which these processes take place are questionable, particularly given the possibility of multiple activation of several brain regions by means of mechanisms described above (and which are schematically shown in figure). It is possible that activation of pain-related neurons at any point within the circuits shown in figure would trigger the pain with strong affective component, as long as the body region of projected pain included that which was involved in the previously experienced intense pain.

In a general sense, all of these observations underscore the critical link between neuroplastic processes related to memory and the affective-motivational dimension of pain. The observation that pain with strong affect can be evoked by microstimulation of sites classically considered to be a part of the sensory-discriminative pathway for pain suggests that the sharp dichotomous division between neural structures and pathways involved in sensory and affective dimensions of pain is an oversimplification and is misleading.

1. Mountcastle, V.B. *Medical Physiology* (Mosby, St. Louis, Missouri, 1968).
2. Fields, H.L. *Pain* (McGraw-Hill, New York, 1987).
3. Melzack, R. and Casey, K. L. Sensory, motivational, and central control determinants of pain: A new conceptual model. in *The Skin Senses* (ed. Kenshalo, D.) pp. 423-443 (Thomas, Springfield, Illinois, 1968).
4. Willis W.D. *The Pain System* (Karger, New York, 1985).
5. Price, D.D. *Psychological and Neural Mechanisms of Pain* (Raven, New York, 1988).
6. Coghill, R.C. *et al.*, Distributed processing of pain and vibration by the human brain. *Neurosci.* **14**, 4095–4108 (1994).
7. Lenz, F.A. *et al.* Stimulation in the human somatosensory thalamus can reproduce both the affective and sensory dimensions of previously experienced pain. *Nature Med.* **1**, 910–913, 1995).
8. Applebaum, A.E., Beall, J.E., Foreman, R.D., & Willis, W.D. Organization and receptive fields of primate spinothalamic tract neurons. *J. Neurophysiol.* **38**, 572–586 (1975).
9. Davis, K.D., Tasker, R.R., Kiss, Z.H.T., Hutchison, W.D. & Dostrovsky, J.O. Visceral pain evoked by thalamic microstimulation in humans. *Neuroreport* **6**, 369–374 (1995).
10. Mao, J. *et al.* Post-injury treatment with GM1 ganglioside reduces nociceptive behaviour and spinal cord metabolic activity in rats with experimental mononeuropathy. *Brain Res.* **584**, 18–27 (1992).
11. Price, D.D. & Harkins, S.W. The affective-motivational dimension of pain: A two stage model. *APS Journal* **4**, 229–239 (1992).
12. Katz, J. & Melzack, R. Pain 'memories' in phantom limbs: Review and clinical observations. *Pain* **43**, 319–336 (1990).

Department of Anesthesiology
Medical College of Virginia
Richmond, Virginia 23298, USA

Immunomodulation to enhance gene therapy

Many obstacles to successful gene therapy remain to be overcome. Now one of them may be less imposing (pages 890–893).

CHRISTOPHER WILSON & MARK A. KAY

As studies in animal models and in limited human trials have begun to reveal, major impediments remain to be overcome if gene therapy is to become a reality. This is becoming increasingly clear in studies related to cystic fibrosis, where two major issues remain to be resolved: (1) the development of gene delivery vectors that transduce the relevant cell types and regions of the airway epithelium with sufficient efficiency to result in functional correction of the ion channel defect and (2) the need to attenuate or modulate the host immune response to the vector (and perhaps the gene product), particularly in the case of adenoviral vectors. It is the second of these two issues that is addressed by Yang and colleagues in this issue of *Nature Medicine*[1].

Replication-defective adenoviral vectors are appealing candidates for *in vivo* gene therapy because of their capacity to transduce a wide variety of cell types, including non-replicating cells; the lack of integration of vector-derived DNA into the host chromosome avoids the potential risk of insertional mutagenesis, while making it likely that long-term correction of the defect will require repeated administration of the vector. However, an efficient antigen-specific immune response is a prerequisite for mammalian survival in a world rich in microbial challenges. In fact, adenoviral vectors have effectively been employed as vaccine vectors to induce humoral and cellular immunity to various microbial pathogens (reviewed in ref. 2). Thus, it is not surprising that both in preclinical animal models[3–6] and limited clinical trials[7], adenoviral vector administration commonly induces inflammation and antigen-specific cellular and humoral immune responses.

