

Synopsis of Research Articles

Streptococcus suis Outbreak with Symptoms of Toxic Shock Syndrome in China

DOI: 10.1371/journal.pmed.0030218

Streptococcus suis is a pathogen with serious economic effects on the pig industry worldwide. The disease is endemic in adult pigs in most countries where pig farming is common. Infections in adult pigs are usually asymptomatic, but infant piglets that get infected through contact with colonized adult females can develop fatal sepsis. Transmission to humans is rare and generally restricted to individuals with occupational exposure to live or dead pigs. The first human case of *S. suis* infection was reported in Denmark in 1968. Most of the approximately 200 previously reported human cases were characterized by meningitis and septicemia, and associated with a mortality of less than 10%.

Now George Gao and colleagues from the Chinese Academy of Sciences, the Chinese Center for Disease Control and Prevention, and other Chinese institutions report details of a recent unusual outbreak in 2005 of *S. suis* that affected over 200 individuals in Sichuan province and killed 38 of them. Besides the large number of individuals infected and the high mortality rate, it was the clinical symptoms associated with this outbreak that attracted interest and worry from scientists and health officials worldwide when the outbreak was first reported in the news last year.

As Gao and colleagues now detail in their article, a large proportion of the individuals infected (including all but one of the patients who died) showed symptoms of streptococcal toxic shock syndrome (STSS). Up to now, most reported cases of STSS had been attributed to group A streptococci. However, as Gao and colleagues show, the etiologic agents in the recent outbreak, as well as in an earlier outbreak in Sichuan province in 1998 that killed 14 of 25 reported patients, were clearly of the serotype 2 strains of *S. suis*. Both human outbreaks were closely linked to outbreaks in the local pig populations, and the researchers report that there are no reasons to believe that any

of the cases had been caused by human-to-human transmission. They also showed that *S. suis* isolated from the human patients caused typical *S. suis* disease in newborn piglets.

Were there other unusual characteristics among the Chinese isolates that could explain their ability to cause STSS? STSS is thought to be caused by bacterial superantigens that overstimulate the human immune system. Gao and colleagues tested the *S. suis* isolates associated with STSS for superantigen production, but were unable to detect any.

One of the key questions that arose when the recent outbreak was first reported was whether a new and more virulent strain of *S. suis* has emerged in China. Gao and colleagues performed an initial genomic survey of the isolates from the Chinese outbreaks to look for unusual characteristics that could explain the virulence of the pathogens. They did find some differences between the isolates from the 1998 and 2005 Chinese outbreaks (which appear very similar to each other) and other virulent strains, but a more detailed sequence analysis and functional studies will be needed before it is clear whether any of these differences have functional consequences for pathogenesis in pigs or humans.

In an accompanying Perspective article (DOI: 10.1371/journal.pmed.0030187), Shiranee Sriskandan and Joshua Slater suggest that *S. suis* infection “should now be in the list of differential diagnoses when clinicians encounter patients with unexplained sepsis who have a history of exposure to pigs.” They conclude that “the emergence of any new zoonotic disease associated with high mortality is of global concern” and call for “international collaboration ... to clarify differences between isolates circulating in different regions of the world.”

Tang J, Wang C, Feng Y, Yang W, Song H, et al. (2006) Streptococcal toxic shock syndrome caused by *Streptococcus suis* Serotype 2. DOI: 10.1371/journal.pmed.0030151

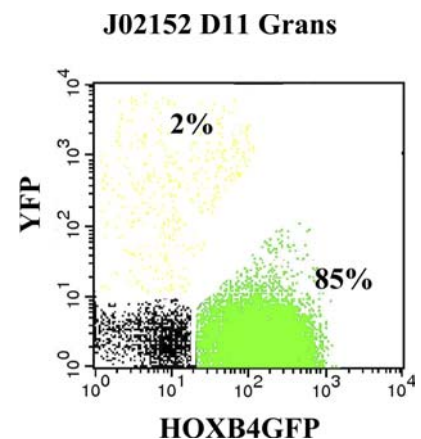
Hematopoietic Stem Cell Expansion: Testing the Potential of HOXB4 in Nonhuman Primates

DOI: 10.1371/journal.pmed.0030243

Throughout life, the body's tissues are maintained and repaired by stem cells—self-renewing cells that differentiate into many mature cell types. Every day, for example, the human body makes billions of white blood cells, red blood cells, and platelets from hematopoietic (blood system) stem cells (HSCs) to replace cells lost by normal wear and tear. This process of hematopoiesis helps to maintain a healthy immune system, enables sufficient oxygen to be carried around the body, and ensures effective blood clotting after wounding.

