

## Synopsis of Research Articles

## Death by Necrosis: The Early Stages of Type 1 Diabetes

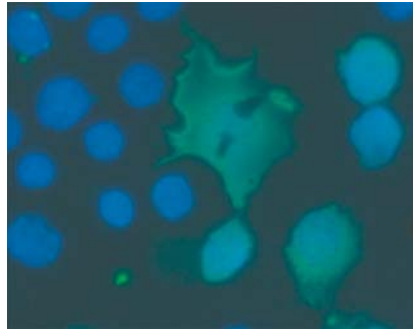
DOI: 10.1371/journal.pmed.0030051

Diabetes mellitus is a major global health problem. The most common form of diabetes—90%–95% of cases—is type 2 or non-insulin-dependent diabetes, which affects mainly adults. The remaining cases are type 1 or insulin-dependent diabetes. Also known as juvenile diabetes, type 1 diabetes usually develops before the age of 30. It is an autoimmune disease in which the insulin-producing or  $\beta$ -cells of the pancreatic islets of Langerhans (groups of specialized cells that regulate blood sugar levels) are destroyed by lymphocytes. Type 1 diabetes affects about one in 500 American children and adolescents; the only treatment is daily insulin injections.

$\beta$ -cell death is the hallmark of type 1 diabetes. An early phase of  $\beta$ -cell death, probably triggered by environmental factors (for example, viral infection) in genetically susceptible individuals, releases  $\beta$ -cell-specific antigens; subsequently, T lymphocytes that specifically recognize these antigens mediate widespread  $\beta$ -cell killing. John Corbett and colleagues believe that by studying the early phase of  $\beta$ -cell death, it may be possible to find ways to prevent this destructive autoimmunity from developing in individuals with a family history of type 1 diabetes.

Cytokines, chemical messengers produced by lymphocytes and macrophages, are thought to contribute to the loss of  $\beta$ -cell function and viability early in autoimmune diabetes. The effect of cytokines on  $\beta$ -cell function is mediated by nitric oxide (NO), but it is not clear if the same is true for  $\beta$ -cell death. In their study, Corbett and colleagues asked whether NO mediates the death of rat  $\beta$ -cells induced in vitro by the macrophage-derived cytokine interleukin-1 (IL-1), and whether the cells are killed by apoptosis or necrosis, two different mechanisms of cell death. Apoptosis, or programmed cell death, is a highly organized process that minimizes the leakage of cell contents and the development of inflammation. Necrosis is much less tidy: the dying cells swell and burst, releasing their contents into the extracellular space where they cause inflammation.

The researchers report that 24–48 hours treatment with IL-1 reduced



DOI: 10.1371/journal.pmed.0030051.g001

**IL-1 causes nuclear membrane breakdown in rat insulinoma cells**

the viability of rat  $\beta$ -cells from two sources—an insulinoma cell line and islets. Then, by inhibiting NO synthesis or by adding an NO donor, they provide evidence that IL-1-induced death of  $\beta$ -cells is mediated in part by NO production. Turning to the mechanism of  $\beta$ -cell death, the researchers show that IL-1 treatment failed to activate caspase 3—an enzyme required for apoptosis—in  $\beta$ -cells, and that a caspase-3 inhibitor did not attenuate IL-1 induced  $\beta$ -cell death. Another marker of apoptotic cell death—lipid accumulation on the cell surface—was also missing in  $\beta$ -cells treated with IL-1.

Having discounted death by apoptosis, the researchers then show that IL-1 stimulated the release of HMGB1 (a

chromatin-binding protein that is released by cells undergoing necrosis but not apoptosis) by rat  $\beta$ -cells. Finally, because human  $\beta$ -cells behave somewhat differently from rat  $\beta$ -cells, the researchers demonstrate that a combination of cytokines (including IL-1) stimulated HMGB1 release from some preparations of human islets in an NO-dependent manner.

Overall, the authors conclude that macrophage-derived cytokines may participate in the early stages of type 1 diabetes by inducing necrotic death in  $\beta$ -cells. Other researchers believe that apoptotic cell death is more important in these early stages, particularly in human cells. But, based on their results, Corbett and colleagues speculate that cytokine induction of necrosis could kick start type 1 diabetes by causing the release of both  $\beta$ -cell antigens and HMGB1, which stimulates inflammatory responses. Although these findings need to be confirmed within the context of the human pancreas—what cells do in vitro may not reflect what happens in the body—they provide new insights into the early stages of type 1 diabetes that could suggest ways to prevent or stop its development.

