

Macrophages in gene therapy: cellular delivery vehicles and in vivo targets

B. Burke,* S. Sumner,[†] N. Maitland,[‡] and C. E. Lewis[†]

*Department of Microbiology and Immunology, University of Leicester, United Kingdom; [†]Tumor Targeting Group, Section of Oncology & Cellular Pathology, Division of Genomic Medicine, University of Sheffield Medical School, United Kingdom; and [‡]YCR Cancer Research Unit, Department of Biology, University of York, United Kingdom

Abstract: The appearance and activation of macrophages are thought to be rapid events in the development of many pathological lesions, including malignant tumors, atherosclerotic plaques, and arthritic joints. This has prompted recent attempts to use macrophages as novel cellular vehicles for gene therapy, in which macrophages are genetically modified *ex vivo* and then reintroduced into the body with the hope that a proportion will then home to the diseased site. Here, we critically review the efficacy of various gene transfer methods (viral, bacterial, protozoan, and various chemical and physical methods) in transfecting macrophages *in vitro*, and the results obtained when transfected macrophages are used as gene delivery vehicles. Finally, we discuss the use of various viral and nonviral methods to transfer genes to macrophages *in vivo*. As will be seen, definitive evidence for the use of macrophages as gene transfer vehicles has yet to be provided and awaits detailed trafficking studies *in vivo*. Moreover, although methods for transfecting macrophages have improved considerably in efficiency in recent years, targeting of gene transfer specifically to macrophages *in vivo* remains a problem. However, possible solutions to this include placing transgenes under the control of macrophage-specific promoters to limit expression to macrophages or stably transfecting CD34⁺ precursors of monocytes/macrophages and then differentiating these cells into monocytes/macrophages *ex vivo*. The latter approach could conceivably lead to the bone marrow precursor cells of patients with inherited genetic disorders being permanently fortified or even replaced with genetically modified cells. *J. Leukoc. Biol.* 72: 417–428; 2002.

Key Words: vector · adoptive immunotherapy · transfection · homing · transcriptional targeting

INTRODUCTION

Macrophages have been implicated in the onset and/or progression of various diseases, mainly via their production of proinflammatory or proangiogenic mediators, but also as a result of their ability to act as hosts and reservoirs for pathogens. Such condi-

tions include the growth and spread of malignant tumors, the pronounced inflammation present in rheumatoid arthritic joints, HIV infection, chronic granulomatous disease (CGD), atherosclerosis, lysosomal storage diseases (LSD), diabetes, lupus erythematosus, and Wiskott-Aldrich syndrome [1].

Many of the events regulating the infiltration and functions of macrophages in such diseased tissues have now been identified. This has prompted attempts to transfer therapeutic gene constructs into macrophages to use these cells in adoptive immunotherapy protocols or to alter their deleterious or defective activities *in vivo*. Most studies have used gene transfer methods to manipulate gene expression in macrophages *in vitro*. However, as is often the case, attempts to replicate these studies in the more complex *in vivo* environment have proved difficult with high-level, macrophage-specific transfection proving problematic.

GENE TRANSFER TO MACROPHAGES

There is now growing interest in the development of efficient methods for the transfection of primary macrophages and macrophage cell lines, partly as a means of investigating their myriad functions, but also with a view to expressing transgenes in these cells in new therapeutic protocols. The non- (or at least extremely low) proliferative nature of primary macrophages precludes the use of viral vectors requiring cell division for efficient transduction and has promoted the search for other forms of gene transfer vehicle. As will be seen later in this review, adenoviruses, lentiviruses, adeno-associated viruses, and poxviruses, as well as a wide array of nonviral methods, have been used. The main advantage of nonviral methods is that the time-consuming process of constructing recombinant viruses is avoided, and a large number of constructs can be more rapidly tested for functionality. However, viral methods generally give higher transfection efficiencies (a higher percentage of cells expressing the transgene) and a longer period of transgene expression. This could be due to the fact that, in many nonviral transfection methods, exogenous DNA enters the macrophage by endocytosis and is likely to be digested by

Correspondence: Dr. B. Burke, Department of Microbiology and Immunology, Maurice Shock Building, University of Leicester, University Road, Leicester LE1 9HN, UK. E-mail: bb14@leicester.ac.uk

Received February 7, 2002; revised April 4, 2002; accepted April 4, 2002.

nucleases, which are abundant in the lysosomes of macrophages [2], following endosome-lysosome fusion.

Viral methods

Adenoviruses

Adenoviruses have become a popular method of gene transfer to primary macrophages due to their ability to infect nondividing cells with high efficiency and reasonable longevity (up to several weeks) of transgene expression [3]. Various studies have reported transfection efficiencies of 10–80% with human macrophages, depending on cell culture conditions and the multiplicity of infection (MOI; number of infectious virus particles per cell) used [4–7]. It is noteworthy that adenoviruses appear to be relatively ineffective in infecting monocytes, presumably due to the paucity of appropriate integrins (the receptors for adenoviruses) on these cells [5]. A recent study using an adenoviral vector to transfer interleukin (IL)-18 antisense into the murine monocytic cell line RAW 264.7 only achieved a transfection efficiency of 20% [8]. One study solved the integrin problem by incubating primary human monocytes in 100 ng/ml macrophage-colony stimulating factor (M-CSF) for 72 h to stimulate expression of the integrins $\alpha_v\beta_3$ and $\alpha_v\beta_5$. With this method, using MOIs of 100 or 50, infection rates of greater than 90% were achieved [9].

Retroviruses

Retroviruses (with the exception of the lentivirus subclass) are generally considered ineffective in the transfection of nonproliferating or poorly proliferating cells such as primary macrophages. One explanation for this may be that such viruses are incapable of entering the nuclei of nonproliferating cells, and thus are unable to integrate their genome into the chromosomes of the infected cell, which is essential for significant levels of retroviral gene expression. Parveen and co-workers [10] have addressed this problem by introducing a nuclear localization signal (NLS) sequence into the matrix protein of the C type retrovirus spleen necrosis virus (SNV). This enabled the SNV vector to penetrate the nucleus of primary human monocyte-derived macrophages, allowing infection of 90% of cells compared with 0% for the control virus without the NLS.

There is some evidence that gene expression from integrated retroviruses often lasts only a few weeks, especially *in vivo*, which may be due to “transcriptional silencing” caused by methylation of DNA near the promoter and by integration of the virus genome into condensed chromatin regions in which the transgene would be inaccessible to the transcription machinery (reviewed in ref [3]). The recent discovery of two types of DNA element, locus control regions and ubiquitously acting chromatin opening elements, which alter the conformation of the surrounding chromatin in such a way as to enhance expression of nearby genes, offers a potential solution to the problem of insertion into transcriptionally inactive regions of chromatin [3].

CD34⁺ bone marrow stem cells, precursors of macrophages (and other leukocytes), are capable of cell division, rendering them susceptible to retroviral infection. Murine leukemia viruses have most often been used for this, but other retroviral vectors have also recently been developed, for example based on human foamy virus, which can give a transfection efficiency

of up to 80% [11]. Other studies have shown that retrovirally transduced CD34⁺ cells continue to stably express transgenes after being induced to differentiate into macrophages. In one study, stable expression of a reporter gene in 7–29% of CD14⁺ macrophages (as well as 4–23% of CD1a⁺/CD14⁻ dendritic cells) was achieved by retroviral transduction of granulocyte (G)-CSF-mobilized peripheral blood CD34⁺ cells. These had been grown in the presence of IL-3, IL-6, GM-CSF, and stem-cell factor prior to being induced to differentiate into macrophages (and dendritic cells) with GM-CSF and tumor necrosis factor α (TNF- α) [12]. The ability to stably transfect these precursor cells opens up the possibility of reinfusing such genetically modified stem cells into a patient, thereby providing a circulating (and possibly bone marrow populating) source of therapeutically modified macrophages (and other leukocytes that would develop from these precursors *in vivo*).

Other retroviruses of the lentivirus group including HIV are able to infect and integrate into the chromosomes of nonproliferating cells including monocyte/macrophages [13, 14], and therefore have the potential to effect stable, long-term gene transfer to such cells. In the case of HIV, sequences in the viral proteins Matrix, Vpr, Integrase, and Pol have been implicated in enabling nuclear import of the viral genome in nondividing cells [15]. However, it should be noted that *productive* HIV infection (i.e., producing progeny virus particles) of monocytes is reported to require cell proliferation [16, 17]. There is also evidence that lentiviral vectors offer the advantage of being less prone to transcriptional silencing than retroviral vectors (reviewed in ref [3]). Although HIV seems to offer the most effective, current method for the stable transfection of DNA into macrophages, there are safety concerns over its use (even when using replication-deficient variants) due to the danger of generating replication-competent viruses by recombination. However, considerable effort is being focused on the development of novel packaging systems that could significantly reduce this risk [18].

Adeno-associated viruses (AAV)

AAV have potential for gene therapy as they exhibit a number of attractive features including their ability to integrate into the genome of host cells and mediate long-term expression of transgenes (up to 2 years in mice), and to target proliferating and nonproliferating cells [3]. AAV have recently been used to efficiently transduce monocytes and dendritic cells [19, 20].

Poxviruses

A highly attenuated poxvirus, modified vaccinia Ankara, has been used to transfer genes expressing tumor-specific antibodies into GM-CSF-activated macrophages *in vitro* [21, 22]. In addition, a herpes simplex virus-1-derived vector has been used to transfer transgenes to macrophage cell lines [23].

