Could a DNA vaccine be useful in the control of tuberculosis?*

JOSÉ MACIEL RODRIGUES JÚNIOR, KARLA DE MELO LIMA,
ARLETE APARECIDA MARTINS COELHO CASTELO, VÂNIA LUIZA DEPERON BONATO MARTINS,
SANDRA APARECIDA DOS SANTOS, LUCIA HELENA FACCIOLI, CÉLIO LOPES SILVA.

The DNA vaccines currently under pre-clinical and clinical development may prove to be important tools in combating infectious diseases, such as tuberculosis, for which no safe and effective form of prevention has yet been developed. In recent years, several studies have aimed to develop a DNA vaccine encoding mycobacterial proteins such as antigen 85 (Ag85) and the 65-kDa mycobacterial heat shock protein (hsp65). The latter is protective against virulent infection with Mycobacterium tuberculosis (including multidrug-resistant strains). The hsp65 DNA vaccine, currently under clinical evaluation in Brazil for cancer therapy, is able to induce the secretion of Th1 cytokines, such as gamma-interferon, associated with disease control. Furthermore, this vaccine stimulates cytotoxic CD8 and CD4 T-cell clones that can be characterized as memory cells, which are responsible for effective and long-lasting immunity against tuberculosis. When used as a therapeutic agent in inoculated mice, the hsp65 DNA vaccine promotes changes in the immunity profile, triggering the secretion of Th1 cytokines and establishing a favorable environment for the elimination of bacilli. The results also demonstrate that the route of administration, as well as the formulation in which the vaccine is administered, fundamentally influence the pattern and duration of the immune response induced. Taking all currently available data into account, we can conclude that a DNA vaccine against tuberculosis could contribute significantly to the control of the disease.

Key words: Tuberculosis/epidemiology. Vaccines, DNA/therapeutic use. Heat shock proteins. Auto-immunity.

* Study carried out by the Rede Brasileira de Pesquisa em Tuberculose (Brazilian Tuberculosis Research Network) at the University of São Paulo Faculdade de Medicina de Ribeirão Preto (Ribeirão Preto School of Medicine), Ribeirão Preto, SP, Brasil.

Correspondence to: Célio Lopes Vieira. Rua Clóvis Vieira, 24 - Campus da USP de Ribeirão Preto. CEP 14049-900 - Ribeirão Preto-SP, Brazil. Email: clsilva@cpt.fmrp.usp.br

Financial support: Rede Brasileira de Pesquisa em TB (Rede-TB)/Grant no. 62.0055/01-4-PACDT-Milenio.
Submitted: 29 April 2004. Accepted, after review: 27 May 2004.

What a DNA vaccine is

In recent decades, advances in vaccine technology have allowed the introduction of new strategies for obtaining and producing antigens, as well as for the optimization of new forms of administering and introducing these antigens into immune system cells. These strategies have opened pathways to innovation, especially in the context of developing vaccines that are safer, more effective and more polyvalent. Among these, there are subunit, or second-generation, vaccines comprised of purified or recombinant antigens obtained from infectious agent cultures or through synthetic production. More recently, genetic, or third-generation, vaccines have been developed. In DNA vaccines, genes or gene fragments encoding potentially immunizing antigens are delivered by plasmid DNA.

Although DNA vaccines are still in the laboratory and clinical testing phase of development, they may prove to be important tools in combating infectious diseases such as herpes, acquired immunodeficiency syndrome (AIDS), malaria, tuberculosis (TB), hepatitis, schistosomiasis, dengue and others for which no safe and effective form of prevention has yet been developed. Moreover, DNA vaccines have shown great potential for the prevention and cure of specific types of cancer.

Gene isolation is a process the scientific community can control today due to significant advances in the development of molecular biology techniques. In order to produce a DNA vaccine, genes that are frequently related to the expression of molecules involved in virulence or pathogenicity of infectious agents are cloned and inserted into a fragment of circular DNA called a plasmid. The replication of a plasmid used in a genetic vaccine must be bacterial in origin, thereby allowing efficient duplication (hundreds of copies per cell). In addition, these plasmids must have a selection gene (which usually confers resistance to an antibiotic, allowing clones of the cells that carry the plasmid to be selected). Furthermore, it is essential that such plasmids possess a multiple-cloning site that can be cleaved with restriction enzymes, allowing the insertion of the gene of interest (which, in the case of DNA vaccines, is a gene that encodes an antigen). Moreover, these plasmids must include a promoter (usually a viral promoter) that allows transcription of the gene of interest in eukaryotic cells. After cloning the gene that encodes the vaccine antigen in the plasmid, the gene is introduced into a host bacterium, usually *Escherichia coli*, using a process called bacterial transformation, in order to produce plasmids on a large scale, providing a sufficient quantity of DNA for vaccination[1].

