vaccinated mice with a single oral dose of a recombinant adeno-associated virus vector expressing the *env* gene from HIV-1 (AAV/HIV*env* vector).[6] The vaccination induced a strong immune response, both locally (in the mucosa) and systemically.

The *Human Gene Therapy* study showed that anti-HIV antibodies (IgG and IgA) were present for 3–5 months after vaccination. Importantly, this means that the oral vaccine induced humoral mucosal immunity. Demonstrable mucosal immunity is crucial when the aim is to protect individuals from pathogens like HIV that are generally acquired via mucosal surfaces.

The researchers also convincingly demonstrated that the vaccine induced a strong HIV-specific cell-mediated immunity. This represents a significant improvement compared to previous vaccines administered by injection into muscle tissue that, typically, did not induce a strong cellular immune response.

However, to really show a vaccine actually works, one must show it induces protective immunity. To do this, the authors challenged immunized mice with a recombinant vaccinia virus expressing HIV-1 *env*. Using a rectal inoculum they observed a decreased viral load (about 2 logs) in immunized animals. These results are encouraging, but, in addition to the surrogate viral challenge used by Okuda and his colleagues, they need to be repeated for HIV itself.

The success of this new approach in mice is exciting because, theroretically, it could be equally successful in humans. We already know that the AAV vector used is safe and effective in humans.[7] This vector is also almost certainly less pathogenic than all vectors presently adopted in animal and human trials.[8] In addition, because the vaccine is a live viral preparation, it is more likely to induce a protective immune response. Now Okuda's group have shown that AAV's resistance to a range of temperatures, proteases and pH variations allows successful oral delivery of an HIV vaccine.

However, we should not crack open the champagne yet. The short duration of the induced immune response in the mouse model suggests that the AAV vector mainly infects rapidly renewing cells of the intestinal lumen. Therefore, in conjunction with the AAV/HIV*env* oral vaccination, we need to boost immunity in other ways. Adoptive immunotherapy with professional antigen-presenting dendritic cells or combined DNA/protein vaccinations with different gene products and schedules are two promising ways this may be achieved.

General use of this approach in humans will also be hampered by the strong anti-AAV immunity induced by the initial vaccination, that curtails the positive effects of subsequent vaccinations.[9] Consequently, AAV vector-based vaccines such as this one may be most effective when used in conjunction with other approaches.

The way to a successful HIV vaccine is paved with difficulties and surely monkeys, and not mice, will have to be used for setting the first standard of protection before human trials. Notwithstanding, the new work by Okuda's group at least represents a promising lead in the quest for the Holy Grail of a vaccine to prevent further misery being inflicted by the plague of AIDS. ■

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Cardiac gene therapy

# Pumping up the heart

RG Crystal

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Numerous studies have shown that gene therapy can transiently forestall heart failure.[1–5] Now, for the first time, Chien and his colleagues have demonstrated that experimental heart failure can persistently be prevented with gene therapy.[1]

If you could give your heart one piece of advice, the most sensible would be to emulate the Energizer bunny – keep the pump going and going. In simple terms, the heart is a regulated pump, each beat providing blood sufficient for the body's demands. While the normal heart has sufficient reserve to maintain the pump function throughout life, the stress of common disorders such as hypertension, atherosclerosis, or diabetes can cause the cardiac pump to deteriorate. The result is heart failure: the faltering pump cannot provide adequate blood flow to other organs and there is an accumulation of fluid in the lungs and lower extremities

The molecular mechanisms underlying heart failure are complex. They involve energy generation and signal transduction pathways, as well as the mechanics of cardiac function.[6,7] The key is contractility. The ability of the heart cell to contract and relax at rest and in response to enhanced demand is controlled at three levels: signaling via activation of G-protein-related receptor pathways, transduction of this signal through cAMP/protein-A-kinase-mediated control of calcium ($Ca^{2+}$) cycling in the sarcoplasmic reticulum, and transduction of the $Ca^{2+}$ cycling signal to the apparatus that mediates muscle contraction (Figure 1). Each of these processes is complex, and abnormalities in each are linked to heart failure in experimental animals and humans.

The new work from the Chien laboratory, published in August's *Nature Medicine*,[1] focuses on correcting abnormalities in $Ca^{2+}$ cycling to treat heart failure. One such abnormality, clearly linked to heart failure,[2,3,6–8] is a decrease in the activity of an enzyme that regulates diastolic and systolic function, sarcoplasmic reticulum $Ca^{2+}$ ATPase (SERCA). Such a decrease leads to reduced $Ca^{2+}$ cycling and thus decreased contractility

SERCA activity decreases as the level of an inhibitory protein, phospholamban, increases.[7,9] Phosphorylation of phospholamban releases the controlled inhibition of the $Ca^{2+}$ pump, resulting in enhanced cardiac function. In heart failure, the phospholamban regulatory pathway goes awry. Phospholamban is chronically under-phosphorylated, resulting in chronic suppression of SERCA and thus reduced contractility.