Data from mice with global or selective genetic immunodeficiencies clearly indicate that T cell-dependent, antigen-specific immunity is the principal factor limiting the duration of transduced gene expression following inoculation of naive animals and largely prevents gene expression following secondary administration[2–4,8]. Further, it appears that the $CD8^+$ T cells, responding largely to the small amounts of newly synthesized viral proteins produced by these vectors, act with $CD4^+$ T cells to limit the duration of gene expression. Neutralizing antibody, produced by B cells in cooperation with $CD4^+$ T cells in response to input and newly synthesized proteins, precludes gene expression following secondary administration of the same or related adenoviral vectors. The capacity of the latter cells to respond vigorously to input viral proteins makes it unlikely that any modifications in vectors will fully eliminate their immunogenicity.

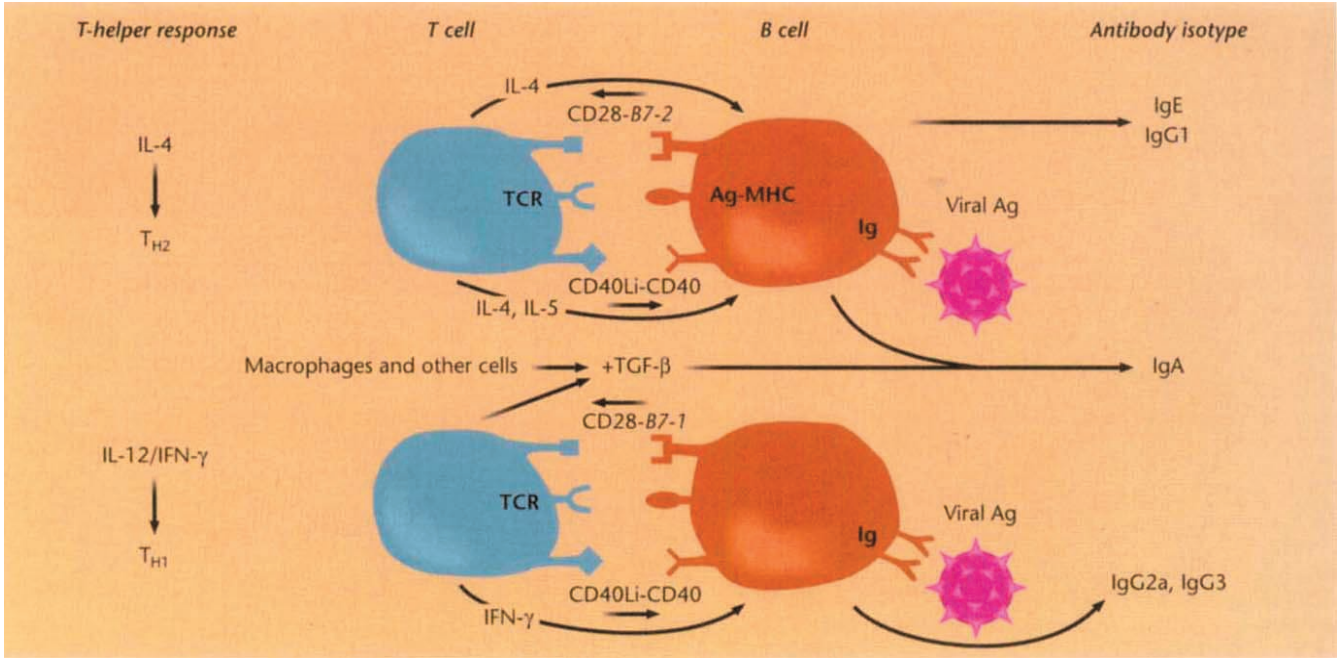
As a prelude to their studies with IL-12, Yang *et al.*[1] first depleted $CD4^+$ T cells at the time of intratracheal administration of a first generation, E1-deleted, type 5 adenoviral (Ad5) β-galactosidase vector (β–gal). This resulted in prolonged β–gal expression, a marked reduction in the appearance of neutralizing antibody to the vector in the bronchoalveolar lavage fluid and robust gene expression following later administration of an Ad5 alkaline phosphatase vector. As noted by others[2], infection of the lung is associated with the development of antigen-specific T and B cells in the local lymphoid tissue and a balanced antibody response consisting of IgA and several IgG isotypes. Although both IgA and IgG may serve to neutralize virus, the marked reduction of neutralizing activity observed in the $CD4^+$ T cell-depleted mice by Yang *et al.*[1] paralleled a marked reduction in IgA in the bronchoalveolar lavage fluid most closely.

