InFocus Article

Ethics, Biotechnology, and Global Health: The Development of Vaccines in Transgenic Plants

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As compared with conventional vaccine production systems, plant-made vaccines (PMVs) are said to enjoy a range of advantages including cost of production and ease of storage for distribution in developing countries. In this article, we introduce the science of PMV production, and address ethical issues associated with development and clinical testing of PMVs within three interrelated domains: PMVs as transgenic plants; PMVs as clinical research materials; and PMVs as agents of global health. We present three conclusions: first, while many of the ethical issues raised by PMVs are familiar, PMVs add a new dimension to old issues, and raise some novel issues for ethicists and policy-makers; secondly, it is premature to promise broad applicability of PMVs across the developing world without having demonstrated their feasibility; thirdly, in particular, proponents of PMVs as a solution to global health problems must, as a condition of the ethical conduct of their research, define the commercial feasibility of PMVs for distribution in the developing world.

Keywords

bioethics

genetic engineering

plant vaccines

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INTRODUCTION

The past century should be celebrated for rapid increases in the ability to protect against disease, primarily through vaccination. Although Edward Jenner's pioneering work on vaccination occurred in the late 18th century, introduction of new vaccines occurred most rapidly during the last several decades. New technologies for attenuating and testing vaccines, in addition to ability for vaccine production in bacteria, yeast, chicken eggs, and mammalian cell cultures ("conventional" systems) have provided ever safer and more efficient ways of generating vaccines, such that many infectious diseases have been eliminated altogether—at least in the Western world.

The success of global immunization programs depends largely on the cooperation of all interested parties, especially local governments and international health organizations (de Quadros 2004). Unfortunately, the cost and distribution of vaccines in developing countries remain major hurdles for global health. Through a combination of economics, comparably poor standards of hygiene, and comparably high risk of exposure, infectious conditions—such as respiratory infections, AIDS, tuberculosis, malaria, and diarrheal diseases—still account for 30–50% of all deaths in low-income countries (Ratzan et al. 2000).

Diseases endemic in developing countries have often been 'orphaned' by drug manufacturers. But the global economy and the higher frequency of international travel have increased concern amongst health officials, travelers, and the military for protection against a wider range of infectious diseases. For instance, the current market for "traveler's vaccines" is approaching $2 billion per year; however, the ability to conduct research and development in this area is far more difficult due to competing demands for vaccines against higher profile diseases and focus of funding agencies on new biowarfare threats. Diarrheal diseases are one instance of product development not being prioritized by the pharmaceutical industry, despite the high prevalence of these diseases globally. Cases of "seemingly mild diarrhea" are responsible for over three million deaths per year globally and 200–800 in the US. The global market specifically for traveler's diarrhea

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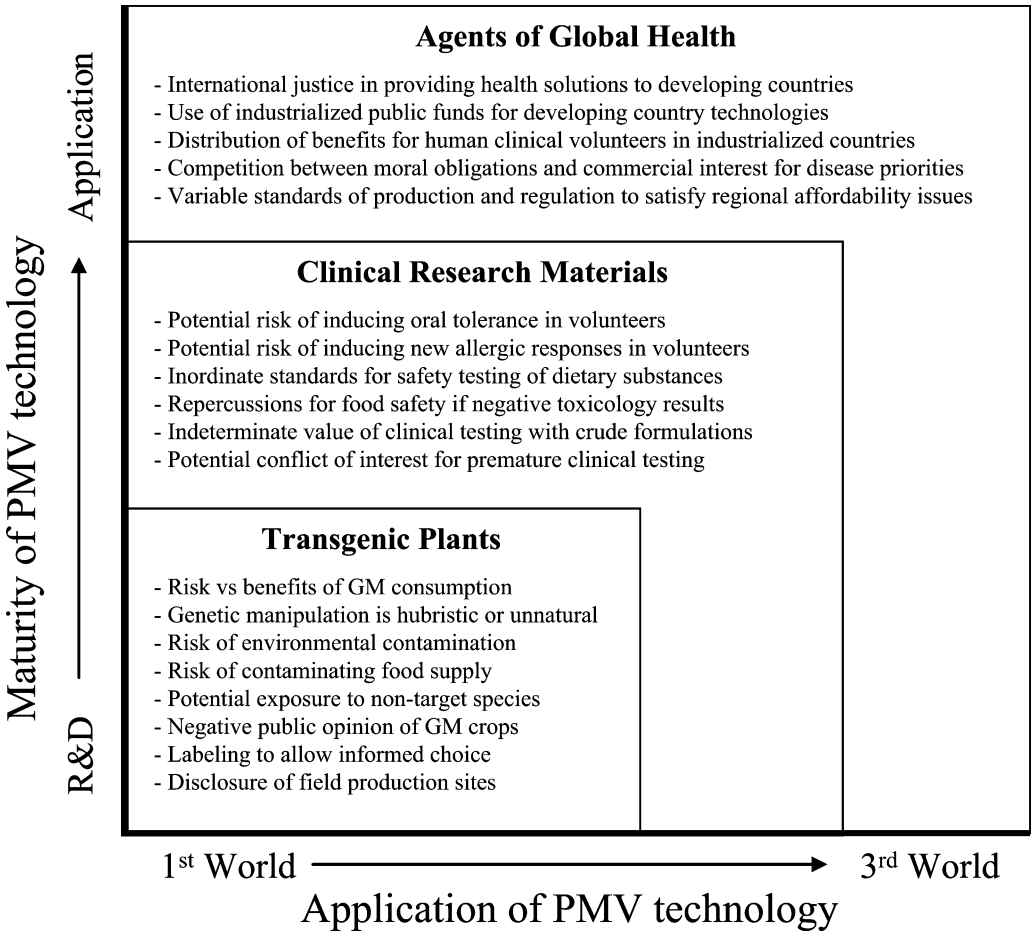

