Murphy, S. J. & Dickson, G. (1999). Cationic lipids and polymers are able to enhance adenoviral infection of cultured mouse myotubes. *Journal of Neurochemistry* **72**, 2105–2112.

Dorai, T., Perlman, H., Walsh, K., Shabsigh, A., Goluboff, E. T., Olsson, C. A. & Buttyan, R. (1999). A recombinant defective adenoviral agent expressing anti-bcl-2 ribozyme promotes apoptosis of bcl-2-expressing human prostate cancer cells. *International Journal of Cancer* **82**, 846–852.

Dorsman, J. C., Teunisse, A. F. A. S., Zantema, A. & van der Eb, A. J. (1997). The adenovirus 12 E1A proteins can bind directly to proteins of the p300 transcription co-activator family, including the CREB-binding protein CBP and p300. *Journal of General Virology* **78**, 423–426.

Doucas, V., Ishov, A. M., Romo, A., Juguilon, H., Weitzman, M. D., Evans, R. M. & Maul, G. G. (1996). Adenovirus replication is coupled with the dynamic properties of the PML nuclear structure. *Genes & Development* **10**, 196–207.

Douglas, J. T., Miller, C. R., Kim, M., Dmitriev, I., Mikheeva, G., Krasnykh, V. & Curiel, D. T. (1999). A system for the propagation of adenoviral vectors with genetically modified receptor specificities. *Nature Biotechnology* **17**, 470–475.

Dunphy, E. J., Redman, R. A., Herweijer, H. & Cripe, T. P. (1999). Reciprocal enhancement of gene transfer by combinatorial adenovirus transduction and plasmid DNA transfection in vitro and in vivo. *Human Gene Therapy* **10**, 2407–2417.

Edgell, C. J., Curiel, D. T., Hu, P. C. & Marr, H. S. (1998). Efficient gene transfer to human endothelial cells using DNA complexed to adenovirus particles. *Biotechniques* **25**, 264–268, 270–272.

Einfeld, D. A., Brough, D. E., Roelvink, P. W., Kovesdi, I. & Wickham, T. J. (1999). Construction of a pseudoreceptor that mediates transduction by adenoviruses expressing a ligand in fiber or penton base. *Journal of Virology* **73**, 9130–9136.

Eischen, C. M., Weber, J. D., Roussel, M. F., Sherr, C. J. & Cleveland, J. L. (1999). Disruption of the ARF–Mdm2–p53 tumor suppressor pathway in Myc-induced lymphomagenesis. *Genes & Development* **13**, 2658–2669.

Eissa, N. T., Chu, C. S., Danel, C. & Crystal, R. G. (1994). Evaluation of the respiratory epithelium of normals and individuals with cystic fibrosis for the presence of adenovirus E1a sequences relevant to the use of E1a— adenovirus vectors for gene therapy for the respiratory manifestations of cystic fibrosis. *Human Gene Therapy* **5**, 1105–1114.

Elkon, K. B., Liu, C. C., Gall, J. G., Trevejo, J., Marino, M. W., Abrahamsen, K. A., Song, X., Zhou, J. L., Old, J. L., Crystal, R. G. & Falck-Pederson, E. (1997). Tumor necrosis factor alpha plays a central role in immune-mediated clearance of adenoviral vectors. *Proceedings of the National Academy of Sciences, USA* **94**, 9814–9819.

Elliott, W. M., Hayashi, S. & Hogg, J. C. (1995). Immunodetection of adenoviral E1A proteins in human lung tissue. *American Journal of Respiratory Cell Molecular Biology* **12**, 642–648.

Emtage, P. C., Wan, Y., Hitt, M., Graham, F. L., Muller, W. J., Zlotnik, A. & Gauldie, J. (1999). Adenoviral vectors expressing lymphotactin and interleukin 2 or lymphotactin and interleukin 12 synergize to facilitate tumor regression in murine breast cancer models. *Human Gene Therapy* **10**, 697–709.

Faha, B., Harlow, E. & Lees, E. (1993). The adenovirus E1A-associated kinase consists of cyclin E–p33cdk2 and cyclin A–p33cdk2. *Journal of Virology* **67**, 2456–2465.

Fallaux, F. J., Bout, A., van der Velde, I., van der Wollenberg, D. J., Hehir, K. M., Keegan, J., Augur, C., Cramer, S. J., van Ormondt, H., van der Eb, A. J., Valerio, D. & Hoeben, R. C. (1998). New helper cells and matched early region 1-deleted adenovirus vectors prevent generation of replication-competent adenoviruses. *Human Gene Therapy* **9**, 1909–1917.

Fallaux, F. J., van der Eb, A. J. & Hoeben, R. C. (1999). Who's afraid of replication-competent adenoviruses? *Gene Therapy* **6**, 709–712.

Fasbender, A., Zabner, J., Chillon, M., Moninger, T. O., Puga, A. P., Davidson, B. L. & Welsh, M. J. (1997). Complexes of adenovirus with polycationic polymers and cationic lipids increase the efficiency of gene transfer in vitro and in vivo. *Journal of Biological Chemistry* **272**, 6479–6489.

Fasbender, A., Lee, J. H., Walters, R. W., Moninger, T. O., Zabner, J. & Welsh, M. J. (1998). Incorporation of adenovirus in calcium phosphate precipitates enhances gene transfer to airway epithelia in vitro and in vivo. *Journal of Clinical Investigation* **102**, 184–193.

Fechner, H., Haack, A., Wang, H., Wang, X., Eizema, K., Pauschinger, M., Schoemaker, R., Veghel, R., Houtsmuller, A., Schultheiss, H. P., Lamers, J. & Poller, W. (1999). Expression of coxsackie adenovirus receptor and αv-integrin does not correlate with adenovector targeting in vivo indicating anatomical vector barriers. *Gene Therapy* **6**, 1520–1535.

Feigenblum, D., Walker, R. & Schneider, R. J. (1998). Adenovirus induction of an interferon-regulatory factor during entry into the late phase of infection. *Journal of Virology* **72**, 9257–9266.

Ferreira, V., Sidenius, N., Tarantino, N., Hubert, P., Chatenoud, L., Blasi, F. & Korner, M. (1999). In vivo inhibition of NF-kappa B in T-lineage cells leads to a dramatic decrease in cell proliferation and cytokine production and to increased cell apoptosis in response to mitogenic stimuli, but not to abnormal thymopoiesis. *Journal of Immunology* **162**, 6442–6450.

Fessler, S. P. & Young, C. S. (1998). Control of adenovirus early gene expression during the late phase of infection. *Journal of Virology* **72**, 4049–4056.

Fisher, L. J. (1997). Neural precursor cells: applications for the study and repair of the central nervous system. *Neurobiology and Disease* **4**, 1–22.

Fisher, K., Stallwood, Y., Ulbrich, K., Mautner, V. & Seymour, L. (2000). Protection and retargeting of adenovirus using a multifunctional hydrophilic polymer. *Molecular Therapy* **1**, S57.

Flomenberg, P., Piaskowski, V., Truitt, R. L. & Casper, J. T. (1995). Characterization of human proliferative T cell responses to adenoviruses. *Journal of Infectious Diseases* **171**, 1090–1096.

Foxwell, B., Browne, K., Bondeson, J., Clarke, C., de Martin, R., Brennan, F. & Feldmann, M. (1998). Efficient adenoviral infection with IκB α reveals that macrophage tumor necrosis factor α production in rheumatoid arthritis is NF-κB dependent. *Proceedings of the National Academy of Sciences, USA* **95**, 8211–8215.

Franklin, C. M. (1999). Clinical experience with soluble TNF p75 receptor in rheumatoid arthritis. *Seminars on Arthritis and Rheumatism* **29**, 172–181.

Fredman, J. N. & Engler, J. A. (1993). Adenovirus precursor to terminal protein interacts with the nuclear matrix in vivo and in vitro. *Journal of Virology* **67**, 3384–3395.

Fujii, T., Garcia-Bermejo, M. L., Bernabo, J. L., Caamano, J., Ohba, M., Kuroki, T., Li, L., Yuspa, S. H. & Kazanietz, M. G. (2000). Involvement of protein kinase C δ (PKCδ) in phorbol ester-induced apoptosis in LNCaP prostate cancer cells. Lack of proteolytic cleavage of PKCδ. *Journal of Biological Chemistry* **275**, 7574–7582.

Gabaglia, C. R., Pedersen, B., Hitt, M., Burdin, N., Sercarz, E. E., Graham, F. L., Gauldie, J. & Braciak, T. A. (1999). A single intramuscular injection with an adenovirus-expressing IL-12 protects BALB/c mice against *Leishmania major* infection, while treatment with an IL-4-expressing vector increases disease susceptibility in B10.D2 mice. *Journal of Immunology* **162**, 753–760.

Gahery-Segard, H., Juillard, V., Gaston, J., Lengagne, R., Pavirani, A., Boulanger, P. & Guillet, J. G. (1997). Humoral immune response to the capsid components of recombinant adenoviruses: routes of immunization modulate virus-induced Ig subclass shifts. *European Journal of Immunology* **27**, 653–659.

Gahery-Segard, H., Farace, F., Godfrin, D., Gaston, J., Lengagne, R., Tursz, T., Boulanger, P. & Guillet, J. G. (1998). Immune response to recombinant capsid proteins of adenovirus in humans: antifiber and anti-penton base antibodies have a synergistic effect on neutralizing activity. *Journal of Virology* **72**, 2388–2397.

Gambotto, A., Tuting, T., McVey, D. L., Kovesdi, I., Tahara, H., Lotze, M. T. & Robbins, P. D. (1999). Induction of antitumor immunity by direct intratumoral injection of a recombinant adenovirus vector expressing interleukin-12. *Cancer Gene Therapy* **6**, 45–53.

Ganz, T. & Lehrer, R. I. (1998). Antimicrobial peptides of vertebrates. *Current Opinions in Immunology* **10**, 41–44.

Gao, G. P., Engdahl, R. K. & Wilson, J. M. (2000). A cell line for high-yield production of E1-deleted adenovirus vectors without the emergence of replication-competent virus. *Human Gene Therapy* **11**, 213–219.

Ghivizzani, S. C., Lechman, E. R., Kang, R., Tio, C., Kolls, J., Evans, C. H. & Robbins, P. D. (1998). Direct adenovirus-mediated gene transfer of interleukin 1 and tumor necrosis factor alpha soluble receptors to rabbit knees with experimental arthritis has local and distal anti-arthritic effects. *Proceedings of the National Academy of Sciences, USA* **95**, 4613–4618.

Ghoda, L., Lin, X. & Greene, W. C. (1997). The 90-kDa ribosomal S6 kinase (pp90rsk) phosphorylates the N-terminal regulatory domain of IκBα and stimulates its degradation in vitro. *Journal of Biological Chemistry* **272**, 21281–21288.

Gilbert, R., Nalbantoglu, J., Petrof, B. J., Ebihara, S., Guibinga, G. H., Tinsley, J. M., Kamen, A., Massie, B., Davies, K. E. & Karpati, G. (1999). Adenovirus-mediated utrophin gene transfer mitigates the dystrophic phenotype of mdx mouse muscles. *Human Gene Therapy* **10**, 1299–1310.

Ginsberg, H. S., Moldawer, L. L. & Prince, G. A. (1999). Role of the type 5 adenovirus gene encoding the early region 1B 55-kDa protein in pulmonary pathogenesis. *Proceedings of the National Academy of Sciences, USA* **96**, 10409–10411.

Goebel, E. A., Davidson, B. L., Graham, S. M. & Kern, J. A. (1998). Tumor reduction in vivo after adenoviral mediated gene transfer of the herpes simplex virus thymidine kinase gene and ganciclovir treatment in human head and neck squamous cell carcinoma. *Otolaryngology and Head and Neck Surgery* **119**, 331–336.

Goodbourn, S., Didcock, L. & Randall, R. E. (2000). Interferons: cell signalling, immune modulation, antiviral responses and virus countermeasures. *Journal of General Virology* **81**, 2341–2365.