Some people, however, do not have a fully functioning hematopoietic system. They may have been born with a genetic alteration that disrupts the function of some blood cells, or they may have had chemotherapy for cancer that has

destroyed their hematopoietic system. One way to help such people, who are often prone to infection, is to provide them with a new supply of HSCs through transplantation. HSCs are found in small numbers in the bone marrow and peripheral blood, as well as cord blood, which is harvested from the umbilical cord at birth. Cord blood is increasingly being used to treat hematopoietic disorders, but the low number of HSCs present in a unit of cord blood means that transplanted cells can be slow to establish themselves (or engraft) in an adult recipient, prolonging the time the patient is susceptible to infections. Consequently, researchers are looking for ways to encourage HSC expansion before transplantation. Xiao-Bing Zhang, Hans-Peter Kiem and colleagues now



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There is a high percentage of HOXB4GFP⁺ cells early after transplantation

report that overexpression of a stem cell self-renewal gene called *HOXB4* in HSCs improves their expansion and engraftment in a nonhuman primate model, particularly early after transplantation.

Overexpression of human *HOXB4* in mouse HSCs is known to induce their ex vivo expansion without compromising their ability to differentiate. It also encourages the expansion of hematopoietic precursor cells from human cord blood, both in culture and when transplanted into mice. However, because immunodeficient mice do not support the differentiation of all human hematopoietic lineages, they are not an ideal model in which to investigate human HSC transplantation. Zhang, Kiem, and colleagues, therefore, turned to nonhuman primates, a well-established preclinical model for HSC transplantation and gene therapy, to investigate further whether treatment with *HOXB4* holds promise for HSC expansion before transplantation.

The researchers first isolated CD34⁺ cells from six experimental animals—CD34 is a protein that is expressed only by lymphoid and myeloid hematopoietic precursor cells, which together differentiate into all the different blood cells. Next, the researchers split the

CD34⁺ cells from each animal into two batches. One batch was treated with a retrovirus expressing *HOXB4* tagged with a green fluorescent protein marker (*HOXB4GFP*⁺); the control batch was treated with a retrovirus expressing a yellow fluorescent protein marker (*YFP*⁺). Three or nine to 12 days later, the batches of cells were mixed and transplanted back into their respective donor animals, whose hematopoietic system had in the meantime been destroyed by irradiation. The researchers then tracked the labeled cells as they repopulated the animals.

Zhang, Kiem, and colleagues showed that, in this competitive repopulation assay, *HOXB4* overexpression greatly improved the engraftment of CD34⁺ cells, particularly when the cells were expanded ex vivo for an additional six to nine days before transplantation. Short-term engraftment (two weeks after transplantation) of *HOXB4GFP*⁺ cells was up to 56-fold higher than that of *YFP*⁺ cells. Over time, the percentage of *HOXB4*-expressing cells in the animals' blood declined, but remained higher than the percentage of control cells even after six months, suggesting that *HOXB4* overexpression might also improve long-term engraftment. Finally, the researchers report that three and six months after transplantation both the

myeloid and lymphoid hematopoietic lineages contained *HOXB4GFP*⁺ and *YFP*⁺ cells. *HOXB4GFP*⁺ cells were more common in the myeloid lineage than *YFP*⁺ cells, but in lymphocytes the pattern was reversed, indicating that *HOXB4* overexpression may have a larger effect on the engraftment and differentiation of myeloid precursors than of lymphoid precursors.

These results suggest that *HOXB4*-mediated ex vivo expansion of stem cells could be one way to accelerate the engraftment of HSCs from sources that contain limited numbers of stem cells (such as cord blood). Because only small numbers of animals were used in this proof-of-principle study, more experiments will be needed before it is clear whether *HOXB4* can be used to improve the expansion and engraftment of CD34⁺ cells in patients whose hematopoietic system has failed. As the researchers point out, the availability of recombinant *HOXB4* protein makes it possible to treat HSCs directly, without the potential problems associated with genetic manipulation of the cells.

Zhang XB, Beard BC, Beebe K, Storer B, Humphries RK, et al. (2006) Differential effects of *HOXB4* on nonhuman primate short- and long-term repopulating cells. DOI: 10.1371/journal.pmed.0030173