Steer SA, Scarim AL, Chambers KT, Corbett JA (2006) Interleukin-1 stimulates  $\beta$ -cell necrosis and release of the immunological adjuvant HMGB1. DOI: 10.1371/journal.pmed.0030017

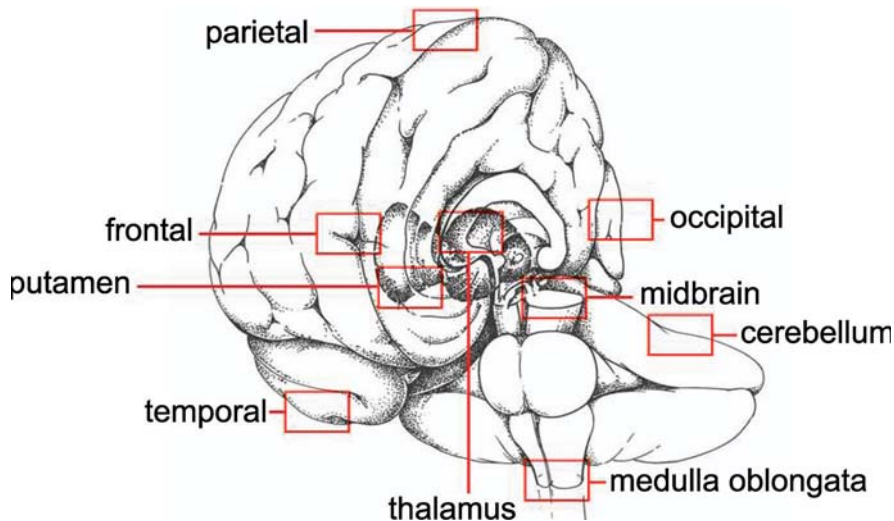
## Toward a Better Understanding of Human Prion Diseases

DOI: 10.1371/journal.pmed.0030055

Misfolding of a single protein, the cellular prion protein (PrP<sup>c</sup>) into the disease-associated form PrP<sup>Sc</sup> is believed to cause fatal prion diseases in humans and other mammals, including sheep, cattle, and deer. The misfolding can occur sporadically or after contact—through inoculation or ingestion—with PrP<sup>Sc</sup> from an external source. There are also familial forms of prion diseases that are associated with certain mutations in *PNRP*, the gene encoding PrP<sup>c</sup>. These abnormal PrP<sup>c</sup> proteins are thought to have a higher probability to misfold than normal PrP<sup>c</sup>. The idea is that a few misfolded molecules can initiate a chain reaction and cause transformation of

many of the other PrP<sup>c</sup> molecules into harmful PrP<sup>Sc</sup> versions. The presence of PrP<sup>Sc</sup> proteins causes widespread cell death, leading to the characteristic spongiform degeneration of the brain that kills patients, most of them within a matter of months.

The most common human prion disease is sporadic Creutzfeldt–Jacob Disease (sCJD). The disease is rare (affecting roughly one to two individuals per a million people, worldwide), and its etiology is unclear; neither exogenous nor endogenous causes have been identified. sCJD is inevitably fatal, but the disease is clinically, pathologically, and genetically heterogeneous. Most patients



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### PrP<sup>Sc</sup> profiling was done in nine different central nervous system areas

have rapidly progressing dementia, often accompanied by involuntary muscle spasms, and death occurs within months of the first clinical symptoms. However, for some patients ataxia is the first clinical sign, while others develop sight problems, and for some the disease duration can be more than two years. In the hope that understanding the heterogeneity will help them to understand what causes sCJD, researchers are trying to systematically collect and catalog data from patients. To do this in a meaningful way, standardized assays that allow results from different patients and different laboratories to be compared in a meaningful way are necessary.

Markus Glatzel and colleagues have developed such an assay, and applied

it as part of the detailed molecular characterization to autopsy samples from 50 patients with sCJD. The new assay, which the researchers call PrP<sup>Sc</sup> profiling, measures the amount of PrP<sup>Sc</sup> in defined brain regions. In the past, PrP<sup>Sc</sup> amounts were routinely only measured in one or two regions by a variety of assays. PrP<sup>Sc</sup> profiling quantifies the amount of PrP<sup>Sc</sup> in nine defined brain regions relative to internal standards, and thereby allows for direct comparison of individual profiles.