Goodrum, F. D. & Ornelles, D. A. (1999). Roles for the E4 orf6, orf3, and E1B 55-kilodalton proteins in cell cycle-independent adenovirus replication. *Journal of Virology* **73**, 7474–7488.

Gorziglia, M. I., Lapcevich, C., Roy, S., Kang, Q., Kadan, M., Wu, V., Pechan, P. & Kaleko, M. (1999). Generation of an adenovirus vector lacking E1, E2a, E3, and all of E4 except open reading frame 3. *Journal of Virology* **73**, 6048–6055.

Graham, F. L. & Prevec, L. (1992). Adenovirus-based expression vectors and recombinant vaccines. *Biotechnology* **20**, 363–390.

Graham, F. L., Smiley, J., Russell, W. C. & Nairn, R. (1977). Characteristics of a human cell line transformed by DNA from human adenovirus type 5. *Journal of General Virology* **36**, 59–72.

Grand, R. J., Turnell, A. S., Mason, G. G., Wang, W., Milner, A. E., Mymryk, J. S., Rookes, S. M., Rivett, A. J. & Gallimore, P. H. (1999*a*). Adenovirus early region 1A protein binds to mammalian SUG1 – a regulatory component of the proteasome. *Oncogene* **18**, 449–458.

Grand, R. J., Parkhill, J., Szestak, T., Rookes, S. M., Roberts, S. & Gallimore, P. H. (1999*b*). Definition of a major p53 binding site on Ad2E1B58K protein and a possible nuclear localization signal on the Ad12E1B54K protein. *Oncogene* **18**, 955–965.

Greber, U. F., Webster, P., Weber, J. & Helenius, A. (1996). The role of the adenovirus protease on virus entry into cells. *EMBO Journal* **15**, 1766–1777.

Greber, U. F., Suomalainen, M., Stidwill, R. P., Boucke, K., Ebersold, M. W. & Helenius, A. (1997). The role of the nuclear pore complex in adenovirus DNA entry. *EMBO Journal* **16**, 5998–6007.

Gropp, R., Frye, M., Wagner, T. O. & Bargon, J. (1999). Epithelial defensins impair adenoviral infection: implication for adenovirus-mediated gene therapy. *Gene Therapy* **10**, 957–964.

Guidotti, J. E., Mignon, A., Haase, G., Caillaud, C., McDonell, N., Kahn, A. & Poenaru, L. (1999). Adenoviral gene therapy of the Tay–Sachs disease in hexosaminidase A-deficient knock-out mice. *Human Molecular Genetics* **8**, 831–838.

Haase, G., Pettmann, B., Bordet, T., Villa, P., Vigne, E., Schmalbruch, H. & Kahn, A. (1999). Therapeutic benefit of ciliary neurotrophic factor in progressive motor neuronopathy depends on the route of delivery. *Annals of Neurology* **45**, 296–304.

Halbert, D. N., Cutt, J. R. & Shenk, T. (1985). Adenovirus early region 4 encodes functions required for efficient DNA replication, late gene expression, and host cell shutoff. *Journal of Virology* **56**, 250–257.

Hale, T. K. & Braithwaite, A. W. (1999). The adenovirus oncoprotein E1a stimulates binding of transcription factor ETF to transcriptionally activate the p53 gene. *Journal of Biological Chemistry* **274**, 23777–23786.

Hall, A. R., Dix, B. R., O'Carroll, S. J. & Braithwaite, A. W. (1998). p53-dependent cell death/apoptosis is required for a productive adenovirus infection. *Nature Medicine* **4**, 1068–1072.

Hamamori, Y., Sartorelli, V., Ogryzko, V., Puri, P. L., Wu, H. Y., Wang, J. Y., Nakatani, Y. & Kedes, L. (1999). Regulation of histone acetyltransferases p300 and PCAF by the bHLH protein twist and adenoviral oncoprotein E1A. *Cell* **96**, 405–413.

Hammond, J. M., McCoy, R. J., Jansen, E. S., Morrissy, C. J., Hodgson, A. L. & Johnson, M. A. (2000). Vaccination with a single dose of a recombinant porcine adenovirus expressing the classical swine fever virus gp55 (E2) gene protects pigs against classical swine fever. *Vaccine* **18**, 1040–1050.

Han, J., Sabbatini, P., Perez, D., Rao, L., Modha, D. & White, E. (1996). The E1B 19K protein blocks apoptosis by interacting with and inhibiting the p53-inducible and death-promoting Bax protein. *Genes & Development* **10**, 461–477.

Harada, J. N. & Berk, A. J. (1999). p53-Independent and -dependent requirements for E1B-55K in adenovirus type 5 replication. *Journal of Virology* **73**, 5333–5344.

Harari, O. A., Wickham, T. J., Stocker, C. J., Kovesdi, I., Segal, D. M., Huehns, T. Y., Sarraf, C. & Haskard, D. O. (1999). Targeting an adenoviral gene vector to cytokine-activated vascular endothelium via E-selectin. *Gene Therapy* **6**, 801–807.

Hardy, S., Kitamura, M., Harris-Stansil, T., Dai, Y. & Phipps, M. L. (1997). Construction of adenovirus vectors through Cre-lox recombination. *Journal of Virology* **71**, 1842–1849.

Harrod, K. S., Hermiston, T. W., Trapnell, B. C., Wold, W. S. & Whitsett, J. A. (1998). Lung-specific expression of adenovirus E3-14.7K in transgenic mice attenuates adenoviral vector-mediated lung inflammation and enhances transgene expression. *Human Gene Therapy* **9**, 1885–1898.

Hart, I. R. (1996). Transcriptionally targeted gene therapy. *Current Topics in Microbiology and Immunology* **213**, 19–25.

Hartigan-O'Connor, D., Amalfitano, A. & Chamberlain, J. S. (1999). Improved production of gutted adenovirus in cells expressing adenovirus preterminal protein and DNA polymerase. *Journal of Virology* **73**, 7835–7841.

Harvey, B. G., Hackett, N. R., El-Sawy, T., Rosengart, T. K., Hirschowitz, E. A., Lieberman, M. D., Lesser, M. L. & Crystal, R. G. (1999). Variability of human systemic humoral immune responses to adenovirus gene transfer vectors administered to different organs. *Journal of Virology* **73**, 6729–6742.

Hateboer, G., Hijmans, E. M., Nooij, J. B., Schlenker, S., Jentsch, S. & Bernards, R. (1996). mUBC9, a novel adenovirus E1A-interacting protein that complements a yeast cell cycle defect. *Journal of Biological Chemistry* **271**, 25906–25911.

Hautala, T., Grunst, T., Fabrega, A., Freimuth, P. & Welsh, M. J. (1998). An interaction between penton base and alpha v integrins plays a minimal role in adenovirus-mediated gene transfer to hepatocytes in vitro and in vivo. *Gene Therapy* **5**, 1259–1264.

Hay, R. T., Freeman, A., Leith, I., Monaghan, A. & Webster, A. (1995). Molecular interactions during adenovirus DNA replication. In *The Molecular Repertoire of Adenoviruses*, pp. 31–48. Edited by W. Doerfler & P. Bohm. Berlin: Springer.

Hay, J. G., Shapiro, N., Sauthoff, H., Heitner, S., Phupakdi, W. & Rom, W. N. (1999*a*). Targeting the replication of adenoviral gene therapy vectors to lung cancer cells: the importance of the adenoviral E1b-55kD gene. *Human Gene Therapy* **10**, 579–590.

Hay, R. T., Vuillard, L., Desterro, J. M. & Rodriguez, M. S. (1999*b*). Control of NF-κB transcriptional activation by signal induced proteolysis of IκB α. *Philosophical Transactions of the Royal Society Series B* **354**, 1601–1609.

Hayes, A. L., Smith, C., Foxwell, B. M. & Brennan, F. M. (1999). CD45-induced tumor necrosis factor alpha production in monocytes is phosphatidylinositol 3-kinase-dependent and nuclear factor-kappaB-independent. *Journal of Biological Chemistry* **274**, 33455–33461.

He, T. C., Zhou, S., da Costa, L. T., Yu, J., Kinzler, K. W. & Vogelstein, B. (1998). A simplified system for generating recombinant adenoviruses. *Proceedings of the National Academy of Sciences, USA* **95**, 2509–2514.

He, Z., Wlazlo, A. P., Kowalczyk, D. W., Cheng, J., Xiang, Z. Q., Giles-Davis, W. & Ertl, H. C. (2000). Viral recombinant vaccines to the E6 and E7 antigens of HPV-16. *Virology* **270**, 146–161.

Hearing, P., Samulski, R. J., Wishart, W. L. & Shenk, T. (1987). Identification of a repeated sequence element required for efficient encapsidation of the adenovirus type 5 chromosome. *Journal of Virology* **61**, 2555–2558.

Hedlund, T. E., Meech, S. J., Srikanth, S., Kraft, A. S., Miller, G. J., Schaack, J. B. & Duke, R. C. (1999). Adenovirus-mediated expression of Fas ligand induces apoptosis of human prostate cancer cells. *Cell Death and Differentiation* **6**, 175–182.

Hehir, K. M., Armentano, D., Cardoza, L. M., Choquette, T. L., Berthelette, P. B., White, G. A., Couture, L. A., Everton, M. B., Keegan, J., Martin, J. M., Pratt, D. A., Smith, M. P., Smith, A. E. & Wadsworth, S. C. (1996). Molecular characterization of replication-competent variants of adenovirus vectors and genome modifications to prevent their occurrence. *Journal of Virology* **70**, 8459–8467.

Heise, C., Sampson-Johannes, A., Williams, A., McCormick, F., Von Hoff, D. D. & Kirn, D. H. (1997). ONYX-015, an E1B gene-attenuated adenovirus, causes tumor-specific cytolysis and antitumoral efficacy that can be augmented by standard chemotherapeutic agents. *Nature Medicine* **3**, 639–645.

Heise, C. C., Williams, A. M., Xue, S., Propst, M. & Kirn, D. H. (1999). Intravenous administration of ONYX-015, a selectively replicating adenovirus, induces antitumoral efficacy. *Cancer Research* **59**, 2623–2628.

Herman, J. R., Adler, H. L., Aguilar-Cordova, E., Rojas-Martinez, A., Woo, S., Timme, T. L., Wheeler, T. M., Thompson, T. C. & Scardino, P. T. (1999). In situ gene therapy for adenocarcinoma of the prostate: a phase I clinical trial. *Human Gene Therapy* **10**, 1239–1249.

Hidaka, C., Milano, E., Leopold, P. L., Bergelson, J. M., Hackett, N. R., Finberg, R. W., Wickham, T. J., Kovesdi, I., Roelvink, P. & Crystal, R. G. (1999). CAR-dependent and CAR-independent pathways of adenovirus vector-mediated gene transfer and expression in human fibroblasts. *Journal of Clinical Investigation* **103**, 579–587.

Hillemann, M. R. & Werner, J. R. (1954). Recovery of a new agent from patients with acute respiratory illness. *Proceedings of the Society for Experimental Biology and Medicine* **85**, 183–188.

Hirschowitz, E. A. & Crystal, R. G. (1999). Adenovirus-mediated expression of interleukin-12 induces natural killer cell activity and complements adenovirus-directed gp75 treatment of melanoma lung metastases. *American Journal of Respiratory Cell and Molecular Biology* **20**, 935–941.

Hirschowitz, E. A., Ohwada, A., Pascal, W. R., Russi, T. J. & Crystal, R. G. (1995). In vivo adenovirus-mediated gene transfer of the *Escherichia coli* cytosine deaminase gene to human colon carcinoma-derived tumors induces chemosensitivity to 5-fluorocytosine. *Human Gene Therapy* **6**, 1055–1063.

Hitt, M. M., Addison, C. L. & Graham, F. L. (1997). Human adenovirus vectors for gene transfer into mammalian cells. *Advances in Pharmacology* **40**, 137–206.

