# Gene Therapy

## Countering Bioweapons

*You need not to fear the terror by night,*
*or the arrow that flies by day,*
*the plague that stalks in the darkness,*
*or the scourge that ravages at noon.*

Psalms 91

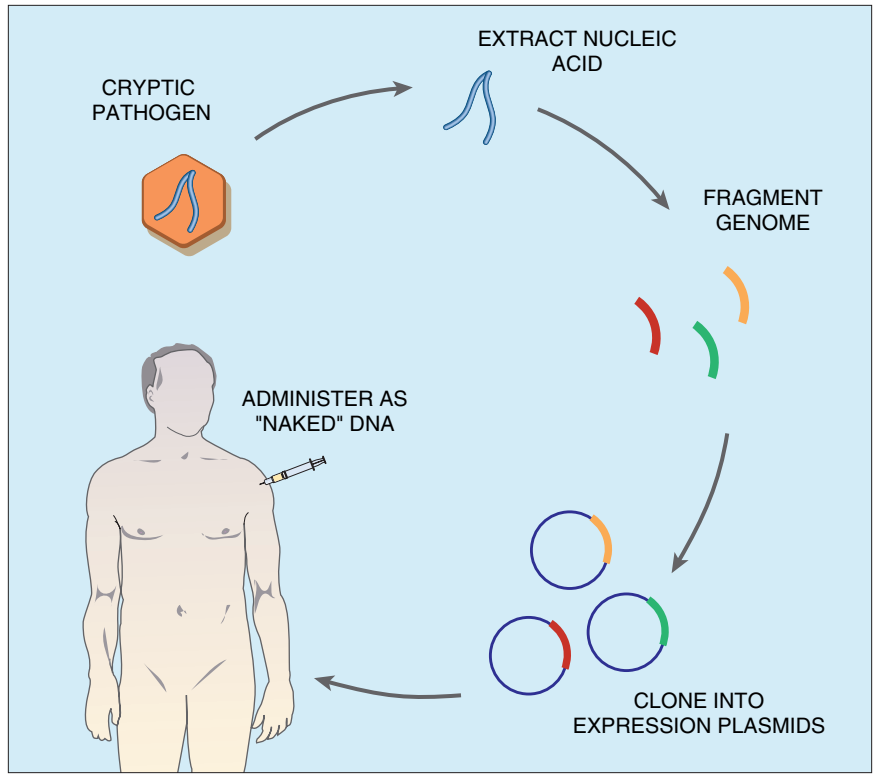
The events of September 11 and beyond have brought into focus the threat posed by biological weapons. While the potential use of typical bioweapons agents has provoked discussion of mass vaccinations for military personnel and the population at large, the agents that have until now been used against Americans are not the most threatening possibility for our defense planners. The power of genetic engineering raises the possibility of advanced-generation bioweapons that are even more virulent and capable of evading our current vaccine defenses.

History teaches us that the greatest instabilities in civilization occur when the power of offensive weapons suddenly outstrips defensive abilities. The advent of iron weaponry at the close of the Bronze Age and the emergence of tanks in the era of equine cavalry are examples of such technological disconnects.

Today's advances in molecular biology have ushered in another such period of potential instability. Recent popular novels (Richard Preston's *The Cobra Event* is one example) illustrate the chilling and real possibilities of a mismatched biological battlefield. The challenge for today's scientists and defense planners is to close this technological gap between the offense and defense.

Any strategy against a bioweapons assault must (1) protect the non-immunized from acute infection, (2) must protect the infected from tissue injury, and (3) must neutralize the overall threat to the public health through long-term immunization. Furthermore, these defenses must be devised not only for conventional bioweapons but also for novel, genetically engineered organisms, which could represent the front line of any bioweapons assault.

Gene therapy offers many tools to assist in this task. In the first instance, strategies to protect the acutely exposed, non-immunized person have traditionally been based on passive immunotherapy – that is, the intravenous delivery of preformed antibodies directed against the microbial agent. This strategy requires the stockpiling of large amounts of preformed antibodies for use in such emergency situations as a bioweapons attack.




**Shotgun immunization** allows immunization against a potential pathogen without knowledge of its structure. The cryptic agent's genome is fragmented and cloned into expression plasmids, which are then employed for naked plasmid DNA immunization via intramuscular administration.

