Plant Biotechnology in the Service of Human Health

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Plea for a Scientific and Technical Approach

It is a cliché to say that the poor countries are becoming poorer and poorer while the rich ones become richer and richer. Previous experience shows that it is an illusion to count on mankind's sense of solidarity to resolve this dilemma. And yet we are dealing with a problem that is real, vast, and urgent. The developed world uses its knowledge and technological opportunities to sustain and increase its wealth, and in the race for scientific and technical advances the "Third World" faces the serious risk of never being able to catch up. Seen in this light, it is understandable that some people have allowed themselves to be seduced by the notion that in order to achieve a better equilibrium in the world all that is required is to return to a "more natural" life in which science and technology no longer play a dominant role. It seems to us, however, that this vision is unrealistic because it does not take account of the fact that human nature pushes us toward competition and that the kind of solidarity that would compensate for, and mitigate, this competitiveness works successfully only on a small scale (e.g., within the family or clan) or in very special circumstances (e.g., wars, natural catastrophes).

Furthermore, this view ignores the fact that we could organize and direct science and technology to tackle the problems with which the "Third World" is confronted. Such an approach would be rational in its essense, and would put knowledge that has been worked out in the so-called "developed" world into the service of the "Third World."

To be sure, this approach is idealistic. Nonetheless, it might be workable, above all because it is one of the principal motives of

scientists and technologists to put their knowledge into practice, if society gives them the means to do so. Moreover, even if this knowledge and these techniques primarily serve the interests of the advanced societies that have developed them, they can – with relatively modest effort – also be redirected to serve the needs of the "Third World."

Here we intend openly to plead for a rational and functional approach to the problems of our planet, even if this approach is utilitarian. It is to be feared that the ancestral fear of the unknown is today being transposed onto science and technology, prompting us to ignore their potential. We, for our part, see science not merely as a source of marvellous discoveries, but also as a base from which we must try to find realistic solutions to certain of the major problems with which human society is confronted.

Plant Biotechnology in the Service of the "Third World"

The demographic explosion in the "Third World" and the pressure to satisfy growing needs result today in an overuse and an abusive use of natural resources. As a consequence we see a growing fragility of the environment which goes hand in hand with a decline in food safety and health of the population. One must make agriculture in the "Third World" more productive and more considerate of natural resources. One must also enable agriculture, at least in part, to satisfy the health requirements of these countries in an economical way that does not presume the creation of new or complicated infrastructure.

Since time immemorial, plants have served humans as a source of pharmaceutical products. This should also be so in the future, although in an unusual way.

To illustrate this point, we will take plant biotechnology as our example, i.e., the use of plants as "bioreactors" for the production of pharmaceuticals. In particular we will analyze progress in the field of the production of vaccines in cultivated plants, vaccines to protect against infectious diseases or to be used as a contraceptive.

Plants – An Economical and Effective Source of Vaccines

In March 1993 the following statement appeared in an American journal:[1] "Plant and plant viruses engineered to produce proteins that stimulate a protective immune response in people may someday serve as inexpensive sources for large quantities of antigens, which could then be purified for use in injectable or oral vaccines." At the limit, it might be possible to envisage the production of oral vaccines in edible plant tissues so that it would be sufficient for humans and domestic animals to eat these plants in order to be immunized. The author of the article cited above rightly emphasizes that for this approach to succeed, the plants must be able to assemble the antigens correctly, and the antigens must remain active after they have passed through the stomach in order to stimulate effectively the cells of the mucosal immune system.

Even if, at best, the idea of an "edible vaccine" can only be realized in the more or less distant future, we must now ask whether the goal can indeed be achieved. What advances have already been made and what questions remain to be answered?

It may be useful at this point to recall some fundamental aspects of immunization. A lexicon of technical terms is provided at the end of this article. Two types of immunization must be distinguished, "active" and "passive." In active immunization, one or more eliciting factors are administered and the recipient, human or animal, produces antibodies which in successful cases provide immune protection. In passive immunization, pre-produced antibodies are administered.