Abbreviations used in this paper:

AIDS – Acquired immunodeficiency syndrome
TB – Tuberculosis
BCG – Bacillus Calmette-Guérin
RT-PCR – Reverse-transcription polymerase chain reaction
WHO – World Health Organization
hsp65 – 65-kDa mycobacterial heat shock protein
PLGA – Poly-lactic-co-glycolic acid
MHC – Major histocompatibility complex
Mtb – Mycobacterium tuberculosis

These DNA vaccines may be administered to various animal species (and to humans) in various forms and using various regimens. The most common form of DNA vaccine delivery is intramuscular injection. In addition, genetic vaccines may be delivered orally, intranasally (as an aerosol), or intradermally by means of bombardment with gold microparticles coated with genetic material. Although intramuscular injection is a simple, inexpensive process, it usually requires a great quantity of plasmids in order to stimulate an appropriate immune response. Hypertonic glucose solution, which causes edema in muscular tissue[2], or a local anesthetic solution, such as bupivacaine, which can injure tissues and cause an inflammatory reaction[3-5], have been used as means of increasing antigen transfection into macrophages and dendritic cells after intramuscular injection. However, due to their adverse effects, these procedures are not acceptable for use in humans. Intramuscular injection of plasmids is a simple technique, but it exposes DNA to various obstacles at the delivery site, such as enzymes (nucleases) that are capable of degrading the plasmid DNA, thereby preventing the induction of an immune response.

The size of the DNA molecule and its superficial negative charge is another relevant factor that may limit its penetration into the target cell, requiring higher DNA doses in order to induced a significant immune response. A prerequisite for the efficacy of the use of DNA as a vaccine or in gene therapy

is efficient liberation of its nucleic acid within the target cell. Nucleic acids must reach the cell nucleus, and this has been achieved by the use of living (bacterial and viral) and non-living (synthetic system) vectors. It is estimated that only one in every 1000 plasmid molecules delivered will reach the nucleus and express the message for the desired protein synthesis. Therefore, the delivery of high doses of plasmid DNA (from hundreds of micrograms to one or more milligrams) is required in a standard treatment. Pharmaceutical-grade recombinant DNA can be obtained by means of inducing its expression in transformed bacteria and purifying it in a series of chromatographic procedures, thereby allowing it to be produced on a large scale(6).

Perpectives on an anti-tuberculosis DNA vaccine

Special attention has been given to the development of more effective TB vaccines. Maintaining the distinction of being the infectious disease with the highest worlwide mortality rate, the annual incidence of new TB cases has recently arrived at 10 million, and the annual mortality rate at 3 million people – 18.5% among adults from age 15 to age 59, corresponding to the most productive portion of the population. According to the World Health Organization (WHO), 32% of the global population is infected. In the last decade, 300 million people were infected, 90 million new cases were reported, and approximately 30 million people died from the disease. In 1993, the WHO declared TB a global emergency, and *Mycobacterium tuberculosis* (Mtb) is now considered the infectious agent responsible for more adult deaths than any other.

Various vaccines enlisted to combat TB have been studied. Notable among such vaccines are those encoding antigen 85 and the 65-kDa mycobacterial heat shock protein (hsp65). These are the two most-studied antigens, and pre-clinical trials (which have generated a great volume of data in the literature) have shown promising results. In the present study, we will focus on results obtained with hsp65.

In order to develop a DNA vaccine against TB, Silva et al. introduced the gene of one of the mycobacterial immunodominant antigens, stress protein hsp65 of *Mycobacterium leprae*, into the plasmids pCDNA3 and pHMG. Both plasmids effectively drive the expression of the mycobacterial hsp65 gene in mammal cells[7-9]. Mice were inoculated with intramuscular injections of plasmids containing the mycobacterial gene. All vaccinated animals were compared to a group of animals inoculated only with intradermal Bacillus Calmette-Guérin (BCG) or only with plasmids not containing the hsp65 gene. Two weeks after the third plasmid dose, production of specific antibodies was observed in the serum of animals immunized with hsp65 DNA. Induction of immune response was detected by a specific test which measured the proliferative capacity of lymphocytes in the lymph nodes when combined with hsp65 antigens. Immune response mediators and regulators, such as interleukins, were also detected in the culture supernatant or in the lymphocytes of the immunized animals using both ELISA and Reverse-transcription polymerase chain reaction (RT-PCR) techniques. In these lymphocytes, there was increased liberation of gamma-interferon (IFN-^{3}) and interleukins (IL-2 and IL-12), which are considered cytokines that stimulate a Th1-type cellular immune response, but not of IL-4, IL-10 or IL-13, which suppress immune response. Therefore, the pattern of induced immune response due to immunization with hsp65 DNA in mice was reported to be of the Th1 type, which is highly effective in the elimination of infectious agents.