Hofmann, C., Loser, P., Cichon, G., Arnold, W., Both, G. W. & Strauss, M. (1999). Ovine adenovirus vectors overcome preexisting humoral immunity against human adenoviruses in vivo. *Journal of Virology* **73**, 6930–6936.

Honda, R. & Yasuda, H. (1999). Association of p19 (ARF) with Mdm2 inhibits ubiquitin ligase activity of Mdm2 for tumor suppressor p53. *EMBO Journal* **18**, 22–27.

Hong, S. S., Karayan, L., Tournier, J., Curiel, D. T. & Boulanger, P. A. (1997). Adenovirus type 5 fiber knob binds to MHC class I α2 domain at the surface of human epithelial and B lymphoblastoid cells. *EMBO Journal* **16**, 2294–2306.

Hong, S. S., Galaup, A., Peytavi, R., Chazal, N. & Boulanger, P. (1999*a*). Enhancement of adenovirus-mediated gene delivery by use of an oligopeptide with dual binding specificity. *Human Gene Therapy* **10**, 2577–2586.

Hong, S. S., Gay, B., Karayan, L., Dabauvalle, M. C. & Boulanger, P. (1999*b*). Cellular uptake and nuclear delivery of recombinant adenovirus penton base. *Virology* **262**, 163–177.

Horridge, J. J. & Leppard, K. N. (1998). RNA-binding activity of the E1B 55-kilodalton protein from human adenovirus type 5. *Journal of Virology* **72**, 9374–9379.

Horwitz, M. S., Evans, C. F., McGavern, D. B., Rodriguez, M. & Oldstone, M. B. (1997). Primary demyelination in transgenic mice expressing interferon-gamma. *Nature Medicine* **3**, 1037–1041.

Howell, J. M., Lochmuller, H., O'Hara, A., Fletcher, S., Kakulas, B. A., Massie, B., Nalbantoglu, J. & Karpati, G. (1998). High-level dystrophin expression after adenovirus-mediated dystrophin minigene transfer to skeletal muscle of dystrophic dogs: prolongation of expression with immunosuppression. *Human Gene Therapy* **9**, 629–634.

Huang, S., Kamata, T., Takada, Y., Ruggeri, Z. M. & Nemerow, G. R. (1996). Adenovirus interaction with distinct integrins mediates separate events in cell entry and gene delivery to hematopoietic cells. *Journal of Virology* **70**, 4502–4508.

Ilan, Y., Attavar, P., Takahashi, M., Davidson, A., Horwitz, M. S., Guida, J., Roy Chowdhury, N. & Roy Chowdhury, J. (1996). Induction of central tolerance by intrathymic inoculation of adenoviral antigens into the host thymus permits long-term gene therapy in Gunn rats. *Journal of Clinical Investigation* **98**, 2640–2647.

Ilan, Y., Jona, V. K., Sengupta, K., Davidson, A., Horwitz, M. S., Roy-Chowdhury, N. & Roy-Chowdhury, J. (1997). Transient immunosuppression with FK506 permits long-term expression of therapeutic genes introduced into the liver using recombinant adenoviruses in the rat. *Hepatology* **26**, 949–956.

Ilan, Y., Sauter, B., Roy Chowdhury, N., Reddy, B. V. N., Thummala, N. R., Droguett, G., Davidson, A., Ott, M., Horwitz, M. S. & Roy Chowdhury, J. (1998). Oral tolerization to adenoviral proteins permits repeated adenovirus-mediated gene therapy in rats with pre-existing immunity to adenoviruses. *Hepatology* **27**, 1368–1376.

Imazu, T., Shimizu, S., Tagami, S., Matsushima, M., Nakamura, Y., Miki, T., Okuyama, A. & Tsujimoto, Y. (1999). Bcl-2/E1B 19 kDa-interacting protein 3-like protein (Bnip3L) interacts with bcl-2/Bcl-xL and induces apoptosis by altering mitochondrial membrane permeability. *Oncogene* **18**, 4523–4529.

Irie, A., Anderegg, B., Kashani-Sabet, M., Ohkawa, T., Suzuki, T., Halks-Miller, M., Curiel, D. T. & Scanlon, K. J. (1999). Therapeutic efficacy of an adenovirus-mediated anti-H-ras ribozyme in experimental bladder cancer. *Antisense & Nucleic Acid Drug Development* **9**, 341–349.

Iwamoto, H. S., Trapnell, B. C., McConnell, C. J., Daugherty, C. & Whitsett, J. A. (1999). Pulmonary inflammation associated with repeated, prenatal exposure to an E1, E3-deleted adenoviral vector in sheep. *Gene Therapy* **6**, 98–106.

Ji, L., Bouvet, M., Price, R. E., Roth, J. A. & Fang, B. (1999). Reduced toxicity, attenuated immunogenicity and efficient mediation of human p53 gene expression in vivo by an adenovirus vector with deleted E1–E3 and inactivated E4 by GAL4–TATA replacement. *Gene Therapy* **6**, 393–442.

Jones, N. (1995). Transcriptional modulation by the adenovirus E1A gene. *Current Topics in Microbiology and Immunology* **199**, 59–80.

Jooss, K., Ertl, H. C. & Wilson, J. M. (1998*a*). Cytotoxic T-lymphocyte target proteins and their major histocompatibility complex class I restriction in response to adenovirus vectors delivered to mouse liver. *Journal of Virology* **72**, 2945–2954.

Jooss, K., Turka, L. A. & Wilson, J. M. (1998*b*). Blunting of immune responses to adenoviral vectors in mouse liver and lung with CTLA4Ig. *Gene Therapy* **5**, 309–319.

Jooss, K., Yang, Y., Fisher, K. J. & Wilson, J. M. (1998*c*). Transduction of dendritic cells by DNA viral vectors directs the immune response to transgene products in muscle fibers. *Journal of Virology* **72**, 4212–4223.

Ju, D. W., Tao, Q., Cheng, D. S., Zhang, W., Zhang, M., Hamada, H. & Cao, X. (2000). Adenovirus-mediated lymphotactin gene transfer improves therapeutic efficacy of cytosine deaminase suicide gene therapy in established murine colon carcinoma. *Gene Therapy* **7**, 329–338.

Juillard, V., Villefroy, P., Godfrin, D., Pavirani, A., Venet, A. & Guillet, J. G. (1995). Long-term humoral and cellular immunity induced by a single immunization with replication-defective adenovirus recombinant vector. *European Journal of Immunology* **25**, 3467–3473.

Kaneko, S., Hallenbeck, P., Kotani, T., Nakabayashi, H., McGarrity, G., Tamaoki, T., Anderson, W. F. & Chiang, Y. L. (1995). Adenovirus-mediated gene therapy of hepatocellular carcinoma using cancer-specific gene expression. *Cancer Research* **55**, 5283–5287.

Kaner, R. J., Worgall, S., Leopold, P. L., Stolze, E., Milano, E., Hidaka, C., Ramalingam, R., Hackett, N. R., Singh, R., Bergelson, J., Finberg, R., Falck-Pedersen, E. & Crystal, R. G. (1999). Modification of the genetic program of human alveolar macrophages by adenovirus vectors in vitro is feasible but inefficient, limited in part by the low level of expression of the coxsackie/adenovirus receptor. *American Journal of Respiratory Cell and Molecular Biology* **20**, 361–370.

Kannabiran, C., Morris, G. F. & Mathews, M. B. (1999). Dual action of the adenovirus E1A 243R oncoprotein on the human proliferating cell nuclear antigen promoter: repression of transcriptional activation by p53. *Oncogene* **18**, 7825–7833.

Kaplan, J. M. & Smith, A. E. (1997). Transient immunosuppression with deoxyspergualin improves longevity of transgene expression and ability to readminister adenoviral vector to the mouse lung. *Human Gene Therapy* **8**, 1095–1104.

Kaplan, J. M., Armentano, D., Sparer, T. E., Wynn, S. G., Peterson, P. A., Wadsworth, S. C., Couture, K. K., Pennington, S. E., St George, J. A., Gooding, L. R. & Smith, A. E. (1997). Characterization of factors involved in modulating persistence of transgene expression from recombinant adenovirus in the mouse lung. *Human Gene Therapy* **8**, 45–56.

Kaplan, J. M., Armentano, D., Scaria, A., Woodworth, L. A., Pennington, S. E., Wadsworth, S. C., Smith, A. E. & Gregory, R. J. (1999). Novel role for E4 region genes in protection of adenovirus vectors from lysis by cytotoxic T lymphocytes. *Journal of Virology* **73**, 4489–4492.

Kasof, G. M., Goyal, L. & White, E. (1999). Btf, a novel death-promoting transcriptional repressor that interacts with Bcl-2-related proteins. *Molecular and Cell Biology* **19**, 4390–4404.

Kass-Eisler, A., Falck-Pedersen, E., Elfenbein, D. H., Alvira, M., Buttrick, P. M. & Leinwand, L. A. (1994). The impact of developmental stage, route of administration and the immune system on adenovirus-mediated gene transfer. *Gene Therapy* **1**, 395–402.

Keblusek, P., Dorsman, J. C., Teunisse, A. F. A. S., Teunissen, H., van der Eb, A. J. & Zantema, A. (1999). The adenoviral E1A oncoproteins interfere with the growth-inhibiting effect of the cdk-inhibitor $p21^{CIP1/WAF1}$. *Journal of General Virology* **80**, 381–390.

Keicho, N., Higashimoto, Y., Bondy, G. P., Elliott, W. M., Hogg, J. C. & Hayashi, S. (1999). Endotoxin-specific NF-kappaB activation in pulmonary epithelial cells harboring adenovirus E1A. *American Journal of Physiology* **277**, L523–L532.

Kijima, T., Osaki, T., Nishino, K., Kumagai, T., Funakoshi, T., Goto, H., Tachibana, I., Tanio, Y. & Kishimoto, T. (1999). Application of the Cre recombinase/loxP system further enhances antitumor effects in cell type-specific gene therapy against carcinoembryonic antigen-producing cancer. *Cancer Research* **59**, 4906–4911.

Kikuchi, T. & Crystal, R. G. (1999). Anti-tumor immunity induced by in vivo adenovirus vector-mediated expression of CD40 ligand in tumor cells. *Human Gene Therapy* **10**, 1375–1387.

Kim, K. N., Watanabe, S., Ma, Y., Thornton, S., Giannini, E. H. & Hirsch, R. (2000). Viral IL-10 and soluble TNF receptor act synergistically to inhibit collagen-induced arthritis following adenovirus-mediated gene transfer. *Journal of Immunology* **164**, 1576–1581.

Kimura, K. & Gelmann, E. P. (2000). Tumor necrosis factor-alpha and fas activate complementary fas-associated death domain-dependent pathways that enhance apoptosis induced by gamma-irradiation. *Journal of Biological Chemistry* **275**, 8610–8617.

Kirby, I., Davison, E., Beavil, A. J., Soh, C. P., Wickham, T. J., Roelvink, P. W., Kovesdi, I., Sutton, B. J. & Santis, G. (1999). Mutations in the DG loop of adenovirus type 5 fiber knob protein abolish high-affinity binding to its cellular receptor CAR. *Journal of Virology* **73**, 9508–9514.

Kirby, I., Davison, E., Beavil, A. J., Soh, C. P., Wickham, T. J., Roelvink, P. W., Kovesdi, I., Sutton, B. J. & Santis, G. (2000). Identification of

contact residues and definition of the CAR-binding site of adenovirus type 5 fiber protein. *Journal of Virology* **74**, 2804–2813.

Konig, C., Roth, J. & Dobbelstein, M. (1999). Adenovirus type 5 E4orf3 protein relieves p53 inhibition by E1B-55-kilodalton protein. *Journal of Virology* **73**, 2253–2262.

