Can plant biotechnology help break the HIV–malaria link?

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A B S T R A C T
The population of sub-Saharan Africa is at risk from multiple, poverty-related endemic diseases. HIV and malaria are the most prevalent, but they disproportionately affect different groups of people, i.e. HIV predominantly affects sexually-active adults whereas malaria has a greater impact on children and pregnant women. Nevertheless, there is a significant geographical and epidemiological overlap which results in bidirectional and synergistic interactions with important consequences for public health. The immunosuppressive effects of HIV increase the risk of infection when individuals are exposed to malaria parasites and also the severity of malaria symptoms. Similarly, acute malaria can induce a temporary increase in the HIV viral load. HIV is associated with a wide range of opportunistic infections that can be misdiagnosed as malaria, resulting in the wasteful misuse of antimalarial drugs and a failure to address the genuine cause of the disease. There is also a cumulative risk of toxicity when antiretroviral and antimalarial drugs are given to the same patients. Synergistic approaches involving the control of malaria as a strategy to fight HIV/AIDS and vice versa are therefore needed in co-endemic areas. Plant biotechnology has emerged as a promising approach to tackle poverty-related diseases because plant-derived drugs and vaccines can be produced inexpensively in developing countries and may be distributed using agricultural infrastructure without the need for a cold chain. Here we explore some of the potential contributions of plant biotechnology and its integration into broader multidisciplinary public health programs to combat the two diseases in developing countries.

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I n t r o d u c t i o n
HIV/AIDS and malaria are devastating diseases that in 2010 alone caused 1.8 and 1.2 million deaths, respectively, in sub-Saharan Africa alone (Murray et al., 2012; http://www.unaids.org/en/media/unaids/contentassets/documents/unaidspublication/2011/jc2216_worldaidsday_report_2011_en.pdf, 2011). Both diseases are associated with poverty, particularly in developing countries, and place an immense burden on public health, productivity and the economy in general (Hahn et al., 2000; http://www.rbm.who.int/globaladvocacy/docs/gm_guide-en.pdf). Because both diseases are prevalent in tropical and sub-tropical regions, they overlap geographically and there is a persistent danger of co-morbidity, particularly in malaria endemic regions with large HIV-positive populations, such as sub-Saharan Africa. Co-morbidity is promoted by the tendency of both diseases to disproportionately affect
the poorest communities, reflecting the lack of education, nutrition, information, prophylactics (e.g. malaria nets, condoms) and effective drugs (Yuan et al., 2011).

Both diseases increase the severity of each other’s symptoms and can add complications to both diagnosis and therapy (Alemu et al., 2013). For example, although malaria does not increase the frequency of sexually-transmitted HIV infections, it is often treated by blood transfusion which increases the risk of HIV transmission via contaminated blood. Malaria infection is more common and more severe in HIV-positive individuals because HIV has an immunosuppressive effect, whereas acute malaria can temporarily increase the viral load in HIV patients for the same reason, increasing the risk of AIDS (Kublin et al., 2005). HIV also tends to promote opportunistic febrile diseases, which makes it difficult to achieve an accurate malaria diagnosis. Co-morbidity reduces the effectiveness of both antimalarial and antiretroviral drugs, and co-administration can increase the risk of drug-related toxicity (Brentlinger et al., 2006).

The HIV and malaria disease cycles

HIV/AIDS is typically a sexually-transmitted disease, although the virus can also be transmitted via contaminated needles or blood stocks, and by mother-to-child transfer during pregnancy, childbirth or lactation (http://www.unaids.org/en/media/unaids/contentsets/documents/unaidspublication/2011/jc2216_worldaidsday_report_2011_en.pdf). The virus is taken up by T-cells, macrophages and dendritic cells expressing the surface receptor CD4, which is recognized by envelope glycoprotein 120 (gp120). Subsequent conformational changes in gp120 initiate virus binding to the co-receptor (CCR5 or CXCR4) and fusion between the cell membrane and the virus core, mediated by envelope glycoprotein 41 (gp41) (Clapham and McNught, 2001). HIV carries two copies of a single-stranded RNA genome, which are reverse transcribed after uncoating to generate a double-stranded DNA provirus that integrates into the host genome. The integrated provirus is transcribed by cellular RNA polymerases to generate full-length progeny genomic RNAs and spliced mRNAs encoding viral proteins (Shors, 2011). After export from the nucleus, the mRNAs are translated and the resulting proteins are processed by a viral protease before coining with the genomic RNAs to form new virions. These bud from the membrane, acquiring lipid envelopes in the process, and become infectious particles (Este et al., 2008).