However, broadly immunosuppressive or cytoablative regimens are not appealing adjuncts for gene therapy. Rather a regimen causing only transient immunosuppression, which is not cytoablative, minimizes effects on pre-existing immunity and does not result in immunological tolerance to wild-type adenovirus is needed. Importantly, Yang *et al.*[1] take a produc-

tive step toward the development of such a regimen. Reasoning that modulation of CD4+ T-cell function might reproduce at least some of the beneficial effects of CD4+ T-cell depletion without its potential deleterious consequences, mice were treated with one of two cytokines, IL-12 or interferon-γ, at the time of primary vector administration. The results are very encouraging: the amounts of neutralizing and IgA (but not IgG) antibody were reduced in the mice treated with either of these two cytokines and successful gene expression occurred following administration of a second adenoviral vector given 28 days after the initial one. However, unlike CD4+ T-cell depletion, cytokine treatment did not result in more prolonged gene expression from the primary or secondary vector, which was brief. Given this, repetitive gene expression would certainly be necessary to maintain a therapeutic effect. However, this was not assessed in their report.

What is the basis for the effect observed, is it likely to be effective in other tissues and in other species and (because CD4+ T-cell ablation had the dual effect of prolonging gene expression and allowing repetitive treatment) are there additional or alternative approaches that might result in both? To address these questions, it is useful to consider how CD4+ T cells recognize and respond to foreign antigen and in so doing regulate antibody production and the cellular immune response (see figure). CD4+ T cells are activated to produce cytokines and to proliferate in response to recognition of specific antigen, in the form of processed peptides bound to class II MHC proteins on antigen-presenting cells (B cells, dendritic cells and macrophages). Individual murine CD4+ T cells can be broadly grouped into two major classes based on the production of certain representative cytokines in a largely mutually exclusive fashion: T_{H1} cells produce IL-2 and interferon-γ (IFN-γ) and T_{H2} cells produce IL-4, IL-5 and IL-10 (ref. 9); T_{H0} cells produce a mixture of these cytokines. An individual T cell appears to have the potential to acquire either the T_{H1} or T_{H2} phenotype. If activation occurs in an environment rich in IL-12 and IFN-γ, development of the T_{H1} phenotype is favoured, whereas in the presence of IL-4, the T_{H2} phenotype is favoured.

In addition to the effects of cytokines, the initial activation of a CD4+ T cell and subsequent responses by T_{H1} cells are markedly enhanced and, in some cases dependent on the concomitant engagement of CD28 on T cells by the B7-1 or B7-2 molecules on antigen-presenting cells[10]; B7-2 engagement may favour development of T_{H2} cells, whereas, later and more persistent B7-1 engagement may favour T_{H1} development[11]. The other partner in this interaction, and the source of antibody production, is the B cell. B cells bind antigen directly through surface immunoglobulin, which provides a necessary but insufficient signal for efficient antibody production. The other signal is provided by the activated CD4+ T cell, which expresses a protein ligand on its surface (CD40 Li) that engages CD40 on the surface of the B cell. Ligation of CD40 and interaction of antigen with surface immunoglobulin are sufficient to induce robust B cell proliferation, to enhance the amount and affinity of antibody produced and to allow the B cell to switch from IgM to IgG, IgA or IgE production[12,13]. Thus, bidirectional signals between T cell and B cell (and other antigen-presenting cells) are central to the efficient development of *de novo* cellular and humoral immunity to protein antigens; importantly, the costimulatory signals provided by CD28 interaction with B7 and by CD40 ligand interaction with CD40 appear to be less critical in the maintenance of established immunological memory[10,12]. Which isotype the B cell produces is profoundly influenced by the cytokines secreted by the T cell (and other nearby cells). As shown in