Krajcsi, P., Dimitrov, T., Hermiston, T. W., Tollefson, A. E., Ranheim, T. S., Vande Pol, S. B., Stephenson, A. H. & Wold, W. S. (1996). The adenovirus E3-14.7K protein and the E3-10.4K/14.5K complex of proteins, which independently inhibit tumor necrosis factor (TNF)-induced apoptosis, also independently inhibit TNF-induced release of arachidonic acid. *Journal of Virology* **70**, 4904–4913.

Krasnykh, V., Dmitriev, I., Mikheeva, G., Miller, C. R., Belousova, N. & Curiel, D. T. (1998). Characterization of an adenovirus vector containing a heterologous peptide epitope in the HI loop of the fiber knob. *Journal of Virology* **72**, 1844–1852.

Kremer, E. J., Boutin, S., Chillon, M. & Danos, O. (2000). Canine adenovirus vectors: an alternative for adenovirus-mediated gene transfer. *Journal of Virology* **74**, 505–512.

Kuhnel, F., Zender, L., Paul, Y., Tietze, M. K., Trautwein, C., Manns, M. & Kubicka, S. (2000). NFκB mediates apoptosis through transcriptional activation of Fas (CD95) in adenoviral hepatitis. *Journal of Biological Chemistry* **275**, 6421–6427.

Kumar-Singh, R. & Chamberlain, J. S. (1996). Encapsidated adenovirus minichromosomes allow delivery and expression of a 14 kb dystrophin cDNA to muscle cells. *Human Molecular Genetics* **5**, 913–921.

Kvist, S., Ostberg, L., Persson, H., Philipson, L. & Peterson, P. A. (1978). Molecular association between transplantation antigens and cell surface antigen in adenovirus-transformed cell line. *Proceedings of the National Academy of Sciences, USA* **75**, 5674–5678.

Ladiwala, U., Li, H., Antel, J. P. & Nalbantoglu, J. (1999). p53 induction by tumor necrosis factor-alpha and involvement of p53 in cell death of human oligodendrocytes. *Journal of Neurochemistry* **73**, 605–611.

Lanuti, M., Gao, G. P., Force, S. D., Chang, M. Y., El Kouri, C., Amin, K. M., Hughes, J. V., Wilson, J. M., Kaiser, L. R. & Albelda, S. M. (1999). Evaluation of an E1E4-deleted adenovirus expressing the herpes simplex thymidine kinase suicide gene in cancer gene therapy. *Human Gene Therapy* **10**, 463–475.

Lechman, E. R., Jaffurs, D., Ghivizzani, S. C., Gambotto, A., Kovesdi, I., Mi, Z., Evans, C. H. & Robbins, P. D. (1999). Direct adenoviral gene transfer of viral IL-10 to rabbit knees with experimental arthritis ameliorates disease in both injected and contralateral control knees. *Journal of Immunology* **163**, 2202–2208.

Lee, J. H., Zabner, J. & Welsh, M. J. (1999*a*). Delivery of an adenovirus vector in a calcium phosphate coprecipitate enhances the therapeutic index of gene transfer to airway epithelia. *Human Gene Therapy* **10**, 603–613.

Lee, W. C., Wan, Y. H., Li, W., Fu, F., Sime, P. J., Gauldie, J., Thomson, A. W., Fung, J. J., Lu, L. & Qian, S. (1999*b*). Enhancement of dendritic cell tolerogenicity by genetic modification using adenoviral vectors encoding cDNA for TGF beta 1. *Transplantation Proceedings* **31**, 1195.

Legrand, V., Spehner, D., Schlesinger, Y., Settelen, N., Pavirani, A. & Mehtali, M. (1999). Fiberless recombinant adenoviruses: virus maturation and infectivity in the absence of fiber. *Journal of Virology* **73**, 907–919.

Leon, R. P., Hedlund, T., Meech, S. J., Li, S., Schaack, J., Hunger, S. P., Duke, R. C. & DeGregori, J. (1998). Adenoviral-mediated gene transfer in lymphocytes. *Proceedings of the National Academy of Sciences, USA* **95**, 13159–13164.

Leonard, G. T. & Sen, G. C. (1996). Effects of adenovirus E1A protein on interferon-signaling. *Virology* **224**, 25–33.

Leonard, G. T. & Sen, G. C. (1997). Restoration of interferon responses of adenovirus E1A-expressing HT1080 cell lines by overexpression of p48 protein. *Journal of Virology* **71**, 5095–5101.

Leopold, P. L., Kreitzer, G., Miyazawa, N., Rempel, S., Pfister, K. K., Rodriguez-Boulan, E. & Crystal, R. G. (2000). Dynein- and microtubule-mediated translocation of adenovirus serotype 5 occurs after endosomal lysis. *Human Gene Therapy* **11**, 151–165.

Leppard, K. N. (1997). E4 gene function in adenovirus, adenovirus vector and adeno-associated virus infections. *Journal of General Virology* **78**, 2131–2138.

Leppard, K. N. & Everett, R. D. (1999). The adenovirus type 5 E1b 55K and E4 Orf3 proteins associate in infected cells and affect ND10 components. *Journal of General Virology* **80**, 997–1008.

Li, W., Berencsi, K., Basak, S., Somasundaram, R., Ricciardi, R. P., Gonczol, E., Zaloudik, J., Linnenbach, A., Maruyama, H., Miniou, P. & Herlyn, D. (1997). Human colorectal cancer (CRC) antigen CO17-1A/GA733 encoded by adenovirus inhibits growth of established CRC cells in mice. *Journal of Immunology* **159**, 763–769.

Li, E., Stupack, D., Bokoch, G. M. & Nemerow, G. R. (1998). Adenovirus endocytosis requires actin cytoskeleton reorganization mediated by Rho family GTPases. *Journal of Virology* **72**, 8806–8812.

Li, H., Alonso-Vanegas, M., Colicos, M. A., Jung, S. S., Lochmuller, H., Sadikot, A. F., Snipes, G. J., Seth, P., Karpati, G. & Nalbantoglu, J. (1999*a*). Intracerebral adenovirus-mediated p53 tumor suppressor gene therapy for experimental human glioma. *Clinical Cancer Research* **5**, 637–642.

Li, Y., Kang, J., Friedman, J., Tarassishin, L., Ye, J., Kovalenko, A., Wallach, D. & Horwitz, M. S. (1999*b*). Identification of a cell protein (FIP-3) as a modulator of NF-κB activity and as a target of an adenovirus inhibitor of tumor necrosis factor α-induced apoptosis. *Proceedings of the National Academy of Sciences, USA* **96**, 1042–1047.

Li, Y., Pong, R. C., Bergelson, J. M., Hall, M. C., Sagalowsky, A. I., Tseng, C. P., Wang, Z. & Hsieh, J. T. (1999 *c*). Loss of adenoviral receptor expression in human bladder cancer cells: a potential impact on the efficacy of gene therapy. *Cancer Research* **59**, 325–330.

Lieber, A., He, C. Y., Meuse, L., Schowalter, D., Kirillova, I., Winther, B. & Kay, M. A. (1997). The role of Kupffer cell activation and viral gene expression in early liver toxicity after infusion of recombinant adenovirus vectors. *Journal of Virology* **71**, 8798–8807.

Lieber, A., He, C. Y., Meuse, L., Himeda, C., Wilson, C. & Kay, M. A. (1998). Inhibition of NF-κB activation in combination with bcl-2 expression allows for persistence of first-generation adenovirus vectors in the mouse liver. *Journal of Virology* **72**, 9267–9277.

Lieber, A., Steinwaerder, D. S., Carlson, C. A. & Kay, M. A. (1999). Integrating adenovirus–adeno-associated virus hybrid vectors devoid of all viral genes. *Journal of Virology* **73**, 9314–9324.

Lin, J., Chen, J., Elenbaas, B. & Levine, A. J. (1994). Several hydrophobic amino acids in the p53 amino-terminal domain are required for transcriptional activation, binding to mdm-2 and the adenovirus 5 E1B 55-kD protein. *Genes & Development* **8**, 1235–1246.

Linette, G. P., Shankara, S., Longerich, S., Yang, S., Doll, R., Nicolette, C., Preffer, F. I., Roberts, B. L. & Haluska, F. G. (2000). In vitro priming with adenovirus/gp100 antigen-transduced dendritic cells reveals the epitope specificity of HLA-A*0201-restricted CD8+ T cells in patients with melanoma. *Journal of Immunology* **164**, 3402–3412.

Lipinski, K. S., Fax, P., Wilker, B., Hennemann, H., Brockmann, D. & Esche, H. (1999). Differences in the interactions of oncogenic adenovirus 12 early region 1A and nononcogenic adenovirus 2 early region 1A with the cellular coactivators p300 and CBP. *Virology* **255**, 94–105.

Look, D. C., Roswit, W. T., Frick, A. G., Gris-Alevy, Y., Dickhaus, D. M., Walter, M. J. & Holtzman, M. J. (1998). Direct suppression of Stat1 function during adenoviral infection. *Immunity* **9**, 871–880.

Lubberts, E., Joosten, L. A., van den Bersselaar, L., Helsen, M. M., Bakker, A. C., van Meurs, J. B., Graham, F. L., Richards, C. D. & van den Berg, W. B. (1999). Adenoviral vector-mediated overexpression of IL-4 in the knee joint of mice with collagen-induced arthritis prevents cartilage destruction. *Journal of Immunology* **163**, 4546–4556.

Lukashok, S. A. & Horwitz, M. S. (1998). New perspectives in adenoviruses. *Current Clinical Topics in Infectious Diseases* **18**, 286–305.

Lukashok, S. A., Tarassishin, L., Li, Y. & Horwitz, M. S. (2000). An adenovirus inhibitor of tumor necrosis factor alpha-induced apoptosis complexes with dynein and a small GTPase. *Journal of Virology* **74**, 4705–4709.

Lusky, M., Christ, M., Rittner, K., Dieterle, A., Dreyer, D., Mourot, B., Schultz, H., Stoeckel, F., Pavirani, A. & Mehtali, M. (1998). In vitro and in vivo biology of recombinant adenovirus vectors with E1, E1/E2A, or E1/E4 deleted. *Journal of Virology* **72**, 2022–2032.

Lusky, M., Grave, L., Dieterle, A., Dreyer, D., Christ, M., Ziller, C., Furstenberger, P., Kintz, J., Ali Hadji, D., Pavirani, A. & Mehtali, M. (1999). Regulation of adenovirus-mediated transgene expression by the viral E4 gene products: requirement for E4 ORF3. *Journal of Virology* **73**, 8308–8319.

Lutz, P. & Kedinger, C. (1996). Properties of the adenovirus IVa2 gene product, an effector of late-phase-dependent activation of the major late promoter. *Journal of Virology* **70**, 1396–1405.

Lutz, P., Rosa-Calatrava, M. & Kedinger, C. (1997). The product of the adenovirus intermediate gene IX is a transcriptional activator. *Journal of Virology* **71**, 5102–5109.

McDonald, C. & Reich, N. C. (1999). Cooperation of the transcriptional coactivators CBP and p300 with Stat6. *Journal of Interferon and Cytokine Research* **19**, 711–722.

Maestro, R., Dei Tos, A. P., Hamamori, Y., Krasnokutsky, S., Sartorelli, V., Kedes, L., Doglioni, C., Beach, D. H. & Hannon, G. J. (1999). Twist is a potential oncogene that inhibits apoptosis. *Genes & Development* **13**, 2207–2217.

Mahr, J. A. & Gooding, L. R. (1999). Immune evasion by adenoviruses. *Immunological Reviews* **168**, 121–130.

Maini, R., St Clair, E. W., Breedveld, F., Furst, D., Kalden, J., Weisman, M., Smolen, J., Emery, P., Harriman, G., Feldmann, M. & Lipsky, P. (1999). Infliximab (chimeric anti-tumour necrosis factor alpha monoclonal antibody) versus placebo in rheumatoid arthritis patients receiving concomitant methotrexate: a randomised phase III trial. ATTRACT Study Group. *Lancet* **354**, 1932–1939.