Limitations of current viral vectors

A potentially important limitation in the use of adenoviral, retroviral/lentiviral, and AAV vectors is that they impose relatively low size limits (approximately 7.5 Kb, 8 Kb, and 4.5 Kb, respectively) on the amount of foreign DNA that can be incorporated. In contrast, the use of poxviruses such as vac-

cinia is not hampered by this problem because these viruses have much larger genomes. It should be noted that the clinical use of certain viral vectors, notably adenoviruses and AAV, may prove to be limited due to the immunogenicity of the virus-infected cells. In the case of adenoviruses, “gutless” vectors, which lack parts of the viral genome and therefore express fewer potentially immunogenic viral proteins, are under development. Such vectors have the additional advantage of a greater capacity for heterologous DNA, although these vectors present a number of problems in terms of manufacture (reviewed in ref [3]).

A problem limiting the use of viral vectors in transfecting macrophages *in vivo* is their lack of specificity for macrophages—most infect a broad range of cell types. However, promising recent studies have shown progress in altering the cell tropism of adenoviruses to enhance their ability to transduce target cells in specific diseased sites. One of these studies produced a bispecific single chain antibody or “diabody,” which binds a viral protein (the knob domain of the adenovirus fiber proteins on the virus surface, which are responsible for adenovirus-cell interactions) at one end and a cell type-specific surface protein (CD105) at the other [24]. This antibody, effectively an artificial virus cell-binding receptor, acts as an adapter between the virus and the target cell type. Other workers, such as Krasnykh et al. [25, 26] have taken the more direct route of “genetic targeting” adenoviruses by genetically manipulating the genes coding for the adenoviral fiber protein. Such work could potentially lead to the development of adenoviruses targeted to specific cell types such as macrophages.

Nonviral methods

A number of nonviral methods have been tested for the transfer of exogenous nucleic acids (plasmid DNA, oligonucleotides, and ribozymes) into macrophages, including electroporation and nonimmunogenic, synthetic DNA carriers such as liposomes, lipoplexes, and diethylaminoethyl (DEAE)-dextran, and they will be discussed in the following sections. However, these methods usually give low transfection efficiencies and short durations of transgene expression in target cells *in vitro*. They also have only limited use for systemic application. Cationic liposome/plasmid DNA or oligonucleotide complexes, for example, are rapidly cleared from the circulation, with the highest levels of activity usually observed in organs such as the lungs, spleen, and liver—that is, tissues with a known role in the removal of particulate and foreign matter from the bloodstream.

Interactions between macrophages and naked DNA

Macrophages have been shown to take up exogenous DNA, possibly via a specific transport mechanism. Most of this is thought to be degraded in endosomes [27]. However, exogenous DNA has been reported to be transported to the nucleus and expressed by a macrophage cell line (RAW 264.7) *in vitro* when used at very high concentrations. Maximal reporter gene expression was observed at a DNA concentration of 500 $\mu\text{g/ml}$ [28]. Gene transfer to macrophages using naked DNA has also been reported *in vivo* in a murine wound model [29], although in this study there was no cell-type specificity, with adipocytes

being the predominant cell type expressing the transgene, followed by macrophages and fibroblasts.

However, scenarios exist in which constraints such as the lack of target cell specificity and low levels of transgene expression may not be important, as in genetic immunization. For example, Condon and co-workers [30] showed that cutaneous immunization with naked DNA, attached to microscopic particles and projected forcefully into the skin by a burst of gas from a “gene gun,” results in transfection of dendritic cells in the skin, giving potent, antigen-specific, cytotoxic T lymphocyte (CTL)-mediated protective tumor immunity.

Another finding of significance in the transfection of macrophages is that these cells have specific mechanisms that recognize bacterial DNA because of the presence in this of unmethylated CpG dinucleotides (i.e., a cytosine followed by a guanine), which are rare in eukaryotic DNA (reviewed in ref [31]). Macrophages respond to such DNA by undergoing activation similar to that caused by lipopolysaccharide (LPS). This could be a complicating factor in gene therapy protocols using plasmid DNA or even oligonucleotides containing CpG dinucleotides, as unmethylated CpG DNA is possibly the most potent adjuvant known [32]. The problem could possibly be avoided by treating the DNA to be transfected with a methylase before transfection, which has been shown to be effective in the case of plasmid DNA [31].

Electroporation

Electroporation involves passing a brief pulse of electricity through a suspension of cells and exogenous DNA. The electric pulse is thought to cause the formation of pores in the cell membrane, allowing the cells to take up the exogenous DNA, which is believed to move directly into the nucleus, bypassing the endosomal degradation pathway. It has been noted that the conditions applied, such as the voltage and capacitance, ionic strength and temperature of the medium, and quantity of DNA used have to be optimized for each cell type [33]. It has also been reported that the proportion of cells in S phase is important in transfection of granulocyte-macrophage progenitors by electroporation [34].

Electroporation has achieved moderate levels of transfection efficiency for human monocytic cell lines such as MonoMac 6 (40–50%; B. Burke and C. E. Lewis, unpublished observations) and monocyte-derived macrophages (>30%; ref. [33]) *in vitro*, but appears less effective with promonocytic/leukemic cell lines (<3%; refs. [35, 36]). The main disadvantage of electroporation is the high level of cell death. This ranges from 5 to 60% with cell lines within 72 h after electroporation (depending on the electropulsing method used [35, 36]), to 30 to 75% within 24 h using primary cells [33]. However, few studies have been able to confirm that electroporation is an effective way of transfecting primary macrophages *in vitro*.

Li et al. [37] used electroporation to transfect CD34⁺ precursor cells *in vitro* and used a postpulse-pelleting method and caspase inhibitors to reduce osmotic swelling and cell death, achieving a transfection efficiency of approximately 20%. It remains to be seen whether this method will prove effective with macrophages.

Liposomes, lipoplexes, and cationic compounds

These transfection reagents offer a number of advantages. They have low toxicity, deliver DNA of essentially unlimited size, and do not evoke an immunogenic or inflammatory response when used in vivo. However, attempts at using lipid-based reagents to transfect primary macrophages or myeloid cell lines in vitro have generally yielded poor transfection efficiencies (<5%), with transgene expression lasting no more than 24 h [36]. However, when Mack et al. [38] optimized their DEAE-dextran DNA transfection method for adherent primary human macrophages, they found transgene expression could be maintained for up to 56 h.

In one study, which compared the efficiency of various liposomal and nonliposomal agents for transfecting the murine macrophage cell line RAW 264.7, LipofectAMINE (in combination with the DNA-condensing agent protamine sulfate) was found to be the most effective, followed by Lipofectin (fourfold less), DOTAP (tenfold less), and DEAE-dextran (20-fold less) [39]. The percentage of cells transfected was not given, but rather the total luciferase activity expressed by transfected cells was measured. This is a common approach in transfection experiments, but makes it impossible to assess the proportion of cells transfected, as "high" levels of reporter gene activity could arise from a relatively small proportion of the target cells. The use of a reporter gene such as green fluorescent protein (GFP) would allow transfection efficiency to be calculated using flow cytometry or fluorescence microscopy.

Cationic liposome/DNA complexes have also been shown to be capable of transfecting monocytes/macrophages in vivo in the blood, liver, and spleen. However, the transfection was nonspecific, and endothelial cells throughout the body were the main recipient of the transgene, with other leukocytes also being transfected [40, 41].

The use of liposomes is not restricted to the transfer of plasmid DNA. They have also been used to transfect primary macrophages with antisense oligonucleotides and ribozymes in vitro [42]. Cationic lipids have also been used to deliver ribozymes to murine peritoneal macrophages in vitro and in vivo with the aim of blocking TNF- α production, and achieved a 70% decrease in release of this cytokine from macrophages in treated versus untreated mice [43]. However, administration of the lipid/RNA complexes produced a fourfold increase in the numbers of peritoneal exudate inflammatory cells, which could counteract the desired effect of reducing inflammation by reducing TNF- α expression. Also, the duration of the inhibitory effect on TNF- α was not examined, and only 6% of the ribozyme was taken up by macrophages, the rest being taken up by organs such as spleen, lung, liver, pancreas, and intestines.

One recent study has used a novel DNA-condensing cationic peptide, CL22, to transfect monocyte-derived dendritic cells in vitro with genes encoding tumor-associated antigens, and showed that these cells then protected mice from a normally lethal challenge with melanoma cells [44].

Receptor-mediated gene transfer

Following the detailed characterization of receptor expression by macrophages over the last two decades, some transfection methods have been adapted to target specific endocytotic pathways in these cells. Ligands such as mannose and transferrin

have been incorporated into gene transfer vehicles and have been shown to markedly increase the efficacy of transfection for primary macrophages in vitro [45–47]. Erbacher and co-workers [45] observed much higher reporter gene expression with human macrophages in vitro using mannosylated polylysine/DNA complexes than with DEAE-dextran or Lipofectin. Simoes et al. [46] determined that the transfection efficiency for primary human monocyte-derived macrophages in vitro increased from essentially zero to 2% of cells when using transferrin.