DNA vaccine-induced protection

Immunization of mice with hsp65 DNA has been shown to be highly effective in protecting the animals against Mtb infection, and the protective effect was very similar to that presented by animals vaccinated with BCG[10]. Based on these data, other mycobacterial antigens (hsp70, hsp10, 36-kDa, and ESAT6) have been cloned into pCDNA3 and pHMG plasmids and their protective actions determined retrospectively. The DNA sequences encoding the hsp70 and ESAT6 antigens induced significant protection, but less than that obtained from hsp65. On the other hand, those encoding hsp10 and 36-kDa presented no protective activity against Mtb infection. In summary, the results of these studies demonstrate that intramuscular inoculations with vaccines produced from DNA sequences encoding

the genes of the immunogenic proteins hsp65, hsp70 and 36-kDa, combined with promoters of cytomegalovirus and hydroxymethylglutaryl CoA reductase, confer significant protection against Mtb infection in mice[11].

Cells involved in protection and studies on immunologic memory

Silva et al. have carried out various studies in order to characterize more precisely the cellular subpopulations correlated with control of infectious processes, as well as the mediators involved (interleukins), and the effector functions triggered at the onset of the cellular activation process. The comprehension of these factors informs the understanding of the immune mechanisms involved in TB pathogenesis, leading to new perspectives on control of the disease. Studies evaluating animals infected with Mtb and vaccinated with hsp65 DNA have been carried out in order to establish relationships among T-cell subpopulation phenotypes, mediators released by these cells, and the expression of molecules, such as CD44, that have been related to cellular migration and immunologic memory.

The T cells of animals previously vaccinated were transferred to nonimmunized animals challenged with Mtb H37Rv. The latter animals showed better control of the infection when compared to those only infected with the bacilli. The cellular phenotype correlated with protection was characterized by cells presenting high CD44 expression on their surface ($CD44^{hi}$ vs. $CD44^{lo}$). The $CD44^{hi}$ cells obtained from immunized animals were able to control the proliferation of bacilli better than the $CD44^{lo}$ cells from the same immunized animals, as well as conferring better protection than the $CD44^{hi}$ and $CD44^{lo}$ cells from the infected-only animals. Among the $CD44^{hi}$ cells, the $CD8^{+}/CD44^{hi}$ cells were more efficient than the $CD4^{+}/CD44^{hi}$ in infection control, effectively preventing bacillus multiplication. Within this cellular phenotype (that provides protection against Mtb infection), it has been shown that $CD44^{hi}$ cells predominantly produce IFN-^{3}, whereas $CD44^{lo}$ cells preferably produce IL-4[7,12].

In order to define which cellular phenotype provided the best protection against the mycobacterial infection, clones of T $CD4^{+}/CD44^{hi}$, T $CD4^{+}/CD44^{lo}$, T $CD8^{+}/CD44^{hi}$ and T $CD8^{+}/CD44^{lo}$ cells were selected from vaccinated and infected animals. The T $CD4^{+}$ and T $CD8^{+}$ cells were obtained through negative selection and separated into $CD44^{hi}$ and $CD44^{lo}$ subpopulations, using a FACSort flow cytometer. Through the limited dilution technique, 16 T $CD4^{+}$ clones and 16 T $CD8^{+}$ clones were selected from immunized animals and from animals vaccinated with hsp65 DNA. These clones were characterized for CD44 expression ($CD44^{hi}$ or $CD44^{lo}$), IFN-^{3} or IL-4 production, and cytotoxic potential. The results show that the four $CD8^{+}/CD44^{hi}$ clones obtained from immunized animals produced IFN-^{3} and were cytotoxic. These clones conferred higher protection on infected animals when compared to T $CD8^{+}/CD44^{lo}$ clones. All four T $CD4^{+}/CD44^{hi}$ clones also produced IFN-^{3}, and three were cytotoxic. However, their protection potential was lower than that of T $CD8^{+}/CD44^{hi}$ clones. As for the clones obtained from infected animals, only two of the four T $CD8^{+}/CD44^{hi}$ clones produced IFN-^{3} and were cytotoxic, whereas the other two clones produced IL-4 and had no cytotoxic potential[7,12,13].