Figure 1. Three domains of ethical analysis of plant-made vaccines.

vaccines alone is estimated to exceed $600 million (Frost and Sullivan 1999). Traveler's diarrhea has typically been categorized as including a group of agents such as Enterotoxigenic *E. coli*, *Vibrio cholerae* (cholera), rotavirus and noroviruses. With the exception of cholera, there are no licensed vaccines in the United States for any of these disease agents. There are obvious disparities between the supply and demand for both current vaccines and for new vaccines. The increasing cost of development and the difficulties associated with many of the most endemic diseases is reducing the number of suppliers in this field (Offit 2004).

The use of transgenic plants as one alternative to conventional vaccine production systems is seen to enjoy a range of advantages in protein production and product storage, and promises putative advantages for distribution in developing countries; but the commercial feasibility of plant-made vaccines (PMVs) has yet to be demonstrated, and there is as yet virtually no literature on the ethics of PMV research and product development. In this article, we address ethical considerations that may arise during development and clinical testing with transgenic plants as a new production system for vaccine manufacturing. We begin with an introduction to the science and technology of PMVs, and catalogue their many promised advantages over conventional systems for vaccine production. We then explore ethical issues for PMV development in three interrelated domains as depicted in Figure 1: PMVs as transgenic plants; PMVs as clinical research materials subject to regulatory approval; and PMVs as agents of global health—vaccines developed specifically for use in developing countries.

THE HISTORY, SCIENCE, AND TECHNOLOGY OF PLANT-MADE VACCINES

In 1990, the World Health Organization emphasized the need for new technologies to advance immunization programs. The hope was that new

technologies would produce vaccines for diseases with limited preventative options, and improve existing vaccines by reducing cost and resolving logistical difficulties. In particular, new technologies might eliminate the need for needles during immunization, possibly generating heat stabile, oral, multi-component vaccines that require reduced or one-time administration. Several years earlier, Roy Curtiss III and Guy Cardineau began pioneering experiments using transgenic plants to produce vaccines (Curtiss 1999). Transgenic plants containing vaccines are created through integration of new DNA into a plant cell, which is then regenerated into a whole plant. The vaccine can be produced in all tissues of the plant, or targeted to be selectively produced in certain tissues such as the fruit, tubers, or roots. The plant is multiplied through seed or cuttings and grown in a controlled field or greenhouse, or produced through cell culture methods where plant cells in liquid solution can be grown in a bioreactor similar to other fermentation-style systems. The desired tissues are harvested from the plant and processed by methods such as freeze-drying and grinding to achieve a powder for delivery to humans, or recovered from the bioreactor and processed to a high purity. The potential advantages of producing vaccines by this process are shown in Table 1. Note that several of these putative advantages depend on tremendous assumptions, namely that plant-made vaccines will fare better in the court of public opinion than other genetically modified plants; that PMVs will indeed prove both safe and effective through ethically designed and executed clinical trials with appropriate human populations; and that it will prove economically feasible for pharmaceutical companies or major philanthropic organizations to invest extensively in R&D beyond the proof-of-principle stage and that coordinated action problems in distribution can be resolved. We explore ethical and pragmatic dimensions of these assumptions in the ensuing sections.

The early innovative thinking by Curtiss and Cardineau led to the first patent application in this field in 1988 (Curtiss and Cardineau 1990). They achieved expression of the *Streptococcus mutans* surface protein A in transgenic tobacco, and then immunized mice orally with the plant material. The transgenic tobacco was successful in inducing antibody responses which were reactive against the pathogen. The Curtiss group also created transgenic alfalfa for expression of the Enterotoxigenic E. coli heat labile toxin B subunit (LT-B), and successfully induced both mucosal and serum antibody responses (Curtiss 1999). Table 2 provides a timeline of the early developmental events and clinical trials which have been achieved since 1986. There are several aspects regarding the technical status of this technology which can be filtered from Table 2. There has yet to be highly efficacious demonstration in humans with a feasible prototype material—raw, unprocessed materials will not be acceptable to vaccine recipients or consumers. Despite the perception that oral immunization with PMVs will induce mucosal immunity (for enhanced protection

Table 1. Putative Advantages and Disadvantages of PMVs Over Conventional Vaccines, Especially for Achieving Global Health Objectives. (Adapted from Kirk and Webb 2005)

Advantages	Disadvantages
Known	Known
Oral delivery (no injection)	Long development time to establish the platform technology
- reduced cost of needles and highly trained staff	Plant production is restricted to regulated field sites or greenhouses
- remove risk of infection at site of injection	Negative public opinion of GM plants
- greater adherence to vaccination program by recipients	Potential for induction of oral tolerance
No contamination with blood or culture products	Potential for induction of allergenicity by oral adjuvants
Protein glycosylation better than other systems	No financial support from pharmaceutical industry for development
Product stability at ambient temperatures for several years	More difficult to administer to infants
- remove cost of refrigeration during distribution	No approved oral adjuvants to aid in product development
- ease of stockpiling at remote locations	Disease targets are limited to subunit antigen strategies
- reduce wastage of product by heat or contamination	Complex intellectual property environment
Perceived	
Low cost of production	
Less demand for oral toxicity testing	
Less demand for microbial limits testing	

