Archivum Immunologiae et Therapiae Experimentalis, 2000, **48**, 177–182
PL ISSN 0004-069X

Review

Current Status and Future Perspectives of DNA Vaccine Delivery by Attenuated Intracellular Bacteria

GUIDO DIETRICH[1, 2]*

[1] Chiron Behring GmbH & Co, Preclinical Research Vaccines, D-35006 Marburg, Germany, [2] Institute of Hygiene and Microbiology, University of Wuerzburg, D-97080 Wuerzburg, Germany

Abstract. Vaccination by intradermal or intramuscular injection of antigen-encoding plasmid-DNA elicits strong cellular and humoral immune responses. Professional antigen presenting cells (APC) seem to induce these responses, making it, therefore, desirable to deliver the plasmid molecules directly to these cells. The exploitation of attenuated intracellular bacteria as DNA delivery vehicles makes the direct targeting of DNA vaccine vectors to professional APC feasible.

Key words: DNA vaccines; delivery; attenuated intracellular bacteria.

DNA vaccines in general consist of plasmid vectors which encode antigens under the control of eukaryotic promoter sequences[11]. For application, the DNA can either be dissolved in saline and injected intramuscularly or coated on gold particles and introduced into the dermis with a gene gun. Both routes of application have been shown to result in strong cellular as well as humoral immune responses, which can be directed against a wide variety of pathogenic microorganisms, tumor antigens and allergy antigens.

Despite its many advantages, DNA vaccination still faces several problems: the efficiency of the DNA-uptake seems to be quite low and dose-dependant, so that high amounts of DNA have to be injected to elicit protective immune responses[8]. Furthermore, the intramuscular injection of plasmid DNA does not seem to induce immune responses at distal mucosal surfaces[8]. Reports on the successful mucosal application of DNA vaccines are scarce. FYNAN et al.[13] described the application as nose drops of plasmid DNA encoding a very strong antigen which led to protective immunity. Additionally, in muscle tissue there is a very limited number of antigen-presenting cells (APC)[17]; therefore, protection against pathogenic microorganisms might only be possible with highly immunogenic antigens[4]. Bone marrow derived APC, rather than myocytes or keratinocytes, seem to be essential for the immune responses observed after vaccination by both routes[27]. This could be due to either direct transfection of the APC[3] or to cross-priming[28]. Furthermore, the APC have to migrate to the spleen for the induction of the immune responses seen after DNA vaccination[4, 10, 27].

Indeed, it seems to be desirable to deliver DNA vaccine vectors directly to these cells, a task that can be accomplished by the exploitation of attenuated intracellular bacteria. These microorganisms show a pronounced preference for professional APC like macrophages. After being phagocytosed by APC, the bacteria

Abbreviations used: APC – antigen-presenting cell, BMM – bone marrow derived macrophages, CMV – cytomegalovirus, GFP – green fluorescent protein, LPS – lipopolysaccharide, OVA – chicken ovalbumin.

* Correspondence to: Dr Guido Dietrich, University of Wuerzburg, Institute of Hygiene and Microbiology, Josef Schneider Str. 2, D-97080 Wuerzburg, Germany, tel.: +49 931 201 39 01, fax +49 931 201 34 45, e-mail: gdietrich@hygiene.uni-wuerzburg.de

can survive inside these cells either by modification of the phagosomal compartment, e.g. by preventing the fusion with the lysosome, or by egression from the phagosome into the host cell cytosol[18]. Most intracellular bacteria can be transformed with eukaryotic expression vectors. Attenuated mutant strains of these bacteria, which lyse inside the APC, can therefore carry these plasmids into the host cell phagosome or cytosol and release the DNA upon intracellular desintegration. The DNA can subsequently enter the nucleus and plasmid-encoded antigens can be expressed by the APC. These antigens can be presented by the APC together with major histocompatibility (MHC) class I and class II molecules, which should result in efficient cellular and humoral immune responses.