Based on these observations, persistent activation of SERCA is an obvious therapeutic strategy for heart failure. But how can this be accomplished? In an attempt to meet this challenge, the Chien laboratory used a gene therapy approach. They delivered a mutant form of phospholamban to the heart in a vector already shown to be effective at transferring and persistently expressing genes in the heart:[10] recombinant adeno-associated virus serotype 2 vector (AAV2). The mutant phospholamban (S16E) has a serine replaced by a glutamate at one of its two phosphorylation sites, mimicking phosphorylation of the serine (ie pseudo-phosphorylation).

Theoretically, delivering an S16E form of phospholamban to heart cells should increase SERCA activity, and thus increase

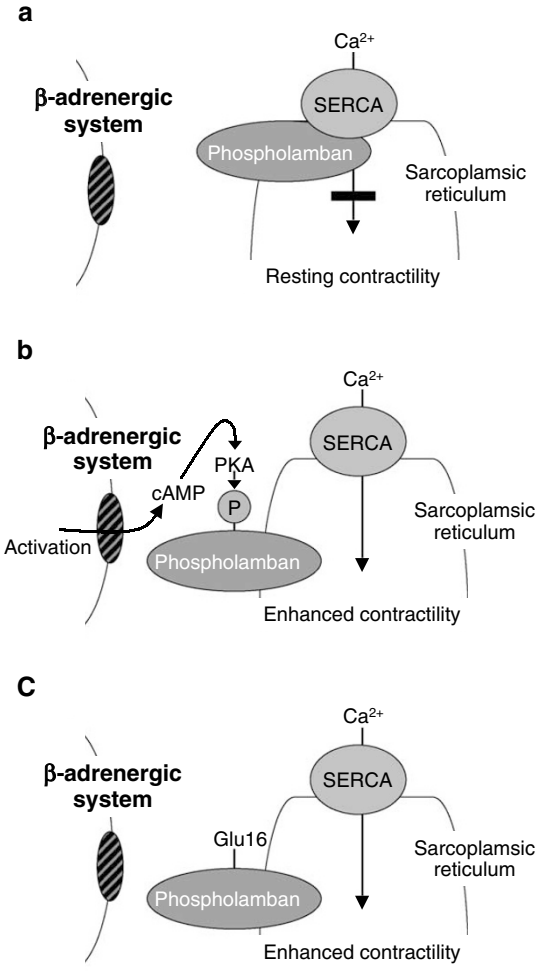


***Figure 1*** *Contractile function of heart cells. (a) Normal heart, resting. Nonphosphorylated phospholamban binds to sarcoplasmic reticulum $Ca^{2+}$-ATPase (SERCA), suppressing $Ca^{2+}$ cycling, maintaining a resting level of contractility. (b) Normal heart, activated. Phosphorylation of phospholamban removes the resting SERCA suppression, enhances SERCA-mediated $Ca^{2+}$ cycling, and thus contractility. (c) Gene therapy with S16E phospholamban. Gene transfer of a pseudophosphorylated form of phospholamban (S16E) results in persistent SERCA-mediated $Ca^{2+}$ cycling, and thus persistent enhanced contractility.*

contractility. A variety of *in vitro* and acute *in vivo* models suggested that this strategy should work.[6,7] However, heart failure is a chronic condition, and thus the critical question is whether this approach would persistently prevent heart failure? To address this question, Chien's group used an animal model of progressive cardiomyopathy and heart failure, the BIO14.6 hamster.[11] With an adeno-associated virus vector, Chien and his co-workers were able to persistently express S16E phospholamban in the heart. They also showed that S16E phospholamban gene therapy, despite not addressing the primary cardiac abnormality in the BIO14.6 hamsters, enhanced a variety of parameters associated with cardiac function.

The achievement of Chien and his colleagues is significant in two ways. First, while heart failure can wax and wane, it is a chronic condition requiring persistent therapy. The new study is the first to demonstrate that gene therapy can achieve this. Second, this study shows that enhancement of SERCA function can be used to treat heart failure caused by other defects. It seems that SERCA is sufficiently central to cardiac function and that enhancing its activity at least partially overrides other abnormalities.

Where do we go next? Should we move toward human trials using this strategy? The answer is clearly not yet by a long shot.

First, heart failure in humans often has a different cause from that addressed in this study. There are multiple pathways that go awry in human heart failure. Therefore, there may be other important abnormalities, not present in this hamster model, that also need to be corrected.[6,7] The lack of success in human trials of an antibody treatment that prevents heart failure in a murine model[12] suggests that we need to be cautious in extrapolating results in specific animal models. To ensure that gene therapy is directed at the dominant pathway (and thus will lead to a therapeutic benefit), more work needs to be done on the causes of human cardiac failure.

Second, if you are going to put a gene into a human heart that will be persistently expressed, the amount you put in and the place you put it must be absolutely correct. Once the gene is inserted, you cannot simply stop administering the therapeutic protein it produces, as is done with conventional drugs. Ways to regulate the expression of the gene of interest or to destroy the cells in which the gene has been placed must be developed. Since a strategy of destroying heart cells is not a good idea, we will need to adapt promoter control strategies to regulate the level of expression of the transferred gene.

Despite these reservations, the study by the Chien group is important and it is a significant step in the steady progression of gene therapy toward developing meaningful therapies for heart failure. If $Ca^{2+}$ cycling is central to the pathogenesis of human heart failure, and if strategies can be developed to control the expression of genes from AAV vectors, then gene therapy may be one way to keep the cardiac pump going and going ■

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