Table 2. Timeline of Early PMV Innovation and Clinical Development, 1986–2005. (HBsAg, Hepatitis B Surface Antigen; IgA, Immunoglobulin A; IgG, Immunoglobulin G; LTB, Enterotoxigenic *E. coli* Heat Labile Toxin B Subunit; NVCP, Norwalk Virus Capsid Protein; PCT, Patent Cooperation Treaty; RVgp, Rabies Virus Glycoprotein; WIPO, World Intellectual Property Organization)

Year	Technology development				
1986	Conception of strategy by Curtiss & Cardineau to produce vaccines in transgenic plants (see Curtiss 1999)				
1988	First plant expression data obtained. PCT patent application filed; US patents issued in 1997 (patents US 5,679,880; US 5,686,079; US 6,194,560)				
1990	First animal immunogenicity data obtained. Patent application published by WIPO (see Curtiss & Cardineau 1990)				
1992	First peer-reviewed publication describing plant expression of antigens; HBsAg production in tobacco (see Mason et al. 1992)				
1995	First peer-reviewed publication describing immune response in animals; LTB production in tobacco (see Haq et al. 1995)				
	Clinical Development (Dose of antigen)	Trial Location	Formulation and Schedule[1]	Volunteers	Immunological Response[2]
1997	First human clinical trial (370 υg–1570 υg LTB)	Baltimore, MD, USA	100 g raw, diced potato Days 0, 7 and 21	10 experiment 4 placebo control	100% IgG conversion 50% IgA conversion
1998	Second human clinical trial (215 υg–751 υg NVCP)	Baltimore, MD, USA	100–150 g raw, diced potato Days 0, 7 and 21	20 experiment 4 placebo control	20% IgG conversion 30% IgA conversion
1998	Third human clinical trial (0.15 υg–1 υg HBsAg)	Poland	150–200 g raw lettuce leaves Days 0 and 30–60[3]	3 experiment 2 placebo control	67% IgG conversion – IgA not evaluated
1999	Fourth human clinical trial (640 υg–1060 υg HBsAg)	Buffalo, NY, USA	100–110 raw, diced potato Days 0, 14 and 28	33 experiment 9 placebo control	48% IgG conversion – IgA not evaluated
2001	Fifth human clinical trial (84 υg–700 υg RVgp)	Philadelphia, PA, USA	20–150 g raw spinach[4] Days 0, 14 and 28	14 experiment 10 placebo control	21% IgG conversion 0 % IgA conversion[5]
2003	Sixth human clinical trial (1000 υg LTB)	Baltimore, MD, USA	2.1g corn meal with 5 oz water Days 0, 7 and 21	9 experiment 4 placebo control	78% IgG conversion 44% IgA conversion

[1]Some studies compared the delivery of 2 or 3 doses, in which case, the middle dose is a placebo formulation for the 2-dose group.

[2]For this analysis, seroconversion is considered as at least 4-fold increase in antigen-specific antibody titers after vaccine administration.

[3]The second dose was given within 2 months of the first dose.

[4]The vaccine formulation was created by infection of spinach with alfalfa mosaic virus as the vector for antigen production.

[5]This study was conducted in 2 groups with different study designs. Three volunteers were boosted in group 1 (n = 5) by consuming transfected spinach after receiving commercial vaccine; no volunteers showed antibody responses in group 2 (n = 9) after consuming spinach vaccine prior to receiving commercial vaccine; IgA responses are indicated but 4-fold increases are not evident and therefore a 0% response is listed.

against enteric diseases particularly), the immunological success of these trials as judged by IgA conversion has been disappointing. As noted by Kirk and Webb (2005), the next major milestones for this technology are the conduct of a Phase II clinical trial with a realistic product definition, and the demonstration of protection against disease in human volunteers. We conclude that any proposal for broad acceptance of this technology in developing countries is grossly premature without significant improvements in technical and economic feasibility, neither of which is obvious at this point in time.

The image of how plant-made vaccines would be made, distributed, and consumed, has changed dramatically over time, beginning with the paradigm of "edible vaccines". Based on newspaper headlines but also the pronouncements of scientists, many initially imagined that eating a genetically modified fruit or two, or perhaps a bowl of new-fangled corn flakes, would vaccinate the diner against their disease of choice. Throughout the 1990s, researchers proposed widespread, local production of such edible vaccines, conjuring images of the world's poorest people consuming vaccines through fresh produced obtained from selected farmers or even from their own garden (Prakash 1996). But as researchers elaborated the technology, it became evident that in order to control the level of exposure (dosage), there would need to be restrictions on delivery. The paradigm has since evolved further away from such images of edible vaccines, such that PMVs are now envisioned as prescribed pills rather than as fresh produce. Experimental PMVs today are products derived from batch-processed, freeze-dried, plant tissue; they are often not recognizable as a plant material, but are rather a powdered material packaged as a pill or capsule to be administered by a health care worker. This is in part-because because plant-made vaccines must meet standard requirements for pharmaceuticals and must be produced within an appropriate framework for quality assurance.