The severest form of malaria is caused by the unicellular parasite Plasmodium falciparum, which is transmitted to humans by female mosquitoes during a blood meal (http://www.cdc.gov/malaria/about/biology/mosquitoes/) resulting in high levels of parasitemia (Miller et al., 2002). The parasite is introduced as an infectious form (sporozoite) which travels through the blood to the liver where it multiplies asexually to produce multiple merozoites, which can invade red blood cells (http://www.niaid.nih.gov/topics/malaria/pages/lifecycle.aspx). These may continue to multiply asexually, prolonging the infection, but can also form gametocytes that are taken up by feeding mosquitoes and complete the cycle by fusing to form zygotes (ookinetes) that develop into new sporozoites. Clinical malaria occurs during the asexual blood stage when the parasite leaves the liver and begins to invade and multiply within red blood cells.

The immune response against malaria is not fully understood, although both humoral and cell-mediated immunity are involved and various T-cell subsets are required (Troye-Blomberg et al., 1999). Because HIV infects and destroys CD4+ T-cells, which regulate other immune cells, as well as the macrophages that normally destroy parasites by phagocytosis, HIV infections are likely to reduce the impact of adaptive immunity against malaria and cause prolonged and more severe infections, particularly in individuals who are already immunocompromised. Some studies have also shown that malarial antigens and pigments released during cell-burst induce the production of cytokines that promote HIV replication (Hoffman et al., 1999). However, other reports have shown that malarial pigments can inhibit HIV. For example, the processing of heme by P. falciparum releases the derivative pigment hemozoin that is taken up by macrophages, and this interferes with the replication of HIV-1 (Dio et al., 2009). Furthermore, the different clinical manifestations of malaria are associated with different states of immune dysfunction (Akamori et al., 2000). These data suggest that there are multiple points of intersection between the two diseases.

The impact of HIV and malaria in different population groups

Many investigators have studied the association between HIV and malaria in different population groups (e.g. children, adults, pregnant women and their infants) and have compared stable and unstable malaria transmission areas. The balanced or elevated transmission of P. falciparum indicates a stable transmission area, in which malaria is endemic regardless of environmental changes, and this is often accompanied by the development of immunity in the population. The low-level transmission of P. falciparum indicates an unstable transmission area in which malaria may be epidemic under favorable environmental conditions, resulting in sporadic outbreaks due to the minimal immunity in the population (Guerra et al., 2008).

Although early reports found no association between P. falciparum, HIV and malaria in children from stable malaria areas (Grimwade et al., 2003), more recent reports have shown that HIV is associated with greater mortality (all causes) and malaria-related mortality (Chintu et al., 1995), a greater prevalence of severe malarial anemia (Otieno et al., 2006), higher parasite densities, malnutrition and invasive bacterial infection (Berkley et al., 2008). Co-morbidity also increases the risk of severe anemia and mortality (Davenport et al., 2010). A comparison of children and adults in stable transmission areas showed that negative malaria diagnoses (blood smears) were associated with a greater likelihood of HIV infection in children but that positive diagnoses were associated with a greater likelihood of HIV infection in adults, after controlling for age and gender. This was attributed to either an increase in non-malarial febrile illnesses or a recent exposure to anti-malarial drugs in HIV-infected children resulting in a negative blood smear. The greater likelihood of HIV infection in adults was attributed to the loss of acquired malaria-specific immunity (Bebell et al., 2007).

