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Genetic, cellular and immune approaches to disease therapy: past and future

Gary J Nabel

Advances in immunology and molecular genetics have accelerated our understanding of the genetic and cellular basis of many diseases. At the same time, remarkable progress in recombinant DNA technology has enabled the development of molecular and cellular treatments for infectious diseases, inherited disorders and cancer. This Perspective is intended to give a sample of the progress over the past ten years in cellular, genetic and immune therapy of disease. During this time, monoclonal antibody technology and cellular transplantation have begun to come of age in biomedicine. Innovations in gene delivery have not only catalyzed the nascent field of human gene therapy, but may also ultimately impact human health by advancing recombinant vaccine technology.

The year 2003 occasioned a landmark celebration: the fiftieth anniversary of the discovery of the structure of DNA. This was an opportunity to appreciate the extraordinary accomplishments of modern biology. The discovery paved the way for a renaissance in understanding basic biological principles, which is beginning to have direct application to the treatment of human disease. The year 2004 marks another anniversary: ten years of *Nature Medicine*, during which time the information derived from basic research has increasingly found its way into clinical practice. Advances in the powerful disciplines of immunology and molecular genetics have been synergistic, and our strengthened understanding of disease pathogenesis has allowed us to conceive and generate new therapies. During the past decade, several technologies once thought promising, but technically challenging, have proven tractable and are making significant inroads into the practice of medicine.

Recombinant antibodies

The development of the monoclonal antibody technique[1]—the fusion of spleen-derived B cells with dividing cancer cells, creating an antibody-generating 'hybridoma'—was greeted with enthusiasm in 1975, and monoclonal antibodies were predicted to have a profound effect on the treatment of human disease. Indeed, these antibodies quickly became of great practical use in basic research and diagnostic analysis. For at least two decades, monoclonal antibodies have provided fundamental insights into the antigenic structure of proteins and have helped reveal the roles of certain proteins in human disease. But the early promise of monoclonal antibodies as therapeutic agents was slow to materialize. Progress in this area was hampered by a number of technical hurdles: therapeutically relevant targets were difficult to identify, and the cost of producing purified biological reagents was prohibitively high. Worse still, it became evident that the first-generation antibodies derived from mouse hybridomas were themselves subject to immune responses that limited the efficacy and duration of their therapeutic effect.

The past decade has seen several major breakthroughs in monoclonal antibody therapeutics. The realization that antibodies to tumor necrosis factor-α (TNF-α) can ameliorate the symptoms of autoimmunity in diseases including rheumatoid arthritis and Crohn disease was a catalyst for further antibody research[2]. Indeed, antibodies to TNF-α provided a means to define the role of the cytokine in the immunopathogenesis of rheumatoid arthritis, while simultaneously becoming a therapeutic intervention. Three monoclonal antibodies have recently been evaluated and licensed for the treatment of rheumatoid arthritis[3–5], and further studies are in progress for psoriasis and psoriatic arthritis[6,7]. Substantial improvements in production technology, including the ability to make fully humanized monoclonal antibodies that evade the immune response, have facilitated progress in this field[3].

Efforts to humanize the monoclonal antibodies capitalized on an understanding of the structure of the immunoglobulin gene family. This knowledge enabled mouse 'complementarity-determining regions' (the regions that bind antigenic epitopes) of the immunoglobulin molecule to be engineered into a human variable- and constant-region framework (**Fig. 1**). Remicade, one of three licensed monoclonal antibodies to TNF-α, is such a humanized antibody, but it still elicits immune responses to the residual mouse complementarity-determining regions, which affect its potency and half-life.

Because even humanized antibodies can still elicit limiting undesirable immune responses, efforts have focused on the development of complete human monoclonal antibodies. The early hybridoma partners (cancer cells fused to spleen-derived B cells) were incapable of supporting the development of human antibodies, but a variety of technical improvements have now made it possible to generate completely humanized therapeutic antibodies (such as the recently licensed TNF-α-specific antibody adalimumab) that minimize the likelihood of generating anti-immunoglobulin responses. Among these technical improvements

Gary J. Nabel is at the Vaccine Research Center, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bldg. 40, Room 4502, MSC-3005, 40 Convent Drive, Bethesda, Maryland 20892-3005, USA.
e-mail: gnabel@nih.gov
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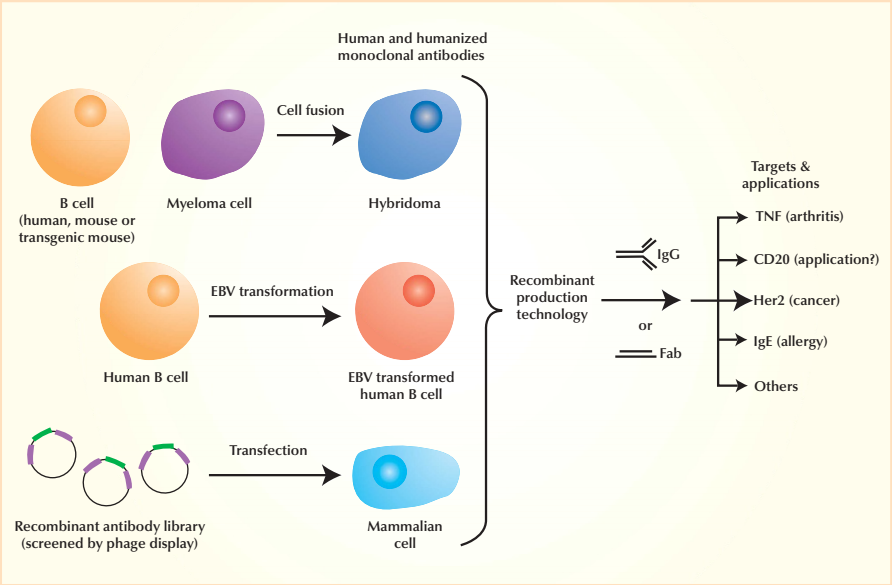