Cytokines and costimulatory molecules governing CD4+ T-cell activation, differentiation and provision of help to B cells. CD40Li (CD40 ligand, gp39), IFN-γ (interferon-γ), TGF-β (transforming growth factor-β), Ag-MHC (peptide antigen bound to major histocompatibility complex class II molecule), TCR (T-cell receptor).

figure, IFN-γ from T_{H1} cells enhances production of IgG2a and IgG3, whereas IL-4 from T_{H2} cells enhances production of IgG1 and IgE. Interestingly, a balanced combination of T_{H1} and T_{H2} cells appears to favour IgA production[14]. This may reflect the complexity and balance of factors needed for optimal IgA production in the mouse: ligation of CD40 by CD40 ligand, transforming growth factor-β (TGF-β), IL-4 and IL-5 (ref. 15). What is provided by the T_{H1} cells to facilitate IgA production is not clear, but may include TGF-β (ref. 14).

What was the mechanism of action of IL-12 and IFN-γ in the studies of Yang *et al.*[1]? Although it may have been due in part to an increase in T_{H1} versus T_{H2} cells, the increase in IFN-γ secretion observed was modest and seems unlikely to account fully for the nearly complete loss of airway IgA. Rather much of the effect of the administered IFN-γ (or IL-12-induced IFN-γ) may have been due to a direct inhibition of B-cell switching to IgA production. It is also somewhat difficult to reconcile the presence of similar quantities of IgG antibodies but markedly reduced amounts of neutralizing antibodies to adenovirus in the airway fluid of IFN-γ and IL-12-treated mice compared to control mice, as both IgG and IgA antibodies would be expected to possess neutralizing activity. Thus, it is possible that the assessment of IgG and IgA antibodies by ELISA at a single 1:10 dilution was misleading, and that IgG was actually present in much lower amounts than IgA in the controls. If so, the reduction of IgA in the treated mice could fully account for the reduction in neutralizing antibody. Also, an undetected alteration in the relative amounts of one or more specific IgG isotypes or a diminution of average antibody avidity could have contributed to the disproportionate reduction of neutralizing antibody.

Without a clearer understanding of the basis for the effects of IL-12 and IFN-γ, it is difficult to predict the degree to which this approach can be generalized to adenoviral-mediated gene therapy in other contexts (such as after systemic administration). For example, if due to an overall inhibition in production of neutralizing antibody, it might be generally applicable. If not, this approach is likely to be useful only in airway-directed therapy, and then only in the absence of airway inflammation sufficient to allow entry of neutralizing IgG antibodies. This is most likely the case. In sum, although a valuable step in modulation of the immune response to enhance lung gene therapy, this approach did not enhance expression after initial vector administration and may not be useful in the context of systemic therapy.

It is not surprising that both in preclinical animal models and limited clinical trials, adenoviral vector administration commonly induces inflammation and antigen-specific cellular and humoral immune responses.

However, the bidirectional contact-dependent costimulatory signals between T cells, B cells and other antigen-presenting cells (see figure) provide additional targets for intervention. Reagents that specifically interfere with the interactions between CD28 and B7-1/B7-2 and between CD40 and its ligand have been found to attenuate antibody production and cellular immune reactions[10,13]. Our group has found that blockade of CD28 interaction with B7-1/B7-2 results in markedly prolonged (greater than 5 months) expression of adenoviral transduced genes in the liver and markedly attenuates antibody production[16]. Thus, alteration of the cytokine profile and interdiction of costimulatory interactions along with improvement in vector design to minimize or eliminate residual production of adenoviral proteins may allow the potential of these vectors to be more fully realized. As a caution to this optimistic note, it is worth remembering that mice are not human beings, and those individuals most in need of gene therapy are likely to pose additional obstacles (for example, pre-existing immunity and lung inflammation) not encountered in these murine studies. Nonetheless, the needs of these patients and these initial encouraging results with immunomodulation should serve as an impetus to explore additional approaches.

