Chapter 1

General introduction

Chapter 1

Context

  History has taught us that vaccines are suitable for controlling many infectious diseases. The worldwide eradication of smallpox is an example of a successful vaccination campaign. The level of other diseases like polio, diphtheria, tetanus, pertussis, and rabies is significantly reduced by vaccination. But infectious diseases still pose major health problems, especially in the more impoverished parts of the world and the veterinary world in which vaccines are too costly or not available [1]. Furthermore, there is much concern about new and re-emerging infectious diseases in the developed world. The increased (widespread) use of antibiotics has significantly increased the emergence of antibiotic-resistant organisms (e.g. *Streptococcus pneumoniae*, enterococci and gram-negative enteric pathogens) worldwide [2]. In addition, immunodeficient people and travellers can be a carrier of old or foreign infectious diseases. Therefore, development of vaccines and vaccination strategies remains important.

  Two types of vaccines can be discerned: live vaccines (composed of live, attenuated micro-organisms) and non-living vaccines (composed of inactivated micro-organisms, subunits thereof, recombinant DNA products or chemically synthesised peptides or oligosaccharides). Live vaccines possess many of the biological characteristics of the virulent microorganism like receptor binding, passage through physical barriers and production of active components. They are presented via the major histocompatibility (MHC) class-I-pathway. Although they are highly immunogenic, the microorganisms in live vaccines have the risk of return to virulence [1,3]. Obviously, non-living vaccines do not hold this risk. Since they lack certain characteristics of the alive counterpart, they are in general less immunogenic and are presented via the MHC class-II-pathway. When antigens are reduced into peptides, the biological complexity of the original microorganism is also reduced and this is accompanied by a further loss in immunogenicity.

  In the last two decades, considerable scientific progress has been made, which has revolutionised the way both live and non-living vaccines can be designed, formulated and produced. Moreover, new immunisation strategies are being explored in order to simplify the administration of vaccines.

Mucosal vaccination

  Most living pathogens enter the body via the mucosal tissues of the gastrointestinal (GI), urigenital or respiratory tract. Specific and non-specific defence mechanisms must limit the consequences. Specific immunity at mucosal tissues is brought about by both the local (mucosal) and systemic immune system and the first is the most important one

(box 1.1). Parenteral vaccination induces the systemic immune system, but hardly the mucosal immune system [4]. Vaccines administered by the mucosal route can induce both immune systems [2,5-8].

The mucosal tissues represent the interface between the host and its environment and mucosal vaccines can be applied at any point of the mucosal system. From a practical point of view, intranasal and oral vaccinations are most attractive, but intravaginal and intrarectal vaccinations are considered also (e.g. for protection against HIV [9]). All

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Box 1.1   The mucosal immune system

The mucosal immune system (MIS) is stimulated by uptake of antigens (micro-organisms and particles) and initiates an immunological cascade that primes the mucosa-associated lymphoid tissue (MALT). The MALT represents a compartmentalised, interconnected system of lymphoid tissue with various induction and effector sites, like the lamina propria of the upper and lower respiratory tract, the intestine, the genitourinary tract, and the salivary, mammary and lacrimal glands [2]. The inductive sites of the gut-associated lymphoid tissue (GALT) are the Peyer's patches (PP). PPs are found on the follicle-associated epithelium (FAE) and are covered with specialised cells called microfold or M-cells. M-cells sample luminal antigens by receptor-mediated uptake and transcytose them for presentation by antigen presenting cells (APC), which are located in the dome-area of the follicle [64]. Mucosal epithelial cells express major histocompatibility complex (MHC) class-I molecules and low levels of MHC-class II molecules and can also present antigen to CD4+ and CD8+ T-cells [1]. The dome area contains mainly B-cells and CD4+ T-cells. The MALT is best primed by local application of antigen. Once the mucosal immune system has been triggered, oral boost immunisations induce more vigorous responses than parenteral boost immunisations [69].

Upon stimulation, primed antigen-specific B- and T-cells migrate via the thoracic duct to the draining mesenteric lymphnodes (MLN) and spleen where clonal expansion and isotype-switch takes place [65]. The most important effector molecule of the MIS is secreted dimeric immunoglobulin of the IgA isotype, sIgA. sIgA is produced in large quantities in both animals and humans ($\sim$ 3 g/day in human) [66,67], which is more than all other immunoglobulins together [30].