Figure 1 Evolution of recombinant antibody technology and clinical applications. Identification of relevant target proteins, modification of immunogenicity and improvements in production technology have enabled clinical applications of treatments for rheumatoid arthritis, Crohn disease, psoriasis, cancer therapy, allergy, asthma and other indications.

are Epstein-Barr virus–transformed cell lines, newer myeloma fusion partners that support the production of human monoclonal antibodies, the use of transgenic animals that express human immunoglobulin genes, the development of recombinant technologies for screening human libraries by phage display, and more sophisticated replacement strategies to minimize the animal determinants residual in the monoclonal antibodies.

Improvements in production technology have also advanced the field. For example, immunoglobulins can now be produced on a large scale in mammalian cells, selected immunoglobulin fragments can be expressed in bacteria, and antibody Fab fragments can be chemically modified (PEGylated) to increase their half-life *in vivo*. These advances have allowed manufacture at the appropriate scale at decreased expense. Finally, the identification of monoclonal antibodies with different affinities or specificities with respect to the target antigen has led to reagents with alternative properties.

In addition to the success for rheumatoid arthritis, monoclonal antibodies have been used with increasing success for the treatment of malignancy. One of the first advances in this field was the targeting of the interleukin-2 receptor (IL-2R) for the treatment of T-cell leukemia. This approach grew from basic research that used antibodies to IL-2R to define its role in T-cell proliferation (reviewed in ref. 8). These studies showed that T-cell signaling through IL-2R is a key process in the development of this form of cancer. The development of monoclonal antibodies to this receptor, as well as the use of the IL-2 protein fused to a toxin[9], have provided proof of concept that targeting of a cell-specific growth factor receptor can induce a significant clinical response.

The IL-2R approach provided a paradigm for the development of several other antitumor monoclonal antibody therapies, including rituximab and Herceptin for the treatment of lymphoma and breast cancer, respectively, as well as other candidates directed toward different tumor cell types[10–12]. The number of monoclonal antibodies under development for other disease indications is growing rapidly, with more than 200 different antibodies now in clinical trials[13]. Beyond the evident contributions to cancer therapy, recent progress has been made in the treatment of allergic diseases and asthma by targeting IgE or chemokine receptors[14,15].

But all is not clear sailing from now on for antibody therapy—a number of challenges remain. For example, it has become clear that individual monoclonal antibodies for the treatment of rheumatoid arthritis have different kinetics and side effects that may affect their efficacy *in vivo*. As with conventional drugs, side effects of monoclonal antibodies must be dealt with, although they are more likely to be related to the mechanism of action than to unknown side effects often seen with pharmacotherapy. Such complications have already been observed with anti-TNF therapy, in which a recognizable increase in the incidence of infectious complications, particularly reactivation of tuberculosis, has occurred in patients undergoing this treatment.

Despite these problems, monoclonal antibodies will undoubtedly find ever increasing uses in the future treatment of disease, as well as in validating signaling pathways and disease targets for which conventional drug therapies can be directed and developed.

Lymphocyte transfer and cell transplantation

Several types of cellular transplantation have shown progress in the last ten years. Lymphocyte cell transfer, for malignancy, provided the first indications that cellular immunotherapy might prove beneficial. The recognition that tumors often contain lymphocytes that can be expanded with lymphocyte growth factors such as IL-2, then adoptively transferred into recipients, suggested that cell-based immune therapies could have an important role in the treatment of cancers. Indeed, an early clinical trial of such lymphocyte transfer to treat melanoma resulted in regression in 15–25% of individuals (reviewed in ref. 16).

These early observations prompted intensive research to define the immune effector cells that mediate the effects, identify the target tumor antigens, and understand the cytokine regulatory networks that might limit the efficacy of such immune responses. For example, the ability of cytolytic T cells to recognize processed peptides derived from cellular genes, such as those encoding Mart-1 or tyrosinase in melanoma, led to the recognition that protective immune responses are often directed toward tumor-associated, rather than tumor-specific, antigens. In addition, a variety of immune-suppressive factors, such as IL-1, transforming growth factor-β or Fas ligand (CD95L) can inhibit protective immunity. This understanding has stimulated efforts to overcome these impediments to effective antitumor immunity.

Lymphocyte transfer methodology has also been used to induce immune responses or deliver recombinant genes in T-lymphocyte populations that have been applied to cancer, inherited genetic diseases and infectious diseases (discussed in more detail in the next section). Particularly impressive has been the use of antigen-specific T lymphocytes for the treatment of viral infections,

including Epstein-Barr virus lymphoma[17–20] and cytomegalovirus *in vivo*[17,21,22].

The success of such immune therapies stimulated efforts to develop other cancer immunotherapies, including cell- and gene-based vaccines. Dendritic cell transfer is among the most promising of cellular strategies, and it has generated considerable excitement. Intensive research over the past 30 years has revealed the considerable complexity of dendritic cells (reviewed in ref. 23). Different types of dendritic cells, such as myeloid or plasmacytoid, as well as different stages of maturation, have been described. Scientists have also learned how to maintain and expand these cells using cytokines *in vitro*. Dendritic cells are now being evaluated for their effects on a variety of immunotherapy disorders, including cancer and HIV infection, where they are likely to make significant contributions in the future.

