Synopses of Research Articles

How Group A Streptococci Hide in Macrophages

In the past 10 years, evidence has suggested that group A streptococci (GAS) adhere to epithelial cells and invade them. In 1994, LaPenta and colleagues found that GAS could invade human epithelial cells, in some cases better than classical intracellular bacterial pathogens such as *Listeria* and *Salmonella* spp. can.

Several studies quickly confirmed this landmark finding and produced further evidence to support this mechanism. One study showed that high-frequency invasion needed expression of M protein (a surface protein, variation in which provides the basis of Lancefield's method of serotyping GAS) and/or fibronectin-binding proteins such as SfbI. Another study found that the M1 serotype of GAS was particularly adept at intracellular invasion of epithelial cells.

It appeared, therefore, that GAS could invade human cells using several mechanisms, which suggested that intracellular invasion played an important role in the disease pathogenesis. The most direct evidence for intracellular invasion came from studies of patients with recurrent tonsillitis, when it was found that antibiotics failed to eradicate streptococci from the throat in about 30% of cases of pharyngotonsillitis. Osterlund and colleagues showed that tonsils excised from such patients contained intracellular GAS.

The reason for invasion of host cells is not clear, although it was suggested that streptococci might find the intracellular environment a good place to avoid host defense mechanisms. Other theories suggested that internalization played a role in the carriage and persistence of streptococci. Internalization might also be involved in the invasion of deeper tissues, although some studies have found that low virulence was associated with internalization.

In this month's *PLoS Medicine*, Pontus Thulin, Anna Norrby-Teglund, and colleagues try to further elucidate the mechanism behind GAS pathogenesis by examining the in vivo interactions between GAS and cells involved in innate immune responses. The team used human biopsies collected from 17 patients with soft-tissue infections. They found that host phagocytic cells, primarily macrophages, were being used as reservoirs for intracellular bacteria during the acute tissue infection.

The data suggest that this could be a mechanism to avoid antibiotic eradication, which might explain why a high bacterial load was present even in tissues collected after prolonged intravenous antibiotic therapy. The authors also note that the localization of GAS varied and depended on the severity of tissue infection; intracellular localization was most frequent in noninflamed tissue, which also had a low bacterial load. Osterlund's study had already shown that intracellular GAS was present in pharyngeal epithelial and macrophage-like cells of patients with tonsillitis. However, the current study by Thulin and colleagues shows for the first time that this occurred in vivo during severe invasive GAS soft-tissue infections in humans, even while patients are on antibiotic therapy.

The theoretical implication of this study is that if intracellular bacteria are most commonly found in newly involved tissue with low inflammation and low bacterial load, it might be the case that internalization could promote the spread of bacteria within the tissue. This so-called Trojan horse approach is also seen in the fish, and sometimes human, pathogen *Streptococcus iniae*,
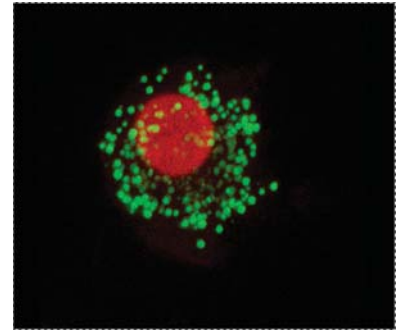
Human macrophages are host cells for intracellular group A streptococci. The figure shows viable (green) bacteria within a macrophage (cell nuclei in red).

which invades macrophage-like cells in a similar way. Studies are currently exploring whether this also occurs in GAS soft-tissue infections. But in any case, the clinical implication of this finding is that alternate therapies will be required if clinicians are going to improve the morbidity and mortality associated with severe GAS soft-tissue infections.

Thulin P, Johansson L, Low DE, Gan BS, Kotb M, et al. (2006) Viable group A streptococci in macrophages during acute soft tissue infection. DOI: 10.1371/journal.pmed.0030053

## Failures in the Management of Elderly Women with Breast Cancer

Breast cancer is one of the highest-profile diseases in women in developed countries. Although the risk for women younger than 30 years is minimal, this risk increases with age. One-third of all breast cancer patients in Sweden, for example, are 70 years or older at diagnosis. Despite these statistics, few breast cancer trials take these older women into account. Considering that nowadays a 70-year-old woman can expect to live for at least another 12–16 years, this is a serious gap in clinical knowledge, not least because in older women breast cancer is more likely to be present with other diseases, and doctors need to know whether cancer treatment will affect or increase the risk for these diseases.

In 1992, guidelines were issued to the Uppsala/Örebro region in Sweden (with a population of 1.9 million) that all women with breast cancer should be able to receive equal treatment. At the same time, a breast cancer register was set up to record details about patients in the region, to ensure that the guidelines were being followed. Sonja Eaker and colleagues set out to assess data from the register to see whether women of all ages were receiving equal cancer treatment.