Proliferating B- and T-cells enter the circulation and home preferentially to all mucosal effector sites and also to peripheral lymphoid organs where, in human, they can differentiate into IgA- and, to a lesser extent, IgG-secreting plasma cells [8,13,14]. The interconnection between all mucosal tissues is also known as the common mucosal immune system (CMIS) [6,13,65]. The secretion of IgA across intestinal mucous membranes is excellently reviewed by MacPherson *et al.* and Mestecky *et al.* [67-68].

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mucosal tissues are connected via the common mucosal immune system (CMIS) (box 1.1). Live mucosal vaccines based on invading microorganisms are able to penetrate the mucosal tissues due their biological characteristics [10]. Non-living vaccines lack the feature of passing the physical barrier and are presented by MHC class-II instead of MHC class-I like live vaccines. Irrespective of the type of mucosal vaccine, the major obstacle in their development is their low and short lasting efficacy [6,11,12].

Oral vaccines

Oral vaccination is the most attractive route of mucosal vaccination because of its simple way of administration. In theory, oral vaccines are capable of inducing both mucosal and systemic immune responses [13,14]. Progress in the development of oral vaccines is, however, limited. At present, there are a few commercially oral vaccines available. In the Netherlands, a polio (OPV, Sabin®) and a typhoid vaccine (Ty21a, Vivotif Berna®) are the only prescribed oral vaccines. Both are composed of live, attenuated microorganisms. Non-living oral vaccines are still in phase of research and development and in the last decade, no major breakthrough has been reported.

Oral immunisation
Besides the nature of the antigen (living/non-living) [12,15], there are several other factors affecting the outcome of oral immunisation. Factors that may be of influence in the induction of oral immune responses or oral tolerance instead, are the dose of antigen, frequency of administration and immunisation protocol, age of first exposure, immune status of the host towards the specific antigen, species, delivery systems and use of adjuvants [4]. Non-living oral vaccines preferably must be designed according to the following criteria: 1) protection of the antigen from enzymatic digestion. Exposure of the vaccine to low pH and proteolytic enzymes during passage through the GI tract results in degradation of the antigen or loss of conformation [11]; 2) enhancement of antigen uptake by M-cells or epithelial cells in the GI tract and facilitation of passage through the epithelial barrier; 3) activation of the innate and/or specific immune system instead of induction of oral tolerance; 4) induction of immunological memory [16,17]. For a detailed overview of the pro's and con's of oral vaccines we refer to an excellent textbook [4] and recent publications [1,2].

Oral tolerance
Oral tolerance is the phenomenon of systemic immunological unresponsiveness that occurs after oral intake of antigens. This subject has been recently reviewed by Iijima *et*

al. [18]. The primary task of the GI tract is absorption of nutrition out of food and under normal conditions and the body is tolerant against dietary antigens. Immunological reactions are preferably prevented [7]. Perturbation of the mucosal immune response may lead to unwanted diseases like food allergy and coeliac disease as a result of breakdown in oral tolerance [19]. There are several possible mechanisms for the induction of mucosally induced tolerance. High doses of antigen can induce clonal deletion and anergy of T-cells whereas low doses of antigen can generate regulatory cytokines with suppressive effects on many aspects of the immune response [2,18-20]. Both mechanisms lead to suppression of antigen-specific immune responses. Recently, reports have been published which revealed possible roles of $\gamma\delta$T-cells, dendritic cells and intestinal epithelial cells (IECs) in the induction of oral tolerance. The exact mechanisms by which these cells establish oral tolerance are still under investigation [18,19,21]. Once a T-cell is tolerised, tolerance can be spread via the cognate interaction between antigen presenting cells (APC) and/or T-cells. It has been proposed that tolerised cells may mediate their suppressive effects directly via the production of inhibitory cytokines or indirectly by competing for growth factors, MHC-peptide complexes, or co-stimulatory molecules on APC and thus pass on tolerance [19].