The possibilities of cellular therapy have also been realized in the field of transplantation. Among the most impressive advances in recent years has been the use of purified pancreatic β-cells, together with specific immune suppression, for the treatment of juvenile-onset diabetes. This autoimmune disease triggers destruction of endogenous pancreatic β-cells at an early age, and the adaptation of cellular therapy to replace insulin in a physiological manner—an approach thought quite challenging only a few years ago—has shown great promise[24,25]. Besides the use of specific immune suppression to promote cellular engraftment, a potent regulatory T cell, the $CD4^+$ T_{reg} cell, may suppress immune activation in a variety of physiologic settings. Increased understanding of the mechanism of action of these cells and improved methods for their isolation may provide yet other approaches to cellular therapy for autoimmune and allergic diseases[26,27].

The next frontier for cellular transplantation lies in the realm of human stem cells. With the notable exception of bone marrow transplantation, this technology has yet to yield therapies for disease, but selected embryonic or adult stem cell populations could potentially be used for cell transplantation therapy for a variety of diseases such as diabetes, Parkinson disease, inherited genetic deficiencies and sickle-cell anemia (**Fig. 2**). The ability to develop embryonic stem cell lines and the successful cloning of several animal species have in recent years stimulated considerable interest in using embryonic stem cells. The use of embryonic stem cells in medical practice remains highly controversial and is currently premature, but it remains an important area of laboratory research considering its therapeutic potential.

A number of studies have demonstrated the therapeutic potential of stem cells. Mesenchymal stem cells, for example, are under evaluation in preclinical and early clinical studies of myocardial infarction for their ability to limit infarct size[28–32]. Whether some aberrant stem cells have the capacity for self-renewal but also for unregulated growth control that might be desirable targets for the treatment of malignancy is another intriguing idea[33] with significant implications for cancer chemotherapy.

Bone marrow transplantation has already proven the success of a specialized committed progenitor, the hematopoietic stem cell, as a highly effective cell-based therapy. But if progress is to be made with committed adult stem cells of other lineages, major questions remain to be addressed, including understanding the plasticity of adult stem cells, the control of self-renewal and the differentiation capacity of committed progenitors. The delineation of the genetic programs that lead to commitment and to specific pathways of differentiation will likely facilitate the development of these new therapies. Further characterization of specific cell types, as well as the use of defined media that allow the expansion of specific and homogeneous cell types, might considerably improve translation into the clinic.

Gene therapy

Gene therapy holds promise that progress in molecular genetics can be applied directly to the treatment of human disease. Inherited genetic disorders were originally considered natural targets for such therapies, but considerable difficulties have arisen in targeting delivery to specific cell types *in vivo*, regulating expression of recombinant genes and controlling vector immunogenicity (reviewed in ref. 34). As a result, gene therapy has not yet fulfilled its potential. The development of this field may well parallel that of monoclonal antibody therapy in its early stages. While apparently simple in concept, the practical realities of translating these ideas into clinical practice have proven more difficult than first imagined. And though gene therapy may face greater technical

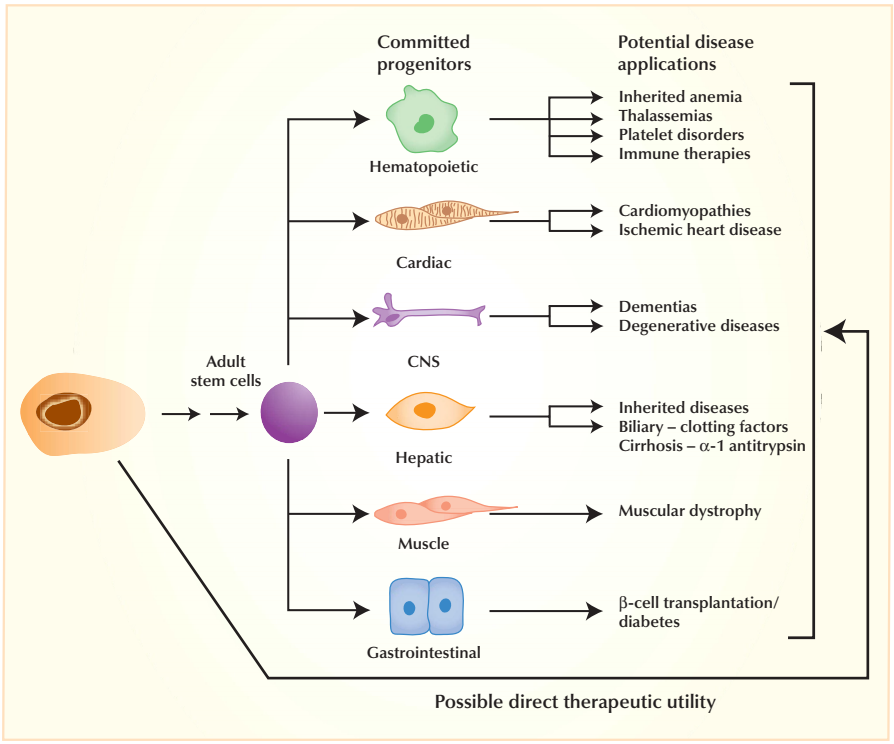

Figure 2 Diverse applications of cellular transplantation. Characterization and isolation of various cell types, including lymphocytes, pancreatic β-cells and bone marrow progenitors, have allowed the development of new therapies for cancer, diabetes and cardiovascular and other diseases.