DNA-Vaccine Delivery by Gram-Negative Bacteria

Attenuated mutant strains of the invasive bacteria *Shigella flexneri*, *Salmonella typhimurium* and *S. typhi* were successfully exploited for the delivery of eukaryotic antigen-expression vectors *in vitro* as well as *in vivo*[2, 6, 12, 20, 24–26]. Attenuation of these strains was achieved by deletions of genes involved in the synthesis of metabolites which are essential for cell wall synthesis. The auxotrophic bacteria are thus able to enter the mammalian cells, but they lyse inside their eukaryotic prey due to the lack of these metabolites.

Eukaryotic expression vectors containing *Escherichia coli* origins of replication are fully functional also in *S. flexneri*. Since *S. flexneri* is able to enter the host cell cytosol after being phagocytosed, these bacteria are ideally suited for the direct delivery of plasmid vectors to this host cell compartment. In the first such report, a *S. flexneri asd* mutant strain was utilized for plasmid delivery[25]. This strain is unable to synthesize the bacterial cell wall constituent diaminopimelinic acid (DAP) and therefore undergoes lysis inside mammalian cells, resulting in an efficient release of plasmid molecules into the host cell cytosol. Subsequent expression of the model-antigen ß-galactosidase by the mammalian host cells could be demonstrated *in vitro* as well as *in vivo*[25] (Fig. 1). As anticipated, a noninvasive mutant strain of *S. flexneri* was unable to deliver plasmid-DNA to eukaryotic cells[25]. Plasmid delivery by *S. flexneri* is also suitable for the elicitation of strong humoral and cellular immune responses. Intranasal application of the bacteria carrying a ß-galactosidase expression vector led to strong cellular and humoral immune responses against this model antigen in mice[26]. When the *S. flexneri asd* mutant carrying DNA vaccine vectors encoding different measles virus antigens (fusion protein, hemagglutinin and nucleoprotein) was used for intranasal immunization of mice, strong Th1-type T cell responses ($CD8^+$ CTLs and IFN-γ-secreting helper T cells) were observed, which could be boosted by a second and third application of the bacteria[12]. This demonstrated that boosting with intracellular bacteria delivering DNA vaccines is interestingly feasible. The humoral immune responses achieved with this immunization scheme were only weak, though[12]. As an alternative to the *asd* mutant, an auxotrophic and simultaneously spreading-negative mutant strain of *S. flexneri*, which was obtained by chromosomal deletions of the *aroA* and *virG* genes, was used for the delivery of eukaryotic expression vectors into a wide range of cell types *in vitro*[24].

Whereas *S. flexneri* is able to escape into the host cell cytosol, *S. typhimurium* remains in the phago(lyso) somal compartment. In spite of this phagosomal localization of *Salmonella, aroA* mutant strains of *S. typhimurium* can deliver eucaryotic expression vectors into primary macrophages *in vitro* as well as *in vivo* with high efficiency, leading to strong expression of plasmid-encoded reporter genes[6]. The *in vivo* delivery of plasmid molecules to dendritic cells could also be demonstrated[20]. Attenuated *S. typhimurium* was also applied to the *in vivo* delivery of a DNA vaccine vector encoding listeriolysin[6]. Listeriolysin is a major T cell--antigen of the intracellular bacterium *Listeria monocy-*