They compared the 5-year relative survival for 9,059 women with breast cancer aged 50–84 years. They divided them into two age groups: 50–69 years, and 70–84 years. They also categorized the women according to the stage of breast cancer. They looked at differences between the proliferative ability of breast cancer cells, estrogen receptor status, the number of lymph nodes examined, and
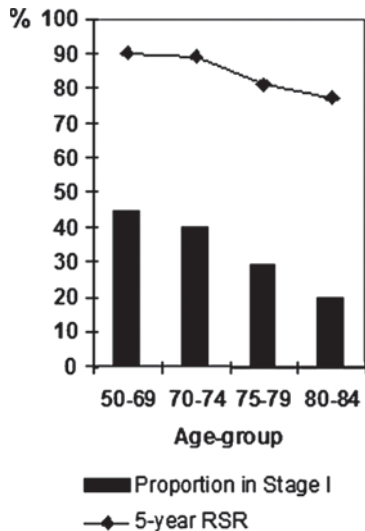
The proportion of women diagnosed in stage I breast cancer and the 5-year cumulative relative survival ratio differ by age group

lymph node involvement. The researchers also compared types of treatment—i.e., surgical, oncological (radiotherapy, chemotherapy, or hormonal)—and the type of clinic the patients were treated in.

They found that women aged 70–84 years had up to a 13% lower chance of surviving breast cancer than those aged 50–69 years. Records for older women tended to have less information on their disease, and these women were more likely to have unknown proliferation and estrogen receptor status.

Older women were less likely to have their cancer detected by mammography screening and to have the stage of disease identified, and they had larger tumors. They also had fewer lymph nodes examined, and had radiotherapy and chemotherapy less often than younger patients. Current guidelines are vague about the use of chemotherapy in older women, since studies have included only a few older women so far, but this did not explain why these women received radiotherapy less often. Older women were also less likely to be offered breast-conserving surgery, but they were more likely to be given hormone treatment such as tamoxifen even if the tumors did not show sign of hormone sensitivity. The researchers suggest that this could be because since chemotherapy tends to be not recommended for older

women, perhaps clinicians believed that tamoxifen could be an alternative.

The researchers admit that one drawback of their study is that there was little information on the other diseases that older women had, which might explain why they were offered treatment less often than younger patients.

However, the fact remains that in Sweden, women older than 70 years are offered mammography screening much less often than younger women—despite accounting for one-third of all breast cancer cases in the country—and those older than 74 years are not screened at all.

Eaker and coworkers' findings indicate that older women are urgently in need of better treatment for breast cancer and guidelines that are more appropriate to their age group. Developed countries, faced with an increasingly aging population, cannot afford to neglect the elderly.

Eaker S, Dickman PW, Bergkvist L, Holmberg L, the Uppsala/Örebro Breast Cancer Group (2006) Differences in management of older women influence breast cancer survival: Results from a population-based database in Sweden. DOI: 10.1371/journal.pmed.0030025

Race/Ethnicity, Lipoproteins, and Antiretroviral Therapy

HIV-1 infection has become a chronic, manageable condition for patients who can get long-term access to highly active antiretroviral therapy (HAART). However, for some patients, HAART causes various metabolic complications, including the development of dyslipidemia. For example, some protease inhibitors (PI), a class of anti-HIV drugs, have been associated with elevated levels of cholesterol, triglyceride (TG), and low high-density lipoprotein cholesterol (HDL-c).

PI-based HAART has also been associated with increased risk for cardiovascular disease, which could, it has been suggested, lead to a future epidemic of cardiovascular disease in patients with HIV-1 treated with this regimen long term.

PIs are not the only anti-HIV drugs thought to play a role in dyslipidemia. Researchers suspect that non-nucleoside reverse transcriptase inhibitors (NNRTIs), a widely used class of antiretroviral, may also contribute to the condition. But the role of NNRTIs is less clear. The relationship between HIV-1 infection and its treatment is a complex one that we still do not completely understand, and it may have a profound impact on the future well-being of this population.

The features of PI-HAART-related dyslipidemia resemble familial combined hyperlipidemia (a lipid disorder that runs in families and which has a genetic basis), which suggests that lipoprotein gene variants might be linked to this condition. For example, apoC-III, a protein whose plasma levels are directly correlated with TGs in the general population, has also been implicated in regulating breakdown of triglyceride-

rich lipoprotein. Several studies have established a complex interaction of genetic variation between apoC-III and apoA-I/C-III/A-IV/AV cluster with plasma TG levels. Two previous studies, restricted almost entirely to White individuals, reported a marked increase in plasma TG levels in patients with HIV-1 on PI-HAART regimens when they also had apoC-III and apoE gene variants.