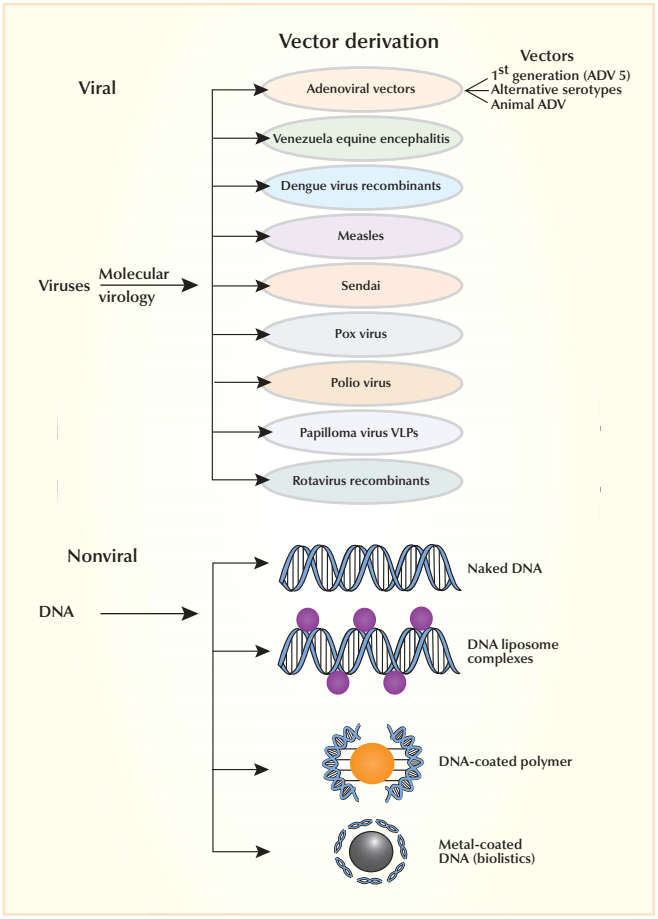

Figure 3 Gene transfer technology and applications to vaccines. Advances in molecular virology have facilitated the generation of diverse viral and nonviral gene transfer techniques that have been used for the development of experimental vaccines for AIDS, Ebola, human papillomavirus, rotavirus, severe acute respiratory syndrome and other infectious diseases.

hurdles, the increasing sophistication of molecular biology may yet allow this approach to make a significant contribution to medical therapy.

The first gene transfer studies, pioneered by Blaese, Anderson and colleagues in 1990, attempted to correct adenosine deaminase deficiency, an inherited genetic disorder. The patient's inability to degrade adenosine causes toxicity to T cells and lymphocyte depletion, resulting in immunodeficiency. In a clinical trial, patient T lymphocytes were genetically modified in the laboratory (*ex vivo*) and then reinfused. Although long-term marking was seen in at least one case[35], no change in immune function was observed, probably because of the low frequency of gene transfer into T cells, the apparent lack of selective expansion of the 'corrected' cells, and possible immunity generated against one of the bacterial resistance genes, neomycin, that was included for positive selection in the retroviral vector. This study and other early gene marking studies[35,36] suggested that recombinant gene transfer could be achieved in a selected subpopulation of cells. These pioneering trials also helped address important regulatory concerns and stimulated research into vector delivery vehicles with applications for inherited and acquired diseases.

Inherited hemophilia was thought to be a particularly promising gene therapy target, perhaps because the deficient protein (factor VIII or factor IX) circulates systemically and can be made, in theory, by any number of cell types. Indeed, preliminary trials have demonstrated gene transfer and expression of human factor IX in animals and human patients[37–39]. But the most encouraging advance so far has been in treatment of an inherited severe combined immunodeficiency (SCID) caused by mutation of the common γ-chain of the growth factor receptor. In this study, bone marrow cells from five children with SCID-X1 were transduced *ex vivo* with a replication-defective retroviral vector expressing the functional γ-chain. When these transduced cells were engrafted back into the patients, they repopulated the lymphoid system and restored immune function[40]. This first therapeutic outcome in a gene transfer trial represented a breakthrough in the field. Similar approaches applied to other immune disorders have been without success, however, probably because of differences in the biology of these diseases. And unfortunately, the feature of SCID-X1 deficiency that led to success conferred an unexpected side effect: the uncontrolled proliferation of the gene-modified T cells in several subjects[41], possibly related to insertional activation of an oncogene in these cells[42]. This experience underscores the need to regulate the expression of recombinant genes and to monitor sites of integration that may induce inadvertent gene activation.

Gene therapy research has grown at an astounding rate over the past ten years. Today, the prototype retroviruses are joined by a large number of different viral and nonviral vectors, as potential gene delivery vehicles. Each system has different properties, advantages and limitations depending on the disease target. Lentiviral vectors, for example, have proven highly effective in achieving stable gene transfer in nondividing cells, but the sites of integration into the genome are not specific, and concerns related to inappropriate activation of neighboring genes have arisen. Adeno-associated virus vectors are considered less immunogenic and have some desirable safety features, but are less efficient at infecting certain nondividing cells and can carry only small therapeutic genes. Adenoviral vectors are highly effective at gene transfer and can be grown to high titer, but they do not integrate and are highly immunogenic; they are thus desirable for immunotherapy and vaccine applications but undesirable for the correction of inherited genetic disorders. Naked DNA, DNA-liposome complexes or DNA-microparticle complexes are proving effective in the induction of immune responses but are not efficient with respect to DNA integration; these nonviral approaches are potentially useful as vectors for cancer vaccines.