PMVs AS GM PLANTS

To date, there has been little discussion of ethical issues for PMV technology beyond the generalized issues raised in response to genetically modified (GM) plants (such as cotton) and GM foods (such as canola, corn, soybean, tomatoes and wheat). While genetic modification *per se* is not a focus of this discussion, it is worth briefly describing the ethical questions which have been raised previously and which provide some intersection with PMVs (Royal Society 1998; Nuffield Council on Bioethics 1999; Deane-Drummond et al. 2001; Royal Society 2002; see also, generally, Robert and Baylis 2003).

Figure 1 lists a number of ethical, social, and safety considerations associated with GM plants and GM foods. The safety concerns include worries about environmental protection from transgene escape, the potential for contamination of food commodities, potential effects on non-target species, and the demand for stewardship by technology developers. These environmental and human health risks have been thoroughly addressed by Kirk et al. (2005) specifically in relation to PMVs; these are wide-ranging concerns, and are important considerations for how and where PMVs should be grown.

While PMVs raise some of the same ethical and social issues as GM plants more generally, many of the ethical and social issues specific to GM foods (as a subcategory of GM plants) bear only superficially on plant-made vaccines. Nevertheless, the general (and generally negative) perception of GM foods may influence the social acceptability of PMVs. While not an ethical issue as such, recent experience with the social unacceptability of GM technologies in some quarters (Lassen et al. 2002) must be considered relevant to assessing future prospects for PMVs. Consider that a key reason that genetically modified foods have not enjoyed widespread public acceptance is that consumption of GM foods carries unknown risks, in a context of little or no motivation for the consumer to accept that risk. The major beneficiaries of GM crops tend to be farmers and seed companies, not supermarket shoppers. Moreover, the sanctity of the food supply was demonstrated to be a major cultural issue in conflict with the introduction of GM foods in Europe. In contrast to GM foods, the chief beneficiary of vaccination is the patient, given that the outcome of not receiving a vaccine could be contraction of an infectious disease. As with any vaccine, and in developed countries at least, the patient makes an initial choice as to whether the risk of being unvaccinated is an acceptable alternative.

Further, as against the case of GM foods generally, the safety—at least over the short term—of any commercially available PMV will have been established through human clinical testing (see "PMVs as clinical research materials", below). The first GM food approved for human consumption was the FLAVR SAVR tomato developed by Calgene and approved by the US Food and Drug Administration (FDA) in 1994. Unlike plant-made vaccines, the FLAVR SAVR tomato falls under the regulatory jurisdiction of food safety agencies (such

as the FDA-CFSAN; Center for Food Safety and Applied Nutrition) rather than drug agencies (such as the FDA-CBER; Center for Biologics Evaluation and Research), and was required to demonstrate a lack of acute toxicity in animal testing and justify the inclusion of genetic elements such as the selectable marker which conferred specific antibiotic (kanamycin) resistance as part of the plant engineering process. While the ethical questions pertaining to demonstration of public health and environmental safety may overlap considerably between foods and drugs, there is significant difference in the approval processes.[1] As described by Kirk et al. (2005) and below, detailed assessment of product safety and efficacy for PMVs must be completed within human clinical trials under extensive regulatory guidelines for new drug approvals. Moreover, unlike GM foods, PMVs will not be part of the food supply (particularly if grown as a suspension culture), but rather will be highly regulated public goods like all other vaccines, requiring a specific exposure regimen.

Even if plant-made vaccines do not fall prey to negative public opinions about genetically modified foods, there is already evidence of further resistance to new advances in genetic modification in agriculture. For instance, in a broad discussion of the 'third generation of agricultural biotechnology'—the era of plant-made pharmaceuticals and plant-made industrial products (such as novel enzymes and epoxies)—Stewart and McLean (Stewart and McLean 2004) cite electronic comments to *Federal Register* notices of regulatory changes for field tests of such crops. [The field tests are the domain of the United States Department of Agriculture, through the Animal and Plant Health Inspection Service.] A large number of the posted responses articulate concerns about risks to the environment and to the food supply; while many of these comments about putative risks may seem ludicrous to scientists, these perceptions form part of the backdrop against which new PMVs are developed and tested, and so should not be summarily dismissed (Kirk et al. 2005).

Among the more amorphous ethical objections to GM plants are concerns that altering living organisms with molecular methods involves transgressing divine laws ('playing God') and/or transgressing the laws of nature. As most new pharmaceuticals are largely engineered or modified using molecular techniques, it is unclear whether PMVs, designed to prevent disease, would be subject to the same moral objection as might arise in food biotechnology. At the very least, it is worth noting that a recent public consultation in New Zealand has revealed popular support for interspecies gene transplants—as long as the research is for medical purposes (Toi te taiao 2004). Even so, the notions that genetic engineering is unnatural, unwise, and hubristic persist, and may impact the regulatory environment for PMV development, testing, and eventual dissemination (Streiffer and Hedemann 2005).