Given the worldwide demographics of HIV-1 infection, the relationship between PI-HAART-related metabolic events and race/ethnicity could be important. The relationship between race/ethnicity and lipoproteins has been well described in the general population. Race/ethnicity has been considered a surrogate for environmental influences on lipids, although recent studies have demonstrated that genetic factors also account for important differences in plasma lipids across racial/ethnic groups. For example, studies have shown that some hepatic lipase single nucleotide polymorphisms (SNPs) present only in African Americans result in lower enzyme activity, and account for a proportion of the racial/ethnic differences in HDL-c levels.

The role of race/ethnicity in metabolic complications in individuals with HIV-1 has not really been considered. In a new study, Andrea Foulkes and colleagues examined the influence of racial/ethnic influences on plasma lipoproteins, and studied the genetic effects on lipids in patients with HIV-1. They did a cross-sectional analysis of race/ethnicity, apoC-III/apoA-I genotypes, and PI exposure on plasma lipids on 626 patients participating in several ongoing AIDS Clinical Trial Group studies. They found that race/ethnicity was a predictor of plasma lipids in

patients with HIV-1 on HAART. Overall, Black patients on HAART had a less atherogenic lipid profile than White and Hispanic patients. The authors believe their finding was consistent with epidemiological data in non-HIV-1 populations, and could be of particular importance given the global spread of HIV-1 infection, especially in sub-Saharan Africa. They also found that in Hispanic, but not in White or Black, patients, apoC-III gene variants were associated with less dyslipidemia in patients exposed to PI-containing HAART.

Foulkes and colleagues say their findings are the first evidence, to their knowledge, for race/ethnicity–specific differences in both the occurrence of dyslipidemia on antiretroviral therapy (ART) and the influence of genetic factors on the prevalence of PI-related lipid abnormalities. But they stress that the findings do not imply that "race" was responsible for functional differences between gene variants. Rather, it is much more likely that differences relate to variation in the linkage disequilibrium (the inheritance pattern of blocks of DNA, or haplotypes, over time)

in apoC-III, which they found to vary with race/ethnicity, or to the confounding influences of additional environmental and/or genetic factors.

Putting race/ethnicity aside, the authors rightly point out that any strategy that identifies individuals with HIV-1 at increased risk of ART-related metabolic complications will improve decision making for selecting appropriate ART regimens and preventive cardiovascular therapies, and ultimately will decrease the long-term cardiovascular risk of these patients. In addition, they emphasize that such strategies have to be assessed in studies that include adequate numbers of individuals within all racial/ethnic groups in order to determine their generalizability and utility in the worldwide population of patients with HIV-1.

Angiopoietin-2: A New Therapeutic Target for Preventing Lung Dysfunction in Sepsis?

Every year, more than 200,000 Americans die from sepsis, a severe illness caused by bacterial infection of the bloodstream. In the United States, 2%–3% of all hospital admissions are for sepsis, which has a mortality rate of about 30%. In sepsis, pathogens that have entered the bloodstream through a wound or from an infected organ induce a systemic inflammatory response, which can cause circulatory system damage, multiple organ dysfunction, and death, particularly in people with other medical conditions.

Treatment for sepsis includes antibiotics to clear the initiating bacteria and to provide medical support for damaged organs—mechanical ventilation for lung dysfunction, for example. However, the prognosis for patients would be much better if organ dysfunction could be prevented, so researchers, including Vikas Sukhatme and colleagues, are investigating how organs become damaged during sepsis. Sukhatme's team now reports results suggesting that a protein called angiopoietin-2 may play a pivotal role in acute respiratory distress syndrome (ARDS), a condition that affects about 40% of patients with sepsis and worsens their prognosis.

ARDS starts with protein-rich fluid leaking out of the blood capillaries of the lung into the alveoli, the terminal airspaces where gas exchange normally occurs. The presence of this fluid in the alveoli impairs gas exchange, and prevents the lungs from expanding
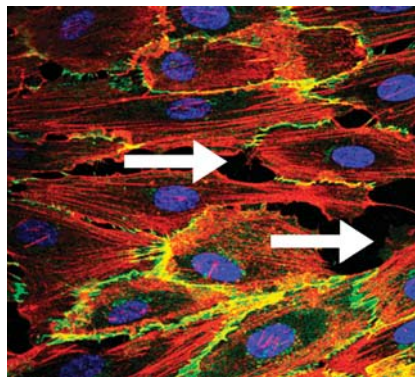
DOI: 10.1371/journal.pmed.0030097.g001