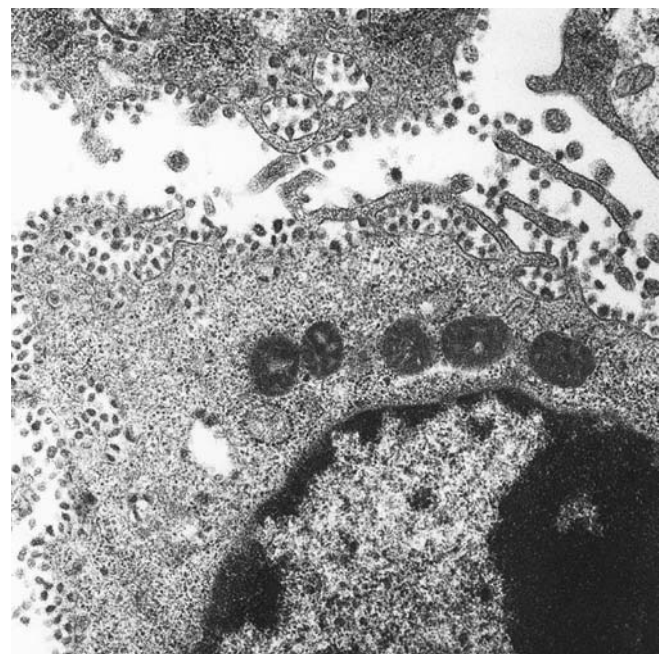
A Macaque Model of SARS

DOI: 10.1371/journal.pmed.0030222

The 2002 SARS outbreak that started in China spread quickly to Hong Kong, Singapore, Vietnam, and Canada. Although the 774 people it killed was a small number compared with the global death toll from other infectious diseases, the outbreak caused widespread panic because of the lack of global preparedness for what could have become a worldwide epidemic. Since then, surveillance and monitoring systems have been put into place and existing ones strengthened, but since another outbreak is always possible, researchers around the globe are still devoting much time to studying the infection. Analyzing the disease in animals to investigate the pathogenesis of the novel coronavirus that causes SARS (SARS-CoV) is crucial to developing vaccines and treatments to tackle the next epidemic.

In adults, SARS first causes flu-like symptoms, then lower respiratory tract disease, and finally severe respiratory disease. But despite having similar levels of viral replication, children tend to have milder symptoms. They do not get chills or myalgias, nor do they need help breathing, as adults tend to need toward the end of the illness.

Several animals—mice, cats, and ferrets—have been tested to see whether they can support replication of SARS-CoV, and others—civets and wild bats—have been investigated as potential viral reservoirs. In studies on nonhuman primates, the focus has been to document histopathological disease rather



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An electron micrograph of SARS-CoV-infected Vero cells

than to look for more advanced symptoms such as radiographic evidence of pulmonary disease, as happens in humans.

In a new study, Jason Paragas, James Lawler, and colleagues now describe what happened when they infected eight macaques with the SARS-CoV Urbani strain; four in the nasal cavities and bronchus; two in the nasal cavities and conjunctiva; and two intravenously.

Although all animals had evidence of viral replication and produced neutralizing antibodies, none of the animals developed fever, and only those in the first two groups had mild-to-moderate symptoms (decreased activity and feeding, and slightly labored breathing). By contrast, the animals that had been intravenously infected showed no clinical symptoms. When tested for the presence of the virus, all animals had viral DNA in nasal swabs and urine samples—irrespective of how they had been infected. Paragas and colleagues also took chest radiographs of six of the animals—never before done in any SARS-CoV study on nonhuman primates. Three nonhuman primates showed signs of pneumonia by radiographs.

More interesting findings came from the fact that some animals were infected with wild-type virus, and others with a recombinant infectious clone. All developed similar disease, indicating that it was just the SARS-CoV that was responsible

for disease, and that no coinfection was required, as has been suggested by some workers. In addition, six animals that were reinfected with SARS-CoV 13 weeks after the first infection were immune—importantly, two of these had initially had the recombinant virus, which means that the molecular clone could induce protection against the wild-type form.

Paragas and colleagues' work differs from previous studies of SARS-CoV in nonhuman primates. Some researchers found more severe clinical disease; others, no overt disease at all. Tests on African green monkeys showed that one monkey had fever on the third day after infection. These differences could have been because of the strain, the dose, or the route of infection.

Ultimately, disease in nonhuman primates is far milder than that in adult humans. What is interesting is that it is similar to SARS-CoV infection in human children. The researchers suggest that the key to the difference in disease severity could lie in the fact that adult humans with SARS-CoV have far higher levels of inflammatory cytokines than do children, or, as this research suggests, nonhuman primates.

Lawler JV, Endy TP, Hensley LE, Garrison A, Fritz EA, et al. (2006) *Cynomolgus macaque as an animal model for severe acute respiratory syndrome*. DOI: 10.1371/journal.pmed.0030149

Drug-Resistant *Leishmania tropica* Parasites Detected in Iranian Cutaneous Leishmaniasis

DOI: 10.1371/journal.pmed.0030230

Leishmaniasis are parasitic diseases that are endemic (constantly present) in many tropical and temperate countries. Every year, 2 million people become infected with one of 20 pathogenic species of *Leishmania* through the bites of infected female sand flies. These pick up parasites by biting an infected animal (zoonotic transmission) or an infected person (anthroponotic transmission). In their human host, *Leishmania* parasites reproduce inside macrophages—white blood cells that usually kill microorganisms, clear up cellular debris, and activate other immune cells. When the macrophages are full of parasites, they burst—this destruction causes the symptoms associated with leishmaniasis—and the released parasites infect further macrophages.