Studies focusing on adults have provided conflicting evidence, with some showing no association between HIV and malaria (Atzori et al., 1993; Colebunders et al., 1990; Quigley et al., 2005) and others indicating a statistically significant positive association (Chalwe et al., 2009; French et al., 2001; Nielsen et al., 2006; Patnaik et al., 2005; Soumare et al., 2008; Whitworth et al., 2000). This association is stronger during co-infection (Francesconi et al., 2001) resulting in a greater frequency of anemia (Diallo et al., 2004). In adults from unstable transmission areas, HIV infection was found to be associated with severe and complicated malaria, requiring parenteral interventions (Grimwade et al., 2004). It was also demonstrated that an existing HIV infection reduces the efficacy of protective immune responses against P. falciparum (Khasnis and Karmad, 2003).

One study comparing pregnant women and their infants in stable and unstable transmission areas revealed no association between HIV and malaria parasitemia (Inion et al., 2003) even though a strong association between HIV and malaria parasitemia (Inion et al., 2003) even though a strong association between HIV and P. falciparum was shown to increase both neonatal (Ticconi et al., 2003) and postnatal mortality (Bioland et al., 1995). HIV-positive women with two or more previous pregnancies were found to have a greater risk of peripheral and placental malaria, higher parasite loads and more cases of febrile illnesses, severe anemia and adverse birth outcomes. HIV-positive women experiencing their first or second pregnancies were also much more likely to suffer severe anemia (van Eijk et al., 2002).
The analysis of mother-to-child HIV transmission has generated complex and conflicting results (Ter Kuile et al., 2004). However, a direct comparison between HIV-positive and HIV-negative women showed that HIV increased the risk of placental malaria (Brahmibhatt et al., 2003). Furthermore, newborns of HIV-positive women had higher rates of parasitemia (Steketee et al., 1996). Two recent studies looking at the association between HIV and malaria in sub-Saharan Africa have also produced contradictory results. The first investigation concluded that malaria is a risk factor for HIV infection, i.e. individuals with high parasitemia were more likely to be HIV-positive (Cuadros et al., 2011a). However, the second study showed no association between HIV and malaria, suggesting that malaria may not play an important role in the spread of HIV in populations where the HIV prevalence is relatively low, e.g. western sub-Saharan Africa, compared to countries such as Cameroon where an association between malaria and HIV has been confirmed (Cuadros et al., 2011b).

The Duffy blood group and the HIV–malaria link

The Duffy blood group factor was discovered in a multiply-transfused hemophiliac patient of the same name (Cutbush and Mollison, 1950; Cutbush et al., 1950). It is a chemokine receptor, also known as FY or CD234, which is normally found on the surface of red blood cells but is missing in many African Americans (Chaudhuri et al., 1993; Horuk et al., 1993). Plasmodium vivax merozoites bind to the Duffy antigen during the blood stage, and antibodies against the parasite ligand can disrupt its ability to infect red blood cells (Grimberg et al., 2007).

A correlation between HIV susceptibility and the expression of the Duffy antigen has been reported (Fig. 1) (University of Texas Health Science Center, San Antonio as reported in The Chronicle (http://www.sfgate.com/health/article/Newfound-genetic-clue-to-HIV-rate-in-blacks-3203944.php)), but the relationship is distorted by the impact of low white cell counts on survival, which also differs among Caucasians and African Americans. HIV susceptibility correlates with genotype — 46C/C, which is associated with the absence of the Duffy antigen and the strength of the association is inversely proportional to the total white cell count (Kulkarni et al., 2009).