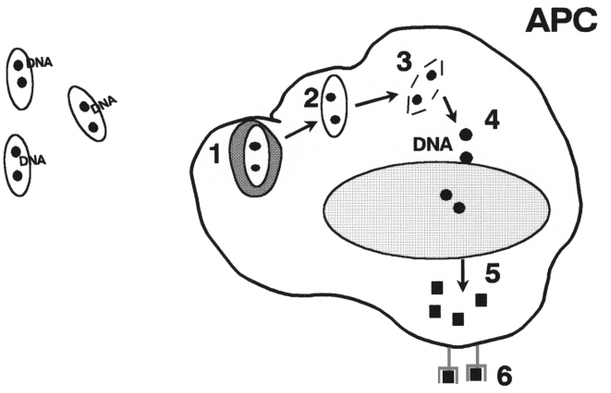

Fig. 1. DNA vaccine delivery to APC by attenuated shigellae, listeriae, invasive *E. coli* and *S. typhimurium* expressing active listeriolysin. 1 – bacteria carrying DNA-vaccine vectors are phagocytosed by an APC, 2 – bacteria lyse the phagosomal membrane, escape into the cytosol and 3 – release the plasmid DNA directly into this host cell compartment upon bacterial desintegration, 4 – the plasmid DNA enters the nucleus, 5 – plasmid-encoded antigens are expressed by the APC, processed and 6 – presented in the context of MHC class I and possibly also MHC class II (either directly or by cross-priming) molecules

togenes[21]. Vaccination of mice via the oral route resulted in $CD8^+$ and $CD4^+$ T cell responses as well as high antibody titers to this antigen. The helper T cell responses were of Th1-type with high levels of IFN-γ and no IL-4. These immune responses were able to protect mice against a lethal challenge with *L. monocytogenes* and could be boosted by oral application of the bacteria. Similarly, attenuated *S. typhimurium* carrying a DNA vaccine encoding a model tumor antigen was demonstrated to induce T cell mediated protection in a mouse tumor model[20]. The *S. typhi* Ty21a strain, which is approved for use as an oral vaccine against typhoid fever in humans, has also been used for the delivery of a DNA vaccine vector coding for the measles virus nucleoprotein[12]. By i.p. injection of this strain into mice, T cell responses were elicited which were comparable to those achieved by i.n. vaccination with the *S. flexneri asd* mutant carrying the same plasmid vector[12].

However, it remains to be elucidated how the plasmid DNA is transported from the phagosomal compartment of the host cell into the nucleus (Fig. 2). Infection with *Salmonella* possibly causes leakage of the phagolysosome. Similarily, infection of primary macrophages with *S. typhimurium* results in the presentation of phagosomal proteins together with MHC class I molecules[22]. Another explanation might be a specific and so far unidentified transport process for the translocation of the plasmid molecules across the phagosomal membrane. Interestingly, *Salmonella* can deliver plasmid DNA only in primary cells, but not in cell lines[6, 25], suggesting that this may be a specific property of mature macrophages.

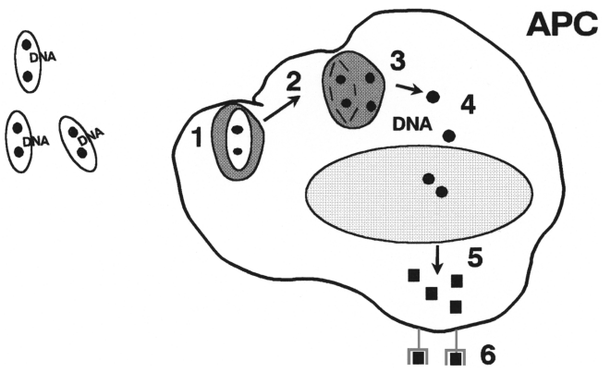

Fig. 2. DNA vaccine delivery to APC by attenuated salmonellae without phagosomal escape function. 1 – salmonellae carrying DNA-vaccine vectors are phagocytosed by an APC, 2 – bacteria lyse inside phagosome, releasing the plasmid molecules into this compartment, 3 – from where they enter the cytoplasm via unknown mechanisms, 4 – the plasmid DNA enters the nucleus, 5 – plasmid-encoded antigens are expressed by the APC, processed and 6 – presented in the context of molecules of the MHC