In light of this, a hsp65 DNA vaccine should comprise a group of CD4 and CD8 cell clones that would express high levels of CD44. Such $CD44^{hi}$ clones could be related with immunologic memory functions and possibly with accelerated migration to the infection site, where they might encounter the appropriate antigen-presenting cell. Therefore, these results show that prior immunization, using appropriate vehicles and administration routes, would be effective in triggering specific local and systemic immune responses that are efficient in mycobacterial infection control.

Use of carriers in genetic vaccines against tuberculosis

A study involving DNA vaccines based on the hsp65 protein, which had previously only been used as naked DNA and administered intramuscularly, was recently conducted[14]. The authors demonstrated that, in an experimental mouse model of TB, multiple doses containing great quantities of plasmid (at least three 100-µg doses) are necessary to promote protection or effect a cure. However, for vaccines still in development, it is important that immunization regimens be

simplified, reducing the number of doses necessary for stimulating protective immunity. Therefore, diverse strategies, involving various types of adjuvants, have been employed[15].

In order to optimize the use of DNA vaccines by reducing the quantity of DNA injected, intradermal administration using a gene gun has been analyzed. However, data from comparative studies have shown that intramuscular injection of DNA preferably induces a Th1-type immune response, whereas gene-gun bombardment triggers a Th2-type immune response. It has been demonstrated that gene-gun administration of hsp65 DNA at a dose level a hundred times lower than that used in conventional intramuscular injection has been shown to stimulate both types of humoral and cellular responses. The gene-gun administration preferably stimulated subclass IgG1 antibodies, and differences were therefore observed between the levels of the IgG1 and IgG2 immunoglobulin subclasses, suggesting a Th2-type response. In addition, BALB/c mice immunized with intramuscular injection of hsp65 DNA produced high levels of IFN-^{3}, whereas the immunization using gene gun does not stimulate IFN-^{3} production – although it does stimulate the production of Th2-type cytokines, such as IL-10 and IL-4. This Th2-type response may be due to the small amount of DNA used in the gene-gun technique. The lower quantity of DNA goes hand in hand with a reduction in the number of CpG motifs. As a matter of fact, mice immunized with intradermal injection of hsp65 DNA presented a reduction in the bacterial loads in the lungs, whereas those immunized using gene gun did not. Therefore, although the gene-gun technique triggered the specific immune response at significantly lower doses of DNA, it is not appropriate for administration of the hsp65 DNA vaccine against TB[16].

A second strategy that has been used to optimize hsp65-DNA vaccination is that of encapsulating the DNA in biodegradable polymer microspheres. The results of studies employing this technique have shown that DNA can be encapsulated into poly-lactic-co-glycolic acid (PLGA) microspheres with no function impairment. This method was able to produce particles of an appropriate size (functioning as vectors into phagocytic cells), as well as efficient in DNA encapsulation. In vitro studies have shown that phagocytic cells capture PLGA microspheres, which release the encapsulated DNA, which in turn coordinates the synthesis of the antigen protein. Therefore, the use of PLGA microspheres may represent an important tool in redirecting DNA into professional antigen-presenting cells after intramuscular or intradermal administration. The capability to directly transfect professional antigen-introducing cells may be interesting since several studies have shown that antigenic peptides, involved in the stimulation of cytotoxic T lymphocytes after DNA vaccination, are introduced by major histocompatibility complex (MHC) class I cells derived from bone marrow and not by transfected myocytes[17-19]. Therefore, when directly transfected *in vivo*, these cells that express co-stimulant molecules on their surface present antigens that provide the necessary signals for the stimulation of T lymphocytes. In addition, following intramuscular injection, the microspheres form a tissue deposit, recruiting antigen-presenting cells to the administration site, allowing the particles to be more easily captured and, consequently, facilitate the transfection of these antigen-presenting cells. Although microspheres are capable of improving antigen presentation, they have no immunostimulating properties. Therefore, formulations with microspheres containing DNA have been shown to stimulate specific immune response, but this response was not sufficient to protect BALB/c mice against infection after Mtb challenge.