Loss-of-function mutations in the human gene encoding the Duffy antigen (DARC) increase susceptibility to HIV-1 infection, while paradoxically prolonging the survival of HIV-1 patients (He et al., 2008). This can be explained by the ability of the Duffy antigen to bind HIV-1 and prevent the virions interacting with CD4+ cells. Loss of the receptor removes this buffering effect, so red blood cells bearing the antigen can no longer absorb an initial HIV inoculum. However, red blood cells can potentially transfer bound viruses to CD4/CCR5+ cells, such as macrophages and dendritic cells (Walton and Rowland-Jones, 2008). Once an infection is established, the absence of the Duffy antigen reduces the efficiency of this transfer process, thus explaining the prolonged survival of HIV-1 patients. The Duffy receptor may also exert its effect by binding pro-inflammatory chemokines, thus its absence would shift the chemokine balance away from an inflammatory response to relevant stimuli and increase the likelihood of HIV infection (Fig. 2) (Walton and Rowland-Jones, 2008).

Because the Duffy antigen is non-essential and capable of influencing both HIV and malaria, it could represent an excellent candidate drug target for both diseases. More than 40% of currently-approved drugs target G-protein-coupled receptors (GPCRs), and although the Duffy antigen is not a canonical GPCR and is not associated with intracellular G-proteins, it is homologous to typical GPCRs and can be screened for interacting ligands in the same manner (Eglen et al., 2007). Such drugs would need to be designed carefully given the polarized effect of the receptor in each disease: the presence of the Duffy antigen increases susceptibility to malaria while helping to prevent HIV infection, whereas its absence protects against malaria but increases susceptibility to HIV (Fig. 2) (He et al., 2008). Ideally, the receptor could be modified in such a way as to prevent interactions with the malaria parasite while preserving or even improving its ability to bind HIV.


**Fig. 1.** The interaction between HIV and malaria based on the ability of both causative pathogens to interact with DARC, the Duffy antigen receptor for cytokines. Redrawn and modified from University of Texas Health Science Center, San Antonio, as reported in The Chronicle (http://www.sfgate.com/health/article/Newfound-genetic-clue-to-HIV-rate-in-blacks-3203944.php).
Drugs used in combination against HIV and malaria

Antimalarial preparations from *Artemisia annua* and *Moringa oleifera* can be combined and used to treat HIV-positive patients that have contracted malaria, because these drugs have also been shown to inhibit HIV (Hirt et al., 2008). Some antiretroviral drugs also inhibit the growth of the malaria parasite, e.g. Lopinavir is a protease inhibitor which presumably targets proteases in both pathogens (Fear G et al., 2007). There appears to be plenty of opportunity for combined interventions that act against both diseases (Dooley et al., 2008).

It is important to identify the potential for interactions between HIV and malaria drugs because human drug metabolism is dependent on many factors, including genotype, environment, concurrent drugs, lifestyle and health indicators (Brentlinger et al., 2006). The biotransformation of drugs and other xenobiotics is divided into two phases (Jancova et al., 2010) and a number of functional polymorphisms affecting both phase I and phase II drug metabolism are prevalent in regions where HIV/AIDS and malaria occur together, although the associated phenotypes and their impact on the disease and their treatment remain to be resolved (Mehlotra et al., 2007). The efficacy of artemisinin in terms of the rate of parasite clearance is reduced in HIV-positive individuals, probably reflecting the increased parasitemia resulting from immunosuppression (Birku et al., 2002).

Plant biotechnology and the HIV–malaria link

Plant biotechnology offers a number of advantages that may help break the link between HIV and malaria, principally by providing safe and inexpensive systems to produce small-molecule drugs and recombinant pharmaceutical proteins. As discussed above, both HIV and malaria are poverty-related diseases, which therefore disproportionately affect the people least able to afford prophylactics and drugs. This is particularly the case for biopharmaceuticals such as recombinant antibodies that are currently produced in expensive fermenter-based systems and must be supplied as a finished product thus requiring an established and stable cold chain (Ma et al., 2003, 2005; Stoger et al., 2000, 2005b).