Ang-2 causes intercellular gaps in cultured endothelial cells

and contracting normally during breathing. But what causes fluid to leak out of the vasculature? Capillaries are normally lined with endothelial cells linked together with adhesive junctions to form an impermeable barrier. The development of this barrier is controlled in part by two peptides called angiopoietin-1 (Ang-1) and angiopoietin-2 (Ang-2). Both peptides bind as ligands to a signaling receptor called Tie-2. Ang-1 functions as an agonist; its binding activates the Tie-2 receptor. In contrast, Ang-2 is an antagonist, whose binding blocks signal transduction. Animal experiments have shown that the two ligands have opposite functions in vascular development: Ang-1 promotes the stabilization of nascent blood vessels; Ang-2 disrupts this action. These

findings, the fact that Tie-2 expression may be abnormal in bronchopulmonary dysplasia (a developmental lung disorder), and the high level of Tie-2 expression in lungs prompted Sukhatme's team to ask if the Tie-2 signaling pathway is affected in sepsis and, specifically, if excess Ang-2 levels might promote the vascular leakage that underlies ARDS.

The researchers measured serum Ang-2 concentrations in 22 patients admitted to the hospital with sepsis, as well as in 29 control individuals hospitalized with other conditions. Admission levels of Ang-2 were higher in patients with septic shock (characterized by low blood pressure) or multiorgan failure than in control patients or in patients with sepsis alone. Over time, Ang-2 levels mirrored the clinical course of illness in individual patients, increasing as they became more sick and declining as they recovered. Finally, Ang-2 levels correlated with impaired lung function. These results are consistent with the idea that increased circulating Ang-2 might contribute to vascular leakage in sepsis.

To test their hypothesis further, the researchers turned to thin sheets (monolayers) of cultured endothelial cells. The addition of patient serum containing high levels of Ang-2 (but not serum containing low levels) to these monolayers caused changes in the actomyosin cytoskeleton (a network of proteins that forms the cellular skeleton). These structural changes, which resulted in the endothelial cells contracting and

gaps forming between them, could be replicated by the addition of Ang-2 alone, and were accompanied by increased permeability of the endothelial monolayer. Adding Ang-1 reversed the effects of Ang-2 on the endothelial cells. Further experiments indicated that Ang-2 activated a protein called Rho-kinase and the subsequent addition of phosphate groups to myosin light chain, a protein involved in cell contraction. In the last set of experiments, the researchers showed that systemic administration of Ang-2 to healthy mice increased vascular permeability and caused fluid extravasation in their lungs, changes reminiscent of early ARDS.

These results support a role of Ang-2 in sepsis-associated ARDS, and suggest new targets for the treatment of sepsis. Up until now, the emphasis has been on damping down the systemic immune response, but drugs that do this often have unwanted side effects. Targeting Tie-2 signaling might be a cleaner way to deal with the leaky vasculature that causes ARDS and other aspects of sepsis. In addition, suggest Sukhatme and colleagues, serial Ang-2 measurements might identify which patients at risk of developing ARDS would benefit most from therapy aimed at preventing vascular leakage. But, they acknowledge, further experiments are needed to prove conclusively that Ang-2 plays a causal role in human ARDS, to clarify why the lungs are especially susceptible to systemic excess levels of Ang-2, and to test whether interfering with Ang-2 function can yield therapeutic benefits.

Parikh SM, Mammoto T, Schultz A, Yuan HT, Christiani D, et al. (2006) Excess circulating angiopoietin-2 may contribute to pulmonary vascular leak in sepsis in humans. DOI: 10.1371/journal.pmed.0030046

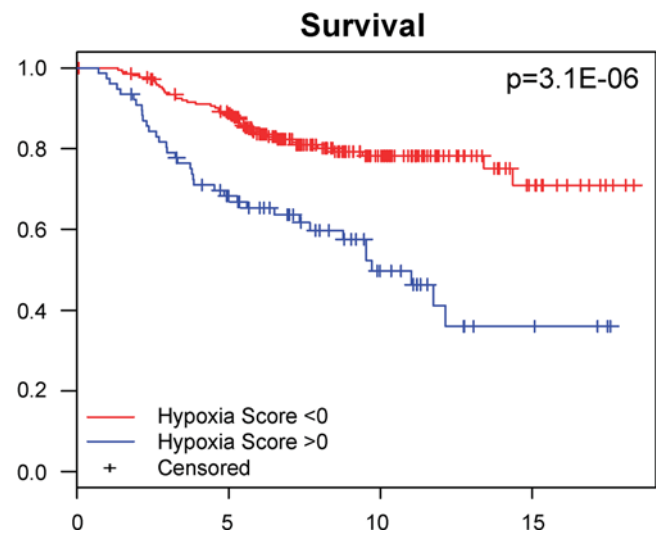# Hypoxia Responses: How Different Cells and Tumors React to Oxygen Shortage

All cells require an adequate supply of oxygen to function. Oxygen is used in aerobic metabolism, which turns carbohydrates into the energy needed to power essential cellular processes such as protein synthesis. Cells, therefore, have to sense and respond to hypoxia—inadequate oxygen levels. In mammalian cells, a family of transcription factors (proteins that control when genes are transcribed) called hypoxia-inducible factors (HIFs) are particularly important in orchestrating cellular responses to hypoxia.