The main advantage afforded by the use of receptor-targeted DNA complexes is that they can be designed to target specific cell types based on the presence of cell type-specific receptors on the cell surface. This has important implications for the clinic as this method could potentially allow direct injection of these complexes into the bloodstream and negates the need for ex vivo gene transfer. Indeed, in vivo gene transfer to macrophages in the liver, spleen, and lungs [48] was achieved using mannosylated polylysine-conjugated plasmids. Kawakami and co-workers [49] showed that this was also possible using mannosylated cationic liposomes.

Microorganisms as vehicles for transfection of macrophages

Various bacterial and protozoan microorganisms have evolved the ability to infect macrophages, evade their antipathogen-defense mechanisms, and establish chronic infections. Thus, these organisms represent a potentially powerful method of transferring therapeutic DNA constructs to macrophages in vivo. Several of these intracellular microorganisms have been exploited for this purpose.

Macrophages are one of the main cellular targets in *Salmonella* infection in humans, and as *Salmonella* can easily be manipulated in vitro to carry plasmid DNA, various workers have attempted to use them to transfect macrophages. For example, Paglia and co-workers [50] developed an attenuated (nonpathogenic) derivative of *Salmonella typhimurium* bearing a plasmid encoding murine interferon- γ (IFN- γ) under the control of the cytomegalovirus (CMV) immediate early promoter. They showed that exposure to such bacteria in vitro or in vivo resulted in the augmented expression of IFN- γ by macrophages. In another report, *S. typhimurium* were successfully used to transfect human monocyte-derived macrophages with transfection efficiencies of 85–95% [51].

A similar approach may be possible using *Leishmania*, a protozoan parasite. Although the wild-type strains of this microorganism can be highly pathogenic, attenuated mutants have been developed that can safely be used as live vaccines [52]. Vaccaro [53] suggested that an attenuated *Leishmania* strain, engineered to express a therapeutic gene, could be used to infect macrophages in individuals suffering from genetic disorders primarily affecting macrophages. As *Leishmania* specifically target the lysosomal compartment of macrophages, it could prove useful in the treatment of lysosomal storage disease (LSD), which involve defects in lysosomal enzymes.

The Gram-positive bacterium, *Listeria monocytogenes*, proved relatively ineffective in transferring reporter genes to the macrophage-like cell lines, J774/A1 and M97, or primary murine macrophages in vitro [54]. This accords well with

earlier reports of low transfection rates using *Listeria* with bone marrow-derived macrophages (BMDM) in vitro and of macrophages in vivo following intraperitoneal (i.p.) injection [55]. The reason(s) for the low transfectability of macrophages using *Listeria* remain to be elucidated.

MACROPHAGES AS GENE DELIVERY VEHICLES

It has been suggested that macrophages could be used to deliver targeted gene therapy to diseased tissues, thus exploiting their natural tendency to migrate into such sites in response to the release of cytokines/chemokines and the up-regulation of certain cell surface proteins in diseased tissues and nearby blood vessels. The need for such a delivery system arises from the fact that few current forms of systemic gene delivery vehicle (viral or nonviral) have been shown to penetrate far from the vasculature into diseased tissues, especially as the vasculature is often disrupted in such tissues.

Ability of macrophages to home to diseased sites after ex vivo manipulation

Adoptive immunotherapy with macrophages has been attempted in several studies with varying degrees of success. In a number of animal studies, infusion of macrophages (following ex vivo activation by such treatments as LPS, IFN, and exposure to medium conditioned by tumor cells) into tumor-bearing mice was shown to induce tumor regression [56–58]. Macrophage adoptive transfer is a relatively risk-free procedure. Cancer patients have been reinfused with up to 3×10^9 ex vivo-activated macrophages with only mild side effects, but unfortunately these studies have shown only minimal therapeutic benefit [reviewed in ref (59)].

Attention began to focus on ways of enhancing the ability of adoptively transferred macrophages to kill tumor cells or ameliorate other disease states by making use of their ability to migrate into diseased tissues to get them to carry therapeutic DNA constructs into such sites. Various studies have looked at macrophage trafficking after injection into a host organism, often after ex vivo manipulation, genetically (e.g., using genes coding for activating cytokines such as IFN- γ), or by treatment with activating agents such as cytokines or LPS. The aim of these studies was to assess their ability to “home” to diseased sites after local or systemic reimplantation into mice, rats, or humans [57–60]. Homing is a fundamental requirement for their use as vehicles to target locally acting gene therapy to specific diseased sites. It is less important for therapeutic strategies involving ex vivo loading of macrophages with, for example, tumor antigens, which utilize the antigen-presenting capabilities of macrophages but do not require them to migrate to specific disease sites to perform this function.

Routes of reimplantation have included intravenous (i.v.), intra-arterial, intraperitoneal (i.p.), and intrapleural [59]. Macrophage homing studies have involved radioactive labeling of macrophages in vitro with isotopes such as Indium 111 or Iodine 125 or expression of a reporter gene [61–64]. Typical findings are that in the short term, up to 2 h after reimplanta-

tion, macrophages accumulate primarily in the lungs and, to a lesser extent, in the liver and spleen rather than in the target diseased tissue. Subsequently, a proportion of labeled macrophages in the lungs returns to the circulation and is carried to the liver and spleen. A small proportion of manipulated macrophages, which appears to vary markedly between studies (ranging from 0.2% [63] to 28.8% [64]) following systemic administration, homes to the diseased site of interest, whether a tumor or a wound. The presence of labeled macrophages in these sites was found to persist for at least 6–7 days in both of these mouse studies. Greater success has been achieved using local administration of macrophages. For example, Chokri and co-workers [64] reported that more than 70% of locally injected macrophages homed to the tumor site in mice carrying a subcutaneous melanoma.

In an intermediate means of administration between local and systemic (sometimes referred to as “directed systemic”), rat BMDM and the rat alveolar macrophage cell line, NR8383, were adenovirally infected to overexpress IL-4 and then infused into the renal arteries of rats suffering from experimentally induced glomerulonephritis. Infused macrophages localized to inflamed glomeruli, and the infusion resulted in a remarkable 75% reduction in albuminuria, a sensitive indicator of glomerular damage [65, 66]. Interestingly, the efficiency with which injected macrophages localized to inflamed kidneys was increased markedly by incubation of cells with LPS prior to injection. This means of infusion is unlikely to be routinely possible in humans, but demonstrates the potential usefulness of macrophages as a gene delivery vehicle if the problem of efficient homing to target sites can be resolved.

Macrophages manipulated ex vivo have also been shown to be capable of homing to experimentally damaged muscle, and bone marrow cells from a mouse transgenic for the lacZ reporter gene were capable of engrafting mice and providing a long-term (up to 2 months) supply of genetically modified macrophages capable of migrating into damaged muscle sites when administered i.v. [67]. One of the potential advantages of macrophage adoptive transfer is the ability to circulate around the body after i.v. injection and target multiple diseased sites, for example, tumor metastases.

Unfortunately, as described above, several studies have shown that the majority of systemically administered macrophages become trapped in organs such as the lungs, liver, and spleen [60, 68, 69]. One of these, an early study using murine peritoneal macrophages, found that the organs to which the macrophages homed were determined by the method originally used to elicit them in the peritoneal cavity. Resident macrophages and those elicited by proteose peptone or thioglycollate broth localized initially to the lungs after i.v. reinfusion and then rapidly disseminated to the liver and spleen, but macrophages elicited by Brewer’s thioglycollate medium localized to the lungs and remained there for at least 72 h with little or no migration to the spleen [60]. The distribution of macrophages was found to be altered by activation of these cells in vivo through the i.p. injection of the pyran copolymer, MVE-2, prior to adoptive transfer. Activated cells contained a highly differentiated, low-density population of macrophages, which became trapped in the lungs for longer periods of time than nonactivated cells.

These cells also exhibited a reduced ability to migrate into the spleen. These different distributions are possibly due to differences in macrophage cell surface proteins induced by the respective treatments. This implies that it might be possible to target macrophages to certain organs or diseased sites by specific pretreatments or even by transfecting them to express particular cell surface-expressed proteins. For example, overexpression of the receptor for macrophage chemotactic protein-1, a cytokine released by many tumors, could possibly be used to maximize migration into such tumors.

Another possible way to enhance macrophage targeting of diseased sites is by using bispecific antibodies. Chokri and co-workers [68] showed that a bispecific antibody, which bound both to Fc receptors on the surface of macrophages and also to an adenocarcinoma antigen, increased the tumor cytotoxicity of the macrophages. They proposed that this approach might also be of value in directing the migration of macrophages to particular diseased sites.

It would seem logical to assume that since monocytes, not macrophages, are taken up across the endothelium into normal and pathological tissues, monocytes would prove more effective in “homing” to such tissues. However, work in a murine model has indicated that relatively mature *ex vivo*-manipulated murine monocytes are capable of migrating into tissues, where they undergo further maturation and cell division [69].