To date more than 600 clinical trials for gene therapy have been initiated or completed[43] (http://www.wiley.co.uk/genetherapy/clinical/). Although it was first conceived as an approach to treating inherited disorders, most gene therapy trials today target acquired diseases. This area seems most likely to produce the next wave of breakthroughs. Though early in development, progress has been made in reconstitution of gene-modified cells in HIV-infected individuals, providing intracellular gene products that exert antiviral effects *in vivo*[43,44].

By far the largest proportion of gene therapy trials target cancer. This focus reflects not only acute unmet medical needs but also the requirements of the therapy. The population of target cells is localized and easy to identify; transient expression of a recombinant gene may induce immune effects that persist; and a variety of approaches can achieve cell cytotoxicity and the generation of antitumor immunity. In fact, the direct administration of

recombinant genes *in vivo* (as opposed to *ex vivo* modification and reinfusion of cells) was first performed in a cancer immunotherapy study[45] that documented the expression of a recombinant gene—an allogeneic histocompatibility gene designed to boost antitumor immunity—and the generation of antitumor immunity in five patients. Local and systemic tumor regression was observed in at least one subject. This approach is currently under evaluation in larger phase 3 efficacy trials.

Another cancer gene therapy approach is the introduction of immunomodulatory genes, such as cytokines and costimulatory molecules, including granulocyte-macrophage colony-stimulating factor or the B7 costimulatory molecule, into malignant cells to boost antitumor immune responses. In other cases, attempts have been made to replace defective tumor oncogenes such as those encoding p53 or retinoblastoma, although delivery of such gene products to all required cells is technically difficult. None of these approaches has yet shown success in phase 3 clinical trials.

Another innovative approach to treating cancer has been the use of conditionally replicating oncolytic viruses such as the ONYX-015 adenovirus, which can only replicate in cells that contain a mutant form of p53. Encouraging preliminary data was reported in a clinical trial using this virus in combination with chemotherapy to induce regression of squamous cell carcinomas of the head and neck[46]. It remains to be seen whether these viruses can infect a sufficient number of tumor cells and avoid immune responses that might otherwise limit the duration of the therapeutic effect.

Although substantial progress has been made over the past decade toward improving gene delivery technology, the field has entered a less exuberant phase. Clearly many challenges remain before the technology can fulfill its promise. These include targeting specific cell populations, achieving integration into the host genome at specific sites that will not affect neighboring genes, achieving appropriate gene regulation and avoiding immune detection. Nevertheless, the technology continues to improve as it incorporates new scientific developments. Among the more promising is RNA interference. The transfer of small interfering RNA molecules offers the opportunity to alter long-term gene expression *in vivo* in ways that might minimize genotoxicity and achieve specificity[47].

In conclusion, although technological improvements will no doubt drive advances, progress is likely to be measured and disease-specific. But the efforts invested in gene therapy and stem cell technologies are likely to be productive and positive as their complexities are more fully appreciated.

Vaccines

Vaccines historically represent one of the most established and cost-effective procedures in medicine, having perhaps the greatest impact on human health of any medical intervention. Jenner's smallpox vaccine, for example, eliminated human infection by variola virus and led to eradication of the naturally transmitted virus whose mortality has exceeded 300 million lives in the last century alone[48]. The impact of other vaccines, such as the polio or influenza vaccines, has reinforced the value of the approach.

Recent developments in molecular and cellular immunology, genetics and gene delivery have stimulated a renaissance in the field of vaccine research. In addition to a variety of modified viral vectors, including poxviruses such as canarypox, fowlpox and modified vaccinia Ankara, researchers have developed novel adenovirus serotypes, alphaviruses, adeno-associated viruses and nonviral vectors such as naked DNA that can be used as vaccine vectors for a variety of infectious diseases (**Fig. 3**). Naked DNA has shown particular promise in a variety of different animal models of infectious disease, including HIV[49], influenza virus[50], malaria[51–54], tuberculosis[55], Ebola virus[56], rabies[57], lymphocytic choriomeningitis virus[58,59] and herpes simplex virus[60]. This technology has been evaluated in phase 1 human clinical trials for HIV, malaria and influenza virus.

Methodologies for defining immunogens and rationally improving immunogen design have been greatly improved by advances in structural biology. In the case of HIV, for example, X-ray crystallographic studies of the gp120 protein[61] have yielded important information regarding antigenic determinants on this molecule. Based on the X-ray structure, specific rational mutations could be designed to alter, or expose potential antigenic determinants. The definition of functional structures, such as the helical coiled-coil regions of viral envelope glycoproteins[62], has also revealed highly conserved functional domains that may be susceptible to neutralizing antibodies or other forms of antiviral therapy. Advances in recombinational PCR[63–65] make it possible to generate large libraries of immunogens that can be selected for desirable immunologic properties.

Undoubtedly the field of vaccine technology will benefit from the sophisticated advances in genomics and bioinformatics. Although these technologies have yet to be applied to large-scale clinical vaccine trials, the large numbers of subjects in such trials and the importance of genetic factors in determining immune responses make this subject a logical focus of investigation. The judicious use of genotyping in human trials is likely to allow the identification of genes that determine responsiveness in human populations. Such information could be used to further optimize vaccines and to test and predict their efficacy in specific human populations.