The researchers determined PrP<sup>Sc</sup> profiles of 50 patients, and tried to correlate the profiles with information on disease types of the patients and prion types present in the different brain areas. sCJD types are determined by a patients' *PRNP* genotype at the

polymorphic position 129 of *PRNP* and by the relative resistance of PrP<sup>Sc</sup> to proteolytic degradation. It is thought that most patients only have one prion type, but previous reports have described coexistence of two different types in some samples.

Analysis of this wealth of data revealed correlations between distinct PrP<sup>Sc</sup> distribution patterns and sCJD subtypes. These results have implications for confirmation of sCJD by brain biopsy. Before doing such biopsies, Glatzel and colleagues suggest, the sCJD subtype should be determined so that the correct brain area is examined. The researchers also found coexistence of two different prion types in 20% of their overall samples, and in more than 50% of the samples from patients who were heterozygous for the 129 polymorphism in the *PRNP* gene. These data lend further support to a link between molecular signature and clinical heterogeneity of the disease.

While many questions remain, this study underlines that the systematic analysis of prion cases can reveal links between molecular pathology, genetic makeup of patients, and disease symptoms. Glatzel and colleagues believe that "PrP<sup>Sc</sup> profiling will be a valuable tool for prion research." In the hope that it will "facilitate comparisons of PrP<sup>Sc</sup> quantities present in defined samples," the researchers will make their PrP<sup>Sc</sup> standard available to the scientific community.

Schoch G, Seeger H, Bogousslavsky J, Tolnay M, Janzer RC, et al. (2006) Analysis of prion strains by PrP<sup>Sc</sup> profiling in sporadic Creutzfeldt-Jakob disease. DOI: 10.1371/journal.pmed.0030014

## Measuring Mortality in Developing Countries

DOI: 10.1371/journal.pmed.0030056

Some of the biggest public health successes in the Western world have come about because of simple records of people's deaths—their age at death, where they lived, and what they died of. Soaring lung cancer rates in the United Kingdom and United States around World War II, for example, led to life-saving research into the effects of smoking.

More than two-thirds of deaths worldwide are in developing countries, yet little is known about the causes of death in these nations. In India, for example, just one-third of deaths are registered, and of these, only one-third provide data on the cause of death. India's HIV/AIDS epidemic is rising—it may already have surpassed South Africa for the highest number of people infected. And like many other developing nations, the numbers of people dying from noncommunicable diseases such as heart disease and cancer are growing. Unlike most infectious

diseases, the causes of noncommunicable ones can be the result of several risk factors, such as smoking, elevated blood pressure, or inherited genetic mutations.

With a population of 1 billion and growing, India urgently needs better data on the causes of death in its people if it is to take further steps to improve public health. To address this need, Prabhat Jha and colleagues designed a prospective study of 1 million deaths in India to run until 2014. They will monitor an expected 1 million deaths in nearly 14 million people across 2.4 million households to find patterns of disease according to gender, age, and region, and to better understand how risk factors such as tobacco and alcohol use and indoor air pollution are related to disease.

The study uses one of India's existing frameworks for measuring mortality, called the Sample Registration System

(SRS). At present, the system covers 50,000 deaths every year. Two independent workers visit the households; one visiting every month and the other every six months. Their reports are collated and any discrepancies reconciled by a third person. To improve the system, Jha and colleagues are using an innovative method called a “verbal autopsy” to record details of death as reported by family or friends to a trained but nonmedical fieldworker. To ensure the robustness of the method, a random 10% of the fieldwork will be repeated by an independent audit team.

After validating the verbal autopsy method, the researchers began the first phase of the study, which ran from 1998 to 2003, and recorded deaths in 6.3 million people across 1.1 million urban and rural households nationwide. As of November 2005, the researchers have collected 140,000 verbal autopsy reports, and 35,000 have been coded and reconciled by two independent and trained physicians. They expect to record a total of about 300,000 deaths in the first phase and 700,000 in the second phase in 2004–2014, which will look at 7.6 million people in 1.3 million households.

Better knowledge of genetic risk factors—about which little is known in developing or developed countries—requires collection of biological samples. Jha and colleagues are also planning to test the feasibility of this by collecting dried blood spots or tubes of blood in SRS units in four to five Indian states.

Studying mortality in 14 million people is a huge challenge, but one that is necessary in view of India’s vast population. As the researchers point out, direct measurement of the causes of death is a great deal more reliable than indirect estimation. By studying diseases that are common in one part of India but not in another, new risk factors should be discovered, and these are likely relevant worldwide. Mortality measurements will be key to the success of one of the world’s largest public health initiatives—the Millennium Development Goals, which were set in 2000 when countries worldwide pledged to reduce by half or more the incidence of many diseases in poor countries. We will only know whether these goals have been met if we have reliable mortality statistics.