Use of transcriptional targeting to overcome the lack of specificity in macrophage homing to diseased sites

The finding that only a small proportion of reimplanted macrophages are likely to home to the target diseased site is perhaps not surprising given the ubiquitous distribution of macrophages around the body [1]. However, it emphasizes the need for a second level of gene targeting, for example, at the transcriptional level, in order to ensure that genetically manipulated macrophages that locate to non-target tissues do not express therapeutic genes in these tissues. This could be achieved using transcriptional control elements responsive to physiological states or to secreted proteins (e.g., cytokines) associated with the diseased tissue to be targeted. The best-explored type of transcriptional targeting is the use of hypoxia-responsive elements (HREs) to target expression to solid tumors, which are known to often contain regions of severe hypoxia (low oxygen) [70], and they may also be suitable for targeting other pathological conditions that are associated with hypoxia, such as wounds and sites of chronic infection. HREs have been used to mediate hypoxia-inducible gene expression in macrophages *in vitro* [71, 72]. The work of Griffiths and co-workers [71] in using the macrophage/HRE system to target tumor spheroids *in vitro* is discussed later in this review. Carta et al. [72] engineered a construct containing the IFN- γ gene under the control of three copies of the HRE from the inducible nitric oxide synthase gene promoter. They used electroporation to transfect the ANA-1 murine macrophage cell line with this and found that although these cells secreted basal levels of IFN- γ in normoxia (normal levels of oxygen), secretion increased more than fivefold when cells were exposed to hypoxia (1% O₂) *in vitro*.

It is not yet known, however, whether hypoxia-responsive enhancers would be sufficiently selective to restrict transgene expression in macrophages strictly to hypoxic areas *in vivo*, especially as transfected macrophages would be exposed to many other stimuli (including hormones such as insulin, which has been shown to up-regulate hypoxia-inducible factor-1 and thus potentially HRE activity) [73] as they pass through body fluids and tissues.

Use of macrophages to deliver therapeutic viruses

An interesting, recent study by Pastorino and co-workers [74] transduced the murine macrophage cell line, WGL5, with a replication-defective retrovirus (bearing the reporter gene eGFP) and a helper virus. They achieved stable integration, retrovirus production, and reporter gene expression. A high-expressing clone was then selected. When the transduced macrophage WGL5 cell line was administered subcutaneously to allogeneic mice, these cells formed solid tumors, as might be expected of an immortalized cell line. CD4⁺ and CD8⁺ T cells within the tumor were shown to be positive for the reporter gene, indicating that recombinant virus had been released from the transduced cells and was capable of infecting other cell types. Transduced WGL5 cells were also administered *i.v.* and were found not to form solid tumors but rather to accumulate initially in organs such as the lungs, spleen, and liver. However, after 24 h, some reporter gene expression was also observed in the brain. Whether this represented trafficking of transduced WGL5 cells or free virus to the brain was not clear.

The advantage of this novel approach of using macrophages as “virus factories” is that relatively few macrophages would need to reach the target organ to have a therapeutic effect, due to the amplification effect of each cell producing large numbers of viruses. However, there are important obstacles to be surmounted before this approach is likely to be viable clinically. For example, to transfer the system to primary macrophages rather than a cell line may be difficult because productive retroviral infections require cell division [16, 17]. Secondly, the DNA sequences regulating virus production would need to be modified to incorporate a second level of targeting (e.g., at the transcriptional level), specific to the disease or tissue being treated, to prevent side effects caused by macrophages localizing to non-target tissues and from viruses released into the circulation. Using hypoxia response elements as an “on/off” switch in such a system could possibly enable virus production to be limited to severely hypoxic tumor sites.

Malignant tumors

Macrophages accumulate in large numbers in avascular, hypoxic (low oxygen) sites in breast [75, 76] and prostate [77] carcinomas. As mentioned previously, hypoxia is widespread in malignant human tumors [70] due to their poorly organized vasculature and the faster growth of tumor cells than blood vessels. Macrophages are thought to be attracted by cytokines released by tumor cells in response to hypoxia and other such physiological stresses imposed by ischemia (reviewed in ref [78]). Exploitation of the tendency of macrophages to accumulate in such tumor sites, by adoptive transfer of macrophages to

cancer patients for therapeutic gene delivery or antigen presentation, has long been proposed [79, 80].

Recently, Griffiths et al. used an adenoviral vector to transduce primary human macrophages with a gene encoding the prodrug activating enzyme cytochrome P450 2B6 under the transcriptional control of a trimer of an HRE [71]. Infiltration of virus-transduced macrophages into tumor spheroids (which contain hypoxic centers) *in vitro* resulted in a sevenfold decrease in viable tumor cells in the presence of the prodrug cyclophosphamide compared with infiltrated spheroids not treated with the drug. However, the question of whether the basal level of cytochrome produced by the transduced macrophages in normoxic tissues would be low enough to also avoid killing cells in these sites was not examined.

LSD

Eto and Ohashi [81] infected murine macrophages derived from the *ex vivo* expansion of bone marrow cells using a recombinant adenoviral vector containing the human B-glucuronidase gene (HBG; a therapeutic gene for one of the LSD) and showed that their glycosaminoglycan accumulation was markedly reduced *in vitro*. They also took macrophages from normal mice (that expressed HBG) and injected them *i.v.* into Sly mice (a mouse model for glucuronidase deficiency). They showed that these cells populate the liver and spleen and, in doing so, raise HBG enzyme levels in these tissues. However, adenovirally infected macrophages have yet to be injected into HBG-negative mice to see whether this is sufficient to correct HBG deficiency.

Alveolar immunodeficiency

Gene therapy for immunodeficiency in the lung is limited, in part, by the difficulty of transfecting lung cells *in vivo*. Many options exist for transfecting cells *in vitro*, but they are not easily adapted for use *in vivo*. To overcome this limitation, macrophages (specifically, the murine macrophage cell line J774A) were transduced *in vitro* with the murine IFN- γ gene and delivered intratracheally into immunocompromised scid (severe combined immunodeficiency) mice. IFN- γ was detected in bronchoalveolar lavage fluid by 48 h, and immune function was partially restored in the lungs, with evidence of enhanced major histocompatibility complex (MHC) class II antigen expression and increased phagocytosis. However, *i.p.* administration of the engineered macrophages did not enhance IFN- γ levels in the lung. This study suggests that airway delivery of genetically engineered macrophages expressing the mIFN- γ gene may partially restore significant immune activity in the lungs of immunodeficient mice [82].

MACROPHAGES AS IN VIVO TARGETS FOR GENE THERAPY

In malignant tumors

As reviewed recently [78], macrophages can exert direct and indirect tumoricidal functions following stimulation with IFN- γ and LPS. They are also capable of phagocytosis of apoptotic

tumor cells and presenting tumor-associated antigens to T cells. Antigen-specific recognition and subsequent destruction of tumor cells are the goals of vaccine-based anticancer immunotherapy. However, macrophage cytotoxicity has been shown to be down-regulated by factors released by tumor cells, and tumor antigen-specific CTLs are often not available or are present in an inactive state. In addition, MHC-I expression by tumor cells is often down-regulated. These situations would allow tumors to evade tumoricidal mechanisms [83].

Genes coding for tumor antigen-specific monoclonal antibodies or fragments of them have been cloned into attenuated poxviruses. GM-CSF-activated human macrophages and cytotoxic T cells infected with such viruses acquire the ability to specifically kill tumor cells expressing those antigens *in vitro* [21, 22]. Whether these novel methods will be applicable *in vivo* awaits further study, but if so, this method could potentially have a role in anticancer gene therapy, although the high mutation rate of cancer cells makes it likely that variants lacking the epitope would arise.

Although IFN- γ is one of the most powerful stimulants for macrophage tumoricidal activity, when this cytokine was evaluated in clinical trials using *ex vivo* adoptive cellular immunotherapy protocols, a major problem encountered was the short duration of *ex vivo* activation of macrophages—repeated injections of *ex vivo*-activated cells were required to obtain a clinical response. Various studies have tried to circumvent this problem by transfecting macrophages to overexpress activating cytokines. Nishihara and co-workers [84] used retroviral vectors to engineer a macrophage cell line to express IFN- γ , IL-4, IL-6, or TNF- α and showed increased *in vitro* and *in vivo* tumoricidal activity by these cells. Ringenbach et al. [85] have used polyethylenimine-mediated transfection of the IFN- γ gene to enhance the tumoricidal activity of human monocytes *in vitro*. Moreover, the cytotoxicity and MHC-II expression of macrophages were augmented following *i.p.* injection of liposome-encapsulated IL-2 and IL-6 DNA into lymphoma-bearing mice [86]. However, little attempt was made in these studies to assess the longevity of gene expression or activation status of transfected cells *in vitro* or *in vivo*.

Evidence for an indirect approach to altering macrophage function in tumors has been provided by Richter et al. [87]. Chinese hamster ovary cells were stably transfected with the gene for the immunosuppressive cytokine IL-10. When grown *i.p.* in mice, these cells showed reduced angiogenesis and tumorigenicity compared with untransfected cells in nude and in SCID mice. The authors suggested that this phenomenon might be linked to the marked reduction in the numbers of macrophages seen in these tumors. Although unproven, this is a plausible hypothesis, as tumor-associated macrophages have been shown to secrete a variety of proangiogenic and prometastatic cytokines and enzymes (reviewed in ref [78]), so their elimination from tumors could result in retarded growth.

Arthritic joints

The abundance and activation of macrophages in the inflamed synovial membrane/pannus correlate closely with the severity of rheumatoid arthritis [88]. These cells exhibit widespread proinflammatory, destructive, and remodeling capabilities and contribute to the progression of acute and chronic disease.

Furthermore, activation of the monocytic lineage is not locally restricted, but extends to systemic parts of the mononuclear phagocyte system. Thus, the selective suppression of macrophage activation is a possible approach to diminishing local and systemic inflammation, as well as the prevention of irreversible joint damage.