In cutaneous leishmaniasis—the most common form of the disease—patients develop skin ulcers a few weeks after being bitten by infected sand flies. These usually heal spontaneously but leave ugly, sometimes disabling, scars. Cutaneous *Leishmania* infections can spread to the nose or mouth to cause mucocutaneous leishmaniasis, which destroys the sensitive linings of these organs. Cutaneous and mucocutaneous leishmaniasis are not life-threatening in themselves, but patients can develop fatal secondary infections. Visceral leishmaniasis, which affects the spleen



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Female sandflies transmit the *Leishmania* parasite

and other internal organs, is often fatal if untreated.

Leishmaniasis are usually treated with pentavalent antimony-containing drugs, such as meglumine antimoniate (Glucantime), but patients are becoming increasingly unresponsive to these drugs. In India, for example, more than 60% of

cases of visceral leishmaniasis do not respond to treatment. Unresponsiveness can be caused by the parasite developing drug resistance, by changes in the host's immunological status, or by suboptimal treatment regimens. Ramtin Hadighi, Mehdi Mohebali, Marc Ouellette, and colleagues have been investigating whether the increased incidence of Glucantime-unresponsive cutaneous leishmaniasis in Iran correlates with parasite resistance to the drug. They now report that treatment failure for cutaneous leishmaniasis in Iran, like the treatment failure seen for visceral leishmaniasis in India, is due to Glucantime-resistant parasites.

The researchers isolated *Leishmania* parasites from 185 skin lesions from untreated patients living in Mashhad, a region of Iran where anthroponotic cutaneous leishmaniasis is endemic. Of these patients, 20 did not respond to Glucantime—their skin ulcers failed to heal. To find out if this was due to drug-resistant parasites, the researchers infected mouse macrophages with all 185 isolates and then treated the infected cells with Glucantime. Several days later, the parasites surviving inside the cells were stained with a dye and then counted using a microscope. The researchers report that although initial infection rates were similar, parasites from the unresponsive patients were

resistant to intermediate or high levels of Glucantime. On average, parasites from unresponsive patients were 4-fold less susceptible to Glucantime than parasites from responsive patients.

Next, the researchers partly characterized the 20 drug-resistant parasite isolates and 11 drug-susceptible isolates. By sequencing the gene for the metabolic enzyme pteridine reductase 1, the researchers discovered that 28 of the isolates were *L. tropica*; the remaining three were *L. major*. Only one unresponsive isolate was *L. major*; the rest were *L. tropica*. The researchers also used pulsed-field gel electrophoresis to separate and study *Leishmania* chromosomes. Because these evolve quickly, the chromosome composition (karyotype) of different isolates indicates

their genetic relatedness. The *L. major* isolates formed one group using this technique but the *L. tropica* isolates fell into three distinct groups, each of which included drug-susceptible isolates and isolates with intermediate and high Glucantime resistance. In other words, susceptible and resistant isolates were often closely related. Finally, the researchers confirmed the drug sensitivity of several closely related strains by testing their ability to grow in a human monocyte cell line in the presence of Glucantime, and also showed that drug resistance was stable over time in resistant isolates but could be reversed by treatment with an inhibitor of glutathione biosynthesis. This last result indicates that thiols (molecules containing a sulphur atom bonded to

a hydrogen atom) may be important for the resistant phenotype, and may suggest a way to reverse drug resistance.

Overall, these results provide the first evidence that *Leishmania* parasites can acquire drug resistance that contributes to treatment failure in cutaneous leishmaniasis. They also indicate that Glucantime-resistant *L. tropica* isolates are now frequent in Iran. Additional work is needed to understand the nature of the resistance mechanisms, with the goal to improve diagnosis and treatment of resistant leishmaniasis.

Hadighi R, Mohebbi M, Boucher P, Hajjarian H, Khamesipou A, et al. (2006) Unresponsiveness to Glucantime treatment in Iranian cutaneous leishmaniasis due to drug resistant *Leishmania tropica* parasites. DOI: 10.1371/journal.pmed.0030162

Childhood Anemia in a Malaria-Endemic Region: The Haptoglobin Genotype Connection

