Chapter 2
Induction of Oral Tolerance to Treat Autoimmune and Allergic Diseases by Using Transgenic Plants

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Abstract In recent years, the use of plants as a green bioreactor for production of recombinant pharmaceutical proteins, a technology known as plant molecular farming or biofarming, has gained increasing attention. This new technology has the potential to produce large quantities of the required protein at competitive low costs. Moreover, edible tissues or organs offer the possibility of direct oral delivery of pharmaceutical proteins expressed by plants with minimal processing, significantly reducing production costs and accelerating product development. To date, a number of recombinant proteins of pharmaceutical interest have been produced in plants, ranging from monoclonal antibodies, vaccines, hormones to enzymes. Furthermore, many plant-made pharmaceutical proteins have been tested in pre-clinical animal models of disease with promising results, with some plant-made vaccines and monoclonal antibodies advanced to human clinical trials. This chapter highlights the progress made towards the utilization of transgenic plants to express and deliver recombinant autoantigens or allergens to induce oral tolerance for the treatment of autoimmunity and allergy.

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2.1 Introduction

In recent years there has been a rapidly growing demand for protein-based therapeutics, largely due to a better understanding of the molecular mechanisms underlying the pathogenesis of various human diseases and the identification of new molecular targets. Moreover, protein-based therapeutics have several advantages over small-molecule drugs, including serving highly specific and complex set of functions that cannot be mimicked by chemical compounds, and having highly specific actions that produce no or minimal interference with normal biological processes (Douthwaite and Jermutus 2006). Today most protein pharmaceuticals, including insulin, growth hormones, and most monoclonal antibodies, are produced through recombinant methods. Bacterial, yeast and mammalian cell cultures are the most commonly used hosts for the expression of recombinant proteins. While these conventional expression systems offer certain advantages, they are generally limited by low yields and high production costs, mainly due to the requirement for large fermenters, sterile conditions, and expensive media. It has become more and more apparent that current expression systems are inadequate to meet the ever increasing demand for protein pharmaceuticals. In recent years, plants have emerged as a promising alternative system for the production of heterologous proteins.

There are several potential advantages of using plants as a protein production system. First, a major advantage of plant systems over conventional cell culture-based production systems is the anticipated cost savings, reflecting the large amount of biomass that can be produced in a short time with no need for specialized equipment or expensive media. Plants can be contained, grown easily and inexpensively in large quantities, can be harvested and processed with available agronomic infrastructures, and have simple, unlimited scalability. It is estimated that protein production in transgenic plants can be as much as four orders of magnitude less expensive than production in mammalian cell culture, on a per gram of unpurified protein basis (Dove 2002). Secondly, in contrast to the bacterial expression systems, plants, being higher eukaryotes, are capable of performing many of the complex protein processing steps, such as glycosylation. Thirdly, plants do not harbor infectious agents such as viruses and prions harmful to humans as these agents cannot replicate in plants. Safety is a primary concern when any therapeutic proteins for human use are prepared from animal tissues or bacterial cells (Tremblay et al. 2010). Finally, pharmaceutical proteins synthesized in the edible tissues of transgenic plants can be delivered by ingestion of transgenic plant tissue without tedious and expensive downstream processing.

Indeed, an increasing number of pharmaceutical proteins have been expressed in plants, ranging from monoclonal antibodies, vaccines, hormones to enzymes. Moreover, many plant-made proteins have been tested in preclinical animal models with promising results, with several plant-derived monoclonal antibodies and vaccines advanced into different stages of human clinical trials (Penney et al. 2011; Paul and Ma 2011). Recently, there has been a growing interest in using transgenic plants as a novel therapeutic strategy for the treatment of autoimmune diseases and allergies.

Autoimmune diseases such as diabetes and multiple sclerosis or allergies such as asthma are manifestations of immunological hypersensitivity. They arise when mechanisms controlling responses to host's self proteins or to innocuous environmental antigens break down (Larché and Wraith 2005). Currently available therapies mainly treat the symptoms of autoimmune or allergic diseases through global immunosuppression and are associated with increased risk of infections. Specific suppression of unwanted autoimmune responses or allergic responses without affecting the normal function of the host's immune system is the major goal of treatment for autoimmune disorders or allergies. Antigen-specific immunosuppression would allow for the inhibition of autoimmune or allergic responses without adversely affecting the function of the immune system. Mucosal administration of protein antigens to induce oral tolerance is an attractive therapeutic option for the treatment of autoimmune diseases and allergies. The use of transgenic plants to express and deliver recombinant autoantigens or allergens represents a novel strategy for oral tolerance induction. This chapter highlights the progress made towards the utilization of transgenic plants to express and deliver recombinant autoantigens or allergens to induce oral tolerance for the treatment of autoimmune diseases and allergies.