Mannervik, M., Fan, S., Strom, A. C., Helin, K. & Akusjarvi, G. (1999). Adenovirus E4 open reading frame 4-induced dephosphorylation inhibits E1A activation of the E2 promoter and E2F-1-mediated transactivation independently of the retinoblastoma tumor suppressor protein. *Virology* **256**, 313–321.

Manome, Y., Abe, M., Hagen, M. F., Fine, H. A. & Kufe, D. W. (1994). Enhancer sequences of the DF3 gene regulate expression of the herpes simplex virus thymidine kinase gene and confer sensitivity of human breast cancer cells to ganciclovir. *Cancer Research* **54**, 5408–5413.

Marshall, E. (1999). Gene therapy death prompts review of adenovirus vector. *Science* **286**, 2244–2245.

Martin-Duque, P., Sanchez-Prieto, R., Romero, J., Martinez-Lamparero, A., Cebrian-Sagarriga, S., Guinea-Viniegra, J., Dominguez, C., Lleonart, M., Cano, A., Quintanilla, M. & Ramon, Y. C. S. (1999). In vivo radiosensitizing effect of the adenovirus E1A gene in murine and human malignant tumors. *International Journal of Oncology* **15**, 1163–1168.

Massie, B., Couture, F., Lamoureux, L., Mosser, D. D., Guilbault, C., Jolicoeur, P., Belanger, F. & Langelier, Y. (1998). Inducible overexpression of a toxic protein by an adenovirus vector with a tetracycline-regulatable expression cassette. *Journal of Virology* **72**, 2289–2296.

Mathews, M. B. & Shenk, T. (1991). Adenovirus-associated RNA and translational control. *Journal of Virology* **65**, 5657–5662.

Mathias, P., Galleno, M. & Nemerow, G. R. (1998). Interactions of soluble recombinant integrin αv $\beta 5$ with human adenoviruses. *Journal of Virology* **72**, 8669–8675.

Matthews, D. A. & Russell, W. C. (1994). Adenovirus protein–protein interactions: hexon and protein VI. *Journal of General Virology* **75**, 3365–3374.

Matthews, D. A. & Russell, W. C. (1995). Adenovirus protein–protein interactions: molecular parameters governing the binding of protein VI to hexon and the activation of the adenovirus 23K protease. *Journal of General Virology* **76**, 1959–1969.

Matthews, D. A. & Russell, W. C. (1998*a*). Adenovirus core protein V is delivered by the invading virus to the nucleus of the infected cell and later in infection is associated with nucleoli. *Journal of General Virology* **79**, 1671–1675.

Matthews, D. A. & Russell, W. C. (1998*b*). Adenovirus core protein V interacts with p32 – a protein which is associated with both the mitochondria and the nucleus. *Journal of General Virology* **79**, 1677–1685.

Matthews, D. A., Cummings, D., Evelegh, C., Graham, F. L. & Prevec, L. (1999). Development and use of a 293 cell line expressing *lac* repressor for the rescue of recombinant adenoviruses expressing high levels of rabies virus glycoprotein. *Journal of General Virology* **80**, 345–353.

Mautner, V. (1989). *Adenoviridae*. In *Andrewes' Viruses of Vertebrates*, 5th edn, pp. 249–282. Edited by J. S. Porterfield. London: Ballière Tindall.

Mazzarelli, J. M., Mengus, G., Davidson, I. & Ricciardi, R. P. (1997). The transactivation domain of adenovirus E1A interacts with the C terminus of human $TAF_{II}135$. *Journal of Virology* **71**, 7978–7983.

Mazzolini, G., Qian, C., Narvaiza, I., Barajas, M., Borras-Cuesta, F., Xie, X., Duarte, M., Melero, I. & Prieto, J. (2000). Adenoviral gene transfer of interleukin 12 into tumors synergizes with adoptive T cell therapy both at the induction and effector level. *Human Gene Therapy* **11**, 113–125.

Meloni, A. R., Smith, E. J. & Nevins, J. R. (1999). A mechanism for Rb/p130-mediated transcription repression involving recruitment of the CtBP corepressor. *Proceedings of the National Academy of Sciences, USA* **96**, 9574–9579.

Mentel, R., Dopping, G., Wegner, U., Seidel, W., Liebermann, H. & Dohner, L. (1997). Adenovirus-receptor interaction with human lymphocytes. *Journal of Medical Virology* **51**, 252–257.

Meredith, J. E., Jr, Winitz, S., Lewis, J. M., Hess, S., Ren, X. D., Renshaw, M. W. & Schwartz, M. A. (1996). The regulation of growth and intracellular signaling by integrins. *Endocrinology Reviews* **17**, 207–220.

Mette, S. A., Pilewski, J., Buck, C. A. & Albelda, S. M. (1993). Distribution of integrin cell adhesion receptors on normal bronchial epithelial cells and lung cancer cells in vitro and in vivo. *American Journal of Research in Respiratory Cell and Molecular Biology* **8**, 562–572.

Michael, S. I., Hong, J. S., Curiel, D. T. & Engler, J. A. (1995). Addition of a short peptide ligand to the adenovirus fiber protein. *Gene Therapy* **2**, 660–668.

Michou, A. I., Lehrmann, H., Saltik, M. & Cotten, M. (1999). Mutational analysis of the avian adenovirus CELO, which provides a basis for gene delivery vectors. *Journal of Virology* **73**, 1399–1410.

Millecamps, S., Kiefer, H., Navarro, V., Geoffroy, M. C., Robert, J. J., Finiels, F., Mallet, J. & Barkats, M. (1999). Neuron-restrictive silencer elements mediate neuron specificity of adenoviral gene expression. *Nature Biotechnology* **17**, 865–869.

Miller, N. & Whelan, J. (1997). Progress in transcriptionally targeted and regulatable vectors for genetic therapy. *Human Gene Therapy* **8**, 803–815.

Miller, C. R., Buchsbaum, D. J., Reynolds, P. N., Douglas, J. T., Gillespie, G. Y., Mayo, M. S., Raben, D. & Curiel, D. T. (1998). Differential susceptibility of primary and established human glioma cells to adenovirus infection: targeting via the epidermal growth factor receptor achieves fiber receptor-independent gene transfer. *Cancer Research* **58**, 5738–5748.

Miyazawa, N., Leopold, P. L., Hackett, N. R., Ferris, B., Worgall, S., Falck-Pedersen, E. & Crystal, R. G. (1999). Fiber swap between adenovirus subgroups B and C alters intracellular trafficking of adenovirus gene transfer vectors. *Journal of Virology* **73**, 6056–6065.

Mizuguchi, H. & Kay, M. A. (1998). Efficient construction of a recombinant adenovirus vector by an improved in vitro ligation method. *Human Gene Therapy* **9**, 2577–2583.

Moorhead, J. W., Clayton, G. H., Smith, R. L. & Schaack, J. (1999). A replication-incompetent adenovirus vector with the preterminal protein gene deleted efficiently transduces mouse ears. *Journal of Virology* **73**, 1046–1053.

Morral, N., O'Neal, W., Rice, K., Leland, M., Kaplan, J., Piedra, P. A., Zhou, H., Parks, R. J., Velji, R., Aguilar-Cordova, E., Wadsworth, S., Graham, F. L., Kochanek, S., Carey, K. D. & Beaudet, A. L. (1999). Administration of helper-dependent adenoviral vectors and sequential delivery of different vector serotype for long-term liver-directed gene transfer in baboons. *Proceedings of the National Academy of Sciences, USA* **96**, 12816–12821.

Morsy, M. A., Gu, M., Motzel, S., Zhao, J., Lin, J., Su, Q., Allen, H., Franlin, L., Parks, R. J., Graham, F. L., Kochanek, S., Bett, A. J. & Caskey, C. T. (1998). An adenoviral vector deleted for all viral coding sequences results in enhanced safety and extended expression of a leptin transgene. *Proceedings of the National Academy of Sciences, USA* **95**, 7866–7871.

Motoi, F., Sunamura, M., Ding, L., Duda, D. G., Yoshida, Y., Zhang, W., Matsuno, S. & Hamada, H. (2000). Effective gene therapy for pancreatic cancer by cytokines mediated by restricted replication-competent adenovirus. *Human Gene Therapy* **11**, 223–235.

Murray, K. D., Etheridge, C. J., Shah, S. I., Matthews, D. A., Russell, W. C., Miller, A. D. & Gurling, H. M. D. (2000). Synthetic viral vector complexes: enhanced cationic liposome-mediated transfection using the viral DNA-binding protein Mu. *Gene Therapy* (in press).

Muruve, D. A., Barnes, M. J., Stillman, I. E. & Libermann, T. A. (1999). Adenoviral gene therapy leads to rapid induction of multiple chemokines and acute neutrophil-dependent hepatic injury in vivo. *Human Gene Therapy* **10**, 965–976.

Ng, P., Parks, R. J., Cummings, D. T., Evelegh, C. M., Sankar, U. & Graham, F. L. (1999). A high-efficiency Cre/loxP-based system for construction of adenoviral vectors. *Human Gene Therapy* **10**, 2667–2672.

O'Connor, M. J., Zimmermann, H., Nielsen, S., Bernard, H. U. & Kouzarides, T. (1999). Characterization of an E1A–CBP interaction defines a novel transcriptional adapter motif (TRAM) in CBP/p300. *Journal of Virology* **73**, 3574–3581.

Ohi, N., Tokunaga, A., Tsunoda, H., Nakano, K., Haraguchi, K., Oda, K., Motoyama, N. & Nakajima, T. (1999). A novel adenovirus E1B19K-binding protein B5 inhibits apoptosis induced by Nip3 by forming a heterodimer through the C-terminal hydrophobic region. *Cell Death and Differentiation* **6**, 314–325.

O'Riordan, C. R., Lachapelle, A., Delgado, C., Parkes, V., Wadsworth, S. C., Smith, A. E. & Francis, G. E. (1999). PEGylation of adenovirus with retention of infectivity and protection from neutralizing antibody in vitro and in vivo. *Human Gene Therapy* **10**, 1349–1358.

Ozawa, H., Ding, W., Torii, H., Hosoi, J., Seiffert, K., Campton, K., Hackett, N. R., Topf, N., Crystal, R. G. & Granstein, R. D. (1999). Granulocyte–macrophage colony-stimulating factor gene transfer to dendritic cells or epidermal cells augments their antigen-presenting function including induction of anti-tumor immunity. *Journal of Investigative Dermatology* **113**, 999–1005.

Pahl, H. L., Sester, M., Burgert, H. G. & Baeuerle, P. A. (1996). Activation of transcription factor NF-κB by the adenovirus E3/19K protein requires its ER retention. *Journal of Cell Biology* **132**, 511–522.

Parks, R. J. & Graham, F. L. (1997). A helper-dependent system for adenovirus vector production helps define a lower limit for efficient DNA packaging. *Journal of Virology* **71**, 3293–3298.

Parks, R. J., Chen, L., Anton, M., Sankar, U., Rudnicki, M. A. & Graham, F. L. (1996). A helper-dependent adenovirus vector system: removal of helper virus by Cre-mediated excision of the viral packaging signal. *Proceedings of the National Academy of Sciences, USA* **93**, 13565–13570.

Parks, E., Strieter, R. M., Lukacs, N. W., Gauldie, J., Hitt, M., Graham, F. L. & Kunkel, S. L. (1998). Transient gene transfer of IL-12 regulates chemokine expression and disease severity in experimental arthritis. *Journal of Immunology* **160**, 4615–4619.

Parks, R., Evelegh, C. & Graham, F. (1999*a*). Use of helper-dependent adenoviral vectors of alternative serotypes permits repeat vector administration. *Gene Therapy* **6**, 1565–1573.

Parks, R. J., Bramson, J. L., Wan, Y., Addison, C. L. & Graham, F. L. (1999*b*). Effects of stuffer DNA on transgene expression from helper-dependent adenovirus vectors. *Journal of Virology* **73**, 8027–8034.