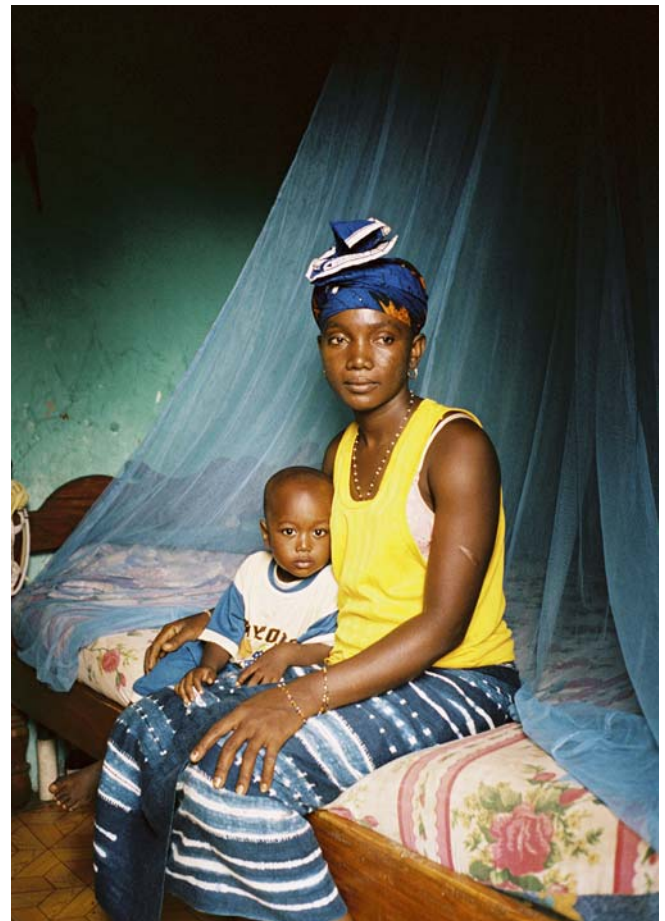
DOI: 10.1371/journal.pmed.0030227

The World Health Organization estimates that malaria kills an African child every 30 seconds. Many of these children die because they develop severe anemia (a deficiency of red blood cells). As many as 5 million cases of severe malarial anemia occur in African children every year, and 13% of these cases are fatal. Turning the statistics around, more than half of young children in African countries where malaria is endemic (constantly present) are anemic. Nutritional deficiencies and various infections account for some of this disease burden, but malaria is one of the most important factors contributing to anemia.

The malaria parasite destroys red blood cells (a process called hemolysis) as part of its life cycle, releasing hemoglobin (Hb)—an iron-containing protein that carries oxygen around the body—into the circulation. Free Hb can cause oxidant stress, which is itself associated with anemia in malaria. An important modulator of such stress is a serum protein called haptoglobin (Hp), which captures Hb during hemolysis.

Hp exists in three molecular forms that are genetically determined by two variants (alleles) of a single gene. People who have two copies of the *Hp*¹ allele make only Hp¹-1, a homodimeric protein. People with two copies of the *Hp*² allele (the *Hp*^{2/2} genotype) make Hp²-2, a large circular polymer, and those with one copy of each allele make the linear polymer Hp¹-2 in addition to these two forms. The functional properties of the three Hp forms are somewhat different. In particular, Hp²-2 binds Hb much less tightly than the other forms. Sarah Atkinson and her colleagues reasoned, therefore, that the *Hp*^{2/2} genotype might be a risk factor for anemia in children in malaria-endemic areas. To test their hypothesis, they measured Hb levels in Gambian children at the start and end of the malaria season, and now report in a new study that, as predicted, the *Hp*^{2/2} genotype is associated with seasonal childhood anemia in this population.

Most cases of malaria in The Gambia occur between September and December, so the researchers recruited 780 children aged two to six years from ten Gambian villages in July 2001, determined their Hp genotypes, assessed their blood Hb and serum Hp concentrations and iron status, and determined whether they were infected with malaria parasites. These



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Bed nets are essential in malaria-endemic regions such as The Gambia

variables were re-measured at the end of the malaria season. In addition, the researchers determined two other genetic polymorphisms that might influence Hb levels over the malaria

season—an *Hb* variant that causes sickle cell anemia (*HbS*), and *glucose-6 phosphate dehydrogenase* (*G6PD*) gene variants associated with hemolytic anemia.

Atkinson and her colleagues first analyzed their study population in terms of their *Hp* genotype. This univariate (single) analysis included 671 children—a few children were not included because of incomplete data. Baseline hb levels were not affected by *Hp* genotype, but the average drop in hb was 8.9 g/l in the 17% of children with the *Hp*^{2/2} genotype compared with only 5.1 g/l in children with the other genotypes. By contrast, the magnitude of the drop in Hb levels over the season was not affected by *HbS* or *G6PD* genotype—two other genetic traits that affect the red blood cells. Because multiple factors influence Hb concentrations (for example, recent infection with malarial parasites and iron status), the researchers also did a multiple regression analysis of their data to test the effect of all such factors on Hb levels at the end of the malaria season. There were 565 children who had data complete enough for this more detailed analysis, and, once again, the *Hp* genotype emerged as a risk factor for anemia, even after adjusting for other factors that affect Hb levels.