Finally, although the field of vaccination has historically focused on the prevention of infectious diseases, this technology provides a broader base for immune modulation of pathologic responses underlying other conditions. Recent studies have explored the possibility of using vaccination or immune intervention for the treatment of neurologic diseases, specifically Alzheimer disease, based on the ability of antibodies to reduce the formation of neurologic lesions in a mouse model of the disease[66]. A vaccine approach has also been developed for the prevention of atherosclerosis through the generation of antibodies to the cholesterol ester transfer protein (CETP)[67,68]. CETP inhibition might increase the ratio of high- to low-density lipoproteins; this more favorable lipid profile is found in individuals with specific inherited mutations of CETP associated with protection against cardiovascular disease. Specific vaccine strategies have also been applied to a variety of allergic and autoimmune diseases. Finally, as noted above, the field of cancer immunotherapy has shown increasing promise as tumor antigens have been better defined, and the mechanisms that prevent immune responses to malignancies better understood. Better understanding of the basic molecular lesions and the delivery technology, as well as a more systematic assessment of human immune responses to tumor antigens will likely yield continued improvements in the success of these approaches.

Over the last several years, advances in gene-based delivery technology arising from the field of gene therapy have helped revitalize the field of vaccine development[69]. There have been advances in the understanding of a number of viruses, and the methodology for making recombinant gene products, both of protein- and gene-based vaccines, has diversified and improved. The ability to

use continuously passaged cell lines to produce viral vaccines provides greater consistency and safety, and represents an important technological improvement. A variety of candidates show promise, including vaccines for human papillomavirus, herpesvirus and rotavirus which are in advanced clinical testing. Should these vaccines alone fulfill their promise, millions of lives would be saved and much needless suffering prevented. The development of vaccines against emerging infectious diseases, including Ebola virus, the severe acute respiratory syndrome coronavirus and West Nile virus, would contribute greatly to biodefense at the same time they would also have profound effects on world health.

Although considerable progress has been made, some major diseases remain recalcitrant to vaccination. AIDS, tuberculosis and malaria continue to extract a major toll on human health. In the next ten years, the confluence of virology and immunology will no doubt advance these fields and help to contain a variety of diseases. Perhaps these advances will constitute the most significant progress that we might expect in the coming decade. Should a fraction of these efforts realize their potential, millions may owe their lives to novel medical interventions made possible only by this golden age of biological discovery.

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COMPETING INTERESTS STATEMENT