**Jha P, Gajalakshmi V, Gupta PC, Kumar R, Mony P, et al. (2006) Prospective study of 1 million deaths in India: Rationale, design, and validation results. DOI: 10.1371/journal.pmed.0030018**

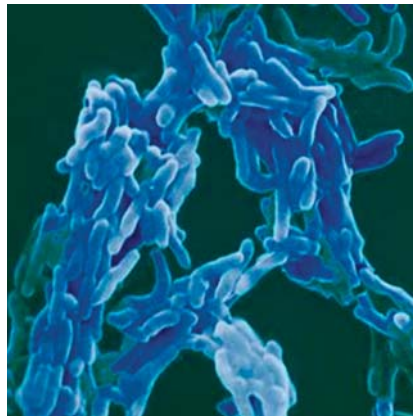
## Variants in the DC-SIGN–Encoding Gene *CD209* and Susceptibility to Tuberculosis

DOI: 10.1371/journal.pmed.0030061

One-third of the world’s population is thought to be infected with *Mycobacterium tuberculosis*, the bacterium that causes tuberculosis. Yet, even though 2–3 million people die from tuberculosis every year, only 5%–10% of individuals infected with the causative agent ever become ill. Many factors affect how well a person’s immune system fights off an attack by *M. tuberculosis*, including HIV infection, age, and malnutrition. It is also becoming clear that, as with other infectious diseases, host genetic factors affect susceptibility to tuberculosis.

One of the first genes to be associated with susceptibility to tuberculosis was *NRAMP1* (natural resistance-associated macrophage protein 1, now renamed *SLC11A1*). Others include the genes for the vitamin D receptor and the mannose receptor, and major histocompatibility complex (MHC) class II alleles. Variants of these genes, all of which encode proteins involved in the immune response, may affect how well the immune system deals with *M. tuberculosis*.

Luis Barreiro, Lluís Quintana-Murci, and colleagues now report that two variants in the gene encoding another immune system molecule—DC-SIGN, which stands for dendritic cell–specific ICAM-3-grabbing nonintegrin—are linked to tuberculosis susceptibility. The first rapid line of defense against *M. tuberculosis* and other pathogens is the innate immune system, where



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### False-color image of *Mycobacterium tuberculosis*

germline-encoded receptors recognize general features on pathogens. DC-SIGN, a C-type lectin that recognizes specific carbohydrate side chains present on the surface of pathogens, is the major receptor of this type for *M. tuberculosis* on human dendritic cells (immune system cells that process pathogens for presentation to the acquired immune system). Consequently, *CD209*—the gene encoding DC-SIGN—is a good place to search for genetic variants associated with tuberculosis susceptibility.

The researchers looked for *CD209* gene variants in 351 individuals with tuberculosis and in 360 healthy controls living in the Cape Town area of South Africa. People living there are known

as South African Colored and have a present-day uniform ethnicity. However, they derive genetically from populations of different ethnic backgrounds with different susceptibilities to tuberculosis. Due to the very high local incidence of tuberculosis, everyone living in this region, even healthy controls, is likely to have been exposed to *M. tuberculosis*. None of the participants were HIV-positive.

Barreiro and colleagues discovered two single-nucleotide polymorphisms (SNPs) or variants in *CD209* whose frequency differed significantly between healthy controls and people with tuberculosis. Both were in the promoter of *CD209* (promoters are noncoding regions that enable genes to be transcribed and proteins to be made). Variants –871G (a guanine 871 nucleotides upstream from the *CD209* coding region) and –336A (an adenosine 336 nucleotides upstream from the coding region), either alone or in combination, were associated with decreased risk of developing tuberculosis in the study population. To make sure that these associations reflected differences in disease susceptibility and not ethnic differences between the control and the diseased groups (a problem called population stratification), the researchers showed that 25 other SNPs located genomewide had similar frequencies in both groups.