Atkinson and her colleagues suggest that the association between *Hp* genotype and seasonal childhood anemia may reflect the reduced ability of the Hp2-2 polymer to scavenge free Hb and its bound iron after malaria-induced hemolysis. They also discuss why *Hp*², a potentially detrimental allele, should be common in The Gambia, where malaria is endemic. *Hp*² arose from *Hp*¹ about 2 million years ago, and its subsequent spread across the world seems to have been driven by a strong genetic pressure, such as exposure to a life-threatening disease. The authors suggest that malaria may be one of the diseases that helped to select for the *Hp*² allele; it is possible that the *Hp*² allele may provide protection from life-threatening malaria, albeit at the expense of impaired hematological recovery from mild and asymptomatic malaria. In a related Perspective (DOI: 10.1371/journal.pmed.0030200), Stephen Rogerson expands on the possible mechanisms of Hp-related anemia, and considers what the wider health implications of this study might be.

Atkinson SH, Rockett K, Sirugo G, Bejon PA, Fulford A, et al. (2006) Seasonal childhood anaemia in West Africa is associated with the haptoglobin 2-2 genotype. DOI 10.1371/journal.pmed.0030172

α^+ -Thalassemia and Protection from Malaria

DOI: 10.1371/journal.pmed.0030221

Over the course of human history, hundreds of thousands of genetic mutations have arisen in the global population. The most harmful ones usually disappear—by affecting an individual's "fitness," i. e., the ability to reproduce, the mutations are lost before carriers can pass them on to their children—whereas most mutations are maintained in the population in low frequencies. Some mutations, however, can give the carrier such a large survival advantage that the mutations become positively selected for, leading to their presence in high frequencies in some populations.

Blood disorders are a good example of this selection process. The sickle cell mutation, for example, is a mutation of the β -globin gene that can cause severe anemia in people who inherit two mutated genes. People with just one mutated hemoglobin (Hb) S gene, however, can be highly protected against malaria. And in Africa, where malaria is one of the biggest killers, up to 40% of people are believed to carry one of the sickle genes.

The thalassemias are also inherited blood disorders that result from mutations in either the α -globin or β -globin genes. α -thalassemias are now the most common genetic disorders of human beings, and this is thought to be because of their protective effect against malaria. People usually have four α -globin genes, two on each Chromosome

16. In Africa, however, a common deletion can remove one of these genes from either chromosome or from both chromosomes; individuals with three or two α -globin genes remaining have anemia, which is more severe the fewer α -globin genes are present but is not life-threatening. This condition, in which at most one α -globin gene is missing from each chromosome, is known as α^0 -thalassemia (α^0 -thalassemia occurs when both genes are removed from a chromosome). Despite the well-known beneficial effect of α^+ -thalassemia against malaria, scientists know little about how exactly the α^+ -thalassemias result in this protection, and whether they protect against all forms of the disease.

In a new study in *PLoS Medicine*, Thomas Williams, Sammy Wambua, and colleagues—researchers from Kenya and Oxford—investigated the effect of α^+ -thalassemia on malaria and other childhood diseases, such as gastroenteritis, in two groups of children in Kenya. The first group comprised children younger than five years old, recruited between September 1998 and August 2001, 301 of whom were analyzed in the study (the mild disease cohort). The second group comprised 2,104 children recruited at birth between May 1992 and April 1995 (the birth cohort). All children analyzed were typed for both HbS and α^+ -thalassemia.

Williams and colleagues found that α^+ -thalassemia (either with one or two α -globin genes lost) was associated with significant reductions in the rate of admission to hospital with malaria (with or without signs of severity) and severe malaria. Both homozygous individuals (with two α genes missing in total, one from each chromosome) and heterozygote individuals (with only one α gene missing in total) had much lower rates of severe malaria anemia than normal children.

However, α^+ -thalassemia had no effect on symptomless parasitemia (defined as the presence of the *Plasmodium falciparum* malaria parasite in the blood of a child, but without fever or other symptoms). And although the occurrence of uncomplicated malaria was lower in both those heterozygous and those homozygous for α^+ -thalassemia compared with normal children, this drop in incidence was not statistically significant.

In general, there were no links between α^+ -thalassemia and the occurrence of nonmalarial illnesses. There were, however, two exceptions. In the birth cohort, fewer heterozygous infants than normal children were admitted to hospital with severe anemia. In the mild disease cohort, both heterozygous and homozygous children had a lower frequency of lower respiratory tract infections than normal children—although, this was not seen in the other cohort.