Pastore, L., Morral, N., Zhou, H., Garcia, R., Parks, R. J., Kochanek, S., Graham, F. L., Lee, B. & Beaudet, A. L. (1999). Use of a liver-specific promoter reduces immune response to the transgene in adenoviral vectors. *Human Gene Therapy* **10**, 1773–1781.

Patel, S. R., Lee, L. Y., Mack, C. A., Polce, D. R., El-Sawy, T., Hackett, N. R., Ilercil, A., Jones, E. C., Hahn, R. T., Isom, O. W., Rosengart, T. K. & Crystal, R. G. (1999). Safety of direct myocardial administration of an adenovirus vector encoding vascular endothelial growth factor 121. *Human Gene Therapy* **10**, 1331–1348.

Paulson, M., Pisharody, S., Pan, L., Guadagno, S., Mui, A. L. & Levy, D. E. (1999). Stat protein transactivation domains recruit p300/CBP through widely divergent sequences. *Journal of Biological Chemistry* **274**, 25343–25349.

Pearson, A. S., Spitz, F. R., Swisher, S. G., Kataoka, M., Sarkiss, M. G., Meyn, R. E., McDonnell, T. J., Cristiano, R. J. & Roth, J. A. (2000). Up-regulation of the proapoptotic mediators Bax and Bak after adenovirus-mediated p53 gene transfer in lung cancer cells. *Clinical Cancer Research* **6**, 887–890.

Peng, Y., Trevejo, J., Zhou, J., Marino, M. W., Crystal, R. G., Falck-Pedersen, E. & Elkon, K. B. (1999). Inhibition of tumor necrosis factor alpha by an adenovirus-encoded soluble fusion protein extends transgene expression in the liver and lung. *Journal of Virology* **73**, 5098–5109.

Perissi, V., Dasen, J. S., Kurokawa, R., Wang, Z., Korzus, E., Rose, D. W., Glass, C. K. & Rosenfeld, M. G. (1999). Factor-specific modulation of CREB-binding protein acetyltransferase activity. *Proceedings of the National Academy of Sciences, USA* **96**, 3652–3657.

Pickles, R. J., McCarty, D., Matsui, H., Hart, P. J., Randell, S. H. & Boucher, R. C. (1998). Limited entry of adenovirus vectors into well-differentiated airway epithelium is responsible for inefficient gene transfer. *Journal of Virology* **72**, 6014–6023.

Piedimonte, G., Pickles, R. J., Lehmann, J. R., McCarty, D., Costa, D. L. & Boucher, R. C. (1997). Replication-deficient adenoviral vector for gene transfer potentiates airway neurogenic inflammation. *American Journal of Research in Respiratory Cell and Molecular Biology* **16**, 250–258.

Poller, W., Schneider-Rasp, S., Liebert, U., Merklein, F., Thalheimer, P., Haack, A., Schwaab, R., Schmitt, C. & Brackmann, H. H. (1996). Stabilization of transgene expression by incorporation of E3 region genes into an adenoviral factor IX vector and by transient anti-CD4 treatment of the host. *Gene Therapy* **3**, 521–530.

Printz, M. A., Gonzalez, A. M., Cunningham, M., Gu, D. L., Ong, M., Pierce, G. F. & Aukerman, S. L. (2000). Fibroblast growth factor 2-retargeted adenoviral vectors exhibit a modified biolocalization pattern and display reduced toxicity relative to native adenoviral vectors. *Human Gene Therapy* **11**, 191–204.

Prives, C. & Hall, P. A. (1999). The p53 pathway. *Journal of Pathology* **187**, 112–126.

Putzer, B. M., Bramson, J. L., Addison, C. L., Hitt, M., Siegel, P. M., Muller, W. J. & Graham, F. L. (1998). Combination therapy with interleukin-2 and wild-type p53 expressed by adenoviral vectors potentiates tumor regression in a murine model of breast cancer. *Human Gene Therapy* **9**, 707–718.

Putzer, B. M., Stiewe, T., Parssanedjad, K., Rega, S. & Esche, H. (2000). E1A is sufficient by itself to induce apoptosis independent of p53 and other adenoviral gene products. *Cell Death and Differentiation* **7**, 177–188.

Qiu, C., De Young, M. B., Finn, A. & Dichek, D. A. (1998). Cationic liposomes enhance adenovirus entry via a pathway independent of the fiber receptor and α_v-integrins. *Human Gene Therapy* **9**, 507–520.

Ramalingam, R., Rafii, S., Worgall, S., Hackett, N. R. & Crystal, R. G. (1999). Induction of endogenous genes following infection of human endothelial cells with an $E1^-E4^+$ adenovirus gene transfer vector. *Journal of Virology* **73**, 10183–10190.

Ramondetta, L., Mills, G. B., Burke, T. W. & Wolf, J. K. (2000). Adenovirus-mediated expression of p53 or p21 in a papillary serous endometrial carcinoma cell line (SPEC-2) results in both growth inhibition and apoptotic cell death: potential application of gene therapy to endometrial cancer. *Clinical Cancer Research* **6**, 278–284.

Rancourt, C., Piche, A., Gomez-Navarro, J., Wang, M., Alvarez, R. D., Siegal, G. P., Fuller, G. M., Jones, S. A. & Curiel, D. T. (1999). Interleukin-6 modulated conditionally replicative adenovirus as an antitumor/cytotoxic agent for cancer therapy. *Clinical Cancer Research* **5**, 43–50.

Rao, L., Perez, D. & White, E. (1996). Lamin proteolysis facilitates nuclear events during apoptosis. *Journal of Cell Biology* **135**, 1441–1455.

Rasmussen, U. B., Benchaibi, M., Meyer, V., Schlesinger, Y. & Schughart, K. (1999). Novel human gene transfer vectors: evaluation of wild-type and recombinant animal adenoviruses in human-derived cells. *Human Gene Therapy* **10**, 2587–2599.

Rauma, T., Tuukkanen, J., Bergelson, J. M., Denning, G. & Hautala, T. (1999). rab5 GTPase regulates adenovirus endocytosis. *Journal of Virology* **73**, 9664–9668.

Rea, D., Schagen, F. H., Hoeben, R. C., Mehtali, M., Havenga, M. J., Toes, R. E., Melief, C. J. & Offringa, R. (1999). Adenoviruses activate human dendritic cells without polarization toward a T-helper type 1-inducing subset. *Journal of Virology* **73**, 10245–10253.

Recchia, A., Parks, R. J., Lamartina, S., Toniatti, C., Pieroni, L., Palombo, F., Ciliberto, G., Graham, F. L., Cortese, R., La Monica, N. & Colloca, S. (1999). Site-specific integration mediated by a hybrid adenovirus/adeno-associated virus vector. *Proceedings of the National Academy of Sciences, USA* **96**, 2615–2620.

Reddy, P. S., Idamakanti, N., Chen, Y., Whale, T., Babiuk, L. A., Mehtali, M. & Tikoo, S. K. (1999). Replication-defective bovine adenovirus type 3 as an expression vector. *Journal of Virology* **73**, 9137–9144.

Reich, N., Pine, R., Levy, D. & Darnell, J. E., Jr (1988). Transcription of interferon-stimulated genes is induced by adenovirus particles but is suppressed by E1A gene products. *Journal of Virology* **62**, 114–119.

Reid, J. L., Bannister, A. J., Zegerman, P., Martinez-Balbas, M. A. & Kouzarides, T. (1998). E1A directly binds and regulates the P/CAF acetyltransferase. *EMBO Journal* **17**, 4469–4477.

Rekosh, D. M., Russell, W. C., Bellet, A. J. & Robinson, A. J. (1977). Identification of a protein linked to the ends of adenovirus DNA. *Cell* **11**, 283–295.

Ridet, J. L., Corti, O., Pencalet, P., Hanoun, N., Hamon, M., Philippon, J. & Mallet, J. (1999). Toward autologous ex vivo gene therapy for the central nervous system with human adult astrocytes. *Human Gene Therapy* **10**, 271–280.

Ridgway, P. J., Hall, A. R., Myers, C. J. & Braithwaite, A. W. (1997). p53/E1b58kDa complex regulates adenovirus replication. *Virology* **237**, 404–413.

Rodriguez, R., Schuur, E. R., Lim, H. Y., Henderson, G. A., Simons, J. W. & Henderson, D. R. (1997). Prostate attenuated replication competent adenovirus (ARCA) CN706: a selective cytotoxic for prostate-specific antigen-positive prostate cancer cells. *Cancer Research* **57**, 2559–2563.

Rodriguez, M. S., Desterro, J. M., Lain, S., Midgley, C. A., Lane, D. P. & Hay, R. T. (1999). SUMO-1 modification activates the transcriptional response of p53. *EMBO Journal* **18**, 6455–6461.

Roelvink, P. W., Lizonova, A., Lee, J. G., Li, Y., Bergelson, J. M., Finberg, R. W., Brough, D. E., Kovesdi, I. & Wickham, T. J. (1998). The coxsackievirus-adenovirus receptor protein can function as a cellular attachment protein for adenovirus serotypes from subgroups A, C, D, E, and F. *Journal of Virology* **72**, 7909–7915.

Rogers, B. E., McLean, S. F., Kirkman, R. L., Della Manna, D., Bright, S. J., Olsen, C. C., Myracle, A. D., Mayo, M. S., Curiel, D. T. & Buchsbaum, D. J. (1999). In vivo localization of [^{111}In]-DTPA-D-Phe1-octreotide to human ovarian tumor xenografts induced to express the somatostatin receptor subtype 2 using an adenoviral vector. *Clinical Cancer Research* **5**, 383–393.

Rogulski, K. R., Wing, M. S., Paielli, D. L., Gilbert, J. D., Kim, J. H. & Freytag, S. O. (2000). Double suicide gene therapy augments the antitumor activity of a replication-competent lytic adenovirus through enhanced cytotoxicity and radiosensitization. *Human Gene Therapy* **11**, 67–76.

Rosenberg, S. A., Zhai, Y., Yang, J. C., Schwartzentruber, D. J., Hwu, P., Marincola, F. M., Topalian, S. L., Restifo, N. P., Seipp, C. A., Einhorn, J. H., Roberts, B. & White, D. E. (1998). Immunizing patients with metastatic melanoma using recombinant adenoviruses encoding MART-1 or gp100 melanoma antigens. *Journal of the National Cancer Institute* **90**, 1894–1900.

Rowe, W. P., Huebner, R. J., Gilmore, L. K., Parrot, R. H. & Ward, T. G. (1953). Isolation of a cytopathic agent from human adenoids undergoing spontaneous degradation in tissue culture. *Proceedings of the Society for Experimental Biology and Medicine* **84**, 570–573.

Russell, W. C., Valentine, R. C. & Pereira, H. G. (1967). The effect of heat on the anatomy of the adenovirus. *Journal of General Virology* **1**, 509–522.

Ryan, K. M., Ernst, M. K., Rice, N. R. & Vousden, K. H. (2000). Role of NF-kappaB in p53-mediated programmed cell death. *Nature* **404**, 892–897.

Sallenave, J. M., Xing, Z., Simpson, A. J., Graham, F. L. & Gauldie, J. (1998). Adenovirus-mediated expression of an elastase-specific inhibitor (elafin): a comparison of different promoters. *Gene Therapy* **5**, 352–360.

Sanchez-Cespedes, M., Reed, A. L., Buta, M., Wu, L., Westra, W. H., Herman, J. G., Yang, S. C., Jen, J. & Sidransky, D. (1999). Inactivation of the INK4A/ARF locus frequently coexists with TP53 mutations in non-small cell lung cancer. *Oncogene* **18**, 5843–5849.

Sandig, V., Loser, P., Lieber, A., Kay, M. A. & Strauss, M. (1996). HBV-derived promoters direct liver-specific expression of an adenovirally transduced LDL receptor gene. *Gene Therapy* **3**, 1002–1009.

Sandy, P., Gostissa, M., Fogal, V., Cecco, L. D., Szalay, K., Rooney, R. J., Schneider, C. & Del Sal, G. (2000). p53 is involved in the p120E4F-mediated growth arrest. *Oncogene* **19**, 188–199.