The researchers also report that the “protective” variants of *CD209* are

more prevalent in Eurasians than in Africans—their presence in the South African Colored population likely reflects the mixing of the two ethnic populations. They suggest that –871G and –336A may have been selected for in Europeans but not in Africans because tuberculosis has been endemic in Europe for several centuries, whereas in Africa it is thought to have arrived much more recently. However, because DC-SIGN is a receptor for many other pathogens, this geographical pattern of variants may reflect exposure to other infectious agents besides *M. tuberculosis*. Indeed,

genetic variation at position –336 of the *CD209* promoter is associated with both the susceptibility to HIV and the severity of dengue fever. Whether it is the polymorphisms at positions –871 and –336 that directly affect host susceptibility (as opposed to other genetic variants of *CD209* that these polymorphisms are closely linked to) is not entirely clear; nor is it known how the variants affect host susceptibility to infectious disease. One possibility is that they alter how much DC-SIGN is made (and results by others suggest that this is the case for at least one of the

variants), which in turn could change the effectiveness of the host immune response. Knowing the functional consequences of the *CD209* variants and the mechanisms by which they affect disease susceptibility, suggest the authors, should ultimately help in the development of new knowledge-based treatments for tuberculosis and other infectious diseases.

**Barreiro LB, Neyrolles O, Babb CL, Tailleux L, Quch H, et al. (2006) Promoter variation in the DC-SIGN-encoding gene *CD209* is associated with tuberculosis. DOI: 10.1371/journal.pmed.0030020**

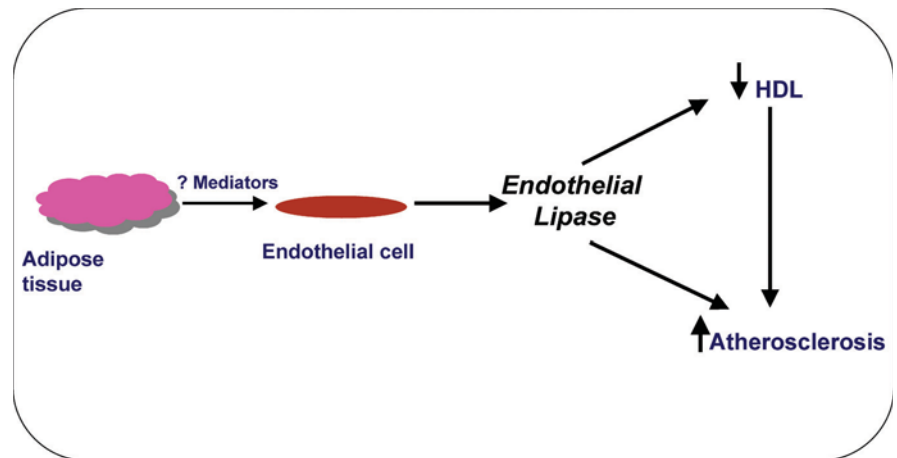
## Endothelial Lipase: A New Risk Factor for Atherosclerosis?

DOI: 10.1371/journal.pmed.0030054

Atherosclerosis, or hardening of the arteries, is the most common cause of death in industrialized countries. The formation in the arteries of deposits or plaques containing cholesterol, calcium, and other materials directly causes heart attacks, strokes, and peripheral circulatory problems by blocking the blood flow to the heart, brain, or limbs, respectively. In addition, plaque rupture causes the formation of blood clots, which can block arterial blood flow anywhere in the body.

Major risk factors for atherosclerosis include high blood pressure, obesity, smoking, and levels of certain cholesterol-containing particles in the blood stream. Cholesterol is carried around the body by particles called lipoproteins, molecules that include both lipids (fats) and proteins. Low-density lipoprotein (LDL) particles transport cholesterol to the arteries and other tissues that take up lipids. High blood concentrations of LDL particles are associated with high risk of atherosclerosis; the cholesterol carried by LDL (LDL-C) is “bad” cholesterol. By contrast, high concentrations of high-density lipoprotein (HDL) particles protect against cardiovascular disease by removing cholesterol from the arteries and taking it to the liver for excretion. Consequently, cholesterol carried by HDL (HDL-C) is “good” cholesterol, and individuals with high plasma concentrations of HDL-C and low concentrations of LDL-C are at lowest risk for cardiovascular disease.

Enzymes known as lipases modulate lipoprotein metabolism, and the activity of two of them—lipoprotein lipase and hepatic lipase—is known to affect the risk



DOI: 10.1371/journal.pmed.0030054.g001

### Endothelial lipase and atherosclerosis

of atherosclerosis. Karen Badellino and colleagues are investigating whether a third lipase—endothelial lipase (EL)—also affects atherosclerosis risk in people, since this enzyme digests HDL and affects the development of atherosclerosis in mouse models.

