be needed to prevent the misuse of this technology, but it is unlikely that the use of stem cells carries any particularly devastating dangers.

Eventually, an 'immortal' population could evolve and that would create its own moral problems

This proposal derives from John Harris who is sufficiently impressed by the promises of stem cell therapy to believe that we may have to face a population that can live two or even more centuries (Harris, 2000). Success on that sort of scale seems a long way off—but it would be an accolade to medicine to have that set of problems to face!

I wish to close with another quotation from the *Microcosmographica Academica*: 'There is only one reason for doing something; the rest are arguments for

doing nothing.' The Luddites can always produce a variety of more or less plausible arguments for resisting technological innovation. But without innovation we would not have moved on from the Stone Age to the Computer Age in only ~100 generations. The present arguments for doing nothing are no more potent than all preceding ones.

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Myth, menace or medical blessing?

The clinical potential and the problems of genetic vaccines • by Wolfgang W. Leitner

Some day in the near future, when children have to see a paediatrician to get their immunizations they may encounter a much less painful procedure than today's children do. Instead of the dreaded needle and syringe, the doctor will hold a gene gun to the child's arm and, with a blast of helium, painlessly shoot tiny gold particles into the skin. These particles are coated with plasmid DNA containing the nucleotide sequences for viral or bacterial surface proteins. Upon their introduction into host cells, the protein is produced which triggers an immune response as it would normally in the case of an infection. As a result, the child will be protected from further attacks by the respective virus or bacterium, as in the case of any vaccination.

This procedure is painless, bloodless, clean and—as far as we know today—completely safe. However, such a scenario is likely to send cold chills down

the backs of most European parents who, in general, tend to be more reluctant in their acceptance of DNA-based technologies than North Americans, who seem to be less concerned about the idea of 'genetic immunization' or 'gene vaccines'. Oddly, the idea of a 'gene transfer' raises irrational fear in many people who do not realize that we are under constant attack by viruses, bacteria and parasites, which—as part of the infectious process—force their genes into our cells and use them as factories to produce their progeny. In the face of all the criticism and opposition that other 'gene-based' products and therapies have spawned in Europe, it may appear premature to believe that genetic vaccines will soon become acceptable to the public.

Indeed, there are some risks involved with genetic vaccines, comparable to those that an infection might have. Some researchers are concerned that vaccine sequences might be integrated into the

genome of the patient's cells. Inserted in the wrong location, these genes could theoretically have oncogenic potential, for instance by disrupting cancer-suppressor genes. It will require extensive clinical trials to test this approach and to convince the public of its safety.

DNA vaccines represent a good example of a successful collaboration between immunology and molecular biology. Since their discovery, plasmid DNA constructs have been extremely useful tools for molecular biologists, but their more recent application as vaccines has dragged them out of the laboratory and into the public spotlight. Plasmids are circular, extrachromosomal pieces of DNA that can be modified to carry genes of interest. When introduced into mammalian cells *in vitro*, they cause these cells to produce the respective protein of interest. For this reason, it was not very surprising to find that injection of plasmid DNA into the leg muscles of mice resulted

in antigen production *in vivo*. Although the first report of this phenomenon dates back to 1962 (Atanasiu, 1962) it had to be 're-discovered' in 1990 (Wolff *et al.*, 1990) before it caught the attention of immunologists.

It was quite a surprise that these injected 'naked' DNA molecules, pure, non-encapsulated DNA without any

humoral and the cellular arm of the immune system. The vaccine can be delivered by various methods, traditionally by injection into the muscle. Injection into the skin has proven to be more effective and the best immune responses have been obtained by immunization with a gene gun, perhaps because the vaccine is directly delivered into the skin's antigen-

erentially stimulate just one arm of the immune system, antibody production, while failing to effectively activate killer cells, which are essential for combating a viral infection. Secondly, recombinant protein vaccines require strong adjuvants and may therefore not be safe for humans.

DNA vaccines, in contrast, are very cheap, easy to construct, and capable of stimulating both cellular and humoral responses. DNA vaccines would also meet the requirements for broad applicability in Third World countries, including easy manufacturing as well as uncomplicated transportation and storage. Nevertheless, DNA vaccines are far from being perfect replacements for conventional ones. While it should not be their primary purpose to replace currently used vaccines with a good track record, i.e. high efficacy and few side-effects, new approaches are urgently needed for diseases that have so far resisted vaccination attempts. Despite success stories, such as the vaccine-based eradication of smallpox in 1980, we are still faced with a large number of pathogens and diseases that kill millions of people every year. And there is still no vaccine available against the big killers—malaria, tuberculosis, HIV and multi-resistant bacteria. Furthermore, as the potential of vaccines to treat and prevent

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adjuvants, triggered an immune response to the encoded protein. Initially, this finding seemed contradictory to one of the dogmas in immunology, according to which the presence of a foreign antigen alone is not sufficient to elicit an immune response. Instead, a 'danger' signal that accompanies the antigen is required to trigger the process. Some of these signals are, for example, provided by bacterial surface molecules, such as lipopolysaccharides, whereas others are produced by host cells responding to a viral infection in order to alert the immune system. In the case of classic vaccination with a protein, the signal is provided by adjuvants—in routine human vaccines aluminium compounds are used (Gupta, 1998)—without which the vaccine would be ineffective. We do not yet fully understand how adjuvants work, but their mechanism of action is likely to include formation of depots, slow release of the vaccine and general stimulation of the cells of the immune system. The phenomenon of 'naked' DNA being capable of stimulating the immune system without any adjuvants has been studied intensively. The only mechanism that has been linked to the 'adjuvant properties' of plasmid DNA vaccines so far is the presence of certain, very specific nucleotide sequences called CpG-motifs on the plasmids, which originate from the bacterial hosts (Krieg *et al.*, 1998). For practical purposes, the fact that DNA vaccines provide their 'own' safe adjuvant is a significant advantage of this technology.