The addition of adjuvants with immunostimulant properties and carriers employing microencapsulation technology has been used in order to improve the immunogenic capacity of microsphere formulations. A new formulation has been devised that contains hsp65-DNA vaccine and an immunostimulant agent, trehalose dimycolate (TDM), both encapsulated in biodegradable microspheres[20].

The capacity of PLGA microspheres to release the encapsulated plasmid slowly suggests a potential for maintaining protein expression for a longer period with no need for booster doses when compared to intramuscular injections of naked DNA. Data from RT-PCR studies have shown expression of the hsp65 RNA messenger in the spleen and lymph nodes of animals that had been immunized

15 days prior with a single dose of DNA encapsulated in microspheres. However, in animals immunized with three doses of naked DNA, no such expression was observed in these secondary lymphoid organs (where lymphocyte stimulation occurs).

Regarding the immune response itself, a significant change in the quality of antibodies has been observed after immunization with microspheres containing hsp65-DNA/TDM. The humoral response in these animals was characterized by high levels of IgG2a, whereas animals immunized with naked DNA presented a mixed profile, with high levels of IgG1 and IgG2a. The cellular immune response was characterized by high levels of IFN-³. Thirty days after the challenge, the animals immunized with microspheres containing hsp65-DNA and TDM presented high levels of IFN-³ in the lungs[21]. This profile may have been promoted by TDM. In 2001, Ryll et al. reported that TDM may stimulate an immune response, evidenced by a proliferation of NK cells, and that TDM may also provoke an early immune response through the release of IFN-³, leading to macrophage activation and upregulation of the expression of MHC class II molecules and CD1[22]. Therefore, TDM may provide excellent conditions for the initiation of an immune response, which makes it a good Th1-type stimulating component for use in TB vaccine formulations under development.

Significant protection against infection with H37Rv Mtb was achieved when mice were vaccinated either with intramuscular injection of naked hsp65 DNA or with hsp65 DNA and TDM encapsulated in microspheres. However, the microsphere formulation was able to stimulate the protective immune response after a single dose, whereas three doses of the injection formulation were required in order to provide the same protection. The vaccine based on microspheres also allowed each plasmid dose to be reduced to one-tenth of that of the naked DNA vaccine. These data showed that, in this particular mouse strain, the immune response to a specific antigen was directly influenced by the method of DNA vaccine administration. The development of formulations involving a combination of adjuvants with various action mechanisms may be an alternative for the development of more effective vaccines.

Exploring the *prime-boost* strategy

The duration of protection provided by a vaccine has been related to the permanence of the antigen in the organism. Therefore, in some vaccination regimens, the administration of multiple doses may be necessary for maintaining the immunity stimulated by the initial dose. Recently, the prime-boost principle has been extensively explored with the context of the heterologous boost concept. In this context, initial and subsequent doses introduced into the immune system contain the same antigen, although in different formulations. It is essential that the formulation used in the initial dose induce the necessary response for the protection of immunized individuals. The most common protocols use DNA or subunit vaccines in the initial dose, and living carriers, such as viruses or recombinant bacteria expressing the specific antigen, in the following doses. Although some results have been promising, this type of protocol reverts to the use of living vectors, the avoidance of which was the motivation behind the development of DNA and subunit vaccines. The prime-boost strategy involves the use of two different vaccines, each encoding the same antigen, administered at intervals of a few weeks. This strategy has been shown to increase cellular immune response in several animal models of infection[23]. As previously mentioned, most prime-boost protocols under development involve induction of the immune response with a DNA vector, such as plasmid DNA, using live-virus vectors[24]. The use of the prime-boost strategy for TB prevention has been evaluated, combining the use of DNA in the initial dose for the induction of the immune response with the use of attenuated BCG or recombinant proteins to maintain that response[25,26]. These protocols typically require that more than one DNA dose be administered in order to induce the initial immune response, and booster doses are administered subsequently.

In order for genetic vaccines to be viable products, the genetic vector must reach the target cells while still in its active form, requiring that it transverse the various barriers within the organism without suffering significant damage. One of the strategies that has been proposed for (and successful in) solving this challenge is that of encapsulating plasmids in biodegradable polymer

microspheres[27,28]. However, it has been shown that the immune response induced by microspheres containing hsp65 DNA diminished 90 days after vaccination in an experimental rat model. In the interest of increasing the duration of the immune response triggered by the microsphere-encapsulated DNA, the effect of a booster dose containing recombinant protein (also encapsulated and administered in conjunction with the DNA) has been evaluated. However, in a formulation with microspheres containing both DNA and protein, it is essential that the DNA be released prior to the protein since the DNA stimulates a Th1-type response. This is not a concern when using microspheres containing recombinant hsp65. In addition, co-encapsulating TDM in order to promote immunostimulation favors the development of a Th1-type response since TDM induces the secretion of cytokines such as IL-12, thereby contributing to the establishment of a favorable microenvironment for the differentiation of Th1 lymphocytes during antigen presentation[20].