HIF-1—the first HIF to be identified—contains two subunits. HIF-1β is expressed all the time; HIF-1α is an oxygen-sensitive inducible subunit. When there is sufficient oxygen (normoxic conditions), enzymes called prolyl hydroxylases add hydroxyl groups to HIF-1α. This leads to the recognition of HIF-1α by von Hippel–Lindau (VHL) protein, which then adds a molecular tag to HIF-1α that targets it for destruction. When oxygen is limiting, the prolyl hydroxylases cannot work, so HIF-1α escapes destruction and forms an active transcription factor by dimerizing with HIF-1β. This induces the expression of numerous target genes that help cells to survive hypoxia. HIF-1α can also escape destruction in normoxic conditions if the VHL protein is defective or missing.

DOI: 10.1371/journal.pmed.0030105.g001

Hypoxia score is associated with patient survival

As well as their important role in normal cells, hypoxia responses are also important in many human diseases. In cancer, the lack of oxygen deep within the tumor or genetic changes can induce a strong hypoxia response that helps the tumor to grow faster, and reduces the efficacy of chemotherapy and radiotherapy. But, despite the importance of the hypoxia response in sickness and in health, it is known that a hypoxia response does not affect all tissues evenly. But little is known about how different cell types respond to hypoxia. DNA microarrays ("gene chips" that can synchronously interrogate thousands of genes for their expression levels) have proven to be very valuable tools to evaluate the similarity and differences between cell types. Jen-Tsan Chi, Zhen Wang, Patrick Brown, and an international team of researchers have remedied this by examining global changes in gene-expression programs in response to hypoxia in several different cell types.

The researchers grew four human cell types in normoxic, hypoxic, or anoxic conditions and determined which genes were expressed in each condition using DNA microarrays. They then used a mathematical approach to identify genes whose expression under hypoxic conditions varied according to cell type. They found that the expression of many more genes was induced in kidney epithelial cells than in breast epithelial cells; fewer genes still were induced in nonepithelial cells. To understand physiological and pathological responses to oxygen variability, it will be necessary to find out exactly what drives these tissue- and cell type–specific differences. For now, though, the researchers report that the vigorous transcriptional response to hypoxia in renal epithelial cells was, in part, due to high levels of HIF-1α expression in these cells. They also suggest that this strong hypoxia response may explain why patients with VHL disease, who have a mutated *VHL* gene, develop mainly kidney tumors.

The researchers next used gene-expression data from renal and breast epithelial cells to derive a gene-expression signature of the epithelial hypoxia response. Because tumor hypoxia is an important factor in human cancer progression, the researchers reasoned that such a signature might help to predict clinical outcomes in patients with various carcinomas (epithelial cell tumors). For kidney, breast, and ovarian cancers, the signature allowed the researchers to divide tumors into a high hypoxia

response group and a low hypoxia response group. For breast and ovarian cancer, a high hypoxia response correlated with poor outcome.

Finally, Chi and colleagues investigated whether the gene-expression signature of hypoxia could indicate the likely outcome for patients any better than existing prognostic factors (for example, tumor size and grade). Their results indicated that the information provided by the hypoxia signature was more predictive than any of the clinical parameters currently in use, and independent of predictive information provided by a "wound" gene-expression signature constructed in a similar way. The researchers conclude that

by integrating gene-expression characteristics of tumors with genetic changes and clinical data, it should be possible to build up a detailed biological profile of individual tumors that could be used both to predict their clinical course and to choose appropriate general or targeted therapies (for example, drugs designed to inhibit HIF activity) when these become available.

Long-Term Outcome of Individuals with Borderline Thrombocytopenia

Many cases of thrombocytopenia in adults are diagnosed incidentally after a routine blood cell count; more efficient laboratory processes have meant that an increasing number of asymptomatic individuals with borderline low platelet counts (100×10^9/l to 150×10^9/l) are now being identified. There are gaps in our knowledge of this condition, and the natural history of thrombocytopenia has not been systematically studied. For example, it's not known how many people might develop other diseases associated with a low platelet count, or how many people can live outwardly healthy lives with borderline normal platelet counts. In the absence of other diseases, current treatment guidelines are based on expert opinion rather than on randomized controlled trials, and patients are treated if they have severe bleeding and/or extremely low platelet counts.