2.2 Oral Tolerance and Its Therapeutic Potential in Autoimmunity and Allergy

Mucosal tolerance is classically defined as a state of hyporesponsiveness to subsequent parenteral injections of proteins to which an individual or animal has been previously exposed via the mucosal route (Faria and Weiner 2006). It is now well recognized that oral tolerance is an immunoregulatory strategy used by the gut and its associated lymphoid tissues to render the peripheral immune system unresponsive to nonpathogenic proteins, such as food, airborne antigens or the commensal bacterial flora (Faria and Weiner 2006; Weiner et al. 2011). The gut-associated lymphoid tissue (GALT) is a well-developed immune network that has not only developed the inherent property of preventing the host from reacting to ingested proteins, but has also evolved to protect the host from ingested pathogens. It is generally agreed that oral tolerance is established and maintained at the T cell level (Faria and Weiner 2006; Weiner et al. 2011).

Oral tolerance can occur via a number of mechanisms, depending on the amount of an antigen administered. Administration of high doses of an antigen induces tolerance via the mechanism of clonal anergy/deletion of effector T cells, whereas low doses of mucosally administered antigen induce tolerance via the mechanism of an active suppression of effector T cells through the induction of antigen-specific regulatory T cells (Treg), which produce downregulatory cytokines such as IL-4, IL-10 and TGF-b, a Th2/Th3 cytokine pattern. After activation in the GALT, the regulatory T cells migrate to the site of inflammation, and on re-encountering the fed antigen, they display their specific suppressive effect, resulting in an attenuated

T cell-mediated immune response. Low-dose and high-dose tolerance may not be mutually exclusive and may have overlapping functionality (Weiner et al. 2011).

The induction of antigen-specific oral tolerance is an attractive therapeutic approach for treatment of autoimmune, allergic, and inflammatory diseases. Oral tolerance has been shown to be effective in treating animal models of autoimmunity and allergy. Examples include experimental allergic encephalomyelitis (EAE), arthritis, diabetes mellitus, uveitis, airway eosinophilia, allergy and food hypersensitivity (Weiner et al. 2011). Oral tolerance has also been studied in humans. In rheumatoid arthritis (RA), patients with active RA were fed dnaJP1, which is a 15-mer dominant epitope heat-shock protein thought to be involved in RA pathogenesis, though independent from the primary trigger of disease. After 6 months of treatment, patients treated with dnaJP1 showed a significant reduction in T cells producing TNF and a trend toward an increase of T cells producing IL-10, indicating a positive effect on the disease (Park et al. 2009; Koffeman et al. 2009). Another human trial conducted involves oral insulin for prevention of type 1 diabetes [Diabetes Prevention Trial 10 (DPT-10)]. Although there were no differences between the oral insulin and placebo groups in the primary outcome, a subset of individuals in the oral insulin prevention trial with high levels of insulin autoantibodies had an apparent several year delay in progression to diabetes ($P = 0.01$), and a follow-up study is planned (Skyler 2008; Weiner et al. 2011). Oral tolerance has also been investigated in food allergy. Clark et al. (2009) reported that oral administration of peanut flour induced clinical tolerance to peanut protein and protected peanut-allergic children from developing severe peanut allergy.

It is likely that the translation of oral tolerance to a realistic therapy for human autoimmunity and allergy will require the use of a mucosal adjuvant to enhance the efficacy of oral tolerance induction (Faria and Weiner 2006; Weiner et al. 2011). Recent studies have shown that oral administration of *Lactacoccus lactis* cells engineered to secrete OVA and/or IL-10 enhances oral tolerance in mice (Huibregtse et al. 2007; Frossard et al. 2007). Oral, nasal, or sublingual administration of antigen coupled to the cholera toxin B subunit (CTB) was also shown to enhance mucosal tolerance (Sun et al. 2010). CTB increases mucosal antigen uptake and presentation to antigen presenting cells by binding to GM1 ganglioside and the induction of regulatory cells (Sun et al. 2010; Weiner et al. 2011).