Plants have evolved to interact with the animals in their environment, e.g. attracting pollinators and warding off pests and herbivores. This is achieved by the synthesis of a diverse array of complex bioactive molecules, many of which have potent pharmacological properties in humans (hence the large number of drugs derived from natural products made by plants). The secondary metabolic pathways that lead to such molecules can be harnessed in plants and plant cells to produce drugs on an industrial scale, e.g. by expressing key rate-limiting enzymes in the corresponding pathways or blocking competitive enzymes that siphon off pathway intermediates (reviewed by Miralpeix et al., 2013; Rischer et al., 2013). Metabolic engineering is preferable when total chemical synthesis is too challenging or expensive, and when the natural source of the drug is scarce (e.g. if the plant is endangered or if the drug is produced at levels too low for economically-feasible extraction). A number of terrestrial plants and marine algae have been reported to produce natural compounds that counteract HIV, e.g. alkaloids and polyphenols, as well as macromolecules such as cyclic depsipeptides, sulfated polysaccharides and lectins (e.g. Zhou et al., 2013; Zofou et al., 2013). There have been no reports thus far of metabolic engineering to increase the production of anti-HIV metabolites, although the production of recombinant lectins is discussed below. In contrast, significant progress has been made towards the efficient production of antimalarial compounds by metabolic engineering, particularly artemisinin, a sesquiterpene lactone that is naturally produced by the glandular trichomes of the Chinese medicinal plant *A. annua* (Srivastava and Akhila, 2011). The commercial production of artemisinin currently involves semi-synthesis from artemisinic acid produced by yeast, with various approaches used to complete the final chemical conversion steps (Paddon et al., 2103; Peplow, 2013). However, the production of artemisinin in transgenic tobacco plants could potentially be achieved at a fraction of the cost by introducing the dedicated steps of the pathway and relying on the endogenous MVA pathway to supply precursors (Farhi et al., 2011). The pathway intermediate amorpha-4,11-diene has already been produced in *Nicotiana benthamiana* using this approach, and efforts to extend the pathway are ongoing (van Herpen et al., 2010). There has also been significant progress in the development of transformation systems for the source species *A. annua* and direct metabolic engineering in this species to increase the production of artemisinin (reviewed by Tang et al., in press). Plants can also be engineered with genes encoding biopharmaceutical proteins, and can be grown on an agricultural scale to produce pharmaceutically useful compounds.
scale (Ramessar et al., 2008a). Molecular pharming in plants is advantageous over traditional fermenter-based platforms because it is safer, more scalable and less expensive (Stoger et al., 2002a, 2002b, 2005a). Biopharmaceuticals are generally produced by fermentation in microbes (which cannot carry out most post-translational modifications) or mammalian cells (which are expensive to grow and can support the propagation of pathogens). Engineered plants producing recombinant pharmaceuticals can be grown in developing countries and the raw material can be processed locally to eliminate the need for a cold chain (Sabalza et al., 2013). Plants are currently the only platform suitable for the production of large-volume commodity-type pharmaceutical proteins (Ramessar et al., 2008a, 2008d). There has been significant progress in the development of plant-derived pharmaceutical products manufactured in containment, including the recent FDA approval of Taliglucerase alfa, a recombinant human enzyme produced in carrot cells for the treatment of Gaucher’s disease (Hollak, 2012). However, the commercialization of transgenic pharmaceutical plants in a field setting will probably take many years due to the unfavorable and onerous regulatory environment (Ramessar et al., 2008b, 2009, 2010; Sparrow et al., 2013).

Neutralizing antibodies are key examples of such commodity pharmaceuticals because they are required in large amounts at a price suitable for the world’s poorest people. This can only be achieved by massive-scale production without the need for transport and chilling, and such a challenging set of prerequisites can only be met by plants. Neutralizing antibodies play an important role in the development of vaccines for passive immunization and as viral entry inhibitors (Reina et al., 2010). Thus far, four HIV-1 neutralizing antibodies have been successfully produced in plants: 2G12, 2F5, 4E10 and b6 (Lotter-Stark et al., 2012). The human monoclonal antibody 2G12 was successfully produced in maize seeds (Rademacher et al., 2008; Ramessar et al., 2008c), Arabidopsis seeds and leaves (Loos et al., 2010) and tobacco leaves (Floss et al., 2009; Strasser et al., 2008), and the efficacy was generally the same as or superior to the same antibody produced in mammalian (CHO) cells. Indeed, 2G12 produced in maize seeds and retained in the endomembrane system achieved an in vitro HIV-binding activity similar to its CHO counterpart but 3–4-fold more potent neutralization activity, probably reflecting the greater proportion of dimers which are known to neutralize the virus more effectively than monomers (Ramessar et al., 2008c).