One cause of an asymptomatic low platelet count is an immune thrombocytopenia (ITP), either primary (idiopathic) or secondary to an autoimmune disorder. Hence, thrombocytopenia might be the start of a systemic autoimmune disease, which can make it difficult to diagnose secondary immune thrombocytopenia from ITP. Systemic autoimmune diseases could be treated if detected early.

In this month's *PLoS Medicine*, Roberto Stasi and colleagues followed the natural history of 260 apparently healthy adults who had low platelet counts and were considered to have "borderline thrombocytopenia." After six months of monitoring to see if the condition persisted, 191 patients were monitored for, on average, 64 months. In 64% of these patients, thrombocytopenia resolved spontaneously or persisted with no other disorders becoming apparent. In the remainder, the most

frequent event was the development of an autoimmune disease. The 10-year probability of developing idiopathic ITP was 6.9% and of developing autoimmune disorders other than ITP was 12%. Among 36 patients (14%) with stable normal platelet counts, most (23/36) improved their counts during the initial six-month assessment. This pattern might be because they had a "silent" viral infection or a minor insult of a different nature to the bone marrow, the authors suggest. Another theory is that these patterns might also be due to seasonal variation of the platelet count.

Interestingly, in this study many patients with a low platelet count who developed autoimmune disease did not have conventional markers of autoimmunity. This finding conflicts with

previous work that has suggested that autoantibodies are present many years before the diagnosis of systemic lupus erythematosus. Specifically, two patients in this study who developed systemic lupus erythematosus did not have detectable autoantibodies.

Many patients with low platelet count also had chronic thyroiditis. Based on previous evidence, the relationship between autoimmune thrombocytopenia and chronic thyroiditis is controversial, and the occurrence of patients with chronic thyroiditis with borderline thrombocytopenia (7%) was not higher than the general population (6.2%). Patients with chronic thyroiditis did not have a higher risk of developing ITP or other autoimmune disorders than the other individuals, the authors say.
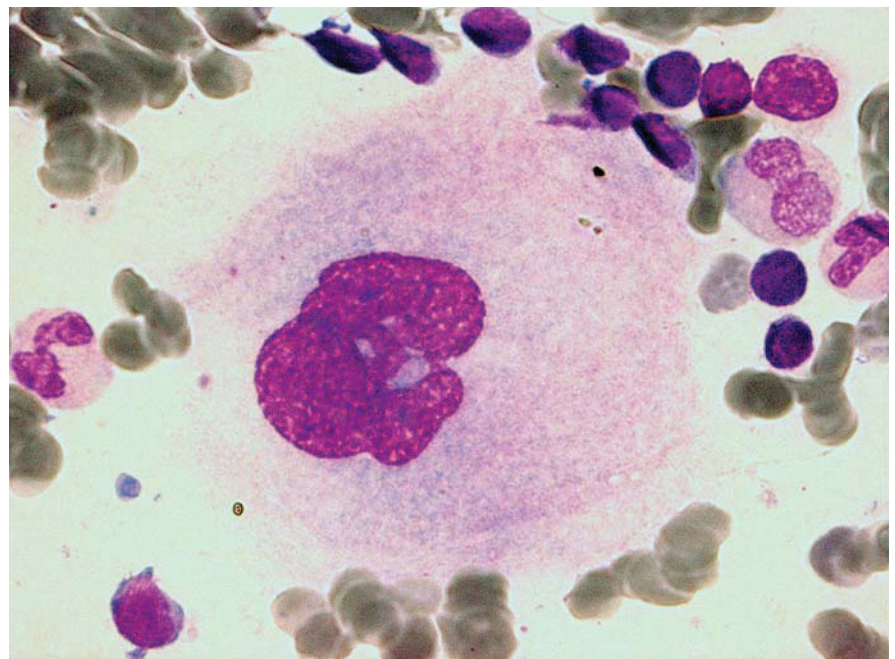
DOI: 10.1371/journal.pmed.0030093.g001

Microphotograph of a megakaryocyte from an individual who developed ITP

This study will improve clinicians' understanding of the natural history of borderline thrombocytopenia, although exactly what these figures mean for individuals is hard to judge because there was no control group. The authors acknowledge that much more needs to be done. For example, for conditions such as ITP, diagnosis is still by exclusion, so other diseases that might cause thrombocytopenia have to be ruled out. Further work will need to be done to establish whether the risk for these patients of developing an autoimmune disease is higher than in the general population and, most practically, whether intensive follow-up has a positive impact on patients' prognosis.