Historically, whole virus or bacteria, made safe by killing or attenuation, were first used for active immunization, later isolated toxins converted to innocuous toxoids. However, for several diseases, whole organisms cannot be used because they cannot be grown in culture (as in the cases of malaria and hepatitis B), or, even if inactive or attenuated, they are considered too dangerous (as in the case of the HIV virus, the causal agent of AIDS). Recent advances in medical biotechnology have made it possible to develop a new approach: the production of subunit vaccines. These contain only a part of the antigen (in itself non infectious) or fragments of antigen (immuno-

genic epitopes) from the infectious agent. They are produced by genetic engineering and subsequent cultivation of the cells (bacteria, yeast, or cells of insects or animals). It is clear that these vaccines are not capable of causing the disease against which they are supposed to protect. This approach is relatively expensive, but we shall see below that there is hope of overcoming this handicap through efficient and large-scale production of subunit vaccines in plants by presenting the immunogenic epitopes on the surface of a plant virus.

The main advantages of using cultivated plants as a source of antigens or immunogenic epitopes for the production of vaccines accrue at four levels:

1. Cost: It is easier to grow plants in large quantities and with traditional methods than to produce cells from bacteria, yeast or animals through fermentation or culture. In the case of "edible vaccines" no purification is required.
2. Safety: The production of vaccines in plants should eliminate the danger of contamination with agents pathogenic for humans and animals as these do not multiply in plants. Furthermore, plant viruses are not infectious for humans and animals.
3. Tradition: Through the use of plants that are traditionally consumed to produce vaccines, we can hope to respect the regional traditions of agriculture. In the particular case of "edible vaccines," humans and domestic animals could be immunized without fundamentally changing their eating habits.
4. Transport of vaccines: Given that fruits, seeds or other plant tissues (leafs or roots) in which vaccines could be produced can – within limits – be transported and conserved without refrigeration, their regional distribution should be relatively easy.

Still, it remains to be shown that the antigenic substances produced from various plants are truly capable of generating a protective immunological response and that, in the case of edible vaccines, their repeated consumption does not result in immunotolerance, i.e., that humans or animals who consume these vaccines continue to respond to the immunological stimulus.

In order to allow evaluation of the merits of producing vaccines by plant biotechnology, particularly in the "Third World," we will give a few examples of what has been realized until now.

The Production of Antigens in Plants for Active Immunization

Infection with the hepatitis B virus and the chronic liver disease that can follow present a major medical problem worldwide. In 1992 an American group showed that it is possible to produce a hepatitis B surface antigen in transgenic tobacco plants.[2] Tobacco is frequently chosen as a model for such experiments because it is particularly well-suited for the introduction and expression of foreign genes.[3] More recently, the same group demonstrated that the hepatitis B surface antigen produced in tobacco is capable of inducing, when injected into mice, the production of specific antibodies and of stimulating a cellular immune response through *in vivo* activation of T lymphocytes.[4] These results, though still preliminary, have encouraged researchers to try to express antigens in edible plants; thus potatoes producing hepatitis B surface antigen have been obtained.[5]

The Production of Antibodies in Plants

Although active immunization is generally preferable because it induces long-term immunity, passive immunization by administering protective antibodies may be valuable in certain instances. Thus, in Britain and the United States,[6] researchers have demonstrated that it is possible to produce antibodies in tobacco that can be added to toothpaste to protect against oral bacteria that are responsible for caries. Such findings are not trivial, but rather of fundamental importance because they show that plants are capable of producing and of correctly assembling complex molecules such as immunoglobulins. In this particular case, a type of immunoglobulin was obtained that is normally produced and secreted by the mucosal immune system.

It should be mentioned at this point that the production of antibodies in plants (sometimes called "plantibodies") has other important applications. In this way it should be possible to develop cultures that are less susceptible to attack by phytopathogenic agents simply by introducing antibodies (e.g., "single chain" antibodies)

that are capable of impeding the proliferation of such agents.[7] Moreover, the expression of specific antibodies in plant tissue should enable us to find answers to basic questions in plant physiology.[8]

The Utilization of Plant Viruses for the Production of Subunit Vaccines

Although traditionally vaccines have been produced from inactive or attenuated forms of infectious agents, one has tended increasingly to develop subunit vaccines. As already mentioned, these vaccines cannot cause the disease against which they are supposed to protect. Unfortunately, because of their reduced size, partial antigens are frequently only weakly immunogenic, i.e., the level of immune protection that they induce is insufficient, and hence they are ineffective vaccines. Their immunogenicity can, however, be increased by attaching the partial antigen to a larger carrier that facilitates the antigen's "presentation" to the immune system. Viral particles are often used for this purpose. While the use of human or animal viruses as carriers of partial antigens could be dangerous, no such danger arises from plant viruses since these are not capable of infecting humans and animals.