Badellino and colleagues have used a new immunoassay to measure plasma EL concentrations in 858 unrelated people enrolled in the Study of Inherited Risk of Atherosclerosis, a cross-sectional study designed to investigate biomarkers and genetic factors associated with coronary atherosclerosis. Participants, who were just examined once rather than followed over time, as happens in a prospective, longitudinal study, had a family history of premature coronary artery disease (CAD) but were asymptomatic and had no other major risk factors for CAD. Because EL is normally bound tightly to heparin and is largely unavailable

for immunoassay, the authors also measured EL concentrations in 510 individuals after heparin treatment to get an accurate measure of total EL mass. They then checked whether pre and postheparin EL levels were linked in any way to the concentrations of specific lipoproteins, components of the metabolic syndrome (a syndrome associated with cardiovascular disease that includes high blood pressure and obesity), or coronary arterial calcification, a noninvasive measure of early coronary atherosclerosis.

The researchers found that although the average postheparin concentration of EL was about three times the average preheparin concentration, pre and postheparin levels in individuals were strongly correlated. This result suggests that measurements of EL levels in untreated individuals will provide a good indication of the total vascular

expression of EL for future studies. Then, they discovered that EL plasma levels both before and after heparin treatment correlated positively with components of the metabolic syndrome. Furthermore, as in mice, high levels of EL correlated with low levels of HDL-C, or “good” cholesterol. This last inverse association was small but statistically significant, as was the positive association between EL concentrations and signs of early atherosclerosis, even

after taking into account other risk factors for atherosclerosis.

Overall, the authors suggest that EL is a proatherogenic factor in people, particularly those with metabolic syndrome. EL concentrations, they suggest, may modulate the composition of lipoproteins in the blood and thus atherosclerosis risk. But, they note, only prospective, longitudinal studies in which EL concentrations are correlated with

subsequent heart attacks, strokes, and other cardiovascular problems can show whether EL concentration really is a risk factor for atherosclerosis, and thus whether interventions designed to reduce EL activity can prevent atherosclerosis.

**Badellino KO, Wolfe MG, Reilly MP, Rader DJ (2006) Endothelial lipase concentrations are increased in metabolic syndrome and associated with coronary atherosclerosis. DOI: 10.1371/journal.pmed.0030022**

## The Cost of Screening Blood Donations for West Nile Virus

DOI: 10.1371/journal.pmed.0030062

West Nile virus (WNV) was first isolated in 1937 from a sick woman living in the West Nile District of Uganda. Since then, it has been found in other parts of Africa, Europe, the Middle East, and central Asia, as well as around the Pacific. Then in 1999, it arrived in the Queens borough of New York City. During this first season, it caused 62 cases of encephalitis and seven deaths. WNV has since then spread across the United States, and can now be found in most states.

WNV is a flavivirus, a type of RNA virus. Like many other flaviviruses, including the dengue and yellow fever viruses, WNV is a blood-borne virus that is passed to people through mosquito bites; in the case of WNV, the mosquitoes acquire the virus predominantly by biting infected birds. Most people infected with WNV have no symptoms, but 20% of them develop West Nile fever, a flu-like disease. About 1% of infected individuals—usually older people or those with a weak immune system—develop severe neuroinvasive disease, either encephalitis or meningitis, which can cause long-term health problems or death. In 2005, about 100 people in the US died after infection with WNV.

Virtually all WNV transmission is through mosquito bites, but a few cases of WNV infection, some of them fatal, have been linked to contaminated blood transfusions. Consequently, in 2004, the US Food and Drug Administration (FDA) mandated that blood donations must be screened for WNV. The FDA did not recommend a specific screening method. In an ideal world, screening would aim to reduce the risk of contracting WNV from a blood transfusion as much as possible, at any cost. In the real world, however, the health benefits of any screening methodology (lives saved and improvements in the quality of life)



DOI: 10.1371/journal.pmed.0030062.g001

**A number of mosquito species can transmit WNV, including *Aedes japonicus*, depicted here**

have to be balanced against the costs of screening. To find out where this balance lies for WNV, Caroline Korves, Sue Goldie, and Megan Murray have estimated the cost-effectiveness of different strategies for screening blood donations for WNV and now report their results.

The researchers used a computer-based mathematical model to compare different screening strategies. The baseline strategy was a donor questionnaire—blood donors reporting a recent fever cannot donate blood. The other strategies tested were nucleic acid testing of pools or individual samples of blood for WNV, universal screening versus screening restricted to donations destined for immunocompromised recipients, and seasonal screening versus screening throughout the year (WNV transmission peaks in late summer/early fall). The researchers modeled the cost-effectiveness of these strategies in areas with high levels of WNV transmission over a long season (as occurred in Mississippi in 2002), high transmission over a short season (Nebraska 2002), and low transmission over a short season (Massachusetts 2002).