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1. Kohler, G. & Milstein, C. Continuous cultures of fused cells secreting antibody of predefined specificity. *Nature* **256**, 495–497 (1975).
2. Feldmann, M. Development of anti-TNF therapy for rheumatoid arthritis. *Nat. Rev. Immunol.* **2**, 364–371 (2002).
3. Bain, B. & Brazil, M. Adalimumab. *Nat. Rev. Drug Discov.* **2**, 693–694 (2003).
4. Smolen, J.S. & Steiner, G. Therapeutic strategies for rheumatoid arthritis. *Nat. Rev. Drug Discov.* **2**, 473–488 (2003).
5. Weinblatt, M.E. *et al.* Adalimumab, a fully human anti-tumor necrosis factor α monoclonal antibody, for the treatment of rheumatoid arthritis in patients taking concomitant methotrexate: the ARMADA trial. *Arthritis Rheum.* **48**, 35–45 (2003).
6. Weinberg, J.M., Saini, R. & Tutrone, W.D. Biologic therapy for psoriasis—the first wave: infliximab, etanercept, efalizumab, and alefacept. *J. Drugs Dermatol.* **1**, 303–310 (2002).
7. Scheinfeld, N. Adalimumab (HUMIRA): a review. *J. Drugs Dermatol.* **2**, 375–377 (2003).
8. Waldmann, T.A. T-cell receptors for cytokines: targets for immunotherapy of leukemia/lymphoma. *Ann. Oncol.* **11** (suppl. 1), 101–106 (2000).
9. Kreitman, R.J. *et al.* Recombinant toxins containing the variable domains of the anti-Tac monoclonal antibody to the interleukin-2 receptor kill malignant cells from patients with chronic lymphocytic leukemia. *Blood* **80**, 2344–2352 (1992).
10 Lin, T.S., Lucas, M.S. & Byrd, J.C. Rituximab in B-cell chronic lymphocytic leukemia. *Semin. Oncol.* **30**, 483–492 (2003).
11. Zelenetz, A.D. A clinical and scientific overview of tositumomab and iodine I 131 tositumomab. *Semin. Oncol.* **30**, 22–30 (2003).
12. Mendelsohn, J. & Baselga, J. Status of epidermal growth factor receptor antagonists in the biology and treatment of cancer. *J. Clin. Oncol.* **21**, 2787–2799 (2003).
13. Breker, O.H. & Sandlie, I. Therapeutic antibodies for human diseases at the dawn of the twenty-first century. *Nat. Rev. Drug Discov.* **2**, 52–62 (2003).
14. Erin, E.M., Williams, T.J., Barnes, P.J. & Hansel, T.T. Eotaxin receptor (CCR3) antagonism in asthma and allergic disease. *Curr. Drug Targets Inflamm. Allergy* **1**, 201–214 (2002).
15. Lanier, B.Q. Newer aspects in the treatment of pediatric and adult asthma: monoclonal anti-IgE. *Ann. Allergy Asthma Immunol.* **90**, 13–15 (2003).
16. Dudley, M.E. & Rosenberg, S.A. Adoptive-cell-transfer therapy for the treatment of patients with cancer. *Nat. Rev. Cancer* **3**, 666–675 (2003).
17. Riddell, S.R. & Greenberg, P.D. T cell therapy of human CMV and EBV infection in immunocompromised hosts. *Rev. Med. Virol.* **7**, 181–192 (1997).
18. Heslop, H.E. *et al.* Transfer of EBV-specific CTL to prevent EBV lymphoma post bone marrow transplant. *J. Clin. Apheresis* **14**, 154–156 (1999).
19. Rooney, C.M., Aguilar, L.K., Huls, M.H., Brenner, M.K. & Heslop, H.E. Adoptive immunotherapy of EBV-associated malignancies with EBV-specific cytotoxic T-cell lines. *Curr. Top. Microbiol. Immunol.* **258**, 221–229 (2001).
20. Heslop, H.E. *et al.* Immune therapy for EBV infections after hemopoietic stem-cell transplant. *Cytotherapy* **4**, 433–434 (2002).
21. Riddell, S.R. & Greenberg, P.D. Cellular adoptive immunotherapy after bone marrow transplantation. *Cancer Treat. Res.* **76**, 337–369 (1995).
22. Greenberg, P.D. *et al.* Genetic modification of T-cell clones for therapy of human viral and malignant diseases. *Cancer J. Sci. Am.* **4** (suppl. 1), S100–S105 (1998).
23. Mellman, I. & Steinman, R.M. Dendritic cells: specialized and regulated antigen processing machines. *Cell* **106**, 255–258 (2001).
24. Titus, T., Badet, L. & Gray, D.W. Islet cell transplantation for insulin-dependant diabetes mellitus: perspectives from the present and prospects for the future. *Expert Rev. Mol. Med.* **2**, 1–28 (2000).
25. Matthews, J.B., Ramos, E. & Bluestone, J.A. Clinical trials of transplant tolerance: slow but steady progress. *Am. J. Transplant.* **3**, 794–803 (2003).
26. Tang, Q. *et al.* Cutting edge: CD28 controls peripheral homeostasis of $CD4^+CD25^+$ regulatory T cells. *J. Immunol.* **171**, 3348–3352 (2003).
27. Bluestone, J.A. & Abbas, A.K. Natural versus adaptive regulatory T cells. *Nat. Rev. Immunol.* **3**, 253–257 (2003).
28. Orlic, D. *et al.* Bone marrow cells regenerate infarcted myocardium. *Nature* **410**, 701–705 (2001).
29. Toma, C., Pittenger, M.F., Cahill, K.S., Byrne, B.J. & Kessler, P.D. Human mesenchymal stem cells differentiate to a cardiomyocyte phenotype in the adult murine heart. *Circulation* **105**, 93–98 (2002).
30. Tomita, S. *et al.* Improved heart function with myogenesis and angiogenesis after autologous porcine bone marrow stromal cell transplantation. *J. Thorac. Cardiovasc. Surg.* **123**, 1132–1140 (2002).
31. Leinwand, L.A. Hope for a broken heart? *Cell* **114**, 658–659 (2003).
32. Prockop, D.J., Gregory, C.A. & Spees, J.L. One strategy for cell and gene therapy: harnessing the power of adult stem cells to repair tissues. *Proc. Natl. Acad. Sci. USA* **100** (suppl. 1), 11917–11923 (2003).
33. Al Hajj, M., Wicha, M.S., Benito-Hernandez, A., Morrison, S.J. & Clarke, M.F. Prospective identification of tumorigenic breast cancer cells. *Proc. Natl. Acad. Sci. USA* **100**, 3983–3988 (2003).
34. Nabel, G.J. The future of gene therapy. *Ernst Schering Res. Found. Workshop* 1–16 (2003).
35. Mullen, C.A. *et al.* Molecular analysis of T lymphocyte-directed gene therapy for adenosine deaminase deficiency: long-term expression *in vivo* of genes introduced with a retroviral vector. *Hum. Gene Ther.* **7**, 1123–1129 (1996).
36. Rosenberg, S.A. *et al.* Gene transfer into humans—immunotherapy of patients with advanced melanoma, using tumor-infiltrating lymphocytes modified by retroviral gene transduction. *N. Engl. J. Med.* **323**, 570–578 (1990).
37. Wang, L., Nichols, T.C., Read, M.S., Bellinger, D.A. & Verma, I.M. Sustained expression of therapeutic level of factor IX in hemophilia B dogs by AAV-mediated gene therapy in liver. *Mol. Ther.* **1**, 154–158 (2000).
38. High, K.A. Gene transfer as an approach to treating hemophilia. *Semin. Thromb. Hemost.* **29**, 107–120 (2003).
39. Kay, M.A. *et al.* Evidence for gene transfer and expression of factor IX in hemophilia B patients treated with an AAV vector. *Nat. Genet.* **24**, 257–261 (2000).
40. Hacein-Bey-Abina, S. *et al.* Sustained correction of X-linked severe combined immunodeficiency by *ex vivo* gene therapy. *N. Engl. J. Med.* **346**, 1185–1193 (2002).
41. Hacein-Bey-Abina, S. *et al.* A serious adverse event after successful gene therapy for X-linked severe combined immunodeficiency. *N. Engl. J. Med.* **348**, 255–256 (2003).
42. Hacein-Bey-Abina, S. *et al.* LMO2-associated clonal T cell proliferation in two patients after gene therapy for SCID-X1. *Science* **302**, 415–419 (2003).
43. Woffendin, C., Ranga, U., Yang, Z., Xu, L. & Nabel, G.J. Expression of a protective gene prolongs survival of T cells in human immunodeficiency virus-infected patients. *Proc. Natl. Acad. Sci. USA* **93**, 2889–2894 (1996).
44. Ranga, U. *et al.* Enhanced T cell engraftment after retroviral delivery of an antiviral gene in HIV-infected individuals. *Proc. Natl. Acad. Sci. USA* **95**, 1201–1206 (1998).
45. Nabel, G.J. *et al.* Direct gene transfer with DNA-liposome complexes in melanoma: expression, biologic activity, and lack of toxicity in humans. *Proc. Natl. Acad. Sci. USA* **90**, 11307–11311 (1993).
46. Khuri, F.R. *et al.* A controlled trial of intratumoral ONYX-015, a selectively-replicating adenovirus, in combination with cisplatin and 5-fluorouracil in patients with recurrent head and neck cancer. *Nat. Med.* **6**, 879–885 (2000).
47. McManus, M.T. & Sharp, P.A. Gene silencing in mammals by small interfering RNAs. *Nat. Rev. Genet.* **3**, 737–747 (2002).
48. Oldstone, M.B.A. in *Viruses, Plagues and History* (Oxford University Press, New York, 1998).
49. Donnelly, J.J., Ulmer, J.B., Shiver, J.W. & Liu, M.A. DNA vaccines. *Annu. Rev. Immunol.* **15**, 617–648 (1997).
50. Ulmer, J.B. *et al.* Heterologous protection against influenza by injection of DNA encoding a viral protein. *Science* **259**, 1745–1749 (1993).
51. Becker, S.I. *et al.* Protection of mice against *Plasmodium yoelii* sporozoite challenge with *P. yoelii* merozoite surface protein 1 DNA vaccines. *Infect. Immun.* **66**, 3457–3461 (1998).