Santis, G., Legrand, V., Hong, S. S., Davison, E., Kirby, I., Imler, J.-L., Finberg, R. W., Bergelson, J. M., Mehtali, M. & Boulanger, P. (1999). Molecular determinants of adenovirus serotype 5 fibre binding to its cellular receptor CAR. *Journal of General Virology* **80**, 1519–1527.

Schmitz, M. L., Indorf, A., Limbourg, F. P., Stadtler, H., Traenckner, E. B. & Baeuerle, P. A. (1996). The dual effect of adenovirus type 5 E1A 13S protein on NF-κB activation is antagonized by E1B 19K. *Molecular Cell Biology* **16**, 4052–4063.

Schneider, D. B., Sassani, A. B., Vassalli, G., Driscoll, R. M. & Dichek, D. A. (1999). Adventitial delivery minimizes the proinflammatory effects of adenoviral vectors. *Journal of Vascular Surgery* **29**, 543–550.

Schowalter, D. B., Himeda, C. L., Winther, B. L., Wilson, C. B. & Kay, M. A. (1999). Implication of interfering antibody formation and apoptosis as two different mechanisms leading to variable duration of adenovirus-mediated transgene expression in immune-competent mice. *Journal of Virology* **73**, 4755–4766.

Segerman, A., Mei, Y. F. & Wadell, G. (2000). Adenovirus types 11p and 35p show high binding efficiencies for committed hematopoietic cell lines and are infective to these cell lines. *Journal of Virology* **74**, 1457–1467.

Shalev, M., Kadmon, D., Teh, B. S., Butler, E. B., Aguilar-Cordova, E., Thompson, T. C., Herman, J. R., Adler, H. L., Scardino, P. T. & Miles, B. J. (2000). Suicide gene therapy toxicity after multiple and repeat injections in patients with localized prostate cancer. *Journal of Urology* **163**, 1747–1750.

Shao, R., Karunagaran, D., Zhou, B. P., Li, K., Lo, S. S., Deng, J., Chiao, P. & Hung, M. C. (1997). Inhibition of nuclear factor-κB activity is involved in E1A-mediated sensitization of radiation-induced apoptosis. *Journal of Biological Chemistry* **272**, 32739–32742.

Shao, R., Hu, M. C., Zhou, B. P., Lin, S. Y., Chiao, P. J., von Lindern, R. H., Spohn, B. & Hung, M. C. (1999). E1A sensitizes cells to tumor necrosis factor-induced apoptosis through inhibition of IκB kinases and nuclear factor κB activities. *Journal of Biological Chemistry* **274**, 21495–21498.

Shih, I. M., Yu, J., He, T. C., Vogelstein, B. & Kinzler, K. W. (2000). The beta-catenin binding domain of adenomatous polyposis coli is sufficient for tumor suppression. *Cancer Research* **60**, 1671–1676.

Shinoura, N., Muramatsu, Y., Yoshida, Y., Asai, A., Kirino, T. & Hamada, H. (2000). Adenovirus-mediated transfer of caspase-3 with Fas ligand induces drastic apoptosis in U-373MG glioma cells. *Experimental Cell Research* **256**, 423–433.

Shisler, J., Duerksen-Hughes, P., Hermiston, T. M., Wold, W. S. & Gooding, L. R. (1996). Induction of susceptibility to tumor necrosis factor by E1A is dependent on binding to either p300 or p105-Rb and induction of DNA synthesis. *Journal of Virology* **70**, 68–77.

Siders, W. M., Halloran, P. J. & Fenton, R. G. (1998*a*). Melanoma-specific cytotoxicity induced by a tyrosinase promoter–enhancer/herpes simplex virus thymidine kinase adenovirus. *Cancer Gene Therapy* **5**, 281–291.

Siders, W. M., Wright, P. W., Hixon, J. A., Alvord, W. G., Back, T. C., Wiltrout, R. H. & Fenton, R. G. (1998*b*). T cell- and NK cell-independent inhibition of hepatic metastases by systemic administration of an IL-12-expressing recombinant adenovirus. *Journal of Immunology* **160**, 5465–5474.

Simos, G. & Georgatos, S. D. (1994). The lamin B receptor-associated protein p34 shares sequence homology and antigenic determinants with the splicing factor 2-associated protein p32. *FEBS Letters* **346**, 225–228.

Smith, T. A., White, B. D., Gardner, J. M., Kaleko, M. & McClelland, A. (1996). Transient immunosuppression permits successful repetitive intravenous administration of an adenovirus vector. *Gene Therapy* **3**, 496–502.

Smith, C. A., Woodruff, L. S., Rooney, C. & Kitchingman, G. R. (1998). Extensive cross-reactivity of adenovirus-specific cytotoxic T cells. *Human Gene Therapy* **9**, 1419–1427.

Somasundaram, K. & El-Deiry, W. S. (1997). Inhibition of p53-mediated transactivation and cell cycle arrest by E1A through its p300/CBP-interacting region. *Oncogene* **14**, 1047–1057.

Souberbielle, B. E. & Russell, W. C. (1995). Human T cell proliferative response to polypeptides from adenovirus type 2. *Journal of Infectious Diseases* **172**, 1421–1422.

Staba, M. J., Wickham, T. J., Kovesdi, I. & Hallahan, D. E. (2000). Modifications of the fiber in adenovirus vectors increase tropism for malignant glioma models. *Cancer Gene Therapy* **7**, 13–19.

Steiner, M. S., Zhang, Y., Farooq, F., Lerner, J., Wang, Y. & Lu, Y. (2000). Adenoviral vector containing wild-type p16 suppresses prostate cancer growth and prolongs survival by inducing cell senescence. *Cancer Gene Therapy* **7**, 360–372.

Steinwaerder, D. S., Carlson, C. A. & Lieber, A. (1999). Generation of adenovirus vectors devoid of all viral genes by recombination between inverted repeats. *Journal of Virology* **73**, 9303–9313.

Sterman, D. H., Treat, J., Litzky, L. A., Amin, K. M., Coonrod, L., Molnar-Kimber, K., Recio, A., Knox, L., Wilson, J. M., Albelda, S. M. & Kaiser, L. R. (1998). Adenovirus-mediated herpes simplex virus thymidine kinase/ganciclovir gene therapy in patients with localized malignancy: results of a phase I clinical trial in malignant mesothelioma. *Human Gene Therapy* **9**, 1083–1092.

Stevenson, S. C., Rollence, M., White, B., Weaver, L. & McClelland, A. (1995). Human adenovirus serotypes 3 and 5 bind to two different cellular receptors via the fiber head domain. *Journal of Virology* **69**, 2850–2857.

Stewart, P. L., Fuller, S. D. & Burnett, R. M. (1993). Difference imaging of adenovirus: bridging the resolution gap between X-ray crystallography and electron microscopy. *EMBO Journal* **12**, 2589–2599.

Stewart, P. L., Chiu, C. Y., Huang, S., Muir, T., Zhao, Y., Chait, B., Mathias, P. & Nemerow, G. R. (1997). Cryo-EM visualization of an exposed RGD epitope on adenovirus that escapes antibody neutralization. *EMBO Journal* **16**, 1189–1198.

Suomalainen, M., Nakano, M. Y., Keller, S., Boucke, K., Stidwill, R. P. & Greber, U. F. (1999). Microtubule-dependent plus- and minus end-directed motilities are competing processes for nuclear targeting of adenovirus. *Journal of Cell Biology* **144**, 657–672.

Suzuki, T., Anderegg, B., Ohkawa, T., Irie, A., Engebraaten, O., Halks-Miller, M., Holm, P. S., Curiel, D. T., Kashani-Sabet, M. & Scanlon, K. J. (2000). Adenovirus-mediated ribozyme targeting of HER-2/neu inhibits in vivo growth of breast cancer cells. *Gene Therapy* **7**, 241–248.

Tan, B. T., Wu, L. & Berk, A. J. (1999). An adenovirus–Epstein–Barr virus hybrid vector that stably transforms cultured cells with high efficiency. *Journal of Virology* **73**, 7582–7589.

Tanaka, N., Sato, M., Lamphier, M. S., Nozawa, H., Oda, E., Noguchi, S., Schreiber, R. D., Tsujimoto, Y. & Taniguchi, T. (1998). Type I interferons are essential mediators of apoptotic death in virally infected cells. *Genes and Cells* **3**, 29–37.

Taniguchi, T., Chikatsu, N., Takahashi, S., Fujita, A., Uchimaru, K., Asano, S., Fujita, T. & Motokura, T. (1999). Expression of p16INK4A and p14ARF in hematological malignancies. *Leukemia* **13**, 1760–1769.

Tao, W. & Levine, A. J. (1999). P19 (ARF) stabilizes p53 by blocking nucleo-cytoplasmic shuttling of Mdm2. *Proceedings of the National Academy of Sciences, USA* **96**, 6937–6941.

Tashiro, F., Niwa, H. & Miyazaki, J. (1999). Constructing adenoviral vectors by using the circular form of the adenoviral genome cloned in a cosmid and the Cre-loxP recombination system. *Human Gene Therapy* **10**, 1845–1852.

Tillman, B. W., de Gruijl, T. D., Luykx-de Bakker, S. A., Scheper, R. J., Pinedo, H. M., Curiel, T. J., Gerritsen, W. R. & Curiel, D. T. (1999). Maturation of dendritic cells accompanies high-efficiency gene transfer by a CD40-targeted adenoviral vector. *Journal of Immunology* **162**, 6378–6383.

Tollefson, A. E., Ryerse, J. S., Scaria, A., Hermiston, T. W. & Wold, W. S. (1996). The E3-11·6-kDa adenovirus death protein (ADP) is required for efficient cell death: characterization of cells infected with adp mutants. *Virology* **220**, 152–162.

Tollefson, A. E., Hermiston, T. W., Lichtenstein, D. L., Colle, C. F., Tripp, R. A., Dimitrov, T., Toth, K., Wells, C. E., Doherty, P. C. & Wold, W. S. (1998). Forced degradation of Fas inhibits apoptosis in adenovirus-infected cells. *Nature* **392**, 726–730.

Toloza, E. M., Hunt, K., Swisher, S., McBride, W., Lau, R., Pang, S., Rhoades, K., Drake, T., Belldegrun, A., Glaspy, J. & Economou, J. S. (1996). In vivo cancer gene therapy with a recombinant interleukin-2 adenovirus vector. *Cancer Gene Therapy* **3**, 11–17.

Tomko, R. P., Xu, R. & Philipson, L. (1997). HCAR and MCAR: the human and mouse cellular receptors for subgroup C adenoviruses and group B coxsackieviruses. *Proceedings of the National Academy of Sciences, USA* **94**, 3352–3356.

Tomko, R. P., Johansson, C. B., Totrov, M., Abagyan, R., Frisen, J. & Philipson, L. (2000). Expression of the adenovirus receptor and its interaction with the fiber knob. *Experimental Cell Research* **255**, 47–55.

Topf, N., Worgall, S., Hackett, N. R. & Crystal, R. G. (1998). Regional 'pro-drug' gene therapy: intravenous administration of an adenoviral vector expressing the *E. coli* cytosine deaminase gene and systemic administration of 5-fluorocytosine suppresses growth of hepatic metastasis of colon carcinoma. *Gene Therapy* **5**, 507–513.

Tsao, Y. P., Huang, S. J., Chang, J. L., Hsieh, J. T., Pong, R. C. & Chen, S. L. (1999). Adenovirus-mediated $p21^{WAF1/SDII/CIP1}$ gene transfer induces apoptosis of human cervical cancer cell lines. *Journal of Virology* **73**, 4983–4990.