Macrophage production of the potent proinflammatory molecule TNF- α has been implicated in the pathogenesis of inflammation in arthritic joints [88]. For this reason, Kisich et al. [43] used cationic, lipid-mediated delivery of ribozymes to selectively inhibit TNF- α production by murine peritoneal macrophages *in vitro* (by 80%). They went on to show that following *i.p.* injection of cationic lipid/ribozyme complexes, elicited peritoneal macrophages accumulated the ribozyme, and their TNF- α release in response to LPS was reduced. It remains to be seen whether such an injection into arthritic joints would suppress TNF- α production by synovial macrophages. Fellowes et al. [89] showed that an IL-10 expression plasmid/liposome complex injected *i.p.* in mice was taken up and expressed by macrophages in a collagen-induced model of arthritis. This led to marked and prolonged (up to 30 days postinjection) amelioration of inflammation in the arthritic joints.

Skin wounds

Macrophages are an important source of mitogenic growth factors and proangiogenic cytokines and enzymes in healing wounds (reviewed in ref [90]). A recent study has shown that it may be possible to accelerate wound healing (or correct defective wound-healing processes) by gene transfer to macrophages in wounds. Meuli et al. [29] were able to transfect macrophages, as well as fibroblasts and adipocytes, with the LacZ reporter gene in surgically wounded mouse skin following local injection of DNA alone or at a much lower level by *i.v.* injection of cationic liposomes/DNA complexes.

LSD

LSD are a group of about 50 monogenic, metabolic disorders caused by a deficiency in the intralysosomal enzymes involved in macromolecule catabolism. These defects are most prominently displayed in macrophages in afflicted patients. CGD is a rare, inherited immunodeficiency syndrome caused by the inability of macrophages to produce sufficient reactive oxygen metabolites. This dysfunction is a result of a defect in reduced nicotinamide adenine dinucleotide phosphate oxidase, the enzyme responsible for the production of superoxide. It is composed of several subunits, two of which, gp91phox and p22phox, form the membrane-bound cytochrome b558, and its three cytosolic components, p47phox, p67phox, and p40phox, have to translocate to the membrane upon activation [91].

gp91phox is encoded on the X-chromosome and p22phox, p47phox, and p67phox, on different autosomal chromosomes, and a defect in any one of these components leads to CGD. Schneider et al. [5] used adenovirus-mediated gene transfer to insert a gp91phox gene expressed from a powerful constitutive promoter (CMV) in gp91phox-deficient macrophages. This caused >70% of transfected cells to show respiratory burst activity *in vitro* and *in vivo*. These data indicate that autologous

macrophages transfected *ex vivo* or *in vivo* to express the gp91phox gene may have use in overcoming the life-threatening infections seen in X-CGD patients.

As most of the genes encoding the normal lysosomal enzymes have now been cloned, and the size of the corresponding cDNAs is found to be generally compatible with their transfer by recombinant vectors, macrophages in various forms of LSD may prove to be a viable and clinically useful target in gene therapy protocols [92].

Silicotic fibrosis

The finding that TNF- α release by alveolar macrophages plays a central role in the development of inflammation in silicotic fibrosis prompted Rojanasakul and co-workers [93] to attempt to inhibit silica-induced TNF- α release by these cells using an antisense oligonucleotide for TNF- α complexed to mannosylated polylysine (which exploits the endocytotic pathway regulated by the mannose receptor on macrophages). This inhibited TNF- α production by alveolar macrophages *in vitro* in the presence of silica. Further studies are now needed to determine whether systemic or local application of such complexes would be effective *in vivo*.

Atherosclerosis

This is an inflammatory disease involving recruitment and activation of macrophages, smooth muscle cells, and T cells. Macrophages accumulate low-density lipoprotein (LDL), which is atherogenic when it undergoes cell-mediated oxidation within the arterial wall [94]. Oxidized LDL promotes vascular dysfunction by exerting direct cytotoxicity toward endothelial cells, increasing the chemotactic properties of monocytes, transforming macrophages into foam cells via scavenger receptors (postulated to enhance their survival), and by enhancing the proliferation of various local cells such as macrophages and smooth muscle cells. These events are recognized as important contributing factors in the development of atherosclerosis. Laukkanen and co-workers [95] used adenoviral gene transfer to make murine macrophages express a secreted form of the human scavenger receptor. This inhibited their ability to degrade acetylated or oxidized LDL by up to 90% and inhibited their ability to form foam cells *in vitro*. Moreover, an adenovirus has been used to introduce a reporter gene into intimal macrophages (as well as intimal cells and smooth muscle cells) in atherosclerotic vessels in organ culture, thereby identifying them as targets for gene transfer *in vivo* [96].

Lung diseases

Ferkol and co-workers [47] recently attempted transfer of the α -1 antitrypsin gene, an inherited defect in which is a cause of the chronic lung disease emphysema, to mouse alveolar macrophages *in vivo* using mannosylated polylysine-conjugated DNA. Significant increases in the level of α -1 antitrypsin were achieved in the lungs of treated mice. In another, more recent study, when an adenovirus encoding heme oxygenase 1 (HO-1) was administered by direct, intratracheal inoculation into a murine model of acute lung injury induced by inhaled pathogen, HO-1 was not only expressed by surface cells in the

respiratory epithelium but also by alveolar macrophages [97].

HIV infection

Monocytes and macrophages are readily infected by HIV and can support viral replication, so there have been attempts to transfect macrophages to render them resistant to this virus. Macrophages derived from the *ex vivo* differentiation of CD34⁺ cells have been retrovirally transduced with the IFN- β gene *in vitro*. This enhanced their resistance to HIV infection and inhibited the replication of the virus in these cells, as well as increasing their release of IL-12 and IFN- γ (which in turn is likely to stimulate other cellular immune responses to HIV) [98].

In another study, macrophages were transfected *in vitro* using liposomes containing antisense oligonucleotide to the Rev response element (responsible for nuclear export of viral mRNAs) or a ribozyme complementary to the HIV-1 5' long-terminal repeat, which contains an important viral promoter. Both resulted in the inhibition of HIV replication by up to 90% in macrophages *in vitro* [42].

Liver disease

Kupffer cells play a significant role in the pathogenesis of many inflammatory liver diseases including early alcohol-induced liver injury. Therefore, a potential therapeutic strategy would be to modulate the activities of macrophages in the liver via a gene delivery system. A recent report has described how adenoviral gene transfer can be used to infect Kupffer cells in the liver *in vitro* and *in vivo* [99]. However, other cell types are likely to be infected using this approach, so some form of macrophage-specific, transcriptional targeting may prove useful to ensure expression of the transgene in Kupffer cells alone. This could involve placing transgenes under promoters for general, macrophage-specific genes such as *c-fms* (the gene encoding the receptor for M-CSF-1) [100], the LPS receptor CD14, CD68 [101], or genes up-regulated by macrophages only in diseased sites. Examples of the latter are the genes for TNF- α , transforming growth factor- β [102], or platelet-activating factor [103], all of which have been shown to be up-regulated at the mRNA level in Kupffer cells in liver disease.

CONCLUDING REMARKS

Gene transfer to macrophages *in vitro*, *ex vivo* for adoptive gene therapy protocols, or *in vivo* to correct their missing or faulty genes, relies on the availability of effective methods for the transfection of these cells. Of those reviewed here, three appear to hold particular promise: replication-defective lentiviruses and adeno-associated viruses, as they offer the advantage of stable gene transfer to nondividing cells such as macrophages, and adenoviruses. It remains to be seen whether any of these viral methods will prove sufficiently effective, safe, and non-immunogenic for use *in vivo*. Macrophages infected using a viral vector *ex vivo* and reinfused or targeted *in vivo* may express viral surface proteins and thus become immunogenic.

For example, although use of adenoviruses achieves very high transfection efficiencies in macrophages *in vitro*, transgene expression is relatively short-lived (several weeks). This could mean that repeated rounds of adenovirally transduced macrophages (or adenoviral vector, in the case of *in vivo* macrophage targeting) would have to be administered. This is unlikely to be feasible due to the immune response mounted by the recipient, although it is possible that the use of less immunogenic "gut-less" virus variants may solve this problem in the future. The use of CD34⁺ cells stably transfected using retroviral vectors holds particular promise as it allows transfection to be carried out *ex vivo* under controlled conditions and provides the recipient with a long-term (possibly even lifelong, if a proportion of these cells repopulate the bone marrow) supply of transgenic macrophages.

Despite interest in the use of macrophages as a gene delivery system, relatively few *in vivo* studies have been carried out to look at the ability of *ex vivo*-transfected macrophages to migrate into (and express transgenes in) target sites. Nor have the effects of different differentiation/activation states of the cells used, cell numbers injected, or routes of administration for reinfusion been fully examined. Such studies are now warranted if the value of such novel, macrophage-based, adoptive gene therapies is to be accurately assessed.

The targeting of macrophages *in vivo*, using various viral and nonviral methods, looks promising, as this utilizes the natural ability of macrophages to take up cell surface ligands (via general phagocytotic or specific receptor-ligand internalization pathways) to ensure uptake of such gene vectors *in vivo*. However, such vectors cannot be relied on to deliver genes exclusively to macrophages, so transcriptional targeting of transgene expression (for example, using macrophage-specific promoters) may prove essential if gene expression in nontarget cells and thus unwanted side effects are to be avoided. Once again, however, the paucity of clinical studies showing the efficacy of *in vivo* targeting of transgenes to macrophages means that possible therapeutic applications remain conjecture rather than fact at this stage.