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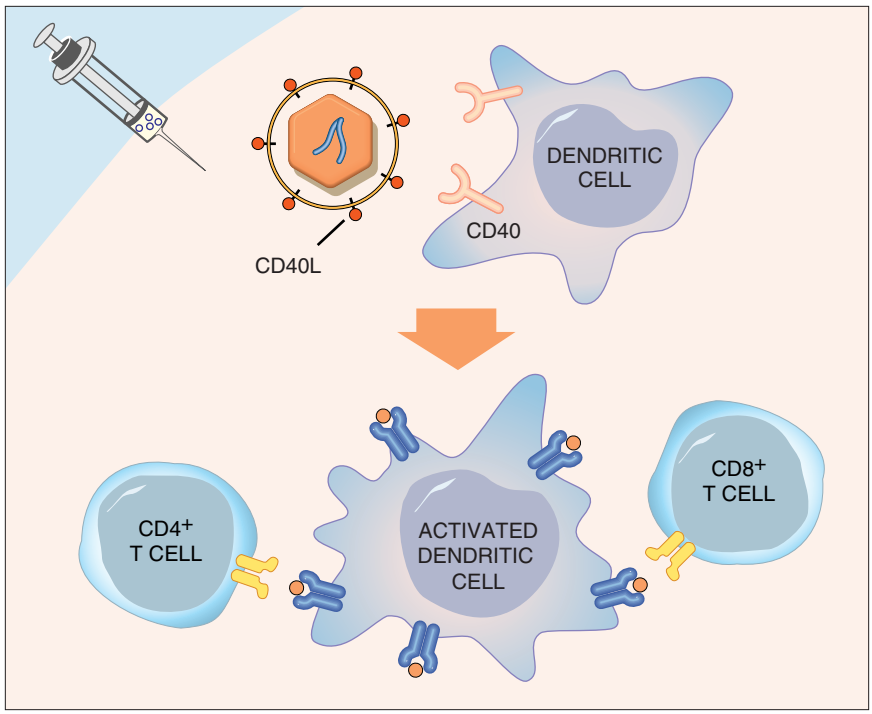


**Active immunization** can be achieved via the selective transduction of dendritic cells. A recombinant adenoviral vector encoding the antigenic epitope of a potential pathogen is injected intradermally. This adenoviral vector has been genetically modified to incorporate into its outer coat the protein CD40 ligand (CD40L). This molecule is recognized by the CD40 receptor characteristic of dendritic cells and thus allows selective delivery of antigen genes to dendritic cells and activates them to present antigen for immunization.

Significant issues with respect to upscaling and storage of large amounts of antibody have limited the full practical employment of this approach. In addition, host immune responses to the administered antibody (frequently derived from non-human sources) have limited the real utility of passive immunotherapy.

Genetic engineering, however, provides the means to derive antibodies against a broad range of target agents. This has been achievable via the derivation of large "libraries" of antibody repertories with selection of desired specificities in the laboratory. With this "biopanning" technique, it is possible to quickly derive antibodies against nearly any candidate agent.

Furthermore, it is also possible to derive genes that encode for these antibodies via genetic engineering methods. These antibody genes may then be delivered to an at-risk person whereby expression would allow the development of a passive immunotherapy.

From the foregoing discussion, it is clear that gene therapy and genetic engineering can provide a rapid means to derive desired antimicrobial antibodies and that gene delivery technology may provide an effective method to administer these antibodies as a passive immunotherapy. Thus, although passive immunotherapy is clearly the intervention needed for aiding acutely exposed, non-immunized persons in a bioweapons attack, gene therapy may provide key technological tools that allow such an approach to be implemented in a practical, effective manner.

In the second instance, persons exposed to a bioweapons microbial may sustain tissue injury from a successful infection. In this case, the antimicrobial will have successfully overwhelmed host defenses and provoked a pathobiologic cascade, with tissue damage resulting in morbidity and mortality.

In many instances, these host defenses have been understood based on a characterization of the defensive strategies that host cells or organs activate to counter the offensive attacks of microbiologic agents. Also, such host defenses are frequently qualitatively appropriate but quantitively inadequate to counter the injurious stimuli. For example, some host cells may express genes that directly counter injurious stresses due to microbial toxins, but they may also express genes that directly counter nonspecific stresses by activating cell death signals.