Korves and colleagues found that in low-transmission areas with a short season, screening by questionnaire alone was the most cost-effective strategy—any other strategy was unlikely to prevent any cases of serious illness despite greatly increasing costs. In areas with high transmission, the best approach was seasonal screening by nucleic acid testing of individual donations earmarked for immunocompromised recipients. The researchers also discovered that seasonal screening of all donations provided little additional clinical benefit and was prohibitively expensive, and that screening throughout the year provided no additional benefit in any setting.

These results indicate that the currently mandated policy of screening donated blood for WNV may not be the best public health strategy. More restricted screening strategies may be preferable, suggest the investigators, with individual states adopting screening strategies that reflect the intensity and duration of their West Nile epidemics. The researchers also note that their estimates of the cost of WNV blood screening strategies were greater than generally accepted cost-effectiveness thresholds for health interventions. Thus, the resources spent in preventing rare cases of WNV infection arising from blood transfusion might be better used to reduce WNV transmission through controlling mosquito vectors. If such an approach were successful, they suggest, it might obviate the need for screening blood for WNV in many areas.

**Korves CT, Goldie SJ, Murray MB (2006) Cost-effectiveness of alternative blood screening strategies for West Nile virus in the United States. DOI: 10.1371/journal.pmed.0030021**

## Pathological Clues to How the SARS Virus Kills

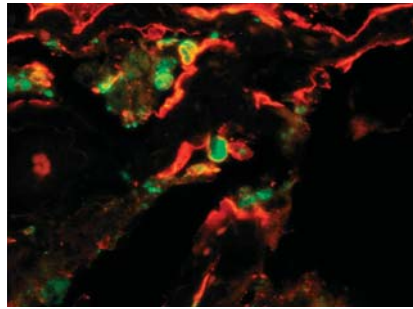
DOI: 10.1371/journal.pmed.0030059

Severe acute respiratory syndrome (SARS) first emerged in Guangdong Province, China, in November 2002. At the end of February 2003, an infected doctor from the province inadvertently took the illness to Hong Kong. From there, a woman staying in the same hotel contracted the disease and took it back with her when she returned to Toronto, Canada. SARS, with its ability to spread by close person-to-person contact and with its 10% death rate, was on the move and was threatening to cause a worldwide epidemic. The World Health Organization responded rapidly to this threat by issuing a global alert, and warning against unnecessary travel to affected areas. This and rigorous local containment efforts meant that only 8,098 people became ill, and only 774 people died in this first SARS epidemic.

The last case of the epidemic was reported in Taiwan in June 2003, and since then there have only been a few isolated cases. But the coronavirus responsible for SARS (SARS-CoV) could cause another epidemic at any time. Consequently, scientists continue to study SARS intensively, including researchers from Hong Kong and Toronto, who have joined forces to examine the organs of people who died from SARS in these two cities and now report their results. As John Nicholls, the leader of the international team, explains, understanding how the SARS virus kills people should help in the treatment of SARS if it re-emerges.

Nicholls and colleagues collected post-mortem material from 32 fatal cases of SARS (the largest such collection to date), and asked three questions about the pathogenesis of SARS. First, was SARS-CoV present in the lungs of these patients throughout their illness? Second, which cells in the lungs contained the virus? Third, did any other tissues contain SARS-CoV? The researchers used three molecular techniques to look for SARS-CoV in their specimens: immunohistochemistry, which detects virus-specific proteins; in situ hybridization, which detects the viral genome; and reverse-transcriptase polymerase chain reaction (RT-PCR), which measures viral load.

The researchers found that SARS-CoV was present only in the lungs of patients who died within two weeks of becoming



DOI: 10.1371/journal.pmed.0030059.g001

### SARS coronavirus (green) in lung cells

ill (four out of seven patients). In 25 patients who died more than two weeks after the onset of symptoms (generally high temperature and lower respiratory tract symptoms, followed by pneumonia), there was no SARS-CoV in post-mortem lung tissue, although in one case the virus had been present in an open lung biopsy taken five days after disease onset. The researchers found no virus in tissues other than the lung in any of the patients, but in the four patients whose lungs contained SARS-CoV, the virus was found in pneumocytes—cells that line the alveoli, the terminal air spaces where gas exchange occurs—and sometimes

in alveolar macrophages, a type of immune cell. No SARS-CoV was found in the cells lining the tubes leading to the alveoli, which explains why patients with SARS only have lower respiratory tract symptoms.