Another salient fact to emerge from this review is that promonocytic and leukemic cell lines are rarely good models to use when assessing gene transfer to macrophages, as methods used for their transfection may prove relatively ineffective in primary macrophages. Moreover, primary cells may express transgenes in a different manner (if at all) compared with these transformed cell lines.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the support of Yorkshire Cancer Research (B. B., N. M., and C. E. L.), and the MRC, BBSRC, EPSRC, and Breast Cancer Campaign (C. E. L.).

REFERENCES

1. Ross, J. A., Auger, M. J. (2002) The biology of the macrophage. In *The Macrophage*, 2nd ed. (B. Burke, C. E. Lewis, eds.), Oxford University Press, Oxford, UK, in press.

2. Nagata, Y., Diamond, B., Bloom, B. R. (1983) The generation of human monocyte/macrophage cell lines. *Nature* 306, 597–599.
3. Mountain, A. (2000) Gene therapy: the first decade. *Trends Biotechnol.* 18, 119–128.
4. Haddada, H., Lopez, M., Martinache, C., Ragot, T., Abina, M. A., Perricaudet, M. (1993) Efficient adenovirus-mediated gene transfer into human blood monocyte-derived macrophages. *Biochem. Biophys. Res. Commun.* 195, 1174–1183.
5. Schneider, S. D., Rusconi, S., Seger, R. A., Hossle, J. P. (1997) Adenovirus-mediated gene transfer into monocyte-derived macrophages of patients with X-linked chronic granulomatous disease: ex vivo correction of deficient respiratory burst. *Gene Ther.* 4, 524–532.
6. De, S. K., Venkateshan, C. N., Seth, P., Gajdusek, D. C., Gibbs, C. J. (1998) Adenovirus-mediated human immunodeficiency virus-1 Nef expression in human monocytes/macrophages and effect of Nef on down-modulation of Fcγ receptors and expression of monokines. *Blood* 91, 2108–2117.
7. Heider, H., Verca, S. B., Rusconi, S., Asmis, R. (2000) Comparison of lipid-mediated and adenoviral gene transfer in human monocyte-derived macrophages and COS-7 cells. *Biotechniques* 28, 260–265, 268–270.
8. Wirtz, S., Becker, C., Blumberg, R., Galle, P. R., Neurath, M. F. (2002) Treatment of T cell-dependent experimental colitis in SCID mice by local administration of an adenovirus expressing IL-18 antisense mRNA. *J. Immunol.* 168, 411–420.
9. Foxwell, B., Browne, K., Bondeson, J., Clarke, C., de Martin, R., Brennan, F., Feldmann, M. (1998) Efficient adenoviral infection with IkappaB alpha reveals that macrophage tumor necrosis factor alpha production in rheumatoid arthritis is NF-kappaB dependent. *Proc. Natl. Acad. Sci. USA* 95, 8211–8215.
10. Parveen, Z., Krupetsky, A., Engelstadter, M., Cichutek, K., Pomerantz, R. J., Dornburg, R. (2000) Spleen necrosis virus-derived C-type retroviral vectors for gene transfer to quiescent cells. *Nat. Biotechnol.* 18, 623–629.
11. Vassilopoulos, G., Trobridge, G., Josephson, N. C., Russell, D. W. (2001) Gene transfer into murine hematopoietic stem cells with helper-free foamy virus vectors. *Blood* 98, 604–609.
12. Chischportich, C., Bagnis, C., Galindo, R., Mannoni, P. (1999) Expression of the nlsLacZ gene in dendritic cells derived from retrovirally transduced peripheral blood CD34+ cells. *Haematologica* 84, 195–203.
13. Miyake, K., Suzuki, N., Matsuoka, H., Tohyama, T., Shimada, T. (1998) Stable integration of human immunodeficiency virus-based retroviral vectors into the chromosomes of nondividing cells. *Hum. Gene Ther.* 9, 467–475.
14. Schroers, R., Sinha, I., Segall, H., Schmidt-Wolf, I. G., Rooney, C., Brenner, M. K., Sutton, R. E., Chen, S. Y. (2000) Transduction of human PBMC-derived dendritic cells and macrophages by an HIV-1-based lentiviral vector system. *Mol. Ther.* 1, 171–179.
15. Follenzi, A., Ailles, L. E., Bakovic, S., Geuna, M., Naldini, L. (2000) Gene transfer by lentiviral vectors is limited by nuclear translocation and rescued by HIV-1 pol sequences. *Nat. Genet.* 25, 217–222.
16. Schuitemaker, H., Miedema, F. (1992) Viral and cellular determinants of HIV-1 replication in macrophages. *AIDS* 10 (Suppl. A), S25–S32.
17. Schuitemaker, H., Kootstra, N. A., Fouchier, R. A., Hooibrink, B., Miedema, F. (1994) Productive HIV-1 infection of macrophages restricted to the cell fraction with proliferative capacity. *EMBO J.* 13, 5929–5936.
18. Wu, X., Wakefield, J. K., Liu, H., Xiao, H., Kralovics, R., Prchal, J. T., Kappes, J. C. (2000) Development of a novel trans-lentiviral vector that affords predictable safety. *Mol. Ther.* 2, 47–55.
19. Liu, Y., Santin, A. D., Mane, M., Chiriva-Internati, M., Parham, G. P., Ravaggi, A., Hermonat, P. L. (2000) Transduction and utility of the granulocyte-macrophage colony-stimulating factor gene into monocytes and dendritic cells by adeno-associated virus. *J. Interferon Cytokine Res.* 20, 21–30.
20. Ponnazhagan, S., Mahendra, G., Curiel, D. T., Shaw, D. R. (2001) Adeno-associated virus type 2-mediated transduction of human monocyte-derived dendritic cells: implications for ex vivo immunotherapy. *J. Virol.* 75, 9493–9501.
21. Paul, S., Bizouarne, N., Dott, K., Ruet, L., Dufour, P., Acres, R. B., Kieny, M. P. (2000) Redirected cellular cytotoxicity by infection of effector cells with a recombinant vaccinia virus encoding a tumor-specific monoclonal antibody. *Cancer Gene Ther.* 7, 615–623.
22. Paul, S., Snary, D., Hoebeke, J., Allen, D., Balloul, J. M., Bizouarne, N., Dott, K., Geist, M., Hilgers, J., Kieny, M. P., Burchell, J., Taylor-Papadimitriou, J., Acres, R. B. (2000) Targeted macrophage cytotoxicity using a nonreplicative live vector expressing a tumor-specific single-chain variable region fragment. *Hum. Gene Ther.* 11, 1417–1428.
23. Paludan, S. R. (2001) Requirements for the induction of interleukin-6 by herpes simplex virus-infected leukocytes. *J. Virol.* 75, 8008–8015.
24. Nettelbeck, D. M., Miller, D. W., Jerome, V., Zuzarte, M., Watkins, S. J., Hawkins, R. E., Muller, R., Kontermann, R. E. (2001) Targeting of adenovirus to endothelial cells by a bispecific single-chain diabody directed against the adenovirus fiber knob domain and human diablo (CD105). *Mol. Ther.* 3, 882–891.
25. Krasnykh, V., Belousova, N., Korokhov, N., Mikheeva, G., Curiel, D. T. (2001) Genetic targeting of an adenovirus vector via replacement of the fiber protein with the phage T4 fibritin. *J. Virol.* 75, 4176–4183.
26. Krasnykh, V. N., Douglas, J. T., van Beusechem, V. W. (2000) Genetic targeting of adenoviral vectors. *Mol. Ther.* 1, 391–405.
27. Bennet, R. M., Gabor, G. T., Merrit, M. (1985) DNA binding to human leukocytes: evidence for a receptor-mediated association, internalization and degradation of DNA. *J. Clin. Investig.* 76, 2182–2190.
28. Stacey, K. J., Sweet, M. J., Hume, D. A. (1996) Macrophages ingest and are activated by bacterial DNA. *J. Immunol.* 157, 2116–2122.
29. Meuli, M., Liu, Y., Liggitt, D., Kashani-Sabet, M., Knauer, S., Meuli-Simmen, C., Harrison, M. R., Adzick, N. S., Heath, T. D., Debs, R. J. (2001) Efficient gene expression in skin wound sites following local plasmid injection. *J. Investig. Dermatol.* 116, 131–135.
30. Condon, C., Watkins, S. C., Celluzzi, C. M., Thompson, K., Faló Jr., L. D. (1996) DNA-based immunization by in vivo transfection of dendritic cells. *Nat. Med.* 2, 1122–1128.
31. Stacey, K. J., Sester, D. P., Sweet, M. J., Hume, D. A. (2000) Macrophage activation by immunostimulatory DNA. *Curr. Top. Microbiol. Immunol.* 247, 41–58.
32. Krieg, A. M. J. (1999) Direct immunologic activities of CpG DNA and implications for gene therapy. *J. Gene Med.* 1, 56–63.
33. Weir, J. P., Meltzer, M. S. (1993) Transfection of human immunodeficiency virus type 1 proviral DNA into primary human monocytes. *Cell. Immunol.* 148, 157–165.
34. Takahashi, M., Furukawa, T., Tanaka, I., Nikkuni, K., Aoki, A., Kishi, K., Koike, T., Moriyama, Y., Shibata, A. (1992) Gene introduction into granulocyte-macrophage progenitor cells by electroporation: the relationship between introduction efficiency and the proportion of cells in S-phase. *Leuk. Res.* 16, 761–767.
35. Liao, H. S., Kodama, T., Doi, T., Emi, M., Asaoka, H., Itakura, H., Matsumoto, A. (1997) Novel elements located at –504 to –399 bp of the promoter region regulated the expression of the human macrophage scavenger receptor gene in murine macrophages. *J. Lipid Res.* 38, 1433–1444.
36. Kusumawati, A., Combes, T., Liautard, J. P., Widada, J. S. (1999) Transfection of myelomonocytic cell lines: cellular response to a lipid-based reagent and electroporation. *Anal. Biochem.* 269, 219–221.
37. Li, L. H., McCarthy, P., Hui, S. W. (2001) High-efficiency electrotransfection of human primary hematopoietic stem cells. *FASEB J.* 15, 586–588.
38. Mack, K. D., Wei, R., Elbagarri, A., Abbey, N., McGrath, M. S. (1998) A novel method for DEAE-dextran mediated transfection of adherent primary cultured human macrophages. *J. Immunol. Methods* 211, 79–86.
39. Dokka, S., Toledo, D., Shi, X., Ye, J., Rojanasakul, Y. (2000) High-efficiency gene transfection of macrophages by lipoplexes. *Int. J. Pharm.* 206, 97–104.
40. Liu, Y., Mounkes, L. C., Liggitt, H. D., Brown, C. S., Solodin, I., Heath, T. D., Debs, R. J. (1997) Factors influencing the efficiency of cationic liposome-mediated intravenous gene delivery. *Nat. Biotechnol.* 15, 167–173.
41. McLean, J. W., Fox, E. A., Baluk, P., Bolton, P. B., Haskell, A., Pearlman, R., Thurston, G., Umemoto, E. Y., McDonald, D. M. (1997) Organ-specific endothelial cell uptake of cationic liposome-DNA complexes in mice. *Am. J. Physiol.* 273, H387–H404.
42. Duzgunes, N., Pretzer, E., Simoes, S., Slepushkin, V., Konopka, K., Flasher, D., de Lima, M. C. (1999) Liposome-mediated delivery of antiviral agents to human immunodeficiency virus-infected cells. *Mol. Membr. Biol.* 16, 111–118.
43. Kisich, K. O., Malone, R. W., Feldstein P. A., Erickson, K. L. (1999) Specific inhibition of macrophage TNF-alpha expression by in vivo ribozyme treatment. *J. Immunol.* 163, 2008–2016.
44. Irvine, A. S., Trinder, P. K., Laughton, D. L., Ketteringham, H., McDermott, R. H., Reid, S. C., Haines, A. M., Amir, A., Husain, R., Doshi, R., Young, L. S., Mountain, A. (2000) Efficient nonviral transfection of dendritic cells and their use for in vivo immunization. *Nat. Biotechnol.* 18, 1273–1278.
45. Erbacher, P., Bousser, M. T., Raimond, J., Monsigny, M., Midoux, P., Roche, A. C. (1996) Gene transfer by DNA/glycosylated polylysine