Stasi R, Amadori S, Osborn J, Newland AC, Provan D (2006) Long-term outcome of otherwise healthy individuals with incidentally discovered borderline thrombocytopenia. DOI: 10.1371/journal. pmed.0030024

Toward an Individual Approach to Methadone Therapy of Heroin Addicts

Between 750,000 and 1 million people in the United States are addicted to heroin, a semisynthetic opioid made from the seeds of opium poppies. This highly addictive, illegal drug is converted in the brain into morphine, which binds to opioid receptors (which normally bind pain-relieving peptides produced by the brain) to produce a euphoric rush or heroin "high." Repeated heroin use causes drug dependency—increasing amounts of the drug are needed to achieve its pleasurable effects, and its removal rapidly produces unpleasant withdrawal symptoms ("lows") that can last for several days to months. Users become addicts when their desire to take heroin outweighs the negative health, social, financial, and legal consequences of their drug habit.

For more than 30 years, the synthetic narcotic (a drug that induces sleep) methadone has been used to treat heroin addiction. Methadone, a powerful pain-relieving drug, binds to the same receptors as heroin but without producing the euphoric rush. Because it lasts much longer in the body than heroin, patients trying to abstain from heroin need to take only a single daily dose of methadone to avoid withdrawal symptoms. Although patients become physically dependent on methadone, the reduction in withdrawal symptoms, together with a reduction in drug cravings, helps heroin addicts in methadone maintenance treatment programs stop using illicit drugs and lead normal lives.

The minimum maintenance dose of methadone recommended in these programs—60mg/day—is derived from randomized trials that have tested the

DOI: 10.1371/journal.pmed.0030128.g001

ability of different doses of methadone to wean populations of addicts off heroin. However, many clinicians report that lower doses of methadone are effective in some patients. To test whether there is scientific evidence for these anecdotal observations, Jodie Trafton, Jared Minkel, and Keith Humphreys undertook a prospective observational study of individual responses to methadone treatment. They now report that setting a standard dose will not optimize therapy for all patients, and recommend that methadone doses be titrated on an individual basis to achieve heroin abstinence.

The researchers studied 222 volunteers addicted to heroin for a year after they started methadone treatment at eight clinic sites. Four of these sites regularly dosed patients with more than the recommended minimum daily methadone dose, and four dosed a significant number of patients with lower doses. Overall, 168 volunteers achieved heroin abstinence for at least a month, as measured by the absence of illicit opioids in their urine. The median effective daily dose of methadone taken by these successful volunteers was 69 mg, but doses ranged from 1.5 to 191.2 mg. Of those who abstained, 16% took daily doses of more 100 mg methadone, 38% remained abstinent on less than the recommended minimum daily dose, and almost half of the patients who did not achieve abstinence received more than 60 mg/day of methadone.

Trafton and colleagues also investigated the factors that might affect the methadone dose needed to achieve heroin abstinence. How long a patient had taken heroin and the amount taken per day did not correlate with the methadone dose associated with abstinence. However, patients who had previously been through drug detoxification treatments appeared to need higher methadone doses, as did those recently diagnosed with depression or posttraumatic stress disorder and those living in areas with lower average heroin purity. In addition, patients who were abstinent on higher doses were more likely to have stayed in treatment longer or attended a clinic where dose reductions were discouraged. Taken together, these factors predicted 40% of the variance in methadone dosage associated with heroin abstinence. The results suggest that only patients with lower methadone needs achieve abstinence in the early titration phase of treatment or at clinics that encourage use of lower doses.

These results provide scientific confirmation that the dose of methadone required to achieve heroin abstinence varies greatly between patients, and indicate that effective and ineffective dose ranges overlap substantially. The researchers suggest that clinicians should be allowed some flexibility in determining methadone dosing and call for research into the most effective way to determine the optimal dose for a particular patient. For now, they suggest, given that patients attending clinics that routinely give at least the recommended minimum dose of methadone do better on average than those attending clinics where lower doses are often given—60 mg/day should be the benchmark for dose titration, which should occur early during treatment.

Trafton JA, Minkel J and Humphreys K (2006) Determining effective methadone doses for individual opioid-dependent patients. DOI: 10.1371/journal. pmed.0030080

Cardiovascular Disease Research: Time to Focus on Minority Ethnic Groups

Long-standing inequities exist in the amount of research on diseases that affect the developed world and those—such as leishmaniasis and schistosomiasis—that affect nonwhite populations who live in the developing world. These inequities have also been perceived to exist when white and nonwhite populations coexist in developed countries.

Ethnic groups differ in their susceptibility to particular diseases. These differences can be genetic—the result of gene mutations that are more prevalent in some ethnic groups. They may also be due to social factors—in industrialized countries, for example, ethnic minorities are often poorer, less educated, and more frequently unemployed than their white counterparts. Studies in white populations, therefore, cannot necessarily be extrapolated to other ethnic groups.