2.3 Transgenic Plants as a Novel Strategy for Oral Tolerance Induction

The induction of oral tolerance requires repeated ingestion of large amounts of autoantigens or allergens. A major consideration for clinical application of oral tolerance is the cost. It is essential to develop such a heterologous expression system that is able to provide recombinant autoantigens or allergens in sufficient quantities and at an affordable cost. While cell culture-based conventional expression systems may allow the production of sufficient quantities of recombinant proteins, they are

unlikely to be cost effective, partly because recombinant therapeutic proteins derived from these systems need to be purified before use. Downstream protein purification and processing are a complex, labour-intensive and expensive process that can eliminate the economic advantage of any production system. The use of transgenic plants for oral tolerance strategy has considerable clinical appeal, not only for efficacy but also for simplicity of production and delivery, advantages of cost, absence of contamination risk with human pathogens, and perhaps, if used in edible plants, increased patient acceptance. Also augmented immune responses to plant produced vaccines may suggest increased stability for plant expressed recombinant proteins to gastrointestinal degradation. There is now increasing evidence that foreign proteins compartmentalized within plant cells are protected in the stomach through bioencapsulation by the plant cell (Verma et al. 2010).

Both nuclear and chloroplast transgenic plants are being exploited to express and deliver recombinant autoantigens and allergens for oral tolerance induction. Transformation of plant nuclear genomes by *Agrobacterium* or biolistic methods has become routine in plant research. The number of plant species amenable to transformation and regeneration is now quite large (Tzfira and Citovsky 2006). The main advantage of using nuclear transgenic plants as an expression platform is its flexibility and the efficiency in scaling up the production of recombinant proteins, which can be achieved simply by planting more acreages of the biotech crops. An additional advantage is the long-term continuous production of recombinant proteins with little to no external input because foreign genes are stably integrated into the nuclear genome of the host plant and are inherited in the next generation and as such, stable and predictable transgene expression can be maintained over many generations. Moreover, the synthesis of foreign proteins in nuclear transgenic plants can be readily targeted to edible plant organs such as leaves, seeds and fruits, allowing for oral delivery of a palatable product. However, a limitation of using nuclear-transformed plants for protein production is the relatively low-level accumulation of recombinant proteins, with typical expression levels within the range of 0.0001 to ~1% of total soluble protein (TSP) (Tremblay et al. 2010).

Chloroplast transformed plants can also be an economic source of autoantigens and allergens. One of the main advantages of chloroplasts as bioreactors is the potential for accumulation of large quantities of recombinant proteins. A typical tobacco leaf cell contains as many as 100 chloroplasts per cell with up to 100 genome copies per chloroplast, and therefore the copy number of any introduced transgene can be amplified by as many as 10,000 per cell, leading to extraordinarily high levels of foreign protein products (Chebolu and Daniell 2009). Other advantages of chloroplast bioreactors include high levels of transgene containment, no transgene silencing and no position effects as often occurred in nuclear transgenic plants, and the ability to express multiple genes in a single transformation event (transgene stacking) (Chebolu and Daniell 2009). A limitation of chloroplast recombinant protein production, however, is that like bacteria they are unable to perform glycosylation essential for proper protein folding and functions.

Recently, the chloroplast of the unicellular green alga *Chlamydomonas reinhardtii* has been gaining increasing attention as a new bioreactor for the production of

autoantigens and other therapeutic proteins (Wang et al. 2008). Compared to chloroplast transgenic plants, the use of chloroplast transgenic algae as a bioreactor offers several additional advantages. Microalgae, such as *C. reinhardtii*, grow and reproduce faster than any other terrestrial or aquatic plant, doubling its biomass in approximately 8 h. Microalgae are non-toxic and non-polluting, thus environmentally friendly for mass cultivation and commercial exploitation. Also, there will be a significant reduction in the time required to generate transgenic algae as compared to the time required to generate transplastomic plants. In general, stable transplastomic algal lines can be obtained in as little as 3 weeks, with the potential to scale up to mass production in an additional 4–6 weeks (Franklin and Mayfield 2004; Rasala and Mayfield 2011). Moreover, *C. reinhardtii* is rich in essential amino acids and protein, with the protein content comprising up to 25% of its dry weight (Franklin and Mayfield 2004). These attributes, and the fact that green algae are generally regarded as safe (GRAS) by the U.S. Food and Drug Administration (FDA) for human consumption, make *C. reinhardtii* a particularly attractive system for oral tolerance induction.