In these cases, gene therapy may provide the means to boost expression of natural host defense genes, thereby shifting the balance of forces and allowing cellular survival. For example, gene therapy-based overexpression of critical endogenous cellular inflammatory mediators, such as cytokines, may allow protection of organs from microbial challenge. If microbial invasion and injury may be understood to reflect a balance between host and microbe forces, gene therapy provides a means to augment host defenses, shifting the balance in favor of organ protection and survival.

Protection of unexposed persons is also of critical importance in providing a prophylactic shield for large, at-risk populations. Clearly, many strategies for vaccination exist, and many of these have been applied in bioweapons scenarios. Here, as well, gene therapy provides special opportunities to bioweapons defense.

The technique of "polynucleotide immunization" is based on the use of DNA encoding an antigen gene as the means to provide immunization against that antigen. This method can provide active immunization for use in infectious disease applications [see the article on DNA Vaccines, by Oyaski and Ertl, in the January/February 2000 issue of *Science & Medicine*].

One special advantage of this method accrues to the fact that genes, and not gene products, are the basis of immunization — it is possible to achieve immunization without a full characterization of the challenge agent. In this regard, the technique of "shotgun immunization" allows a microbial agent's genes to be employed directly as a vaccine agent.

This capacity permits the rapid construction of vaccines against agents for which we lack full characterization and the ability to culture them for vaccine-development purposes. Such speed and flexibility are paramount if researchers are to address the most threatening engineered pathogens of the bioweapons arsenal, which may remain unknown to us until they are used in an attack.

Gene therapy methods may likewise provide practical advantages for traditional vaccine strategies by virtue of advanced vector systems. In this regard, recombinant adenoviral vectors have been used widely as vaccine agents for several decades.

Recent insights into the immune system have identified the dendritic cell as a major mediator of the process of active vaccination. The gene therapy technique of vector "targeting" allows the means to direct gene delivery vehicles to selected cells. This method has thus provided the means to employ adenoviral vaccine agents that have been engineered to selectively deliver antigen genes to dendritic cells.

This ability greatly improves the potency of the basic adenoviral vaccine approach. Thus, gene therapy may provide key technological capabilities that lead to improvements in basic immunization approaches.

The application of gene therapy technologies to bioweapons defense, until now, has been piecemeal and limited. To apply this technology to bioweapons principles will require major re-tooling among gene therapy investigators, as well as collaborations with a new framework of scientists and government personnel.

But the overarching fact, that we already have relevant technologies in place, should not be lost. In the words of Aleksandr Solzhenitsyn, we must come to understand that we are in the "realm of the final inch," the search for perfection in completing a complex and daunting task.

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*[The Optimist, cont. from pg 187]*
ive activity" of seeing. I am sure they are right, but not all medical students are good at drawing, and for that reason the approach at Yale may be more relevant. There, students are asked to describe what they see in a painting or sculpture, in the belief that learning to see will enhance the clinical gaze. Careful if subconscious noting, but not always registering until the observation is required, seems to me to resemble the clinical experience.

Another problem in introducing the medical world through anatomy may be more mystical than scientific. If we rely on the eye for diagnosis as doctors nowadays do, we may teach the habit of putting ourselves at a distance from our patients.

You can see from much farther away than you can ever hear: the "music of the spheres" turns out to be only "solemn silence," however brightly the stars still shine. Surely, to initiate students with a cadaver risks of teaching them that the physician is dominant and the patient simply inert material, like clay in the hands of the Creator.

By focusing on the dead body, we "privilege," as the jargon goes, the body and ignore the spirit and the mind. Never mind that palaver about physician-patient relationships: cadavers never complain.

On the whole, I have convinced myself once more that medical educators should move toward teaching gross anatomy largely by computerized images, which can be looked at again and again, and which, more importantly, reflect modern medical technology.

Now that voice-activated computers are becoming common, and voice-activated computerized surgery seems just around the corner, students should become familiar with the tools that they will rely on in the future. If they are going to operate by computer, and if diagnosis is going to be largely by computer (and whatever follows), it seems prudent to train students now and not look down on the skills that even computer games can enhance.

Having said all this, let me repeat my belief that physicians — living, breathing, and touching — are still needed for our human patients, to listen to their complaints, to make the diagnoses that only intuition can find, and to decide what is important to the patient and what is trivial of all that appears on the screen.

That is what patients of the future will need if they are to be healed.

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