Recombinant hsp65 was initially encapsulated in 75:25 PLGA microspheres, which can provide the release of the protein for 90 days. This enhanced durability is due to the greater number of lactic-acid monomers in the polymer chain, making it less hydrophilic and reducing its hydrolytic degradation when compared to DNA encapsulation in 50:50 PLGA microspheres. The combination of 50:50 PLGA microspheres containing hsp65 DNA and 75:25 PLGA microspheres containing recombinant (prime boost) hsp65 produced high levels of anti-hsp65 antibodies. Serum levels of these antibodies remained elevated 90 days after vaccination. Increased production of IFN-^{3} by splenic cells in vaccinated animals was also reported during the study period (90 days). These results suggest that prime-boost systems based on non-living vectors show great potential for increasing the duration of the immune response and decreasing risks related to toxicity.

Gene therapy for tuberculosis: the therapeutic action of the hsp65-DNA vaccine

Until recently, there was no expectation that the TB bacilli could be eliminated in many out of the great number of individuals infected (one-third of the global population). The bacilli usually reside in the lungs for an indefinite length of time, but they can also infect other organs, remaining within the defense cells of the host. The bacilli can reproduce prolifically if resistance decreases (from malnutrition, undernutrition, stress or comorbidity with other diseases) and may cause death if patients are not properly treated. From this reproduction phase on, the disease is considered established and the individual may infect others. The disease may cause severe lesions that persist even after the elimination of the bacilli, causing a great deal of suffering for the patient.

There is a trend toward greater TB lethality that must be expeditiously investigated in the coming years. Therefore, it is absolutely necessary that appropriate models be devised for combating and controlling TB.

The results of experiments performed in a recent study showed that the administration of hsp65-DNA vaccine in animals previously infected with virulent Mtb not only prevents the development of the disease but eliminates the infection as well[29]. This is extremely relevant if we consider that approximately two billion people are already infected with TB bacilli. In Brazil, the number of people infected is approximately 60 million. In addition to that, the vaccine can also cure even when administered in individuals with more severe presentations of the disease or after the dissemination of the disease throughout the organism. Infection with Mtb prevents the immune systems of animals from responding against the pathogenic agent, allowing the bacilli to grow rapidly and the disease to become entrenched. These authors demonstrated that gene therapy using hsp65-DNA vaccines provokes radical, profound changes, transforming the immune response from the Th2 type to the Th1 type and helping the organism combat bacilli and cure the disease, even if antimycobacterial chemotherapy is not administered[29]. This was the first demonstration of the potential of genetic vaccines for curing infectious diseases.

Immunotherapy for multidrug-resistant tuberculosis

One of the most significant problems in TB control is infection with strains resistant to anti-TB drugs such as isoniazid, pyrazinamide, streptomycin, rifampin, etc. Isolated strains have proven resistant to one or more of these drugs, in

combinations of two, three or even of all them simultaneously. Patients infected with multidrug-resistant strains can expect few, or sometimes no, treatment alternatives. Recent studies have also shown that animals infected with multidrug-resistant bacilli were cured through the administration of genetic vaccines[29].

Immunotherapy for latent tuberculosis

In correlation with the great number of infected individuals, another problem is how easily the TB bacilli adapt themselves to humans. The infection usually results from bacilli being inhaled and entering the defense cells of the host organism. From within the macrophages, which are cells with high microbicidal potential, the bacilli can deactivate the defense systems of the macrophage, surviving and reproducing therein. In humans, the immune defense system identifies the presence of the bacilli and establishes a response, characterized by a chronic granulomatous inflammatory reaction designed to circumscribe and delimit the infection. Under these conditions, the bacilli may survive in a latent or dormant state for years, and the infected individual may not present symptoms of the disease. If this mutual relationship becomes unbalanced, a situation typically associated with suppression of the immune response, the disease may manifest itself. The most common immunosuppression conditions seen concomitantly with TB are, among others, patients who are taking immunosuppressant drugs (such as AIDS patients and patients suffering from stress-related symptoms), drug addicts (such as alcoholics) and malnourished patients.