For oral vaccination, it is important that the immune system can distinguish the proteins within a vaccine from common dietary proteins and reacting with an immune response against the former. When this distinction cannot be made, oral tolerance will be broken and the immune system will react toward all orally administered antigens.

Mucosal adjuvants and delivery systems

The first obstacle mucosally administered antigens encounter is the non-specific defence mechanisms along the mucosal surfaces (e.g. cilia in the nasal system, low pH in the stomach, and proteolytic enzymes in the gut). Then, the antigens have to pass a thick mucosal layer and the epithelial barrier before they finally reach the immune system. Microbial pathogens that colonise the host through mucosal surfaces have evolved strategies to cross these physiological and chemical barriers [12]. Vaccines composed of such microorganisms are more successful in surviving the passage through the GI tract and are efficiently taken up. Live recombinant microorganisms (e.g. Lactobacteria and Salmonella) can thus be appropriate vectors for oral uptake of various proteins or peptides [22]. Virus receptor binding of live vaccines with retained ability to invade the host probably provides a danger signal [23]. This actively stimulates the MIS. In addition, live vaccines form a constant source of antigen and can stimulate the MIS constantly. Non-living vaccines do not have these features and are therefore less successful [10,15,24].

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The immune response can be enhanced by use of appropriate adjuvants and delivery systems. Until today few mucosal adjuvants are known. Delivery vehicles can protect oral antigens from degradation and can enhance vaccine uptake. Commonly used delivery vehicles are microparticles, liposomes, immune stimulating complexes (ISCOMS), and carrier-molecules, which have been reviewed recently [1,25]. This introduction will only summarise the main characteristics of the most thoroughly studied adjuvants.

LT and CT

Escherichia coli heat-labile enterotoxin (LT) and the highly homologous *Vibrio cholerae* toxin (CT) are powerful mucosal immunogens and can also act as powerful adjuvants. Their immunogenicity and adjuvant activity against co-delivered antigens are well documented [6,26,27]. Orally administered LT and CT predominantly induce T-cell responses with Th2-associated cytokines (box 1.1), and IgG1 and IgA antibodies. However, Th1-associated responses like CTL activation are also induced. Both toxins are composed of two structurally and functionally separate A and B subunits [1].

The toxic A-subunit induces ADP-ribosylation, which causes the disease symptoms [28,29]. It is highly immunogenic and can act as an adjuvant towards co-administered antigens. Due to the toxicity, however, LT and CT and their A-subunits are regarded as too toxic for clinical use. Non-toxic forms with retained mucosal adjuvanticity have been created by site-directed mutagenesis [30-35]. These mutant toxins demonstrated that the ADP-ribosylating activity of LT and CT was not a prerequisite for their adjuvant effects since enzymatically inactive mutant toxins retain adjuvant activity after intranasal administration [35]. Nevertheless, ADP-ribosylation appears to enhance the immunogenic and adjuvant potency of orally administered toxins [33].

The non-toxic B-subunit binds as a pentamer with high affinity to gangliosides (mainly GM1) [36-38], and induces apoptosis of CD8$^+$ and CD4$^+$ cells [26]. LTB was taken up predominantly by the IEC rather than the M-cells [25]. Conjugated to antigens, it can act as a carrier molecule and induce mucosal and systemic immune responses [26] while mere mixing with antigen elicits weaker responses [36,39,40]. However, many early studies on the adjuvanticity of the B-subunits have proven to be inconsistent as the presence of traces of holotoxin could not be excluded. The use of recombinant LTB and CTB improved the insight in the adjuvanticity of the B-subunit [30,41]. Furthermore, the degree of cross-linking between different conjugate preparations varies, which may have affected the GM1 binding and decrease the immunogenicity of the conjugate [42]. Some of these problems can be overcome by genetic constructs, but the fusion of genes may also affect the pentamer formation and by consequence the affinity for GM1.

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It is important to note that coupling of antigen to LTB or CTB prior to oral delivery can dramatically decrease the dose required to stimulate tolerance. LTB/CTB then acts as a carrier that shuttle antigen into a tolerance-inducing pathway associated with the gut mucosa, but the precise mechanisms are unknown [36]. This other immunological property of the B subunit can be used as effective therapy against certain diseases. For example, oral administration of CTB-insulin conjugates to nonobese diabetic (NOD) mice could suppress type I diabetes, a model of spontaneous autoimmune disease [12].