In order to enhance the efficacy of plasmid delivery by attenuated *S. typhimurium*, we chose to equip the bacteria with a gene conferring a phagosomal escape function, namely the listeriolysin gene of *L. monocytogenes* which is responsible for the escape of this bacterium from the phagosomal vacuoles of infected mammalian cells[23]. *S. typhimurium* expressing a listeriolysin-hemolysin fusion protein and secreting this fusion protein via the hemolysin transport machinery of *E. coli* is able to escape from the phagosomal vacuoles of infected macrophage cells[14, 16]. We were able to demonstrate that this escape into the cytosol is also enhances the efficiency of plasmid delivery by *S. typhimurium*[2] (Fig. 1). When used for the delivery of a eukaryotic expression vector encoding the T cell antigen chicken ovalbumin (OVA) into murine bone marrow derived macrophages (BMM), we found the expression of the listeriolysin-hemolysin fusion protein to enhance the delivery of the plasmid molecules substantially, as measured by presentation of the major $H\text{-}2K^b$ T cell epitope of $OVA_{257\text{-}264}$[19] together with MHC class I molecules[2]. These results suggest that there is potential for further enhancement of plasmid DNA delivery by attenuated strains of *S. typhimurium*.

Due to their invasive properties, *S. flexneri* and *S. typhimurium* are both well suited for the delivery of macromolecules into eukaryotic cells. In a different approach, a noninvasive and apathogenic bacterium, the genetically well-defined laboratory strain *E. coli* K12, was equipped with genes conferring invasiveness[5, 15]. Again, an auxotrophic cell wall synthesis mutant was used. This strain was transformed either with the 200 kb virulence plasmid of *S. flexneri*, which contains the genes for invasion, intra- and intercellular motility[5], or alternatively with the invasin gene *inv* of *Yersinia pseudotuberculosis* and the *hly* gene of *L. monocytogenes*, coding for listeriolysin[15]. Both strains are able to deliver eucaryotic expression vectors directly to the host cell cytosol with high efficiency *in vitro*[5, 15] (Fig. 1), but have not yet been used in DNA vaccine settings.

DNA-Vaccine Delivery by Gram-Positive Bacteria

Gram-negative bacteria as vehicles for the delivery of DNA vaccines have the disadvantage of producing high amounts of the toxic lipopolysaccharide (LPS), which might be inhibitory for the synthesis of plasmid-encoded antigens by the host cells. Thus, it might be advantageous to apply Gram-positive DNA vaccine

carriers. A promising candidate is the Gram-positive bacterium *L. monocytogenes*. *L. monocytogenes* is found in high numbers in splenic APC shortly after infection through the intestinal mucosa and it is able to enter the cytosol of these APC after phagocytosis. *L. monocytogenes* was attenuated by a deletion of the genes *mpl*, *actA* and *plcB*, which are necessary for intra- and intercellular movement after the bacteria escape from the host cell phagosome into the cytosol[9]. Delivery of the plasmid DNA into the cytosol was achieved by specific autolysis of the bacteria in this compartment (Fig. 1). To this end, we cloned a gene encoding the lysis protein of a *Listeria*-specific bacteriophage under the control of the listerial *actA*-promoter, which is active only in the host cell cytosol. Bacteria carrying this transcription unit undergo rapid lysis upon entering the cytosol of the host cell[9]. This attenuated suicide strain of *L. monocytogenes* was used as a vehicle for the delivery of plasmid vectors carrying either the *gfp*- (coding for the green fluorescent protein – GFP) or the *cat*-gene (encoding chloramphenicol acetyl tranferase – CAT) under the control of the cytomegalovirus (CMV)-promoter. Efficient plasmid delivery as well as GFP- and CAT-expression were obtained in a murine macrophage-like cell line[9] as well as in murine fibroblast cells (DIETRICH et al., unpublished data). Lysin-mediated plasmid-delivery could be demonstrated to be even more efficient than the killing of the intracellular bacteria by treatment with antibiotics[9].