As far as we understand it today, DNA vaccines work by transfecting antigen-presenting cells. In the course of the immunization, these cells display the antigen to T-lymphocytes in the lymph nodes and the spleen, thus activating the

presenting Langerhans cells. Thus, only small amounts of DNA are required. The 'shot' from the gene gun itself may be sufficient to activate antigen-presenting cells and thereby function as an adjuvant, but without the side-effects induced by traditional adjuvants, i.e. fever, allergic reactions, granulomas or irritation at the injection site.

Considering the availability of other vaccines, namely recombinant and attenuated viruses and recombinant protein, one cannot help asking the question: Do we really need such a radically new approach? The simple answer is that all the currently used vaccines have practical

It was quite a surprise that 'naked' DNA molecules, pure, non-encapsulated DNA without any adjuvants, triggered an immune response to the encoded protein

problems. For many decades, a variety of attenuated viruses have successfully protected us against their wild-type brethren. However, such viral vaccines always carry the risk of inadvertent infection when the virus is not sufficiently weakened. A recent outbreak of Polio caused by an insufficiently attenuated vaccine has served as a painful reminder of this problem (Greensfelder, 2000). In general, stronger attenuation makes a given vaccine safer at the cost of decreased efficacy. Furthermore, vaccination with an attenuated virus may be unacceptable in the case of highly pathogenic viruses such as HIV or Ebola, regardless of the degree of attenuation.

Using select purified proteins from the pathogen as the active compound of vaccines carries absolutely no risk of infection, but pure proteins come with two major shortcomings. First, they pref-

cancer is being tested (Walsh *et al.*, 2000), DNA vaccines could also become useful tools in cancer therapy.

Despite the enthusiasm of many researchers over this new generation of vaccines and the progress that has been made so far, DNA vaccines have two major shortcomings. One is their weak immunogenicity, which can limit their efficacy against some pathogens and, especially, against tumour cells. Many strategies are currently being explored to overcome this limitation (Leitner *et al.*, 1999). The most radical approach has been to create a 'self-replicating' nucleic acid vaccine that utilizes a viral-replicase protein derived from RNA viruses that amplifies the viral genome after infection of the host cell. When the organism detects this process, it responds with a strong antiviral immune response. It appears that the activity of the replicase

gene encoded in a DNA or RNA-vaccine is (mis)interpreted by the immunized host as a potentially dangerous viral infection. The result is the same kind of strong anti-viral immune response that the actual virus would elicit. The trick of using a viral replicase may be what is needed to give DNA vaccines the necessary punch to make them sufficiently immunogenic.

While insufficient immunogenicity may be overcome, the bigger problem that DNA vaccines face today cannot be solved in the laboratory: low social acceptance. The idea of being injected with bacterial, viral or parasite genes is likely to trigger instinctive fear and resentment in a large number of people. Many people will also mistake genetic immunization for gene therapy, which suffers from a widely published drawback when a patient died during a gene therapy trial. Gene-transfer technologies lost a lot of credibility in the USA because the investi-

DNA vaccines would also meet the requirements for broad applicability in Third World countries, including easy manufacturing as well as uncomplicated transportation and storage

gation of the incident uncovered serious mishandling of the clinical trial. Indeed, DNA vaccination and gene therapy are closely related approaches. In both cases, a gene is introduced into the host, and the encoding protein is produced by host cells. But in the case of gene therapy, it requires long-term expression of the encoded protein—commonly an enzyme that the patient lacks—and this protein remains immunologically 'silent'. For DNA vaccines, in contrast, the desired outcome is short-term expression of this antigen and a strong immune response directed against it.

In Europe, there is an even greater general resentment against technologies that involve genetic modification of living organisms, particularly humans. This is a response to various consumer scandals, which have made people perceive the life sciences as a threat rather than an enrichment for society and the economy. Mad-cow disease, for example, and its handling in the UK has generated or at least contributed quite significantly to this

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deep rooted distrust of medical science. The false predictions that the 'pathogen will not infect humans' and announcements that 'the disease is contained' have

damaged the reputation of scientists, and have thereby made the task of convincing people that gene technologies are safe and reliable a tough challenge. Despite the incredible potential that these technologies harbour, the public needs to accept them first. Therefore, public education will have to include serious efforts to regain public trust in genetic research.

According to our current knowledge, DNA vaccines represent a clean and safe way to induce an immune response to well-defined antigens. This exciting and promising new technology at least deserves a fair chance to prove itself and should not be sacrificed to a diffuse and irrational fear of gene modification technologies.

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