This suggests LT and CT have distinct immunological activities and that there is some sort of deviation involved that determines the outcome of oral vaccination: an immune response or tolerance.

Microparticles

Microparticles are spheres of polymers with diameters ranging from nanometers up to several micrometers. They may be built from different polymers and are easy to produce and are stable. Depending on their size, they are taken up by M-cells (box 1.1) or even epithelial cells [1,43]. When composed of biodegradable and biocompatible poly (lactide-co-glycolide) (PLG) polymers, microparticles can controllably release incorporated antigens. Incorporation of additional immunomodulators or carrier molecules can improve their potency. There are obvious differences between soluble and particulate antigens. In general, soluble antigens are less immunogenic and tend to induce tolerance rather than immune system activation [4]. Particle size is crucial to its immunogenicity, since small particles are non-specifically taken up by epithelial cells [43,44].

Liposomes

Liposomes are composed of phospholipids and cholesterol. Antigens can be incorporated into liposomes, which are stabile in acidic solutions, bile and pancreatin solutions. Like microparticles, they are actively taken up due to their small size and particulate formulation. Their efficacy can be improved by adding immunomodulatory or carrier molecules [1].

ISCOMS

Immunostimulatory fractions of *Quillaja saponica* (Quil A) have been incorporated into lipid particles and form immune stimulating complexes (ISCOMS). Hydrophobic or membrane-associated proteins are able to incorporate spontaneously into the ISCOMS when present during their assembly. Incorporation of non-hydrophobic proteins is more difficult but also possible. ISCOMS are resistant to both temperature and low

pH. Their particulate formulation facilitates uptake by M- or epithelial cells. ISCOMS can induce CTL and are able to stimulate cells of the innate immune system [1,4,45].

Lectins and ligands

Interaction of antigen with mucosal tissues can be improved by binding to lectin-like structures on IECs. Ligands with affinity for these structures include pili, viral haemagglutinins, many bacterial toxins, lectins, plant toxins and bacterial invasins. These ligands can act as carrier (or transport) molecules or as targeting device to antigens. [1]. Direct targeting to M-cells (box 1.1) further enhances antigen uptake. A few interesting lectins that bind selectively to M-cells have been identified (e.g. *Euonymus europaeus* in canine and *Ulex europeaus* I and II in mice) [46,47]. After binding they are actively taken up by those cells [47-51]. However, these lectins are species specific and at this moment,

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Box 1.2   Construction of edible vaccines

There are several ways to accomplish recombinant gene expression in plants: plastid transformation via particle bombardment (e.g. chloroplast transformation) [70], transient expression by chimeric plant viruses [56,71,72] and *Agrobacterium*-mediated transformation [73].

Plastid transformation results in high expression levels of recombinant protein, and due to their uniparentally maternal inheritance, escape of recombinant genes through pollen can be avoided. It is appropriate for the production of bacterial antigens but not suitable for the production of glycoproteins like viral surface antigens, because of differences in glycosylation [73].

Transient expression by transformed plant viruses (e.g. tobaco and cowpea mosaic virus) also achieves considerable levels of protein. Immunogenic epitopes can be presented on the surface of plant viruses by making translational fusions within or at the 3' terminus of a coat protein. The recombinant gene is not passed down to following generations since it is not incorporated into the plant genome, which takes extra inoculation steps when producing vaccine. The immunogenic epitopes are expressed in virus-like particles (VLP) or linked to viral proteins [56].

Transformation with *Agrobacterium tumefaciens* is the most commonly used method although expression levels obtained with this method are low. The advantage of this method is that foreign genes are stabile incorporated into the nuclear genome (Figure 1), thus enabling large-scale cloning and maintenance of selected high-expressing lines and the ability to sexually cross transgenic lines to obtain multiple proteins expressed in the same plant. One of the major disadvantages of *Agrobacterium*-mediated transformation of plants is the low expression level of the recombinant protein compared to other methods [74].

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no human M-cell binding lectins are identified. Further studies are involved to prove their carrier function [52], and, to our best knowledge, successful immunisations have not been reported yet.