Attenuated suicide *L. monocytogenes* are also suitable for the delivery of plasmid DNA in primary cells. The infection of BMM from BALB/c mice with bacteria carrying a GFP-expression vector led to strong GFP-expression by the macrophage cells (DIETRICH and GENTSCHEV, unpublished data). The expression of heterologous antigens obtained by this procedure results in efficient processing and presentation together with MHC class I molecules. This could be shown with a plasmid construct which codes for the major H-2K^b T cell epitope of ovalbumin, $OVA_{257–264}$, expressed under the control of the CMV-promoter. When this plasmid was delivered to BMM by the attenuated suicide *L. monocytogenes*, the T cell epitope was presented efficiently together with MHC class I molecules, as shown by the activation of a $OVA_{257–264}$-specific T cell hybridoma[9]. Thus, the delivery of DNA by attenuated suicide *L. monocytogenes* leads to efficient expression and presentation of plasmid-encoded antigens. We are currently evaluating the suitability of attenuated suicide *L. monocytogenes* for the elicitation of protective immune responses in different viral and bacterial infection models.

Potential Risks of DNA Vaccine Delivery by Attenuated Intracellular Bacteria

The invasive nature of the employed bacteria is certainly advantageous for DNA vaccine delivery, but it also implies the potential for pathogenicity of these strains for their host, making it necessary to prove their safety. Attenuated *Salmonella* strains which are used as vaccines in human and veterinary medicine have been demonstrated to be sufficiently safe. Attenuated *S. flexneri* and invasive *E. coli* still have to be examined in this respect. Furthermore, the potential endotoxic effect of the LPS expressed by these bacteria has to be evaluated very carefully. Spreading-negative *L. monocytogenes* are highly attenuated in mice[9] and the intracellular expression of the phage lysin should add to the safety of these bacteria.

A very important issue concerning the safety of DNA vaccines is their possible integration into the host cell genome, which might potentiate oncogenesis. Whereas the immunization with naked DNA does not seem to lead to genomic integration[11], the delivery of eukaryotic expression vectors by invasive *E. coli* and attenuated suicide *L. monocytogenes* results in chromosomal integration at least *in vitro*[5, 9]. These findings may be due to the high plasmid copy numbers which are delivered to each single cell by the bacterial carriers; furthermore, they still await an *in vivo* evaluation. Additionally, the cells carrying genomic integrations should ultimately be destroyed by the CTL responses against plasmid encoded antigens[7] and antigens derived from the carrier bacteria[18].

Discussion

The application of DNA vaccines by intramuscular injection or gene gun inoculation leads to the transfection of only very limited numbers of professional APC, which is a possible reason for the low efficiency of DNA vaccination in primates[11]. Delivery of DNA vaccines by suitable intracellular bacteria might enable targeting the plasmid vectors more specifically to the APC in immunologically relevant organs, which should substantially enhance the immune response. *S. typhimurium* and *L. monocytogenes* show a strong preference for macrophages[18]. Bacterial components like LPS or unmethylated sequence motifs in the bacterial DNA might serve as adjuvants and thereby potentiate the immunogenicity of the DNA vaccines. Bacterial carriers also have the advantage of being applicable via the oral route[6], resulting in the transfection of cells of the gut-

-associated lymphoid tissue (GALT), which should result in efficient mucosal immune responses. For the future it should be interesting to see how the efficacy of bacterial delivery of DNA vaccines compares with naked DNA vaccination in a side-by-side study.

There are several approaches to enhancing the delivery of DNA vaccines by intracellular bacteria. Whereas the delivery by auxotrophic cell wall synthesis mutants relies on passive desintegration of the bacteria due to the lack of essential metabolites, the active lysis of the carriers (e.g. by the expression of a phage lysin) might accelerate the process of plasmid transfer and also limit the damage to the host cell. *S. flexneri* and *L. monocytogenes* can transfer plasmid molecules directly to the host cell cytosol due to their ability to escape from the phagosome. Subsequently, the plasmid molecules can enter the host cell nucleus. The efficiency of the plasmid delivery by bacteria which are usually confined to the host cell phagosome can be substantially enhanced by bacterial expression of an enzyme with phagosomal escape function, like listeriolysin[15]. Therefore, *Salmonella* strains which enter the cytosol of infected host cells due to the secretion of active listeriolysin are interesting candidates for the delivery of DNA vaccines[2].