2.4 Treatment of Type 1 Diabetes with Transgenic Plants Expressing Diabetes-Associated Autoantigens

Type 1 diabetes (T1D) is a chronic autoimmune disease in which a loss of self-tolerance to insulin-producing β cells in the pancreatic islets results in impaired glucose homeostasis (Eisenbarth 2004). While insulin replacement therapy has transformed T1D from a fatal disease to a chronic one, it is still associated with significant morbidity. Complications of diabetes lead to kidney failure, blindness, amputations, and increased risk of macrovascular disease. Intensive insulin therapy is associated with significant adverse events related to hypoglycemia (Arabi et al. 2009). Restoration of self-tolerance is considered as the most satisfactory solution to the prevention and cure of T1D. A number of beta-cell-specific autoantigens have been identified including glutamic acid decarboxylase (GAD), insulin, insulinoma-associated antigen (IA-2) and heat shock protein 60 (Hsp60) (Eisenbarth 2004; Jasinski and Eisenbarth 2005). The identification of islet cell autoantigens holds promise as targets for antigen-specific immunotherapy in T1D.

2.4.1 Induction of Oral Tolerance by Mucosal Administration of Transgenic Plants Expressing GAD

GAD is recognized as the major and early islet autoantigen in T1D. There are two isomers of GAD, GAD65 and 67, both of which are implicated in T1D (Bu et al. 1992; Elliott et al. 1994). Rat and human islets express GAD65 predominantly, whereas mouse islets majorly express GAD67 (Elliott et al. 1994). We are the

first group to demonstrate that transgenic plants can be used as a novel strategy to induce antigen-specific oral tolerance for the treatment of type 1 diabetes in non-obese diabetic (NOD) mice. Initially we generated transgenic tobacco and potato plants expressing murine GAD67, with expression levels up to 0.4% of TSP (Ma et al. 1997). To demonstrate that GAD-specific oral tolerance can be induced by administration of GAD67 transgenic plants, young pre-diabetic female NOD mice were fed GAD67 transgenic potato tuber or tobacco leaf tissue as dietary supplementation for a period of 7 months starting at 5 weeks of age. Control mice received an equivalent amount of vector-minus insert transformed tobacco or potato tissue. As expected, GAD67 plant fed mice were protected from diabetes, whereas those fed control plant tissue were not protected from diabetes. The protection was associated with the inhibition of proliferation of GAD-reactive splenic T cells as well as a Th1 to Th2 cytokine profile shift.

We subsequently produced transgenic tobacco plants expressing human GAD65 (Ma et al. 2004). Compared to GAD67 expression in plants, human GAD65 accumulated to a lower level (0.04% of TSP). Oral administration of GAD65 plant tissue delivering approximately 8–10 μg GAD65/per mouse daily was shown not to provide protection against diabetes in NOD mice. To enhance oral tolerance to GAD65, we additionally produced transgenic plants expressing the anti-inflammatory Th2 cytokine IL-4 for use as a mucosal adjuvant. Co-administration of IL-4 plant tissue delivering approximately 1–2 μg IL-4/per mouse daily resulted in suppression of diabetes in NOD mice, suggesting the effectiveness of IL-4 in potentiating induction of oral tolerance, especially in cases where only low expression levels of recombinant autoantigens can be achieved in transgenic plant systems. Frequency analysis of cytokine-secreting cells in spleens showed that the ratio of IL-4/IFN-γ-secreting cells was increased in IL-4 plus GAD65 plant fed mice but was unchanged in control plant treated mice, suggesting a shift in the Th1/Th2 balance toward Th2 dominance. Furthermore, the results of adoptive transfer experiments indicated that the protection from diabetes in IL-4 + GAD65 plant fed mice was associated with the induction of regulatory T cells.

2.4.2 Induction of Oral Tolerance by Mucosal Administration of Transgenic Plants Expressing Insulin

Insulin is another major early autoantigen in T1D (Jasinski and Eisenbarth 2005; Zhang and Eisenbarth 2011). Arakawa et al. (1998) generated transgenic potato plants synthesizing human proinsulin fused to cholera toxin B subunit (CTB-INS). Fusion to CTB was intended to enhance oral tolerance induction to insulin. The plant-derived fusion protein retained the GM1 ganglioside-binding activity of CTB and the antigenicity of insulin. NOD mice fed transgenic potato tubers delivering μg quantities of CTB-INS fusion protein had a reduction in insulitis, and a delay in the progression of clinical diabetes, while control mice fed transgenic potato tubers expressing insulin or CTB protein alone were not protected from diabetes.