In addition to 2G12, 2F5 has been produced in tobacco BY-2 cells (Sack et al., 2007), tobacco leaves (Floss et al., 2008) and maize seeds (Sabalza et al., 2012), and 4E10 has been produced in tobacco roots (Drake et al., 2009). The efficacy of these plant-derived antibodies was similar to their CHO counterparts, but 2F5 was also expressed as an ELP fusion to improve its stability and facilitate recovery, although the presence of this additional protein sequence also reduced the efficacy of HIV neutralization (Floss et al., 2008). Finally, b12 has been expressed in tobacco leaves, alone and as a fusion with the 11-kDa lectin cyanovirin (CV-N) from the blue-green alga Nostoc ellipsosporum. The b12-CV-N fusion protein therefore included two HIV-neutralizing components in one protein and accordingly showed a much greater neutralizing efficacy than either protein acting alone (Sexton et al., 2009). Two other lectins exhibiting anti-HIV activity have been expressed in plants. Griffithsin (GRFT) from the red alga Griffithsia sp. was produced in N. benthamiana leaves and was shown to prevent the infection of cervical explants by HIV strains representing different clades and co-receptor specificities (O’Keeffe et al., 2009). Actinohivin, a lectin from the actinomyete Longispora abida was also produced in N. benthamiana and was shown to bind gp120 and inhibit syncytium formation (Nobuyuki et al., 2010). Among the small number of HIV-neutralizing antibodies and peptide lectins that have been produced in plants, two have already entered clinical trials (Twyman et al., 2012). MAPP66 is a cocktail of several antibodies targeting HIV and Herpes simplex virus (HSV), and was produced using the magnICON system in N. benthamiana. The second clinical candidate is 2G12 produced in tobacco leaves, which completed phase-1 clinical trials in 2013 (http://www.pharma-plantana.org). The plant-derived 2G12 was able to survive for more than 8 h in the vagina and proved to be safe and well tolerated without any serious adverse effects.

Several relevant vaccine candidates have also been produced using plant viruses as expression platforms or display vectors. An HIV-1 gp41 peptide has been expressed on the surface of Potato virus X (PVX) (Marusic et al., 2001) and Cowpea mosaic virus (CPMV) (Durrani et al., 1998), and both demonstrated the ability to induce neutralizing antibodies and HIV-specific secretory IgA antibodies (slgAs) following nasal delivery to mice (Webster et al., 2005). Functional HIV-1 p24 has been produced using Tomato bushy stunt virus (TBSV) vectors in tobacco leaves (Zhang et al., 2002), and the V3 loop of HIV gp120 has been expressed using Alfalfa mosaic virus (AMV) in tobacco leaves and was immunogenic following intraperitoneal administration to mice (Yusibov et al., 1997). The HIV-1 Tat protein has been expressed in several different plants, including tomato fruits (Ramirez et al., 2007), tobacco leaves (Webster et al., 2005), spinach leaves (Karasev et al., 2005) and potato tubers (Kim and Langridge, 2004; Kim et al., 2004) using stable transformation or plant virus expression systems. The spinach–derived Tat protein induced a slight increase in Tat-specific antibodies following immunization with a Tat DNA vaccine (Karasev et al., 2005) and the tomato-derived antigen induced both systemic and mucosal immunity in mice (Ramirez et al., 2007).