Next to macrophages, which are the preferential first habitat of *Salmonella* and *Listeria*, it should be advantageous to target the bacterial carriers also to dendritic cells, which have a much stronger capacity in antigen presentation when compared to other monocytes[1].

Despite the numerous advantages, the risk factors associated with the delivery of DNA vaccines by attenuated intracellular bacteria, such as safety of the bacterial strains and possible genomic integration of the plasmid DNA, have to be addressed very carefully. Furthermore, the broad applicability of these systems to a wide range of antigens and animal models still awaits further proof.

If these issues can be resolved, though, the delivery of DNA vaccines by attenuated intracellular bacteria has the capacity to combine the advantages of live vaccine vectors with those of naked DNA vaccines.

Acknowledgment. I would like to thank I. Gentschev, J. Hess, S. Spreng and W. Goebel for many helpful discussions and M. Dietrich for critical reading of the manuscript.

References

1. BANCHERAU J. and STEINMAN R. (1998): Dendritic cells and the control of immunity. Nature, **392**, 245–252.
2. CATIC A., DIETRICH G., GENTSCHEV I., GOEBEL W., KAUFMANN S. H. E. and HESS J. (1999): Introduction of protein or DNA delivered via recombinant *Salmonella typhimurium* into the major histocompatibility complex class I presentation pathway of macrophages. Microbes Infect., **2**, 113–121.
3. CONDON C., WATKINS S. C., CELLUZZI C. M., THOMPSON K. and FALO L. D. JR. (1996): DNA-based immunization by *in vivo* transfection of dendritic cells. Nat. Med., **2**, 1122–1128.
4. CORR M., LEE D. J., CARSON D. A. and TIGHE H. (1996): Gene vaccination with naked plasmid DNA: mechanism of CTL priming. J. Exp. Med., **184**, 1555–1560.
5. COURVALIN P., GOUSSARD S. and GRILLOT-COURVALIN C. (1995): Gene transfer from bacteria to mammalian cells. CR Acad. Sci., **318**, 1202–1207.
6. DARJI A., GUZMAN C. A., GERSTEL B., WACHHOLZ P., TIMMIS K. N., WEHLAND J., CHAKRABORTY T. and WEISS S. (1997): Oral somatic transgene vaccination using attenuated *S. typhimurium*. Cell, **91**, 765–775.
7. DAVIS H. L., BRAZOLOT-MILLAN C. L. and WATKINS S. C. (1997): Immune-mediated destruction of transfected muscle fibers after direct gene transfer with antigen-expressing plasmid DNA. Gene Ther., **4**, 181–188.
8. DECK R. R., DEWITT C. M., DONNELLY J. J., LIU M. A. and ULMER J. B. (1997): Characterization of humoral immune responses induced by an influenza hemagglutinin DNA vaccine. Vaccine, **15**, 71–78.
9. DIETRICH G., BUBERT A., GENTSCHEV I., SOKOLOVIC Z., SIMM A., CATIC A., KAUFMANN S. H. E., HESS J., SZALAY A. A. and GOEBEL W. (1998): Delivery of antigen-encoding plasmid DNA into the cytosol of macrophages by attenuated suicide *Listeria monocytogenes*. Nat. Biotechnol., **16**, 181–185.
10. DOE B., SELBY M., BARNETT S., BAENZIGER J. and WALKER C. M. (1996): Induction of cytotoxic T lymphocytes by intramuscular immunization with plasmid DNA is facilitated by bone marrow-derived cells. Proc. Natl. Acad. Sci. USA, **93**, 8578–8583.
11. DONNELLY J. J., ULMER J. B., SHIVER J. W. and LIU M. A. (1997): DNA Vaccines. Ann. Rev. Immunol., **15**, 617–648.
12. FENNELLY G. J., KHAN S. A., ABADI M. A., WILD T. F. and BLOOM B. R. (1999): Mucosal DNA immunization against measles with a highly attenuated *S. flexneri* vector. J. Immunol., **162**, 1603–1610.
13. FYNAN E. F., WEBSTER R. G., FULLER D. H., HAYNES J. R., SANTORO J. C. and ROBINSON H. L. (1993): DNA vaccines: protective immunizations by parenteral, mucosal and gene-gun inoculations. Proc. Natl. Acad. Sci. USA, **90**, 1478–1482.
14. GENTSCHEV I., SOKOLOVIC Z., MOLLENKOPF H.-J., DEMUTH A., HESS J., KAUFMANN S. H. E., KROHNE G. F. and GOEBEL W. (1995): *Salmonella* secreting active listeriolysin changes its intracellular localization. Infect. Immun., **63**, 4202–4205.
15. GRILLOT-COURVALIN C., GOUSSARD S., HUETZ F., OJCIUS D. M. and COURVALIN P. (1998): Functional gene transfer from intracellular bacteria to mammalian cells. Nat. Biotechnol., **16**, 862–866.
16. HESS J., GENTSCHEV I., MIKO D., WEIZEL M., LADEL C., GOEBEL W. and KAUFMANN S. H. E. (1996): Superior efficacy of secreted over somatic antigen display in recombinant *Salmonella* vaccine induced protection against listeriosis. Proc. Natl. Acad. Sci. USA, **93**, 1458–1463.
17. HOHLFELD R. and ENGEL A. G. (1994): The immunobiology of muscle. Immunol. Today, **15**, 269–273.