Another concern is that if PMVs prove efficacious, marketing and regulatory personnel will need to choose how to label the vaccine; such a decision may have ethical and practical consequences. In the heat of the debate over GM foods, labeling of genetically altered foodstuffs was one of the basic demands from public interest groups to improve the ability for consumers to make an informed choice. Biotechnology companies were resistant to the introduction of a labeling system due to concerns that consumer choice would be predominantly based on reaction to negative publicity, the added cost of labeling food commodities, and the difficulty in assessing the transgenic content for all products. Pharmaceutical manufacturers do not routinely label vaccine vials or bulk packaging with information pertaining to the production system. Note that conventionally manufactured vaccines have been associated with a varied degree of risk (such as reversion, natural infection or contamination with other blood or cultured products), and plant production may actually reduce this risk. Accordingly, if safety were to be considered by the patient in a direct comparison with other vaccines, transgenic plants may be more highly favored than current systems such as live viruses, modified bacteria, or blood products. The level of concern by physicians for the safety of PMVs is unlikely to match the general response which occurred for GM foods, but potential manufacturers should consider labeling the vaccine to avoid accusations of non-disclosure. Physicians would then have the ability to inform the patient prior to vaccination, although it is unclear whether vaccinees would have any desire to learn about the production system, or whether PMVs would be considered to be better or worse than existing vaccines which are genetically engineered and/or known to cause adverse events.

A final consideration during commercial manufacturing is whether the disclosure of production

1. For further information regarding CFSAN approval of the FLAVR SAVR tomato, see http://www.cfsan.fda.gov/~acrobat2/bnfMFLV.pdf; for a complete listing of CFSAN consultations on GM food safety see http://www.cfsan.fda.gov/%7Elrd/biocon.html.

sites should be required, in the event that greenhouse containment is not utilized and field production is approved by the US Department of Agriculture (USDA) and Food and Drug Administration (FDA). In such circumstances, public interest groups would desire the details of production sites for monitoring gene escape and other social concerns. Unlike GM foods, however, a PMV should be provided much greater protection from the general public for the sake of protecting the manufacturer's investment in such a high-value commodity, and also to decrease the potential for deliberate contamination of the pharmaceutical product.

PMVs AS CLINICAL RESEARCH MATERIALS

It is evident that plant-made vaccines are not only genetically modified plants, but also genetically engineered clinical research materials. As such, they are subject to the regulatory process that applies to all investigational agents. As noted above, and discussed in more detail in the next section, the commercial feasibility of PMVs is yet to be proven. Until greater feasibility is demonstrated in the plant engineering and regulatory aspects of PMV technology and commercial justification can be formulated, it is worth considering the ethical issues of entering into human testing, and the ethical issues which may arise as a result of such testing.

In the quest for investigational new drug (IND) approval, researchers submit immunogenicity, safety, and toxicity data from preclinical research with animal models. Food and Drug Administration investigators evaluate these submissions, often requesting additional data, before granting an IND and, accordingly, license to proceed to human clinical studies. Human clinical trials are divided into four phases. *Phase I* trials are mainly to determine the safety of a drug, and usually involve a small number of healthy participants who are monitored for side effects and for data about how the body absorbs and uses the experimental drug. As summarized by Table 2, six Phase I trials have been conducted with PMVs, generally with very crude formulations (Tacket et al. 1998; Kapusta et al. 1999; Tacket et al. 2000; Yusibov et al. 2002; Tacket et al. 2004; Thanavala et al. 2005). Kirk and Webb (2005) have underscored the need to consider the complete path for product development before any further Phase I clinical trials. *Phase II* trials are for drugs that have so far proven safe in healthy volunteers, but now must be determined both to be safe and to show promise of being effective. A Phase II trial involves several hundred participants, whether members of the general population or the target population (depending on the disease and other factors); in the case of vaccines, immune response markers or protection from disease are used as the clinical endpoints. Only about 1/3 of all experimental drugs successfully pass both Phase I and II trials, and enter into the next phase of trials. No PMVs have yet progressed to Phase II clinical testing. A *Phase III* trial usually involves thousands of participants; they are monitored over a period of many months to several years to further ensure the safety and efficacy of the experimental drug. Only after a drug passes Phase III can it be marketed and sold. If the FDA approves the product, a *Phase IV* clinical trial may be implemented to further observe the safety and efficacy of the product once introduced to the market.

Among the most important potential welfare risks for patients are that PMVs may cause oral tolerance (defined as loss of immune sensitivity and ability to mount response to the disease itself), or, when used in conjunction with adjuvants to increase immune response through oral delivery, PMVs may induce allergic reactions to other dietary proteins. These are both hypothetical effects; we consider them improbable with just 2-3 doses of an orally delivered vaccine. But, if such negative reactions were to manifest, they may be significant for a human subject, especially in terms of dietary tolerability. The risk of ingesting GM plants is unknown and very difficult to quantify, though to date there is little evidence that ingesting GM crops is actually harmful to humans (Jaffe 2004).

One of the perceived advantages of producing vaccines in plants is the expectation that the level of required safety testing would be greatly reduced. Many substances in our current diet are considered by the FDA as Generally Regarded as Safe (GRAS), based on long term acceptance in the community and the absence of any significant negative effects on human health. Substances which are granted GRAS status are generally exempt from toxicology testing, even though their use by alternative routes (such as by injection) might have very high probability of causing adverse effects. Until recently, PMVs entering Phase I clinical trials under supervision of the FDA have not required the same level of toxicology and pharmacology required for other new drugs.