52. Doolan, D.L. *et al.* Circumventing genetic restriction of protection against malaria with multigene DNA immunization: CD8+ cell-, interferon γ-, and nitric oxide-dependent immunity. *J. Exp. Med.* **183**, 1739–1746 (1996).
53. Gardner, M.J. *et al.* DNA vaccines against malaria: immunogenicity and protection in a rodent model. *J Pharm. Sci.* **85**, 1294–1300 (1996).
54. Sedegah, M., Hedstrom, R., Hobart, P. & Hoffman, S.L. Protection against malaria by immunization with plasmid DNA encoding circumsporozoite protein. *Proc. Natl. Acad. Sci. USA* **91**, 9866–9870 (1994).
55. Tascon, R.C. *et al.* Vaccination against tuberculosis by injection. *Nat. Med.* **2**, 888–892 (1996).
56. Xu, L. *et al.* Immunization for Ebola virus infection. *Nat. Med.* **4**, 37–42 (1998).
57. Lodmell, D.L. *et al.* DNA immunization protects nonhuman primates against rabies virus. *Nat. Med.* **4**, 949–952 (1998).
58. Martins, L.P., Lau, L.L., Asano, M.S. & Ahmed, R. DNA vaccination against persistent viral infection. *J. Virol.* **69**, 2574–2582 (1995).
59. Yokoyama, M., Zhang, J. & Whitton, J.L. DNA immunization confers protection against lethal lymphocytic choriomeningitis virus infection. *J. Virol.* **69**, 2684–2688 (1995).
60. Manickan, E., Yu, Z., Rouse, R.J., Wire, W.S. & Rouse, B.T. Induction of protective immunity against herpes simplex virus with DNA encoding the immediate early protein ICP 27. *Viral Immunol.* **8**, 53–61 (1995).
61. Kwong, P.D. *et al.* Structure of an HIV gp120 envelope glycoprotein in complex with the CD4 receptor and a neutralizing human antibody. *Nature* **393**, 648–659 (1998).
62. Chan, D.C., Fass, D., Berger, J.M. & Kim, P.S. Core structure of gp41 from the HIV envelope glycoprotein. *Cell* **89**, 263–273 (1997).
63. Crameri, A., Raillard, S.A., Bermudez, E. & Stemmer, W.P. DNA shuffling of a family of genes from diverse species accelerates directed evolution. *Nature* **391**, 288–291 (1998).
64. Smith, G.P. Applied evolution. The progeny of sexual PCR. *Nature* **370**, 324–325 (1994).
65. Stemmer, W.P. Rapid evolution of a protein *in vitro* by DNA shuffling. *Nature* **370**, 389–391 (1994).
66. Schenk, D. Amyloid-β immunotherapy for Alzheimer's disease: the end of the beginning. *Nat. Rev. Neurosci.* **3**, 824–828 (2002).
67. Davidson, M.H. *et al.* The safety and immunogenicity of a CETP vaccine in healthy adults. *Atherosclerosis* **169**, 113–120 (2003).
68. Rittershaus, C.W. *et al.* Vaccine-induced antibodies inhibit CETP activity *in vivo* and reduce aortic lesions in a rabbit model of atherosclerosis. *Arterioscler. Thromb. Vasc. Biol.* **20**, 2106–2112 (2000).
69. Rappuoli, R. & Nabel, G. Vaccines: ideal drugs for the 21st century? *Curr. Opin. Invest. Drugs* **2**, 45–46 (2001).