18. KAUFMANN S. H. E. (1998): Immunity to intracellular bacteria. In PAUL W. E.: Fundamental immunology. Lippincott-Raven Ltd., New York, 1335–1371.
19. KOVACSOVICS-BANKOWSKY M. and ROCK K. L. (1995): A phagosome-to-cytosol pathway for exogenous antigens presented on MHC class I molecules. Science, **267**, 243–246.
20. PAGLIA P., MEDINA E., ARIOLI I., GUZMAN C. A. and COLOMBO M. P. (1998): Gene transfer in dendritic cells, induced by oral DNA vaccination with *Salmonella typhimurium*, results in protective immunity against a murine fibrosarcoma. Blood, **92**, 3172–3176.
21. PAMER E. G., HARTY T. J. and BEVAN M. J. (1991): Precise prediction of a dominant class I MHC-restricted epitope of *Listeria monocytogenes*. Nature, **353**, 852–855.
22. PFEIFER J. D., WICK M. J., ROBERTS R. L., FINDLAY K., NORMARK S. J. and HARDING C. V. (1993): Phagocytic processing of bacterial antigens for class I MHC presentation to T cells. Nature, **361**, 359–362.
23. PORTNOY D. A., CHAKRABORTY T., GOEBEL W. and COSSART P. (1992): Molecular determinants of *Listeria monocytogenes* pathogenesis. Infect. Immun., **60**, 1263–1267.
24. POWELL R. J., LEWIS G. K. and HONE D. M. (1996): Introduction of eukaryotic expression cassettes into animal cells using a bacterial vector delivery system. In Vaccines 96: Molecular approaches to the control of infectious disease. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 183–187.
25. SIZEMORE D. R., BRANSTROM A. A. and SADOFF J. C. (1995): Attenuated *Shigella* as a DNA delivery vehicle for DNA-mediated immunization. Science, **270**, 299–302.
26. SIZEMORE D. R., BRANSTROM A. A. and SADOFF J. C. (1997): Attenuated bacteria as a DNA delivery vehicle for DNA-mediated immunization. Vaccine, **15**, 804–807.
27. TORRES C. A. T., IWASAKI A., BARBER B. H. and ROBINSON H. L. (1997): Differential dependence on target site tissue for gene gun and intramuscular DNA immunizations. J. Immunol., **158**, 4529–4532.
28. ULMER J. B., DECK R. R., DEWITT C. M., DONNELLY J. J. and LIU M. A. (1996): Generation of MHC class I restricted cytotoxic T lymphocytes by expression of a viral protein in muscle cells: antigen presentation by non-muscle cells. Immunology, **89**, 56–67.

Received in July 1999
Accepted in October 1999