- complexes into human blood monocyte-derived macrophages. *Hum. Gene Ther.* 7, 721–729.
46. Simoes, S., Slepishkin, V., Pretzer, E., Dazin, P., Gaspar, R., Pedrosa de Lima, M. C., Duzgunes, N. (1999) Transfection of human macrophages by lipoplexes via the combined use of transferrin and pH-sensitive peptides. *J. Leukoc. Biol.* 65, 270–279.
 47. Ferkol, T., Perales, J. C., Mularo, F., Hanson, R. W. (1996) Receptor-mediated gene transfer into macrophages. *Proc. Natl. Acad. Sci. USA* 93, 101–105.
 48. Ferkol, T., Mularo, F., Hilliard, J., Lodish, S., Perales, J. C., Ziady, A., Konstan, M. (1998) Transfer of the human alpha-1-antitrypsin gene into pulmonary macrophages in vivo. *Am. J. Respir. Cell Mol. Biol.* 18, 591–601.
 49. Kawakami, S., Sato, A., Nishikawa, M., Yamashita, F., Hashida, M. (2000) Mannose receptor-mediated gene transfer into macrophages using novel mannoseylated cationic liposomes. *Gene Ther.* 7, 292–299.
 50. Paglia, P., Terrazzini, N., Schulze, K., Guzman, C. A. (2000) Colombo MP. In vivo correction of genetic defects of monocyte/macrophages using attenuated Salmonella as oral vectors for targeted gene delivery. *Gene Ther.* 7, 1725–1730.
 51. Montosi, G., Paglia, P., Garuti, C., Guzman, C. A., Bastin, J. M., Colombo, M. P., Pietrangelo, A. (2000) Wild-type HFE protein normalizes transferrin iron accumulation in macrophages from subjects with hereditary hemochromatosis. *Blood* 96, 1125–1129.
 52. Titus, R. G., Gueiros-Filho, F. J., de Freitas, L. A. R., Beverley, S. M. (1995) Development of a safe live Leishmania vaccine line by gene replacement. *Proc. Natl. Acad. Sci. USA* 92, 10267–10271.
 53. Vaccaro, D. E. (2000) Symbiosis therapy: the potential of using human protozoa for molecular therapy. *Mol. Ther.* 2, 535–538.
 54. Hense, M., Domann, E., Krusch, S., Wachholz, P., Dittmar, K. E., Rohde, M., Wehland, J., Chakraborty, T., Weiss, S. (2001) Eukaryotic expression plasmid transfer from the intracellular bacterium *Listeria monocytogenes* to host cells. *Cell. Microbiol.* 3, 599–609.
 55. Spreng, S., Dietrich, G., Niewiesk, S., ter Meulen, V., Gentschev, I., Goebel, W. (2000) Novel bacterial systems for the delivery of recombinant protein or DNA. *FEMS Immunol. Med. Microbiol.* 27, 299–304.
 56. Fidler, I. J. (1974) Inhibition of pulmonary metastasis by intravenous injection of specifically activated macrophages. *Cancer Res.* 34, 1074–1078.
 57. Fidler, I. J. (1994) Therapy of cancer metastasis by systemic activation of macrophages. *Adv. Pharmacol.* 30, 271–326.
 58. Ben-Efraim, S., Tak, C., Romijn, J. C., Fieren, M. J., Bonta, I. L. (1994) Therapeutic effect of activated human macrophages on a human tumor line growing in nude mice. *Med. Oncol.* 11, 7–12.
 59. Andreesen, R., Hennemann, B., Krause, S. W. (1998) Adoptive immunotherapy of cancer using monocyte-derived macrophages: rationale, current status, and perspectives. *J. Leukoc. Biol.* 64, 419–426.
 60. Wiltrout, R. H., Brunda, M. J., Gorelik, E., Peterson, E. S., Dunn, J. J., Leonhardt, J., Varesio, L., Reynolds, C. W., Holden, H. T. J. (1983) Distribution of peritoneal macrophage populations after intravenous injection in mice: differential effects of eliciting and activating agents. *Reticuloendothel. Soc.* 34, 253–269.
 61. Abreo, K., Lieberman, L. M., Moorthy, A. V. (1985) Distribution studies of 111 IN-oxine-labelled peritoneal mononuclear cells in tumor-bearing rats. *Int. J. Nucl. Med. Biol.* 12, 53–55.
 62. Faradji, A., Bohbot, A., Frost, H., Schmitt-Goguel, M., Siffert, J. C., Dufour, P., Eber, M., Lallot, C., Wiesel, M. L., Bergerat, J. P. (1991) Phase I study of liposomal MTP-PE-activated autologous monocytes administered intraperitoneally to patients with peritoneal carcinomatosis. *J. Clin. Oncol.* 9, 1251–1260.
 63. Audran, R., Collet, B., Moisan, A., Toujas, L. (1995) Fate of mouse macrophages radiolabelled with PKH-95 and injected intravenously. *Nucl. Med. Biol.* 22, 817–821.
 64. Chokri, M., Lallot, C., Ebert, M., Poindron, P., Bartholeyns, J. (1990) Biodistribution of indium-labelled macrophages in mice bearing solid tumors. *Int. J. Immunol.* 1, 79–84.
 65. Kluth, D. C., Erwig, L. P., Pearce, W. P., Rees, A. J. (2000) Gene transfer into inflamed glomeruli using macrophages transfected with adenovirus. *Gene Ther.* 7, 263–270.
 66. Kluth, D. C., Ainslie, C. V., Pearce, W. P., Finlay, S., Clarke, D., Anegon, I., Rees, A. J. (2001) Macrophages transfected with adenovirus to express IL-4 reduce inflammation in experimental glomerulonephritis. *J. Immunol.* 166, 4728–4736.
 67. Parrish, E. P., Cifuentes-Diaz, C., Li, Z. L., Vicart, P., Paulin, D., Dreyfus, P. A., Peschanski, M., Harris, A. J., Garcia, L. (1996) Targeting widespread sites of damage in dystrophic muscle: engrafted macrophages as potential shuttles. *Gene Ther.* 3, 13–20.
 68. Chokri, M., Girard, A., Borrelly, M. C., Oleron, C., Romet-Lemonne, J. L., Bartholeyns, J. (1992) Adoptive immunotherapy with bispecific antibodies: targeting through macrophages. *Res. Immunol.* 143, 95–99.
 69. Kennedy, D. W., Abkowitz, J. L. (1998) Mature monocytic cells enter tissues and engraft. *Proc. Natl. Acad. Sci. USA* 95, 14944–14949.
 70. Vaupel, P., Kallinowski, F., Okunieff, P. (1989) Blood flow, oxygen and nutrient supply, and metabolic microenvironment of human tumors: a review. *Cancer Res.* 49, 6449–6465.
 71. Griffiths, L., Binley, K., Iqbal, S., Kan, O., Maxwell, P., Ratcliffe, P., Lewis, C., Harris, A., Kingsman, S., Naylor, S. (2000) The macrophage—a novel system to deliver gene therapy to pathological hypoxia. *Gene Ther.* 7, 255–262.
 72. Carta, L., Pastorino, S., Melillo, G., Bosco, M. C., Massazza, S., Varesio, L. (2001) Engineering of macrophages to produce IFN-gamma in response to hypoxia. *J. Immunol.* 166, 5374–5380.
 73. Zelzer, E., Levy, Y., Kahana, C., Shilo, B. Z., Rubinstein, M., Cohen, B. (1998) Insulin induces transcription of target genes through the hypoxia-inducible factor HIF-1alpha/ARNT. *EMBO J.* 17, 5085–5094.
 74. Pastorino, S., Massazza, S., Cilli, M., Varesio, L., Bosco, M. C. (2001) Generation of high-titer retroviral vector-producing macrophages as vehicles for in vivo gene transfer. *Gene Ther.* 8, 431–441.
 75. Leek, R. D., Lewis, C. E., Whitehouse, R., Greenall, M., Clarke, J., Harris, A. L. (1996) Association of macrophage infiltration with angiogenesis and prognosis in invasive breast carcinoma. *Cancer Res.* 56, 4625–4629.
 76. Lewis, J. S., Landers, R. J., Underwood, J. C., Harris, A. L., Lewis, C. E. (2000) Expression of vascular endothelial growth factor by macrophages is up-regulated in poorly vascularized areas of breast carcinomas. *J. Pathol.* 192, 150–158.
 77. Burton, J., Wells, M., Corke, K. P., Maitland, N., Hamdy, F. C., Lewis, C. E. (2000) Macrophages accumulate in avascular, hypoxic areas in prostate tumours: implications for hypoxia-targeted gene delivery to such sites. *J. Pathol.* 192, 8A (abstr.).
 78. Bingle, L., Brown, N. J., Lewis, C. E. (2002) The role of tumor-associated macrophages in the growth and metastasis of malignant human tumors. *J. Pathol.* 196, 254–265.
 79. Bartholeyns, J., Lopez, M. (1994) Immune control of neoplasia by adoptive transfer of macrophages: potentiality for antigen presentation and gene transfer. *Anticancer Res.* 14, 2673–2676.
 80. Bartholeyns, J., Romet-Lemonne, J. L., Chokri, M., Lopez, M. (1996) Immune therapy with macrophages: present status and critical requirements for implementation. *Immunobiology* 195, 550–562.
 81. Eto, Y., Ohashi, T. (2000) Gene therapy/cell therapy for lysosomal storage disease. *J. Inher. Metab. Dis.* 23, 293–298.
 82. Wu, M., Hussain, S., He, Y. H., Pasula, R., Smith, P. A., Martin W. J. (2001) Genetically engineered macrophages expressing IFN-gamma restore alveolar immune function in scid mice. *Proc. Natl. Acad. Sci. USA* 98, 14589–14594.
 83. Speiser, D. E., Ohashi, P. S. (1998) Activation of cytotoxic T cells by solid tumours? *Cell Mol. Life Sci.* 54, 263–271.
 84. Nishihara, K., Barth, R. F., Wilkie, N., Lang, J. C., Oda, Y., Kikuchi, H., Everson, M. P., Lotze, M. T. (1995) Increased in vitro and in vivo tumoricidal activity of a macrophage cell line genetically engineered to express IFN-gamma, IL-4, IL-6, or TNF-alpha. *Cancer Gene Ther.* 2, 113–124.
 85. Ringenbach, L., Bohbot, A., Tiberghien, P., Oberling, F., Feugeas, O. (1998) Polyethylenimine-mediated transfection of human monocytes with the IFN-gamma gene: an approach for cancer adoptive immunotherapy. *Gene Ther.* 5, 1508–1516.
 86. Wang, Q., Cao, X., Wang, J., Zhang, W., Tao, Q., Ye, T. (2000) Macrophage activation of lymphoma-bearing mice by liposome-mediated intraperitoneal IL-2 and IL-6 gene therapy. *Chin. Med. J.* 113, 281–285.
 87. Richter, G., Kruger-Krasagakes, S., Hein, G., Huls, C., Schmitt, E., Diamantstein, T., Blankenstein, T. (1993) Interleukin 10 transfected into Chinese hamster ovary cells prevents tumor growth and macrophage infiltration. *Cancer Res.* 53, 4134–4137.
 88. Kinne, R. W., Brauer, R., Stuhlmuller, B., Palombo-Kinne, E., Burmester, G. R. (2000) Macrophages in rheumatoid arthritis. *Arthritis Res.* 2, 189–202.
 89. Fellowes, R., Etheridge, C. J., Coade, S., Cooper, R. G., Stewart, L., Miller, A. D., Woo, P. (2000) Amelioration of established collagen induced arthritis by systemic IL-10 gene delivery. *Gene Ther.* 7, 967–977.
 90. Crowther, M., Brown, N. J., Bishop, E. T., Lewis, C. E. (2001) Macrophage regulation of angiogenesis in wound healing and malignant tumors: role of common microenvironmental stress factors? *J. Leukoc. Biol.* 70, 478–490.