Recently, Ruhlman et al. (2007) expressed human proinsulin fused to CTB (CTB-Pins) in chloroplasts of both lettuce and tobacco, with accumulation levels up to ~16% of TSP in tobacco and up to ~2.5% of TSP in lettuce. In a short-term feeding study, 5-week-old female NOD mice were fed 8 mg of powdered tobacco leaf material expressing CTB-Pins or, as negative controls, CTB–green fluorescent protein (CTB-GFP) or interferon–GFP (IFN-GFP), or untransformed leaf, each week for 7 weeks. Histological analysis of the pancreatic islets from CTB-Pins treated mice showed decreased lymphocytic infiltration in the islets (insulitis) compared with the controls. Moreover, increased expression of immunosuppressive cytokines, such as IL-4 and IL-10, was observed in the pancreas of CTB-Pins-treated NOD mice. Serum levels of immunoglobulin G1 (IgG1), but not IgG2a, were also elevated in CTB-Pins-treated mice. Taken together, the prevention of pancreatic insulitis in CTB-Pins treated mice may be likely due to the induction of Th2 lymphocyte-mediated oral tolerance.

2.5 Treatment of Arthritis with Transgenic Plants Expressing Arthritis-Associated Autoantigens

Rheumatoid arthritis (RA) is an autoimmune disease that causes chronic inflammation of the joints. Rheumatoid arthritis can also cause inflammation of the tissue around the joints, as well as in other organs in the body. Numerous antigenic targets have been identified in RA patients including type II collagen (CII), human chondrocyte glycoprotein 39, and various members of the heat-shock protein family (Ichim et al. 2008). To date, treatment interventions have all been associated with non antigen-specific inhibition of inflammatory processes, causing concerns regarding increased susceptibility to infections. Induction of oral tolerance offers the promise of antigen-specific immunotherapy for the treatment of RA.

2.5.1 Induction of Oral Tolerance by Mucosal Administration of Transgenic Plants Expressing Type II-Collagen Peptides

Hashizume et al. (2008) produced a fusion protein, consisting of glutelin and four tandem repeats of a $CII_{250–270}$ peptide (GluA-4XCII$_{250–270}$) containing a human T cell epitope, in transgenic rice and conducted feeding experiments to determine whether oral administration of transgenic rice seeds can provide protection against RA in a mouse model of RA. In these experiments, DBA/1 mice were fed either transgenic rice or wild-type rice for 2 weeks before immunization with type II collagen, and an average daily intake of the $CII_{250–270}$ peptide as a diet was estimated to be about 25 μg per mouse. Mice treated with GluA-4XCII$_{250–270}$ transgenic rice showed lower delayed serum specific-IgG2a response against challenge with type II collagen.

2.5.2 Induction of Oral Tolerance by Mucosal Administration of Transgenic Plants Expressing Mycobacterial Hsp65

Heat shock proteins (Hsp) are a group of highly conserved proteins that serve as intracellular chaperons that are induced under stress conditions. Recent research has implicated Hsp65 as an autoantigen in RA (Massa et al. 2007). To develop a novel oral antigen therapy for RA using plant-based systems, Rodriguez-Narciso et al. (2011) produced transgenic tobacco plants expressing recombinant mycobacterial Hsp65 protein and examined the potential of orally administered plant-made Hsp65 protein for treating adjuvant-induced arthritis in Lewis rats. The results showed that oral feeding of Lewis rats with transgenic tobacco leaf tissue delivering 10 μg of Hsp65 per rat daily, which was started 10 days post induction of arthritis with a M. tuberculosis strain, significantly promoted recovery of the body weight and reduced joint inflammation.

