PLASTID TRANSFORMATION

Over the past decade, data have been published on the transformation and expression of transgenes in plant chloroplasts, thereby taking advantage of the semiautonomous genetic machinery of these organelles (8,74–76). This approach to expression of biopharmaceuticals in crops was initiated primarily to meet perceived environmental transgene containment targets to ensure that genes are not lost to outcrossing. The concept relies on the strictly maternal inheritance of plastids in most species. Chloroplast transformation is an environmentally friendly way to engineer plants minimizing transfer to weeds or crops and decreasing the potential toxicity of pollen to insects (77). Other potential advantages of this system are high ploidy state, high transcription and translation rates, and the lack of gene silencing, all of which can contribute to high levels of foreign protein accumulation. While site-directed integration through homologous recombination appears to be a requirement, it might also provide more control of genetic engineering and increased uniformity of transgene expression (8,75,78,79). Additionally, one can make use of polycistronic operons, much like a bacterial system, to permit coordinated expression of multiple genes (79,80).

Direct transformation of chloroplasts results in high levels of protein accumulation, up to several percent of total soluble protein, which is considerably more than that reported for other systems. In one case involving an operon from *Bacillus thuringiensis*, more than 40% was reported (79). Pharmaceutically important proteins expressed in plastids include human somatotropin (75), a biodegradable synthetic polymer (81), and also CT-B as a vaccine candidate (52).

Plastid transformation has been extended to the experimental model plant *A. thaliana* (82) and two important solanaceous crop species, potato (83) and tomato (76). In relation to the latter two crops, transgene expression and recombinant protein accumulation were observed to occur in plastids that are specific for these two plants: potato tuber amyloplasts and tomato fruit chromoplasts. Unique advantages and disadvantages of plastid expression depend on the prokaryotic nature of the organelle, but so do its shortcomings. For example, N-glycosylation strictly depends on the endomembrane system. However, in the case of prokaryotic antigens or proteins and antigens that do not need to be glycosylated, plastids could possibly offer some distinct advantages.

OTHER TARGETS OF PLANT-EXPRESSED ANTIGENS

Plant-expressed proteins have been proposed for other applications beyond prophylactic vaccines. For example, transgenic plants have been proposed for the production of autoantigens. Human autoantigens could be used to treat autoimmune diseases by inducing tolerance of the immune system rather than by stimulating it. An autoantigen implicated in diabetes, glutamic acid dehydrogenase (GAD), was produced in plants and fed to nude obese diabetic (NOD) mice, which have a particular susceptibility to the development of diabetes. This resulted in a reduction in pancreatic islet inflammation, an indication that immunotolerance had occurred (84). Arakawa et al. (85) used a similar approach feeding plant tissues expressing either proinsulin or a CT-B/proinsulin fusion to NOD mice, and they also observed a reduction in pancreatitis. This result suggested that they had immunotolerized the mice against this type of cytotoxic T cell–mediated autoimmune disease. In this case, reduction of pancreatic inflammation coincided with increase in anti-insulin antibodies, mostly of the IgG1 isotype, leading to the conclusion that the cytotoxic T-cell response is suppressed. They were able to enhance this effect with the addition of a second antigen fusion, CT-B/GAD. It is interesting to note that the reduction in pancreatitis was considerably greater with the fusions than with the autoantigens alone, supporting targeting or adjuvant activity due to the CT-B component. We should also mention the differences in feeding protocols used in these two experiments. Ma et al. (84) fed very large amounts of recombinant GAD (1–1.5 mg per mouse per day) daily for four weeks, a more frequently used toleration protocol. Arakawa et al. (85), on the other hand, fed potato containing 20 μg of the CT-B-proinsulin fusion protein in five doses over a four-week period, which is almost identical to the feeding regimen they had reported to be effective in eliciting protective immune responses against foreign antigens (48). As Arakawa et al. suggested in their paper, the fusion to CT-B may facilitate the presentation of the antigen to the gut-associated lymphoid tissue to enhance the response.

Transgenic plant-derived antigens have also been proposed for immunotherapy of malignant disease. These include, for example, plant-derived personalized human antibodies directed against non-Hodgkin's lymphoma (NHL) (86) (see further discussion in sect. ''Cancer Vaccines''). Other groups have studied a plant-derived tumor-associated colorectal cancer antigen EpCAM that stimulated antibodies that inhibited the growth of colorectal cancer cells xenografted on nude mice (87). A third example is a plant-derived rabbit papilloma virus L1 antigen that stimulated protection against tumor challenge in rabbits (88).

The control of overpopulations of wild mammalian species in a humane and effective manner through the use of immunocontraceptive plant-derived vaccines is another application of this technology (89,90). The approach is to express in plants a protein or a carrier protein harboring an antigenic epitope of an essential component of the mammalian reproductive system with the intention of inducing a humoral response following repetitive ingestion by the animal that results in sterility. The animal would remain sterile coincident with immune memory or until the next boosting vaccination. Antigenic targets reportedly under investigation include the gonadotropin releasing hormone and ZP3 from the zona pellucida of the mammalian ovulated egg (90,91). While effectiveness is still under study, this application is bound to meet considerable objections because of the danger that these broadly cross-reacting vaccines may be ingested by nontargeted mammalian species.

CANCER VACCINES

An exciting recent development in the use of plant biotechnology for vaccine production has come from the manufacture of patient-specific vaccines against follicular B-cell lymphoma (86,92). Follicular lymphomas are a subtype of NHL, a malignant disease of the lymphatic system that is the seventh leading cause of cancer-related deaths in the United States (93). The administration of a tobacco-derived NHL vaccine in a human clinical trial resulted in immune responses in more than 70% of the patients, a majority of which showed a cellular response, suggesting that the vaccine will specifically direct the immune system to attack cancer cells. This was the first report on the clinical safety and immunogenicity of plant-made idiotypic