Gene therapy has also been used as a means of analyzing the state of latency or dormancy of the mycobacteria, which can become reactivated and manifest the disease during immunosuppressive states. An experimental mouse model was developed that mimics the conditions observed in the development of the human form of the disease in immunosuppressed individuals. In the control group animals, which were not vaccinated but were infected, given antibacterial drugs (to establish a state of latency) and then treated with corticosteroids to induce immunosuppression, reactivation and establishment of the disease was observed. In the study group animals, which were treated with the DNA vaccine, no reactivation or development of the disease was observed, especially when three doses of the vaccine were administered[29]. The ability of DNA vaccines to eliminate dormant bacteria may be of considerable benefit in the control, or even eradication, of TB.

Genetic vaccines have been used for treatment, which is conceptually different than the manner in which traditional vaccines are used (only to prevent diseases). These DNA vaccines cure infection, cure the disease and prevent disease reactivation, while maintaining their prophylactic nature. Innumerable practical and strategic benefits are derived from the development of this therapeutic vaccine against TB. It is safe and efficacious, and it can be administered in a single dose, stimulating an ample immune response. It has a long-lasting protective effect and may significantly contribute to decreasing incidence of the disease.

Reducing the tuberculosis treatment period with the use of drugs and DNA vaccine

Bactericidal drugs eliminate susceptible bacilli that are multiplying normally, but immunologic changes seen in clinical TB prevent sufficient destruction of the remaining bacilli. These surviving bacteria are responsible for the reactivation of the disease, prolonging the treatment period. The strategy of combining DNA-vaccine immunotherapy with conventional chemotherapy is aimed at restoring immune system function after the majority of the bacilli have been eliminated. Immune mechanisms should recognize and destroy persisting bacilli, thereby reducing the length of the period during which anti-relapse chemotherapy is required. Several therapeutic protocols and regimens, using chemotherapeutic and immunotherapeutic drugs (such as hsp65 DNA) in concert, have been devised in order to reduce the length of the TB treatment period. Animals have been infected with intravenous, intratracheal or aerosolized Mtb. After the infection became established (bacilli observed in spleen, lungs and liver) these animals were treated with a combination of hsp65-DNA vaccine and drugs such as rifampin, streptomycin, isoniazid, and pyrazinamide. A BCG vaccine was used to compare responses. Evaluation was carried out through the enumeration of bacilli present in the organs, especially in the lungs, of the animals, and the immune response was quantified through determination of cytokine levels. In a pilot

experiment, it was shown that the use of an hsp65-DNA vaccine concomitantly with isoniazid and pyrazinamide was efficacious and significantly reduced the length of the treatment period in animals infected with Mtb.

Practical and strategic benefits resulting from the development of genetic vaccines are innumerable, making these vaccines highly desirable in the context of the public health problems existing in developing countries. The impact on infectious disease control will probably be one of the most important aspects of mastering this new technology, since many serious infectious diseases may eventually be prevented through genetic immunization. The development of new vaccines that may soon prevent the uncontrolled increase in the dissemination of infectious diseases is of fundamental importance for mankind. A DNA vaccine against TB could contribute significantly to the control of the disease.