As described by Kirk and Webb (2005), the FDA has recently requested that additional toxicology testing be conducted on a new formulation which comprises a single transgene (previously tested in human clinical trials) and two existing

food substances (tomato, quillaja saponaria extract) which both have GRAS status. There are a number of important ethical issues that arise in this situation. First, the FDA prefers that animals should be exposed to the same dose that is intended to be delivered to human volunteers. In this case, a 4 g dose of powdered transgenic tomato is proposed to be delivered in gelatin capsules (Kirk, unpublished studies). As a suitable protocol for acute toxicology, 4 g of the material should be delivered by gavage to rats, four times over a period of four weeks. Four grams of powdered tomato is equivalent to approximately 64 g of raw tomato prior to dehydration, which is approximately 20–25% of the body weight of a rat. An equivalent extrapolation of exposure per body weight in humans would require human volunteers to consume the equivalent of 15–20 kg of tomato fruit. This dose range is approximately 10-fold higher than that undertaken by Calgene in acute toxicity studies (also in rats) to demonstrate safety of the FLAVR SAVR tomato as a food commodity. It remains to be seen whether a rat will respond adversely to 4 g of powdered tomato; however, it is not difficult to imagine that the equivalent exposure in humans (to this volume of any single food substance) might certainly cause a wide range of adverse reactions. This raises a dilemma in how to conduct toxicology testing in a practical manner so as to gain safety data beyond reasonable limits, but without forcing impractical comparisons between humans and animal models when the vaccine is delivered orally.

Second, it is interesting to speculate about the ethical dilemmas which might arise if adverse reactions are observed in rats. Proteins such as Lyc e 1 and Lyc e 2 are allergens known to be present in tomato (Westphal et al. 2003; Westphal et al. 2004). If extensive allergenic reactions or even toxigenic responses to proteins such as these are observed in the rats, are researchers obligated to inform the FDA of potential consequences of ingesting tomato in the human diet, even though the toxicology results are not otherwise normally reported outside of an IND? Is FDA then obligated to conduct additional toxicology testing and, if so, what level of consumption should be required by the animal model? It is probable that FDA would use a toxicology protocol where exposure is metered by bodyweight extrapolation, which raises the question why the same standard should not be applied for oral pharmaceutical testing. The different protocols place a higher and severe standard for demonstration of safety on pharmaceuticals, possibly at the risk of discontinuing technologies which cannot meet the criteria. The inconsistent standards for foods and pharmaceuticals should be of concern for vaccine regulators, especially since PMVs are likely to be consumed just 2–3 times, compared to the same substances which might be consumed daily in the general diet. Developers of PMVs would make the argument that more appropriate standards should be applied; however, public agencies and opponents of pharmaceutical companies are likely to be critical of any reduction in safety criteria.

Regulatory agencies should consider reducing the current standards for toxicology testing for oral vaccines such as PMVs, to avoid inconsistencies with existing dietary practices and to reduce the potential that PMV technology will be rejected despite extensive safety for the same materials when consumed as food. Regulatory agencies should also consider whether production standards and prototype products should be consistent worldwide, or whether regional allowances for production methods should be made to account for the relative disease and economic environments where PMVs might be used.

Even after an IND has been approved by the FDA for clinical trials, researchers must seek approval from an ethics review committee (an Institutional Review Board or IRB in the United States) to proceed with enrolment of human subjects. Synthesizing and interpreting a large and complex international regulatory and ethical literature, Emanuel et al. (2000) have clearly articulated the ethical conditions for clinical research: social or scientific value; scientific validity; fair subject selection; favorable ratio of risks to potential benefits; independent ethics review; valid and informed consent; and respect for subjects. In the case of PMVs, the small ethics literature to date has focused only on risk assessment and informed consent (Castle 2003; Castle and Dalgleish 2005; Kirk et al. 2005; but cf. Kirk and Robert 2005). Many commentators appear to assume both the scientific and social value of PMVs, and even argue rhetorically in favor of the development of this technology on putatively moral grounds (e.g., Daar et al. 2002; Acharya et al. 2003). In the remainder of this section and the next, we explore ethical aspects of the social value, scientific value, and scientific validity of PMV trials, and attend to several 'upstream' ethical determinants of ethical PMV trial design and conduct. Given the inevitability of unknown risks in any clinical research involving human subjects who receive an experimental drug, device, or treatment, what is the

extent of the scientific and social justification that should be required before exposing human subjects to the drug, device, or treatment?

The scientific value of research will be defined in many different ways depending upon one's definition of science, perhaps as any advancement in knowledge, or perhaps, in a more applied vein, as a means for derivation of particular technologies. Our understanding of the social value of research is also subject to variation depending on the sorts of social values that are highly esteemed; for instance, there may be disagreements between those who value commercial innovation and those who value health outcomes. By contrast, the scientific validity of research may be seen to be less prone to divergent interpretations: ethicists, biostatisticians, and others have expended great effort in interpreting the validity of research as a matter of the disturbance of clinical equipoise (Freedman 1987; Miller and Weijer 2003), such that only research that disturbs equipoise (in one way or another) is to count as scientifically valid research. But of course the social, institutional, and economic contexts of research together help to determine what counts as a valid research question in the first place, such that scientific validity cannot be so easily disentangled from the social and scientific value of the research.