91. Meischl, C., Roos, D. (1998) The molecular basis of chronic granulomatous disease. *Springer Semin. Immunopathol.* 19, 417–434.
92. Poenaru, L. (2001) From gene transfer to gene therapy in lysosomal storage diseases affecting the central nervous system. *Ann. Med.* 33, 28–36.
93. Rojanasakul, Y., Weissman, D. N., Shi, X., Castranova, V., Ma, J. K., Liang, W. (1997) Antisense inhibition of silica-induced tumor necrosis factor in alveolar macrophages. *J. Biol. Chem.* 272, 3910–3914.
94. Hegyi, L., Hardwick, S. J., Siow, R. C., Skepper, J. N. (2001) Macrophage death and the role of apoptosis in human atherosclerosis. *J. Hematother. Stem Cell Res.* 10, 27–42.
95. Laukkanen, J., Lehtolainen, P., Gough, P. J., Greaves, D. R., Gordon, S., Yla-Herttuala, S. (2000) Adenovirus-mediated gene transfer of a secreted form of human macrophage scavenger receptor inhibits modified low-density lipoprotein degradation and foam-cell formation in macrophages. *Circulation* 101, 1091–1096.
96. Rekhter, M. D., Simari, R. D., Work, C. W., Nabel, G. J., Nabel, E. G., Gordon D. (1998) Gene transfer into normal and atherosclerotic human blood vessels. *Circ. Res.* 82, 1243–1252.
97. Inoue, S., Suzuki, M., Nagashima, Y., Suzuki, S., Hashiba, T., Tsuburai, T., Ikehara, K., Matsuse, T., Ishigatsubo, Y. (2001) Transfer of heme oxygenase 1 cDNA by a replication-deficient adenovirus enhances interleukin 10 production from alveolar macrophages that attenuates lipopolysaccharide-induced acute lung injury in mice. *Hum. Gene Ther.* 12, 967–979.
98. Cremer, I., Vieillard, V., De Maeyer, E. (2000) Retrovirally mediated IFN-beta transduction of macrophages induces resistance to HIV, correlated with up-regulation of RANTES production and down-regulation of C-C chemokine receptor-5 expression. *J. Immunol.* 164, 1582–1587.
99. Wheeler, M. D., Yamashina, S., Froh, M., Rusyn, I., Thurman, R. G. (2001) Adenoviral gene delivery can inactivate Kupffer cells: role of oxidants in NF-kappaB activation and cytokine production. *J. Leukoc. Biol.* 69, 622–630.
100. Himes, S. R., Tagoh, H., Goonetilleke, N., Sasmono, T., Oceandy, D., Clark, R., Bonifer, C., Hume, D. A. (2001) A highly conserved c-fms gene intronic element controls macrophage-specific and regulated expression. *J. Leukoc. Biol.* 70, 812–820.
101. Greaves, D. R., Quinn, C. M., Seldin, M. F., Gordon, S. (1998) Functional comparison of the murine macrophage and human CD68 promoters in macrophage and non-macrophage cell lines. *Genomics* 54, 165–168.
102. Kamimura, S., Tsukamoto, H. (1995) Cytokine gene expression by Kupffer cells in experimental alcoholic liver disease. *Hepatology* 22, 1304–1309.
103. Mizuno, S., Izumi, T., Isaji, S. (2001) Role of PAF in acute liver injury after extended hepatectomy: overexpression of PAF receptor mRNA in Kupffer cells. *Dig. Dis. Sci.* 46, 1299–1304.