REFERENCES

1. Silva CL. Vacinas gênicas. Biotecnologia - Ciên. Desenvolv. 1997;3:32-4.
2. Davis HL, Whalen RG, Demeneix BA. Direct gene transfer in skeletal muscle in vivo: factors affecting efficiency of transfer and stability of expression. Human Gene Ther. 1993;4:151-6.
3. Vitadello M, Schiaffino MV, Picard A, Scarpa M, Schiaffino S. Gene transfer in generating muscle. Human Gene Ther. 1995;5:11-2.
4. Wang B, Ugen K, Srikantan V, Agadjanyan MG, Dang K, Rafaeliu J, et al. Gene inoculation generates immune responses against HIV-1. Proc Natl Acad Sci USA. 1993;90:4156-60.
5. Wells DJ. Improved gene transfer by direct plasmid injection associated with regeneration in mouse skeletal muscle. FEBS Lett., 1993;332:179.
6. Ferreira GNM, Monteiro GA, Prazeres DMF, Cabral JMS. Downstream processing of plasmid DNA for gene therapy and DNA vaccine applications. Tibtech. 2000;18:380-7.
7. Bonato VLD, Lima VMF, Tascon RE, Lowrie DB, Silva CL. Identification and characterization of protective T cells in hsp65 DNA-vaccinated and Mycobacterium tuberculosis infected mice. Infec Immunol. 1998;66:169-75.
8. Lowrie DB, Tascon RE, Colston MJ, Silva CL. Towards a DNA vaccine against tuberculosis. Vaccine. 1994:12:1537-40.
9. Lowrie DB, Silva CL, Colston MJ, Ragno S, Tascon RE. Protection against tuberculosis by plasmid DNA.Vaccine. 1997;15:834-8.
10. Lowrie DB, Tascon RE, Silva CL Vaccination against tuberculosis. Int Arch Allergy Immunol. 1995;108:309-12.
11. Lowrie DB, Colston MJ, Tascon RE, Silva CL. DNA encoding individual mycobacterial antigens protects mice against tuberculosis. In: Brown F, Burton D
12. Silva CL. Characterization of T cells that confer a high degree of protective immunity against tuberculosis in mice after vaccination with tumor cells expressing mycobacterial hsp65. Infect Immunol.,1996;64:2400-7
13. Silva CL; Characterization of the memory/activated T cells that mediate the long-lived host response against tuberculosis after bacillus Calmette-Guerin or DNA vaccination. Immunology 1999;97:573-81.
14. Lowrie DB, Silva CL, Tascon RE. DNA vaccines against tuberculosis. Immunol. Cell Biology unol. 1997;75:591-4.
15. Morein B. Novel adjuvants and vaccine delivery systems. Vet. Immunol Immunopatol. 1996;54:373-84.
16. Lima KM, dos Santos SA, Santos Jr. RR, Brandão IT, Rodríguez Jr. JM, Silva CL. Efficacy of DNA-hsp65 vaccination varies for tuberculosis varies with method of DNA introduction in vivo. Vaccine. 2003;22:49-56.
17. Doe, B. Induction of cytotoxic T lymphocytes by intramuscular immunization with plasmid DNA is facilitated by bone marrow-derived cells. Proc Natl Acad Sci USA. 1996;93:8578-83.
18. Akbari O, Panjwani N, Garcia S, Tascon R, Lowrie D, Stockinger B. DNA vaccination: transfection and activation of dendritic cells as key events for immunity. J Exp Med. 189:169-77
19. Klinman DM. Contribution of cells at the site of DNA vaccination to the generation of antigen-specific immunity and memory. J Immunol. 1999;160:2388-92.
20. Lima VMF. Role of trehalose Dimycolate in recruitment of cells and modulation of production of cytokines and NO in tuberculosis. Infec Immun. 2001;69:5305-12.
21. Lima KM, Santos AS, Lima VMF, Coelho-Castelo AAM, Rodrigues Jr JM, Silva CL. Single-dose of a vaccine based on DNA encoding mycobacterium hsp65 protein plus TDM-loaded microspheres protects mice against a virulent strain of Mycobacterium tuberculosis. Gene Ther. 2003;10:678-85.
22. Ryll R. Mycobacterium cord factor, but not sulfolipid, causes depletion pf NKT cells and upregulation of CD1d1 on murine macrophages. Microbiol Infect. 2001;3:611-9.
23. McShane H. Prime-boost immunization strategies for infectious diseases. Curr Opin Mol Ther. 2002;4:23-7
24. Robinson, H.L. Prime boost vaccines power up in people. Nat Med. 2003;9:642-3.
25. Skinner MA, Buddle BM, Wedlock DN, Keen D, de Lisle GW, Tascon RE, et al. A DNA prime-Mycobacterium bovis BCG boost vaccination strategy for cattle induces protection against bovine tuberculosis. Infect Immunol. 2003;71:4901-7.
26. Vordermeier HM, Lowrie DB, Hewinson RG. Improved immunogenicity of DNA vaccination with mycobacterial HSP65 against bovine tuberculosis by protein boosting. Vet Microbiol. 2003;93:349-59.
27. Lunsford L, McKeever U, Eckstein V, Hedley ML. Tissue distribution and persistence in mice of plasmid DNA encapsulated in a PLGA-based microsphere delivery vehicle. J Drug Target. 2000;8:39-50.
28. Briones M, Singh M, Ugozzoli M, Kazzaz J, Klakamp S, Ott G, O'Hagan D. The preparation, characterization, and evaluation of cationic microparticles for DNA vaccine delivery. Pharm Res. 2001;18:709-12.
29. Lowrie DB, Tascon RE, Bonato VLD, Lima VMF, Faccioli LH, Stavropoulos E, et al. Therapy of tuberculosis in mice by DNA vaccination. Nature. 1999;400:269-71.