These results indicate that the human immune system can stop SARS-CoV replicating within two weeks of infection. By that time, however, the damage to the lungs in some patients appears to be so great that they die even without continued viral replication. This time course of events indicates that antiviral drugs are likely to be useful only during the early stages of SARS. In addition, the absence of virus outside the lungs suggests that death is the result of SARS-CoV replicating in the lungs alone. Whether SARS-CoV fatally damages lung tissue directly or whether macrophages recruited to the lungs in response to infection with SARS-CoV cause fatal immunopathological changes remains an open question.

Nicholls JM, Butany J, Poon LLM, Chan KH, Beh SL, et al. (2006) Time course and cellular localization of SARS-CoV nucleoprotein and RNA in lungs from fatal cases of SARS. DOI: 10.1371/journal.pmed.0030027

## Minority Participation in Health Research—Facts and Fiction

DOI: 10.1371/journal.pmed.0030040

It is widely believed that racial and ethnic minority groups, especially in the US, are less willing to participate in health research than non-minority groups. According to this view, minority groups' comparative unwillingness to participate is due to a lack of trust in health research and health researchers, which traces to past abuses, particularly the Tuskegee Syphilis Study. Conducted from 1932–1972, the US government-funded Tuskegee study examined the natural course of syphilis. The participants, 399 African-American men in the late stages of syphilis, were enrolled and offered free medical care but kept in the dark about the nature of their illness and the purpose of the study. Participants were told that they were being treated for “bad blood,” but the doctors had no intention of curing them and even withheld penicillin treatment when it became available. When the experiment was finally ended—after public outcry following

exposure in the media—28 of the men had died directly of syphilis, 100 were suffering from related complications, 40 of their wives had become infected, and 19 of their children had been born with congenital syphilis.

Given that the study was not halted until 1972, it would not be surprising if its memory influenced the attitudes of minority individuals toward health research today. This is a potential problem, because it is essential that participants in health research are as diverse as the population whose health should be improved as a result of the research. (And in the US today, one in five people is from a minority group). Only representative participation ensures that the outcomes of health research can be generalized to a diverse population.

Is participation in health research representative of the population? A number of studies suggest that in the US it is not; minority groups are often

under-represented in US research studies. But what are the reasons? Are minority groups less willing to participate, or are they given fewer opportunities to participate? Answering this question is vital for efforts to increase minority participation in health research. Should these efforts focus on changing minority attitudes or on removing potential barriers to participation, such as whether minorities are adequately informed of research opportunities?

In a systematic review in this month's *PLoS Medicine*, David Wendler and colleagues assessed whether individuals from minority groups who were eligible and invited to participate in health research were indeed less likely to consent to participate than non-minority individuals. The authors identified 20 health research studies that reported consent rates by race or ethnicity. Eighteen were single-site studies conducted exclusively in the US or multi-site studies conducted primarily or exclusively in the US. The 20 studies collectively report the enrollment decisions of over 70,000 individuals for a broad range of health research studies.

For the three non-intervention studies, African-Americans had a non-significantly lower overall consent rate than non-Hispanic whites, while Hispanics had a non-significantly higher overall consent rate than non-Hispanic whites. For the ten intervention studies, African Americans' overall consent rate was non-significantly higher than that of non-Hispanic whites, while Hispanics had a statistically significant higher overall consent rate than non-Hispanic whites. For the seven surgery trials, minorities as a group had a non-significantly higher overall consent rate than non-Hispanic whites.

Although Wendler and colleagues found only small differences in consent rates by race or ethnicity, they found substantial differences by race or ethnicity in the number of individuals invited to participate. For example, one study of medical versus surgical management of angina offered enrollment to 2,065 non-Hispanic whites but to only 30 individuals from minority groups.

This study, Wendler and colleagues say, "suggests that racial and ethnic

minorities are as willing as non-Hispanic whites to participate in health research." Indeed for some kinds of studies, minority individuals seem more willing to enroll than non-minority individuals. The authors acknowledge their study's limitations, particularly the fact that most individuals were from the US and the willingness of minority groups outside the US to participate in health research may be very different. Other important questions not addressed by this study are what motivates people to participate in health research, and whether motives differ between majority and minority groups. Despite these and other remaining questions, the results of this study suggest that efforts to increase minority participation in health research should concentrate on ensuring better access to health research for all groups, rather than on changing the attitudes of minority groups.

**Wendler D, Kington R, Madans J, Van Wye G, Christ-Schmidt H, et al. (2006) Are racial and ethnic minorities less willing to participate in health research? DOI: 10.1371/journal.pmed.0030019**

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