Miscellaneous

In addition to the vehicles described above, the mucosal immune response upon oral vaccination can be enhanced by adjuvants such as avridine, muramyl dipeptide, saponins or derivatives thereof [53], aluminum salts [54], or cytokines such as IL-12 possibly in combination with a suitable delivery system [8]. Ryan *et al.* gave an excellent overview of the choice of adjuvant or delivery system and their effect on the polarisation of the immune response [1]. The immunostimulatory capacities of unmethylated CpG motifs of bacterial DNA currently receive great attention. CpG oligodeoxynucleotides were shown to enhance the local and systemic antibody responses to oral, intrarectal or intranasal immunisation with tetanus toxoid or influenza virus vaccines [1]. CpG motifs are detected, like bacterial or viral DNA, as a danger signal by the vertebrate immune system [55]. They cause B-cells to proliferate and secrete immunoglobulin, which directly synergise with the antigen-specific effects mediated through the B-cell receptor. In addition, CpGs improve antigen presentation by up regulation of co-stimulatory molecules and MHC class II molecules [54].
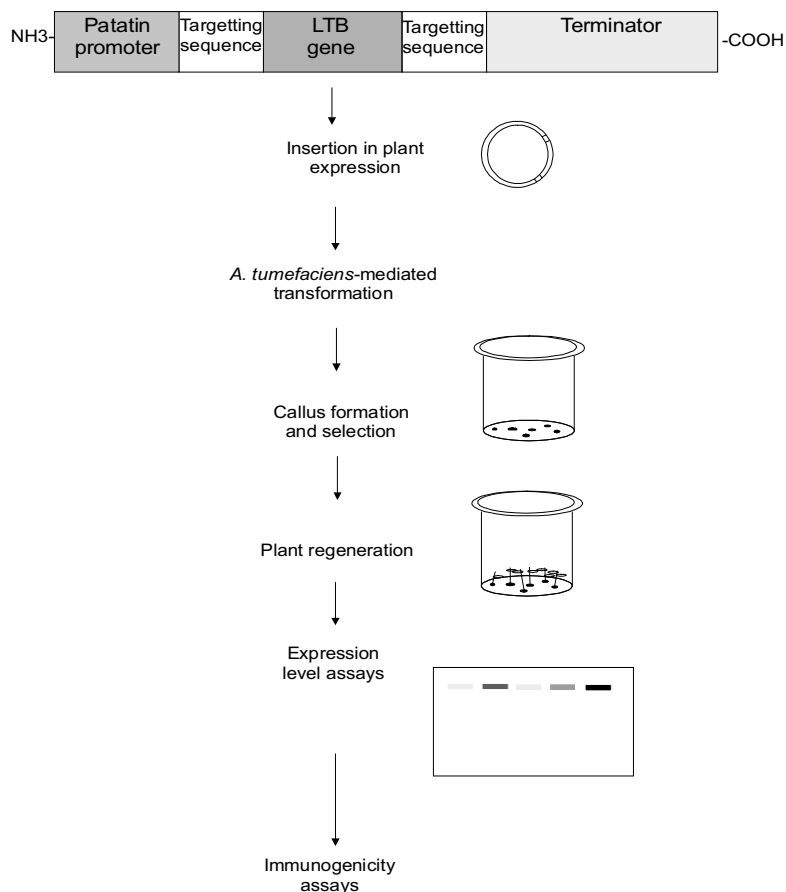
Plants as edible vaccines

The easiest way to deliver oral vaccines is by mixing them with food. This is the basic concept of edible vaccines. The increased knowledge on molecular biology made way for a novel type of farming, namely molecular farming, which uses ordinary plants as factories for the production of inexpensive factories for the production of expensive drugs and vaccines. Production of vaccines in edible plants or plant parts thereof gave rise to a new concept: edible vaccines.