2.6 Induction of Oral Tolerance to Treat Allergic Diseases with Transgenic Plants Expressing Allergens

Allergic diseases have become one of the most important health problems throughout the world. Allergic diseases are caused by inappropriate immunological responses to harmless antigens driven by a Th2-mediated immune response. Currently, the major therapies used to control allergic diseases are glucocorticoids. These steroid hormones act nonselectively and their extended use can lead to numerous side effects such as increased risk of infection (Moghadam-Kia and Werth 2010). Allergen-specific immunotherapy is considered the preferred approach for treating allergy. A major problem with specific allergen immunotherapy is that the allergen extracts that are currently used for vaccination are unfractionated preparations (Niederberger and Valenta 2004). The inclusion of non-allergenic materials may elicit new allergenicity. Recombinant allergen–based immunotherapy will improve current immunotherapy practice and may open possibilities for prophylactic vaccination. Induction of oral tolerance by mucosal administration of transgenic plants expressing specific recombinant allergens may provide a novel and effective therapeutic strategy against allergy.

2.6.1 Induction of Oral Tolerance by Mucosal Administration of Transgenic Plants Expressing a Full-Length Antigen

The sunflower seed methionine-rich 2S albumin (SSA) is an IgE-binding protein and has been identified as the major allergen in sunflower seed (Asero et al. 2002). Smart et al. (2003) produced transgenic lupin (Lupinus angustifolius L.) synthesizing

SSA and investigated whether oral administration of SSA-lupin could protect against the development of experimental asthma. They showed that oral consumption of SSA-lupin seed meal attenuated mucus hypersecretion, pulmonary eosinophilic inflammation, and airway hyperreactivity following subsequent allergen exposure in a mouse model of allergic airway disease. The suppression of experimental asthma was associated with the production of $CD4^+$ T cell-derived IFN-γ and IL-10, and the induction of $CD4^+$ $CD45^{low}$ regulatory T cells. This work provides a proof of concept for a plant-based new approach to treating allergy.

Recently, Lee et al. (2011) expressed Der p2 protein in transgenic tobacco plants for oral tolerance induction as a strategy to treat house dust mite-induced airway allergy. Der p2 is a major allergen from Dermatophagoides pteronyssinus, the main species of house dust mite and a major inducer of asthma by activating cells in the respiratory tract (Smith et al. 2001). To determine the effect, mice were fed total protein extracted from Der p2 transgenic plants once per day over 6 days. Der p2-treated mice showed a decrease in serum Der p2-specific IgE and IgG1 titers, a decrease in IL-5 and eotaxin levels in bronchoalveolar lavage fluid, and a decrease in eosinophil infiltration into the airway. Hyper-responsiveness was also decreased in mice treated with Der p2 containing total protein, but the number of CD4 + CD25 + Foxp3+ regulatory T cells were significantly increased in mediastinal and mesenteric lymph nodes. Furthermore, splenocytes isolated from Der p2-treated mice exhibited decreased proliferation and increased IL-10 secretion following stimulation with yeast-derived recombinant Der p2.

2.6.2 Induction of Oral Tolerance by Mucosal Administration of Transgenic Rice Expressing Allergen-Specific T Cell Epitopes

Takagi et al. (2005) produced transgenic rice expressing mouse dominant T cell epitope peptides of Japanese cedar (Cryptomeria japonica) pollen allergens (Cry j I and Cry j II), fused to soybean seed storage protein glycinin. Animal studies showed that oral administration of transgenic rice seeds inhibited the proliferative activity of allergen-specific CD4+ T cells, decreased serum IgE levels and reduced clinical allergy symptoms such as sneezing in mice. Moreover, the levels of Th2 cytokines such as IL-4, IL-5 and IL-13 as well as IgE-mediated histamine release were also significantly decreased in treated mice. Transgenic rice synthesizing human dominant T cell epitopes of Cry j 1 and Cry j 2 allergens was also produced (Takaiwa 2007).

To further enhance the efficiency of rice seed-based vaccines in inducing oral tolerance against allergy, this group have recently expressed T-cell epitopes of Cry j 1 and Cry j 2 as a fusion protein with the mucosal adjuvant CTB in rice seed (Takagi et al. 2008). They showed that feeding mice with rice seeds containing CTB-fused T-cell epitopes suppressed allergen-specific IgE responses and pollen-induced clinical symptoms at 50-fold lower doses of T-cell epitopes compared with the same

results obtained when control rice seeds expressing T-cell epitopes fused to rice glutelin acidic subunit were used as feed.