Additionally, we should consider potential conflicts of interest which can lead to PMV clinical testing regardless of ultimate feasibility. The opportunity for academic notoriety and advancement is significant for researchers who wish to remain at the forefront of any new technology. It is not difficult to imagine that reputation, compensation, or grant opportunities might outweigh commercial or even technical feasibility as the driving force for researchers to rush into human clinical testing (the general pattern is described by Krimsky 2003). Indeed, given the data summarized in Table 2, one might judge that already researchers have jumped the gun with human subjects research for PMVs. An obvious, but disingenuous, objection to this view is that clinical evaluation of these materials contributes to basic science regardless of the ultimate feasibility. Basic science as described by some philosophers of science is conducted for the sake of understanding nature and not swayed by considerations beyond the simple pursuit of knowledge. But in the more recent images of science contemplated by Kitcher (2001), among others, a purely epistemic approach can be corralled only when the researcher has a clearly justified lack of interest in the practical applications. To consider clinical testing as an instance of basic science would seem incongruous with the PMV technology which is intentionally proposed as a practical solution. In light of the high potential for conflict of interest within such an unproven, yet high-profile technology, and given the particular risks of clinical testing with PMVs, a deeper contemplation of the ethical issues is required.

PMVs AS AGENTS OF GLOBAL HEALTH

Beyond their instantiation of genetically modified plants as clinical research materials, plant-made vaccines are also specifically described as a potential agent of global health. Almost all popular and scientific literature describing PMVs proposes that primary use of the technology will be in developing countries (e.g., Daar et al. 2002; Acharya et al. 2003; Vermij 2004), largely because of the initial strategies for production on local acreage with anticipated reduction in costs. Castle and Dalgleish (2005) have recently claimed that the time is ripe to proactively cultivate the social reception of plant-made vaccines, in order to facilitate the putative global health benefits of PMVs. While we agree to some extent, we also suspect that Castle and Dalgleish have underestimated the enormity and complexity of the project of using biotechnologies to improve global health and reduce global health inequities; particularly the amount of product and clinical development which is still required to meet the basic proof of feasibility for safe and effective use of this technology in humans. The questions of whether, how, when, and where to further investigate PMVs in human subjects must be posed within the context of their promised use. Only by framing and exploring the ethical issues in the context of the developing world can we get beyond the rhetoric and toward the realities of the genuine potential of PMVs to achieve global health outcomes.

As discussed by Kirk and Webb (2005) it is likely that a majority of the product development and clinical testing of PMVs will occur in the public research sector. Use of public funds and human clinical testing will raise new questions of ethics for this technology. No clinical testing of PMVs has yet occurred in developing country locations, and funding from US public agencies has borne the greater part of development and testing costs to date. One ethical consideration in further developing this technology is when to move clinical testing outside of North America and whether US public funds should continue to support a technology whose benefits

are promised to be felt largely in non-developed nations.

Industrialized countries might gain some benefit by reducing infection for travelers and reduced burden of disease globally; however, assuming technical and commercial feasibility, the vaccine recipients in developing countries would stand to receive the greatest direct benefits. We do not wish to suggest that pharmaceutical developers in the US and Europe should starve R&D for developing world vaccines, but contemplation of justice and the distribution of benefits indicates a current ethical mismatch in the use of US funding and US citizens for human clinical testing for the sake of vaccines intended for use almost exclusively outside of the US. Conclusions regarding safety and efficacy may also be quite different if these vaccines are tested in developed countries where natural exposure to the disease (especially diseases contracted by the oral route) is low, routine exposure to GM foods is much higher, and dietary intake is more diverse. The effects of these parameters on oral immunogenicity or allergenicity are not well known. As demonstrated by the worldwide withdrawal of Rotashield™ (Wyeth Laboratories Inc.) due to higher rates of intussusception in developed countries, application of new vaccines may involve different thresholds for acceptable adverse reactions depending on the disease burden of different locations. There is inherent risk when vaccine development and testing occurs in isolation or separation from the most applicable locations. Conversely, there is much public criticism when pharmaceutical developers conduct clinical testing in developing nations where there may be less regulatory oversight (Angell 1997; Varmus and Satcher 1997; Resnik 1998). Researchers in the US might argue that the location of Phase I and II trials is not particularly important, since the knowledge gained is broadly applicable to oral immunology and vaccine development in general, and that both short and long term safety monitoring are more difficult in remote testing locations.

The issues described so far in this section are not unique to vaccines made from plants. However, the probable dependency on public funds for R&D well beyond the stage when conventional vaccines might be normally supported by the pharmaceutical industry will introduce an important ethical concern regarding how developed countries might be subsidizing the reduction of disease burden for other countries, and more so for PMVs than for other health technologies—including other health technologies that may in fact have a much greater impact on global health.

The availability of public funds for clinical testing is expected to be critical for further development of PMV prospects (Kirk and Webb 2005). We believe that PMVs should be further supported by efficient use of public funds, but the clinical testing sites should be aligned with locality for the disease of interest on a case-by case basis to derive the maximum value from those funds. For certain diseases such as diarrheal diseases, clinical testing would be best conducted in regions where diarrheal infection is a common problem. Similarly for a disease such as malaria, clinical testing in tropical regions of the developing world would be preferred as a compromise between the distribution of risk and benefits to participants, use of public funds, and attainment of safety and efficacy data in an authentic environment. That said, the ethical dimensions of research in developing countries, as discussed by many others, require further negotiation (for a start, see, e.g., Angell 1997; Varmus and Satcher 1997; Resnik 1998; Benatar 2001, 2002; Wendler et al. 2004). In the few cases where PMVs might be equally applicable to industrialized countries, such as norovirus or measles vaccines, there would be little ethical concern with conducting the clinical testing in the developed world.