An overview of the synthesis methods in edible vaccines is given in box 1.2 and Figure 1.1. Plants have several advantages above traditional productions systems. They only require simple growth circumstances and large-scale production is easy, which makes them inexpensive production factories. As with all oral vaccines, administration of edible vaccines does not require trained personnel and avoids needle-use and its associated risks [56]. Still, degradation in the stomach and gut is a major concern. Transgenic plant tissue can possibly act as a natural delivery system by encapsulation of the vaccine in plant cells with their tough outer wall [3]. Plant tissue may also contain possible molecules with adjuvant capacities (e.g. Quil A) [57].

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Plants can correctly process and express complex foreign proteins (Table 1.1 and 1.2). The complex sIgA molecule has been produced successfully in potato and tobacco plants, including the correct assembly of the two IgA molecules, the J-chain and the

Figure 1.1

Construction of an edible vaccine using *Agrobacterium tumefaciens* mediated transformation.
Gene transfer into a plant genome is mediated by the plant-infecting bacterium *A. tumefaciens*. This bacterium contains a low copy number of a tumour inducing (Ti) plasmid. The Ti-plasmid transfers part of its DNA (T-DNA) stabile into the plant genome via a wounded plant cell and the T-DNA. Callus that is formed is allowed to grow out into mature plants. The LBT-gene is expressed under control of the tuber-specific promoter patatin. After tuber formation, LTB is expressed and transported to the endoplasmatic reticulum.

secretory component [58,59]. Other research groups reported the correct expression of pentameric LTB or CTB with GM1 binding activity [60-62].

Choice of plant species and plant part

The ideal edible vaccine should have high protein contents with high expression levels of the recombinant protein. It should grow fast and should be easy to multiply for bulk production. Finally, the ideal edible vaccine should not be toxic when given at the required amounts and should be edible as raw, uncooked food. At this moment, the list of food plants that have been transformed is rather long (Table 1.1, 1.2, and 1.3) and several plants have been tested already for oral immunisation. From this list the most suitable plant can be chosen from this list.

Bananas are regarded to be ideal vaccine plants as they do not require cooking and can be grown in developing countries. Growth, however, is slow and the fruit decays quite rapidly after ripening. Tomatoes grow faster and can be cultivated throughout the world, but they too decay rapidly. Drying might preserve these foods and overcome decay [3], but may also alter the vaccine or its bioavailability. Other plausible plants are maize, carrots, peanuts, rice, wheat, and soybeans [63].

With the availability of in-depth knowledge on the molecular biology of potatoes and its transformation possibilities within our research group, we decided to use this plant as model. Potato plants can be propagated rapidly from tubers, produced easily and at large scale, and can be stored for long periods without special precautions [3]. The use of potatoes has also several drawbacks. Consumption of raw potatoes might give complications and cooking may denature the antigens. Being a member of the family of solanaceae, they contain several toxic glycoalkaloids of which the highest levels are found in the foliage, blossoms and sprouts, followed by the peel and the tuber flesh.

Aim and outline of this thesis

The goal of the present thesis is to determine the feasibility of edible vaccines for oral immunisation. Can edible vaccines provide immunological protection? What immunisation protocol is most suitable for edible vaccines? Is LTB a suitable adjuvant for edible vaccines? What are the possibilities of potatoes as vaccine-production system? For this purpose, the research was focussed on a model with LTB produced in potato tubers and tested in mice. Protection was determined by measuring systemic and local antibody responses.