Tuting, T., Steitz, J., Bruck, J., Gambotto, A., Steinbrink, K., DeLeo, A. B., Robbins, P., Knop, J. & Enk, A. H. (1999). Dendritic cell-based genetic immunization in mice with a recombinant adenovirus encoding murine TRP2 induces effective anti-melanoma immunity. *Journal of Genetic Medicine* **1**, 400–406.

van Deutekom, J. C., Cao, B., Pruchnic, R., Wickham, T. J., Kovesdi, I. & Huard, J. (1999). Extended tropism of an adenoviral vector does not circumvent the maturation-dependent transducibility of mouse skeletal muscle. *Journal of Genetic Medicine* **1**, 393–399.

van Ginkel, F. W., McGhee, J. R., Liu, C., Simecka, J. W., Yamamoto, M., Frizzell, R. A., Sorscher, E. J., Kiyono, H. & Pascual, D. W. (1997). Adenoviral gene delivery elicits distinct pulmonary-associated T helper cell responses to the vector and to its transgene. *Journal of Immunology* **159**, 685–693.

Vollmer, C. M., Ribas, A., Butterfield, L. H., Dissette, V. B., Andrews, K. J., Eilber, F. C., Montejo, L. D., Chen, A. Y., Hu, B., Glaspy, J. A., McBride, W. H. & Economou, J. S. (1999). p53 selective and non-selective replication of an E1B-deleted adenovirus in hepatocellular carcinoma. *Cancer Research* **59**, 4369–4374.

von Herrath, M. G., Efrat, S., Oldstone, M. B. & Horwitz, M. S. (1997). Expression of adenoviral E3 transgenes in beta cells prevents autoimmune diabetes. *Proceedings of the National Academy of Sciences, USA* **94**, 9808–9813.

Von Seggern, D. J., Chiu, C. Y., Fleck, S. K., Stewart, P. L. & Nemerow, G. R. (1999). A helper-independent adenovirus vector with E1, E3, and fiber deleted: structure and infectivity of fiberless particles. *Journal of Virology* **73**, 1601–1608.

Walters, R. W., Grunst, T., Bergelson, J. M., Finberg, R. W., Welsh, M. J. & Zabner, J. (1999). Basolateral localization of fiber receptors limits adenovirus infection from the apical surface of airway epithelia. *Journal of Biological Chemistry* **274**, 10219–10226.

Wan, Y., Emtage, P., Foley, R., Carter, R. & Gauldie, J. (1999). Murine dendritic cells transduced with an adenoviral vector expressing a defined tumor antigen can overcome anti-adenovirus neutralizing immunity and induce effective tumor regression. *International Journal of Oncology* **14**, 771–776.

Wang, X. & Bergelson, J. M. (1999). Coxsackievirus and adenovirus receptor cytoplasmic and transmembrane domains are not essential for coxsackievirus and adenovirus infection. *Journal of Virology* **73**, 2559–2562.

Wang, K., Huang, S., Kapoor-Munshi, A. & Nemerow, G. (1998). Adenovirus internalization and infection require dynamin. *Journal of Virology* **72**, 3455–3458.

Watson, G., Burdon, M. G. & Russell, W. C. (1988). An antigenic analysis of the adenovirus type 2 fibre polypeptide. *Journal of General Virology* **69**, 525–535.

Weber, J. (1976). Genetic analysis of adenovirus type 2. III. Temperature sensitivity of processing viral proteins. *Journal of Virology* **17**, 462–471.

Weber, J. D., Taylor, L. J., Roussel, M. F., Sherr, C. J. & Bar-Sagi, D. (1999). Nucleolar Arf sequesters Mdm2 and activates p53. *Nature Cell Biology* **1**, 20–26.

Webster, A., Russell, S., Talbot, P., Russell, W. C. & Kemp, G. D. (1989). Characterization of the adenovirus proteinase: substrate specificity. *Journal of General Virology* **70**, 3225–3234.

Weiden, M. D. & Ginsberg, H. S. (1994). Deletion of the E4 region of the genome produces adenovirus DNA concatemers. *Proceedings of the National Academy of Sciences, USA* **91**, 153–157.

Weigel, S. & Dobbelstein, M. (2000). The nuclear export signal within the E4orf6 protein of adenovirus type 5 supports virus replication and cytoplasmic accumulation of viral mRNA. *Journal of Virology* **74**, 764–772.

Wickham, T. J., Mathias, P., Cheresh, D. A. & Nemerow, G. R. (1993). Integrins $\alpha v\ \beta 3$ and $\alpha v\ \beta 5$ promote adenovirus internalization but not virus attachment. *Cell* **73**, 309–319.

Wickham, T. J., Roelvink, P. W., Brough, D. E. & Kovesdi, I. (1996). Adenovirus targeted to heparan-containing receptors increases its gene delivery efficiency to multiple cell types. *Nature Biotechnology* **14**, 1570–1573.

Wickham, T. J., Lee, G. M., Titus, J. A., Sconocchia, G., Bakacs, T., Kovesdi, I. & Segal, D. M. (1997). Targeted adenovirus-mediated gene delivery to T cells via CD3. *Journal of Virology* **71**, 7663–7669.

Wildner, O., Morris, J. C., Vahanian, N. N., Ford, H., Jr, Ramsey, W. J. & Blaese, R. M. (1999). Adenoviral vectors capable of replication improve the efficacy of HSVtk/GCV suicide gene therapy of cancer. *Gene Therapy* **6**, 57–62.

Willcox, N. & Mautner, V. (1976). Antigenic determinants of adenovirus capsids. I. Measurement of antibody cross-reactivity. *Journal of Immunology* **116**, 19–24.

Wills, K. N., Maneval, D. C., Menzel, P., Harris, M. P., Sutjipto, S., Vaillancourt, M.-T., Huang, W.-M., Johnson, D. E., Anderson, S. C., Wen, S. F., Bookstein, R., Shepard, H. M. & Gregory, R. J. (1994). Development and characterization of recombinant adenoviruses encoding human p53 for gene therapy of cancer. *Human Gene Therapy* **5**, 1079–1088.

Wold, W. M. & Chinnadurai, G. (2000). Adenovirus proteins that regulate apoptosis. In *DNA Virus Replication*, pp. 200–232. Edited by A. J. Cann. Oxford: Oxford University Press.

Wolf, L. A. & Laster, S. M. (1999). Characterization of arachidonic acid-induced apoptosis. *Cell Biochemistry and Biophysics* **30**, 353–368.

Worgall, S., Wolff, G., Falck-Pedersen, E. & Crystal, R. G. (1997 *a*). Innate immune mechanisms dominate elimination of adenoviral vectors following in vivo administration. *Human Gene Therapy* **8**, 37–44.

Worgall, S., Leopold, P. L., Wolff, G., Ferris, B., Van Roijen, N. & Crystal, R. G. (1997 *b*). Role of alveolar macrophages in rapid elimination of adenovirus vectors administered to the epithelial surface of the respiratory tract. *Human Gene Therapy* **8**, 1675–1684.

Worgall, S., Singh, R., Leopold, P. L., Kaner, R. J., Hackett, N. R., Topf, N., Moore, M. A. & Crystal, R. G. (1999). Selective expansion of alveolar macrophages in vivo by adenovirus-mediated transfer of the murine granulocyte-macrophage colony-stimulating factor cDNA. *Blood* **93**, 655–666.

Yang, L., Lochmuller, H., Luo, J., Massie, B., Nalbantoglu, J., Karpati, G. & Petrof, B. J. (1998). Adenovirus-mediated dystrophin minigene transfer improves muscle strength in adult dystrophic (MDX) mice. *Gene Therapy* **5**, 369–379.

Yarosh, O. K., Wandeler, A. I., Graham, F. L., Campbell, J. B. & Prevec, L. (1996). Human adenovirus type 5 vectors expressing rabies glycoprotein. *Vaccine* **14**, 1257–1264.

Ye, J., Xie, X., Tarassishin, L. & Horwitz, M. S. (2000). Regulation of the NF-κB activation pathway by isolated domains of FIP3/IKKγ, a component of the IκB-α kinase complex. *Journal of Biological Chemistry* **275**, 9882–9889.

Yew, N. S., Marshall, J., Przybylska, M., Wysokenski, D. M., Ziegler, R. J., Rafter, P. W., Li, C., Armentano, D. & Cheng, S. H. (1999). Increased duration of transgene expression in the lung with plasmid DNA vectors harboring adenovirus E4 open reading frame 3. *Human Gene Therapy* **10**, 1833–1843.

Yu, D. C., Chen, Y., Seng, M., Dilley, J. & Henderson, D. R. (1999). The addition of adenovirus type 5 region E3 enables calydon virus 787 to eliminate distant prostate tumor xenografts. *Cancer Research* **59**, 4200–4203.

Yuasa, K., Miyagoe, Y., Yamamoto, K., Nabeshima, Y., Dickson, G. & Takeda, S. (1998). Effective restoration of dystrophin-associated proteins in vivo by adenovirus-mediated transfer of truncated dystrophin cDNAs. *FEBS Letters* **425**, 329–336.

Yun, J., Chae, H. D., Choy, H. E., Chung, J., Yoo, H. S., Han, M. H. & Shin, D. Y. (1999). p53 negatively regulates cdc2 transcription via the CCAAT-binding NF-Y transcription factor. *Journal of Biological Chemistry* **274**, 29677–29682.

Zabner, J., Freimuth, P., Puga, A., Fabrega, A. & Welsh, M. J. (1997). Lack of high affinity fiber receptor activity explains the resistance of ciliated airway epithelia to adenovirus infection. *Journal of Clinical Investigation* **100**, 1144–1149.

Zabner, J., Chillon, M., Grunst, T., Moninger, T. O., Davidson, B. L., Gregory, R. & Armentano, D. (1999). A chimeric type 2 adenovirus vector with a type 17 fiber enhances gene transfer to human airway epithelia. *Journal of Virology* **73**, 8689–8695.

Zakhartchouk, A. N., Reddy, P. S., Baxi, M., Baca-Estrada, M. E., Mehtali, M., Babiuk, L. A. & Tikoo, S. K. (1998). Construction and characterization of E3-deleted bovine adenovirus type 3 expressing full-length and truncated form of bovine herpesvirus type 1 glycoprotein gD. *Virology* **250**, 220–229.

Zantema, A. & van der Eb, A. (2000). Control of transcription by adenovirus-E1A proteins. In *DNA Virus Replication*, pp. 173–199. Edited by A. J. Cann. Oxford: Oxford University Press.

Zhang, W.-W. (1999). Development and application of adenoviral vectors for gene therapy of cancer. *Cancer Gene Therapy* **6**, 113–138.

Zhang, R. & DeGroot, L. J. (2000). Gene therapy of established medullary thyroid carcinoma with herpes simplex viral thymidine kinase in a rat tumor model: relationship of bystander effect and antitumor efficacy. *Thyroid* **10**, 313–319.

Zhang, F., Andreassen, P., Fender, P., Geissler, E., Hernandez, J. F. & Chroboczek, J. (1999). A transfecting peptide derived from adenovirus fiber protein. *Gene Therapy* **6**, 171–181.

Zheng, C., Baum, B. J., Iadarola, M. J. & O'Connell, B. C. (2000). Genomic integration and gene expression by a modified adenoviral vector. *Nature Biotechnology* **18**, 176–180.

Zhong, L., Granelli-Piperno, A., Choi, Y. & Steinman, R. M. (1999). Recombinant adenovirus is an efficient and non-perturbing genetic vector for human dendritic cells. *European Journal of Immunology* **29**, 964–972.

Zuckerman, J. B., Robinson, C. B., McCoy, K. S., Shell, R., Sferra, T. J., Chirmule, N., Magosin, S. A., Propert, K. J., Brown-Parr, E. C., Hughes, J. V., Tazelaar, J., Baker, C., Goldman, M. J. & Wilson, J. M. (1999). A phase I study of adenovirus-mediated transfer of the human cystic fibrosis transmembrane conductance regulator gene to a lung segment of individuals with cystic fibrosis. *Human Gene Therapy* **10**, 2973–2985.

Published ahead of print (11 August 2000) in JGV Direct as DOI 10.1099/vir.0.17217-0