These findings appear to contradict previous results from a study conducted by the same group in the Pacific islands of Vanuatu that showed α^+ -thalassemia might increase the frequency of uncomplicated malaria. The authors say this might partly be explained by the fact that, unlike Kenya, in Vanuatu two species of malaria, *P. vivax* and *P. falciparum*, both cause disease at similar frequencies. Furthermore, there may be major genetic differences in both human and parasite populations between these regions.

Whether the mutation has a protective effect against other diseases is still unresolved. The authors maintain that such an effect is plausible—indeed, the protection from lower respiratory tract infections in some children was of a similar magnitude to that for malaria. The authors have also previously recorded protection against nonmalarial diseases in Papua New Guinea.

These findings have important implications for researchers looking

for “antimalarial” genes and for those searching for potential vaccine candidates: they will need to use a carefully focused approach that differentiates between uncomplicated, or symptomless, disease and the complicated, or severe, form.

Wambua S, Mwangi TW, Kortok M, Uyoga SM, Macharia AW, et al. (2006) The effect of α^+ -thalassaemia on the incidence of malaria and other diseases in children living on the coast of Kenya. DOI: 10.1371/journal.pmed.0030158

Modeling the Impact of Intermittent Preventative Treatment on the Spread of Drug-Resistant Malaria

DOI: 10.1371/journal.pmed.0030181

Until the mid-20th century, malaria occurred in most temperate, subtropical, and tropical countries of the world. Then, the introduction of powerful insecticides, including DDT, made it possible to eliminate this human parasitic disease in many temperate countries by controlling the mosquitoes that transmit malarial parasites between people. Elsewhere, eradication efforts were less successful, but the use of inexpensive antimalarial drugs such as chloroquine and sulfadoxine-pyrimethamine (SP) further reduced global morbidity and mortality from malaria. Sadly, the rapid spread of resistance to chloroquine (and more recently to SP) has resulted in a resurgence of malaria over the past three decades. Nowadays, 40% of the world's population is at risk of contracting malaria, and every year, it kills at least 1 million people—mainly children. Pregnant women and their unborn children are particularly vulnerable to malaria, for whom it is a major cause of perinatal mortality, low birth weight, and maternal anemia.

One way to reduce malaria morbidity and mortality is to treat asymptomatic individuals, regardless of their infection status, with regular therapeutic doses of antimalarial drugs. Intermittent preventative (or presumptive) treatment (IPT) is currently used in pregnant women (IPTp) in malaria-endemic areas, and IPT for infants (IPTi) is also being considered. However, before an intervention of this type is widely introduced, its potential impact on the spread of drug-resistant parasites needs to be investigated. A badly designed intervention could increase the speed at which malaria parasites become resistant to new drugs, an outcome that public health officials want to avoid. Ideally, such information would come from field trials, but in practice such trials are rarely undertaken, so researchers, including Wendy Prudhomme O'Meara, David Smith, and Ellis McKenzie, have turned instead to mathematical modeling. O'Meara and colleagues now describes a model that has allowed them to evaluate the possible impact of IPTp and IPTi on the spread of drug-resistant malaria parasites. Their analysis highlights the importance of carefully choosing which drugs to use for IPTi, and indicates which conditions are most likely to encourage the spread of drug resistance.

Drug use patterns—how quickly the body removes each drug, how well an individual's immune response deals with malaria parasites, and how often each person gets bitten by an infected mosquito (the transmission intensity)—all affect the spread of

drug-resistant parasites. Prudhomme O'Meara and colleagues built these factors into a composite model that incorporates a human and a parasite population model. They then used their model to predict the potential for drug-resistant parasites to spread in low- and high-transmission settings, and to predict how the use of IPT in adults and infants, the time taken for drug elimination, and the treatment of infections (instead of asymptomatic individuals alone) might affect the spread of drug resistance.

One prediction of their model is that whereas fully resistant parasites (which can survive a full therapeutic dose of an antimalarial drug) are more likely to spread under conditions of high transmission, partially resistant parasites (which survive at intermediate drug concentrations) are more likely to spread in low-transmission areas, a result supported by epidemiological observations. The model also predicts that the use of a drug for IPT to which there is no existing resistance in a high-transmission area will accelerate the appearance of partial resistance, followed by an explosion of full resistance. Another analysis indicates that drugs that are rapidly eliminated from the body (e.g., chlorproguanil-dapsone) may be preferable to those that linger (e.g., SP). This latter type of drug maximizes the period of protection from each treatment but also maximizes the time when enough drug is present to allow selection of resistant parasites (the window for selection). Finally, comparing IPTp with IPTi, the model predicts that partially resistant parasites will spread faster when IPT is given to infants (who have little or no immunity to malaria) than when given to adults (who often are immune to some degree).