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Table 1.1 Vaccine proteins

Plant expression system	Source of the protein	Protein/ peptide expressed	Transformation method	Maximum expression level *in planta*	Integrity of the protein	Tested species	Immunogenicity and protective capacity	References
Alfalfa	Footh-and-mouth-disease virus	VP1	tobacco mosaic virus	not known		mice	immunogenic and protective upon injection and oral administration	Wigdorovitz, 1999
Arabidopsis	Footh-and-mouth-disease virus	VP1	not mentioned	not known		??	immunogenic and protective upon injection	Carillo, 1998
Arabidopsis	Transmissible gastro-enteritis coronavirus	Glycoprotein S	not mentioned	0.06% TSP		??	immunogenic upon injection	Gomez, 1998
Lettuce	Hepatitis B virus	surface protein	*Agrobacterium*	< 0.01% FW		mice	orally immunogenic	Kapusta, 1999
Lupin	Hepatitis B virus	surface protein	*Agrobacterium*	< 0.01% FW		mice	orally immunogenic	Kapusta, 1999
Maize	*E. coli*	LTB	not mentioned	not known		mice	orally immunogenic and protective	Streatfield, 2000
Maize	Transmissible gastro-enteritis coronavirus	Glycoprotein S	not mentioned	< 0.01% FW		??	orally protective	Streatfield, 2000
Potato	*E. coli*	LTB	*Agrobacterium*	0.19% TSP	GM1-binding multimers	mice, human	orally immunogenic and protective	Haq, 1995; Mason 1998; Tacket, 1998
Potato	*Vibrio cholerae*	CTB	*Agrobacterium*	0.30% TSP	GM1-binding	??	orally immunogenic and protective	Arakawa, 1997; Arakawa, 1998
Potato	Hepatitis B virus	surface protein	*Agrobacterium*	< 0.01% FW		mice	orally immunogenic	Richter, 2001
Potato	Norwalk virus	capsid protein	*Agrobacterium*	0.37% TSP	VLP form	mice	orally immunogenic	Mason, 1995
Potato	Rabbit hemorrhagic disease virus	VP60	*Agrobacterium*	0.30% TSP		rabbit	immunogenic and protective upon injection	Streatfield, 2001
Tobacco	*E. coli*	LTB	chloroplast	< 0.01% FW	multimers	mice	orally immunogenic	Haq, 1995
Tobacco	Hepatitis B virus	Surface protein	chloroplast	< 0.01% FW	VLP form	mice	immunogenic upon injection	Mason, 1992; Thanavala, 1995
Tobacco	Norwalk virus	capsid protein	not mentioned	0.23% TSP	intact protein and VLP form	mice	orally immunogenic	Mason, 1996
Tobacco	Rabies virus	Glycoprotein	not mentioned	1% TSP	intact protein	??	not known	Streatfield, 2001
Tobacco	Transmissible gastro-enteritis coronavirus	Glycoprotein S	not mentioned	0.20% TSP	intact protein	>>	immunogenic upon injection	Streatfield, 2001

Transformed plants that produce vaccine proteins
(TSP – total soluble protein; FW = fresh weight)

The first part of the thesis describes the efforts to optimise the immunisation protocol for transgenic potatoes, and addresses the following questions: can the immune response be increased by the immunisation schedule? (Chapter 2); can the immune response be increased by modifying the immune status of the host? (Chapter 3); and is the optimised immunisation strategy suitable for an antigen produced in potato tuber? (Chapter 4). Chapter 5 describes the efforts to explore whether LTB-fusion proteins can be produced in potato tubers and whether LTB was a suitable adjuvant in edible vaccines. The final chapters of this thesis discuss the feasibility of edible vaccines. Are the speculations about edible vaccines justified? (Chapter 6) and what are the consequences of feeding a vaccine and are potato tubers suitable vaccine delivery systems?(Chapter 7).

Table 1.2 Biopharmaceuticals and plantibodies

Plant expression system	Application
Arapdopsis	Human enkephalins
Alfalfa	Plantibodies
Canola	Human hirudin
Maize	Human aprotinin
Potato	Human lactoferrin
Rice	Plantibodies; human interferon-α; human-α-1-antitrypsin
Soybean	Plantibodies; human protein C, - somatropin, -erythropoietin, -epidermal growth factor, -interferon-β, -serum albumin, -hemoglobin α/β, -homotrimeric collagen
Tobacco	Angiotensins-converting enzyme, glucocerebrosidase
Tomato	Angiotensins-converting enzyme
Wheat	Plantibodies

Transformed plants that produce biopharmaceuticals and plantibodies (adapted from Streatfield, 2001).

Table 1.3   Various transformed plants

Miscellaneous plant expression systems	
Asparagus	Papaya
Banana	Pea
Barley	Peanut
Cabbage	Pepper
Cantaloupe	Plum
Carrot	Raspberry
Cauliflower	Serviceberry
Cranberry	Squash
Cucumber	Strawberry
Eggplant	Sugar beet
Flax	Sugarcane
Grape	Sunflower
Kiwi	Sweet potato
melon	Walnut

Transformed plants with unknown transformation products
(adapted from Richter, 1999)

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