One consideration for public agencies and philanthropic organizations is whether to fund PMVs to advance a prototype vaccine aimed at the developed world as the first vaccine target for this technology, rather than focus on the neglected diseases already discussed above. As an example, noroviruses are one of the leading causes of gastroenteritis, second only to the common cold in frequency of infection in North America, and a potential market of sufficient profit margin to attract corporate investors. A disease target such as noroviruses might be sufficient to entice pharmaceutical or bioscience companies to include PMVs in their R&D portfolio. This would provide the financial and manufacturing support which is essential to address many of the hurdles for a new technology during creation of the first product, which is not possible in the academic and non-profit sectors. It would seem counterintuitive to provide preferential funding to deliberately benefit industrialized countries through a technology which is proposed almost exclusively as a solution for poorer countries. Researchers in this field would be faced with criticism that the needs of the developing countries were placed second to commercial interests. As discussed by Resnik (2001),

pharmaceutical companies may be ignoring an important, though not absolute, moral obligation to devise affordable drugs for use in the developing world. It is unclear how the technology platform will be able to meet the enormous costs of new drug development in the first instance without corporate support. But maturation of this technology may actually enable pharmaceutical companies to discharge their moral obligation to assist developing countries meet their health needs (see also Resnik 2004).

Another consideration is whether PMVs should be manufactured to replace existing vaccines. More specifically, once the PMV technology is proven with release of a first product, health organizations might consider the subsidization of PMV development to replace existing injectible products made by other companies in order to attain greater advantages in product stability, oral administration and cost of goods. The ability to reduce the cost of individual vaccines would result in wider coverage on the global scale. But when use of public funds is contemplated as a means to out-compete existing companies, a wide range of business ethics issues intervene.

A further potential aspect of PMV production that will raise ethical concerns is the manner in which the plants might be grown in different regions of the world. Much of the current development by academic researchers is occurring under containment, typically in greenhouse facilities. The cost of greenhouse construction and maintenance may be a necessary part of the production cost for PMVs. It is estimated that the containment aspects will contribute to no more than 20% of the total cost per dose for PMV production (Kirk, unpublished data). Therefore, the many advantages of containment in dealing with environmental protection may be feasibly integrated for any PMVs intended for commercial sale in North America or other industrialized markets. While this is not expected to be demanded by regulatory agencies such as the USDA or the FDA, it is expected that product approval will be significantly simpler if containment is provided. Conversely, the cost of greenhouse construction either in the US or on location in a developing country is likely to be a significant burden to the overall affordability of the vaccines for developing countries. It is not clear whether regulation in developing countries would require containment as an essential condition of manufacturing. An ethical dilemma arises if different manufacturing standards are contemplated between first and third world locations. If absence of containment allows production of a malaria vaccine in Africa at a price that is affordable to UNICEF, is that of greater benefit and social justice than the alternative of imposing measures which might make manufacturing (and thus access) cost prohibitive? Given the very low margin of profit for vaccines in developing countries, this aspect could make a tremendous difference in the feasibility of manufacturing in those locations. Again we can draw comparison to Rotashield™, where standards in industrialized countries ultimately determined the fate of what might otherwise be a highly valuable vaccine in developing countries. Environmental and food safety concerns in the US and Europe may have the same effect in denying field production of PMVs in regions where they are of greatest need.

CONCLUSIONS

Our discussion has focused on plant-made vaccines as a new emerging technology that raises ethical issues with regard to the production of genetically modified plants, the development of pharmaceuticals, and the promise to achieve global health goals. While PMVs are not simply GM plants, ethical considerations germane to genetic modification of plants generally are nonetheless relevant. And while PMVs are not simply yet another vaccine under development, ethical considerations germane to the ethical design and conduct of pharmaceutical clinical trials are nonetheless centrally important. What is especially novel about PMVs is that the basic research and clinical trials are being conducted with the express purpose of improving health in developing countries, and this context raises numerous ethical issues that have been largely downplayed by proponents of PMVs, and overshadowed by the putative altruism inherent in global health promotion.

The commercial feasibility of PMVs is largely unproven, and the risk of human clinical testing with these materials is not insignificant (and many additional ethical questions may arise as PMV clinical development continues on the global stage). Public funds are likely to be required to advance the development of PMVs to the stage at which corporate investors will become involved. Clinical trials which are supported by public funds should be evaluated to ensure that disease target and locations are appropriate to gain the most value from those funds to promote technology development rather than further demonstration of proof of concept. In

parallel, we believe that demonstration of commercial feasibility of PMVs should be part of regulatory approval of clinical trials (Kirk and Robert 2005).

The potential for plant-made vaccines to improve global health may be enormous, but this depends entirely on whether plant-made vaccines actually work reliably, whether the crops can be grown in safe and appropriate ways, and whether the production of PMVs can be demonstrated to be commercially feasible, either for pharmaceutical companies, philanthropic organizations, or governments in either the developed or developing worlds. These contingencies—and many others—remain to be worked out, by ethicists, regulators, IRB members, policy makers, corporate executives, and the scientists themselves. The technology needs to be developed one step at a time, and promises of global salvation from infectious disease through plant-made vaccines are both entirely premature and morally bereft.

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