Indeed, plant viruses have properties that make them particularly interesting for the production of immunogenic epitopes (and thus potentially for vaccines). To begin with, due to their relatively simple structure, viral particles (virions) are very stable. Thus, certain plant viruses remain structurally intact and capable of infecting new plants after several years in dead or dried leaves. These virions are also very stable when they are in a semi- or highly purified state. Moreover, plants that have been infected with certain viruses produce viral particles in large quantities and it is relatively easy to separate those particles from the plant tissue in pure form. For example, in tobacco leaves that are infected by tobacco mosaic virus, the viral capsid (external envelope) accounts for 10-40 per cent of the total proteins, and it only requires a few hours to separate, in a three-stage process, these viral proteins in pure form. Finally, plant viruses that contain vaccines can be conserved and transported without refrigeration.

Tobacco mosaic virus[9] and cowpea mosaic virus[10] have been modified by genetic engineering so that they present immunogenic epitopes on their surfaces in the form of linear extensions of the coat proteins or loop structures that protrude from the surface of the virion.

A British research team has developed a chimeric cowpea mosaic virus that is genetically stable and capable of providing large quantities of viral particles containing an antigenic site of the human virus HRV-14 (the rhinovirus responsible for colds) in the infected plants. The antigenic site that is inserted into the plant virus envelope induces the production of specific antibodies in rabbits.[11]

Infection with the HIV virus (AIDS virus) is the cause of major problems throughout the world and in particular in certain "Third World" countries. Despite considerable efforts, no protective vaccine has yet been produced. All vaccines prepared from material that might contain an infectious form of the HIV virus are potentially dangerous. Antibodies directed against certain partial antigens from the viral coat neutralize virus infectivity *in vitro* and neutralizing antibody can protect against experimental infection in chimpanzees *in vivo*. Researchers at the British John Innes Institute and at the University of Warwick have succeeded in expressing an immunogenic epitope of the HIV virus in cowpea using a cowpea mosaic virus vector. A purified preparation of the chimeric virus, injected into mice, elicits antibodies that are capable of neutralizing the HIV virus *in vitro*.[12] It might be possible to construct plant virus vectors expressing several immunogenic epitopes of the HIV virus and thus increase the efficacy of the vaccine.

A vaccine effective against the HIV virus must induce not only circulating antibodies with a view to systemic protection, but also secretory antibodies at mucosal surfaces of the genital tract. One could even imagine that such a vaccine – composed of immunogenic plant virus particles – could be effective if administered orally. In the case of the simian immunodeficiency virus (SIV), it has been possible to show that an orally administered vaccine, microencapsulated to prevent degradation in the stomach, induces a protective immune response against vaginal challenge with SIV in rhesus monkeys.[13]

Rapid population growth engenders problems of the greatest urgency in the "Third World." It is therefore encouraging that a

team of researchers at the Scripps Research Institute in La Jolla, California, is attempting to develop a plant-based contraceptive vaccine.[14] One of the essential steps in fertilation is the attachment of the sperm to the egg. In mice the gene responsible for one of the proteins essential for this attachment is known. A fragment of this gene was inserted into tobacco mosaic virus and the modified plant virus preparation was injected into mice. These mice produced specific antibodies that could be shown to bind the surface of the egg, thus hindering subsequent attachment of the sperm and hence preventing fertilization. In order to know whether this vaccine is effective as a contraceptive, we will have to wait for the results of future research.

Edible Vaccines

In the above-mentioned examples we have described the progress that biotechnology has made in the production of antigens or antibodies in plants, potentially effective as vaccines. However, the route of administration must also be considered. Clearly, if the vaccine is to be injected, it is essential that the antigens or immunogenic epitopes be completely separated from all other components in the plant. Purification is just as necessary as when the vaccine is produced in bacterial or animal cells. As already noted, purification makes vaccine production very expensive. In contrast, and by virtue of their physical characteristics, the purification of plant virus particles is often easy. This is one of the reasons that justify choosing plant viruses as vectors for the production of vaccines to be injected.