The researchers stress that their model provides a qualitative, not a quantitative, assessment of how partial and fully resistant malaria parasites will spread in different communities under different drug use strategies. But, they say, the model can be used as a tool to determine the critical questions that need to be addressed before broad implementation of IPT. In particular, they note, their model highlights the importance of carefully selecting the drug to be used in IPTi programs in different settings so that protection is maximized while minimizing the chances of antimalarial drug resistance emerging.

Prudhomme O'Meara W, Smith DL, McKenzie FE (2006) Potential impact of intermittent preventative treatment (IPT) on spread of drug-resistant malaria. DOI: 10.1371/journal.pmed.0030141

HPV Vaccination: Predicting Its Effect on Cervical Cancer Rates

DOI: 10.1371/journal.pmed.0030202

Each year, nearly 500,000 new cases of cervical cancer are diagnosed around the world, and more than 250,000 women die from the disease. Most of these cases occur in developing countries where there is no routine screening for precancerous lesions. By contrast, in developed countries, national screening programs have greatly reduced the number of women dying from this cancer—between 1955 and 1992 in the US, for example, cervical cancer deaths dropped by 74%.

Infection with a sexually transmitted human papillomavirus (HPV) is a precondition for the development of cervical cancer. Of the 35 HPV types that can infect the genital tract, about half have oncogenic potential—the rest cause benign warts. The immune system clears most HPV infections but persistent infection with HPV type 16 accounts for approximately 55% of cervical cancers. Because of the strong association between cervical cancer and HPV infection, several HPV type-specific vaccines are being developed. Early results suggest that these vaccines can prevent almost 100% of persistent infections with the relevant HPV type, raising the possibility of reducing the incidence of cervical cancer by prophylactic vaccination. But what would the impact of such vaccines be in countries that already have cervical cancer screening programs? To find out, Ruanne Barnabas and colleagues have developed a dynamic transmission model of HPV 16 infection and progression to cervical cancer using epidemiological data from Finland. Their analyses indicate that high coverage of women alone over many decades with a vaccine that provides long-term protection would greatly reduce type-specific cancer incidence, a reduction that would be maximized by combining vaccination with routine screening.

The researchers' model is represented by a flow chart in which susceptible women acquire an HPV infection that, in most cases, is cleared by their immune system. In some women, persistent infection induces precancerous lesions that can progress to invasive cervical cancer, regress spontaneously, or be screened and treated. HPV infection in men is represented by a simpler flow chart—they simply become infected and then develop immunity. The researchers incorporated values for parameters such as sexual activity, screening protocols, and treatment rates obtained from published Finnish studies in their model and calibrated it using historical data on the proportion of the Finnish population with antibodies to HPV 16.

To allow them to model how vaccination will affect the incidence of cervical cancer, Barnabas and colleagues first estimated the transmission probability of HPV in the Finnish population. This probability provides a measure of how easily HPV spreads—if it were 1.0, every sexual partnership a woman had with a man infected with HPV would result in her also becoming infected. The researchers' transmission probability estimate of 0.6 is high, which indicates that universal coverage with a very effective vaccine will be needed to eliminate HPV infection in the population. The researchers put this value (which is subject to great uncertainty) and estimated values for age at sexual debut and the annual number of sexual partners into their



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Potential impact of HPV vaccination

model; they also assumed that vaccination takes place before sexual debut, is 100% effective, and gives life-long protection.

The model predicts that vaccinating both men and women will be little better than vaccinating women alone, irrespective of whether vaccine coverage is high or low. Furthermore, although vaccinating 90% of young women before sexual debut could decrease HPV type-specific cervical cancer incidence by 91%, delaying vaccination until after sexual debut could decrease the impact of vaccination. The model also predicts that if 90% of women were vaccinated without screening, there would be 0.6 cases of cervical cancer per 100,000 women per year (compared with seven out of 100,000 with no intervention); vaccination plus screening every five years would reduce this incidence further, by two-thirds. Finally, the researchers investigated how the duration of vaccine-conferred protection might affect invasive cervical cancer rates. The model predicts that—unintuitively—short-lived protection will marginally increase cervical cancer rates compared with no vaccination if, as some people believe, older women are more susceptible to the persistent HPV infections that progress to cervical cancer than are younger women. Booster vaccinations would avoid this potential problem.

The researchers conclude that the most effective strategy for the reduction of cervical cancer in developed countries in which incidence is already low is widespread vaccine coverage (both in terms of the HPV type targeted and the fraction of the population vaccinated), combined with current screening protocols. Whether this recommendation is adopted will depend on how vaccines perform in ongoing phase III trials and on a detailed economic assessment of the options available.

Barnabas RV, Laukkanen P, Koskela P, Kontula O, Lehtinen M, et al. (2006) The epidemiology of HPV 16 and cervical cancer in Finland and the potential impact of vaccination: Mathematical modelling analyses. DOI: 10.1371/journal.pmed.0030138