Although an efficacious malaria vaccine has not been developed, several candidate malaria vaccine antigens have been expressed in plants addressing different stages of the parasite life cycle. Eight of these candidates target the asexual blood stage and are therefore considered asexual blood stage antigens (Clemente and Corigliano, 2012). Promising candidates against P. falciparum include MSP1α, expressed in transgenic tobacco leaves albeit with no reported functionality (Ghosh et al., 2002), MSP1α, expressed in transgenic Arabidopsis seeds, which was immunoreactive against sera from malaria-infected patients (Lau et al., 2010), and an AMA1–MSP1 fusion expressed in tobacco and lettuce chloroplasts, which showed reactivity against native parasite proteins and the ability to induce specific antibodies following subcutaneous and oral delivery to mice (Davoodi-Semiromi et al., 2010). Promising candidates for P. yoelii include MSP4/5 expressed in transgenic tobacco leaves, which was immunogenic following intraperitoneal and oral delivery to mice (Wang et al., 2008), MSP4/5 expressed in tobacco using the magnICON system, which induced specific antibodies following oral delivery to mice or when primed with a DNA vaccine (Webster et al., 2009) and MSP1α expressed in tobacco leaves, which was immunogenic following intraperitoneal delivery with Freund’s adjuvant or oral administration without adjuvant (Ma et al., 2012). Promising candidates against P. vivax include MSP1 and circumsporozoite protein (CSP) expressed in transgenic rapeseed, which induced antigen-specific IgG1 and the Th1-related cytokines IL-12 (p40), TNF and IFN-γ in orally immunized mice (Davoodi-Semiromi et al., 2010).

Two further candidates against P. falciparum target the sexual stage and are thus considered sexual stage antigens (Clemente and Corigliano, 2012). These are P230 and P25 expressed in tobacco by agroinfiltration, which showed immunoreactivity against native parasite proteins and induced transmission-blocking antibodies following subcutaneous and/or intramuscular delivery to mice (Farrance et al., 2011a, 2011b). Another approach specifically for P. falciparum is the use of CSP peptides representing selected B-cell epitopes, which were produced using Tobacco mosaic virus (TMV) in tobacco leaves (Turpen et al., 1995).

Once an efficacious vaccine for malaria and/or HIV has been developed, the next major challenge will be to supply vaccines at a cost that can be met by developing country health systems. Since much of the production costs for vaccines are taken up by processing, purification and formulation, one strategy that could reduce the price of vaccines substantially is the use of plants for bioencapsulation, i.e. supplying vaccines as components of edible crops. This would maintain vaccine stability during storage and distribution, would allow direct oral
administration and the food matrix would provide limited protection in the digestive tract so that each antigen has a greater opportunity to induce oral immunity (Davoodi-Semiromi et al., 2010).

Costs could also be reduced by developing combined vaccines targeting both diseases, which could be achieved by the expression of fusion proteins that can be handled as a single product. Vaccine candidates have already been developed in which malaria antigens from different stages of the life cycle of the parasite have been combined into a single fusion protein (Pathairoty et al., 2012) or combined with the choler toxin B chain as an adjuvant. Indeed, AMA1 and MSP1 have been expressed as fusions with the choler toxin B chain in tobacco and lettuce chloroplasts exhibiting dual immunity against cholera and malaria either by oral or injectable delivery (Davoodi-Semiromi et al., 2010).

Conclusions

HIV/AIDS and malaria are two of the greatest global health challenges of our time, and evidence is accumulating to show a strong association between them. Synergistic prevention and control strategies are therefore required to tackle both diseases, but any intervention strategy is likely to require the large-scale production of inexpensive and stable pharmaceutical products that can be distributed to the world’s poorest communities. Plant biotechnology offers a number of platforms to tackle this challenge, including the inexpensive production of small-molecule drugs, and the inexpensive and agricultural-scale production of bulk pharmaceutical proteins such as HIV-neutralizing antibodies, candidate vaccines targeting multiple stages of the malaria parasite life cycle, anti-Plasmodium antibodies that can be used for passive immunization, and ligands that bind proteins implicated in the spread of both diseases and which could therefore be used to reduce the transmission of both pathogens simultaneously. Remaining barriers include the complex, onerous and expensive regulatory framework for genetically engineered crops particularly when they are grown in the field, but the many advantages of molecular pharming may help to improve the acceptability of the technology especially when targeted towards humanitarian applications.

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