With edible vaccines it may be possible to avoid purification altogether. This being said, the vaccines must be produced in plants that humans and animals are used to consuming. The chosen edible plants must be consumed raw, must be appetizing and be part of the habitual diet. It is for this reason that the American researchers who are trying to produce edible vaccines for humans have focused on their production in bananas.[15]

Active oral immunization is particularly important in the case of enteric diseases, for example cholera or other diarrheal diseases

such as that caused by enterotoxigenic *Escherichia coli*. These diseases are often dangerous, especially to children in the "Third World." Recently a team led by C.J. Arntzen succeeded in expressing a subunit vaccine of the enterotoxin (LT-B) of *E. coli* in potatoes. Mice fed these transgenic potato tubers developed specific antibodies in serum and secretory IgA immunoglobulins in mucosal tissue.[16]

However, it remains to be demonstrated that the stimulation of the immune system by the edible vaccine offers effective protection. To achieve this one must prevent the antigens or immunogenic epitopes of edible vaccines from being degraded in the stomach. Moreover, if the edible vaccine has not been purified, one cannot exclude the possibility that the plant's components may interfere with the induction of effective immunological protection. To get around these problems, one will probably have to produce antigens in large quantities in plant tissues. An effective vaccine might also be obtained by targeting the expression of the immunogenic epitopes to the plant's chloroplasts. This approach would, furthermore, have the advantage of protecting the antigens against attack by the gastric fluids. Another problem to be resolved is that of immunotolerance. It is well-known that the immune system is rarely stimulated by proteins that are commonly found in the diet. It is to be hoped that by presenting antigens on the surface of plant virus particles it will be possible to avoid immunotolerance.

Conclusion

Plant biotechnology could play a major role in the service of the "Third World," not only by rendering its agriculture more productive and less dependent on phytosanitary aids from the developed world, but also by allowing the "Third World" to respond better to the problems of human and animal health and of population growth with which it is confronted. The "Third World" could thus benefit from the research and the investments taking place in the developed world. This requires that national and international institutions direct their research in the field of plant biotechnology

toward the health problems of the "Third World" and toward the use of the crops that are part of their agronomic and dietary traditions. However, it must be admitted that, at the moment, biotechnology is not moving in this direction.

A policy of redirection seems to us realistic as economic interests push the developed countries to invest in biotechnological research in any case, and because it would take no more than relatively modest means for this research to benefit "Third World" countries as well. This viewpoint is particularly valid for plant biotechnology, as we have been tried to show by focusing on potential applications of this technology to medical problems in the "Third World."

We believe that the main ethical question raised in this context is that of the consequences of neglecting the opportunity to contribute to the improvement of health and to the regulation of population growth in the "Third World." There is no doubt that many researchers in our countries are capable of achieving these objectives if they are given the means. Moreover, this task will engage their sense of responsibility and, indeed, will not fail to inspire them.

Lexicon

Active immunization: Stimulation of the immune system with an appropriate antigen. This stimulation induces both an immediate response and immunological memory, thus providing long-term protection.

Antibodies: Proteins produced in response to an antigen that combine specifically with this antigen.

Antigen: Entity that induces the formation of an antibody.

Chimeric virus: Modified virus containing an exogenous protein.

Chloroplasts: Organelles that are responsible for photosynthesis (light-induced biosynthesis) in plant cells.

Immunogenic epitope: That part of the antigen that actually elicits the immune response, e.g., the formation of antibodies.

Immunotolerance: A process that eliminates or suppresses all immune cells (lymphocytes) that could respond to constituents considered as non-foreign.

Passive immunization: Passive transfer of immune protection through the injection of specific antibodies. This immunization does not confer long-term protection.

Phytopathogenic agents: Biological agents (viruses, bacteria, fungi) responsible for plant diseases.

Protective antibodies: Antibodies that confer protection against a disease.

Single-chain antibodies: Antibody fragments produced by genetic engineering that contain a single chain of amino-acids.

Subunit vaccine: Vaccine in which the eliciting factor is a fragment of the complete antigen molecule.

Virion: Complete virus particle.

Notes

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