1. Yang, Y., Trinchieri, G. & Wilson, J.M. Recombinant IL-12 prevents formation of blocking IgA antibodies to recombinant adenovirus and allows repeated gene therapy to mouse lung. *Nature Med.* **1**, 890–893 (1995).
2. van Ginkel, F.W. *et al.* Intratracheal gene delivery with adenoviral vector induces elevated systemic IgG and mucosal IgA antibodies to adenovirus and β-galactosidase. *Hum. Gene Ther.* **6**, 895–903 (1995).
3. Yang Y. *et al.* Inactivation of E2a in recombinant adenoviruses limits cellular immunity and improves the prospect for gene therapy of cystic fibrosis. *Nature Genet.* **7**, 362–369 (1994).
4. Yang, Y., Li, Q., Ertl, H.C.J. & Wilson, J.M. Cellular and humoral immune responses to viral antigens create barriers to lung-directed gene therapy with recombinant adenoviruses. *J. Virol.* **69**, 2004–2015 (1995).
5. Simon, R.H. *et al.* Adenovirus-mediated transfer of the CFTR gene to lung of non-human primates: Toxicity study. *Hum. Gene Ther.* **4**, 771–780 (1993).
6. Zabner, J. *et al.* Safety and efficacy of repetitive adenovirus-mediated transfer of CFTR cDNA to airway epithelia of primates and cotton rats. *Nature Genet.* **6**, 75–83 (1994).
7. McElvaney, N.G. & Crystal, R.G. IL-6 release and airway administration of human CFTR cDNA adenovirus vector. *Nature Med.* 1, 182–184 (1995).
8. Barr D. *et al.* Strain related variations in adenoviral mediated transgene expression in mouse hepatocytes *in vivo*: Comparisons between immunocompetent and immunodeficient inbred strains. *Gene Ther.* **2**, 151–155 (1995).
9. Seder, R.A. & Paul, W.E. Acquisition of lymphokine-producing phenotype by CD4$^+$ T cells. *Annu. Rev. Immunol.* **12**, 635–673, 1994.
10. Bluestone, J.A. New perspectives of CD28-B7-mediated T cell costimulation. *Immunity* **2**, 555–559 (1995).
11. Freeman, G.J. *et al.* B7-1 and B7-2 do not deliver identical costimulatory signals, since B7-2 but not B7-1 preferentially costimulates the initial production of IL-4. *Immunity* **2**, 523–532 (1995).
12. Clark, E.A. & Ledbetter, J.A. How B and T cells talk to each other. *Nature* **367**, 425–428 (1994).
13. Foy, T.M. *et al. In vivo* CD40-gp39 interactions are essential for thymus-dependent immunity. II. Prolonged suppression of primary and secondary humoral immune responses by an antibody targeted to the CD40 ligand, gp39. *J. exp. Med.* **178**, 1567–1576 (1994).
14. Rizzo, L.V., de Kruygg, R.H., Umetsu, D.T. & Caspi, R.R. Regulation of the interaction between Th1 and Th2 T cell clones to provide help for antibody production *in vivo*. *Eur. J. Immunol.* **25**, 708–716 (1995).
15. McIntyre, T.M., Kehry, M.R. & Snapper, C.M. Novel *in vitro* model for high-rate IgA class switching. *J. Immunol.* **154**, 3156–3161 (1995).
16. Kay, M.A. *et al.* Long-term hepatic adenovirus mediated gene expression in mice following CTLA4Ig administration. *Nature Genet.* (in the press).

Departments of Pediatrics and Immunology
University of Washington School of Medicine
Seattle, Washington 98195, USA

Departments of Medicine, Pediatrics, Pathology and Biochemistry
University of Washington School of Medicine
Seattle, Washington 98195, USA