Recently, Suzuki et al. (2011) reported the production of a subunit vaccine consisting of a fragment (p45–145) of mite allergen (Der p 1) containing immunodominant human and mouse T cell epitopes in transgenic rice for oral tolerance induction to treat asthma. They showed that prophylactic oral vaccination of mice with transgenic rice seeds reduced the serum levels of allergen-specific IgE and IgG. Allergen-induced CD4+ T cell proliferation and production of Th2 cytokines in vitro, infiltration of eosinophils, neutrophils and mononuclear cells into the airways and bronchial hyperresponsiveness were also inhibited by oral vaccination. The immune response induced by the rice vaccine was antigen-specific, because the levels of specific IgE and IgG in mice immunized with control antigen Der f 2 or ovalbumin were not significantly suppressed by oral vaccination with the Der p 1 expressing transgenic rice.

2.7 Treatment of Inflammatory Bowel Disease by Oral Administration of Transgenic Plants Expressing Interleukin-10

Inflammatory bowel disease (IBD), which includes Crohn's disease (CD) and ulcerative colitis (UC), is a relapsing and remitting condition characterized by chronic inflammation at various sites in the gastrointestinal (GI) tract, which results in diarrhea and abdominal pain (Kozuch and Hanauer 2008). Inflammation results from a T cell-mediated immune response in the GI mucosa. The precise etiology is unknown, but evidence suggests that IBD may result from a breakdown of immunological tolerance towards the gut flora in genetically susceptible hosts, resulting in an inappropriate immune response responsible for the chronic inflammatory process that characterizes CD and UC (Bouma and Strober 2003). Both UC and CD are characterised by the activation of macrophages and dendritic cells with production of pro-inflammatory cytokines such as IL-1, IL-6 and tumour necrosis factor-alpha (TNF-a) (Bouma and Strober 2003; Balding et al. 2004). At present, there is no cure, and therapeutics are primarily aimed at suppressing inflammatory responses. Treatment with anti-TNF-α antibodies has met with some success, however, its therapeutic limitations, elevated cost, and the side effects of other conventional immunosuppressive drugs underscore the need for novel therapeutic strategies (Kalischuk and Buret 2010).

Menassa et al. (2007) expressed human IL-10 in transgenic tobacco plants and investigated the potential of the plant-made cytokine as a luminal therapy for IBD in animal models of colitis. IL-10 is a potent anti-inflammatory cytokine with therapeutic applications in several autoimmune and inflammatory diseases (Beebe et al. 2002). IL-10 −/− mice, which spontaneously develop colitis, were used for feeding experiments. Mice were fed IL-10 tobacco leaf tissue delivering up to 9 μg of human

IL-10 per mouse daily for 4 weeks. Mice treated with IL-10 had reduced severity of colitis. The TNF-α expression at the sites of inflammation was also down- regulated in IL-10 treated mice. Gut histology was significantly improved relative to controls ($P = 0.002$) and was correlated with a decrease in small bowel TNF-α mRNA levels and an increase in IL-2 and IL-1β mRNA levels.

Recently, Bortesi et al. (2009) expressed viral and murine IL-10 in transgenic tobacco. *In vitro* characterization showed that both plant-derived molecules formed stable dimers, were able to activate the IL-10 signaling pathway and to induce specific anti-inflammatory responses in mouse macrophage cells. Their long-term goals are to treat type 1 diabetes and other inflammatory diseases through oral delivery of plant-made IL-10.

2.8 Conclusions

There is growing evidence to support the therapeutic value of oral tolerance induction in the treatment of autoimmune disease, allergy, organ transplant rejection and many other inflammatory diseases. The use of transgenic plants as an oral tolerance induction strategy offers a number of advantages, including simplicity of production and delivery and low-costs. The number of recombinant autoantigens or allergens delivered to intestinal mucosa by transgenic plants for successful oral tolerance induction is increasing. There are a number of approaches that one could use to further increase the efficiency of transgenic plant-based approach for oral tolerance induction. One is to improve the yield of recombinant autoantigens or allergens in transgenic plants, as this will lead to increased delivery of a target protein without increasing the amount of transgenic plant tissue consumed. Recently, Morandini et al. (2011) reported the expression of GAD65 at a significantly higher level in transgenic plants, accounting for 7.7% of TSP when expressed in Arabidopsis seeds. Another approach is to express autoantigens or allergens as fusion proteins containing a mucosal delivery-enhancing molecule, such as human serum transferrin, to improve plant-based mucosal delivery of target proteins (Brandsma et al. 2010). Transgenic plants are believed to hold great promise for oral tolerance therapy against autoimmune disease, allergy, and many other inflammatory diseases.

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