Cardiovascular disease is a major cause of death for all ethnic groups in developed countries, and the risk is especially high in those originating from South Asia. In the UK, for example, early deaths from coronary heart disease in Indians, Bangladeshis, Pakistanis, and Sri Lankans are about 50% higher than the national average. For Caribbeans and West Africans, on the other hand, the rates are much lower than average.

Meghna Ranganathan and Raj Bhopal systematically reviewed the scientific literature over the past decades to assess the extent to which different ethnic minorities were included in cardiovascular cohort studies in Europe and North America.

They identified 72 studies, 39 of which started after 1975 (at that time, it was well-known that different ethnic groups have different risk factors, and quite a bit was known about causes and control of the disease in white populations). Forty-one studies were conducted in Europe and 31 in North America, and one study involved participants from both continents.

Overall, the researchers found, there is little information on cardiovascular research in ethnic populations—just ten studies compared white and nonwhite populations, and only five focused on one nonwhite ethnic group. All 15 of those were conducted in the United States. Despite the high risk of cardiovascular disease in ethnic minorities in Europe, not one European study so far has investigated the disease specifically in these populations.

In general, it seems that issues of race or ethnicity have rarely been taken into account. Decisions about which ethnic groups to include were not often a part of the study design nor were they made explicit in the study report, and few articles gave details of the ethnic composition of the study population. Even when
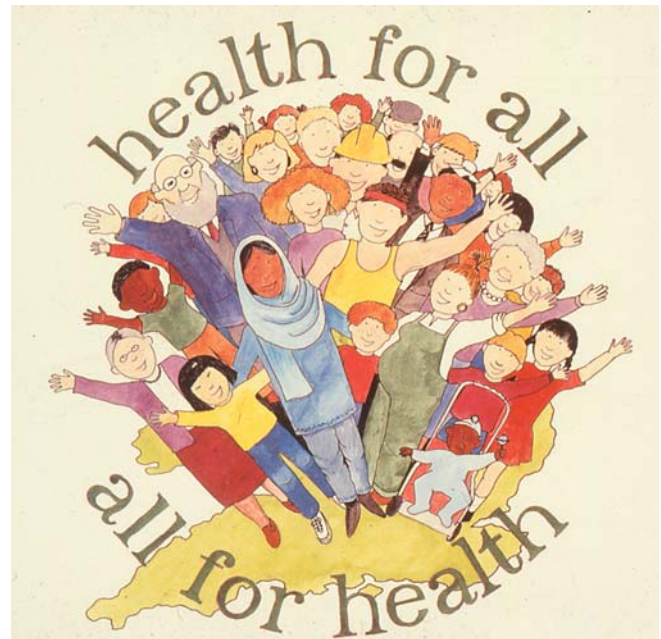
nonwhite participants were included in studies, there were often too few of them to allow for analysis by ethnicity.

In some cases, researchers were open about their aim to study only white populations. In others, by selecting participants based on employment status or by conducting studies in rural settings, researchers were unlikely to include many participants from nonwhite ethnic groups that tend to cluster in cities.

In cardiovascular disease, in particular, clear ethnic variations in risk mean that studying nonwhite populations is crucial if their health needs are not to be neglected, say the authors.

The first step toward better understanding why some ethnic groups are more susceptible to disease is acknowledging the need for studying the question. Because such studies are expensive and challenging, say Ranganathan and Bhopal, they will only be done when there is a demand for them.

Detecting Clusters of MRSA

The incidence of methicillin-resistant *Staphylococcus aureus* (MRSA) in hospitals continues to rise globally. *S. aureus* infections, including MRSA, occur most frequently in patients with weakened immune systems in hospitals and healthcare facilities, such as nursing homes and dialysis centers. Healthcare-associated *S. aureus* infections include surgical-wound infections, bloodstream infections, and pneumonia. Factors associated with the spread of MRSA infections in hospitals include skin-to-skin contact, wounds, contaminated items and surfaces, and poor hygiene. Control measures have focused on hand hygiene, restriction of antibiotics, and the detection and isolation of colonized and infected patients.

Strains resistant to methicillin are a major concern in hospitals because of the high mortality rate associated with infections caused by them and the stringent hygienic requirements needed for patients who carry MRSA. Experts say the focus of most national guidelines is the detection and isolation of colonized and infected patients. However, developing rapid detection techniques has not been easy.

Outbreaks are usually identified from laboratory test results and patients' charts—a time-consuming procedure. In established outbreaks, molecular typing (e.g., pulsed-field gel electrophoresis [PFGE]) is usually used to track the outbreak. To improve the speed of typing, DNA sequence-based approaches,
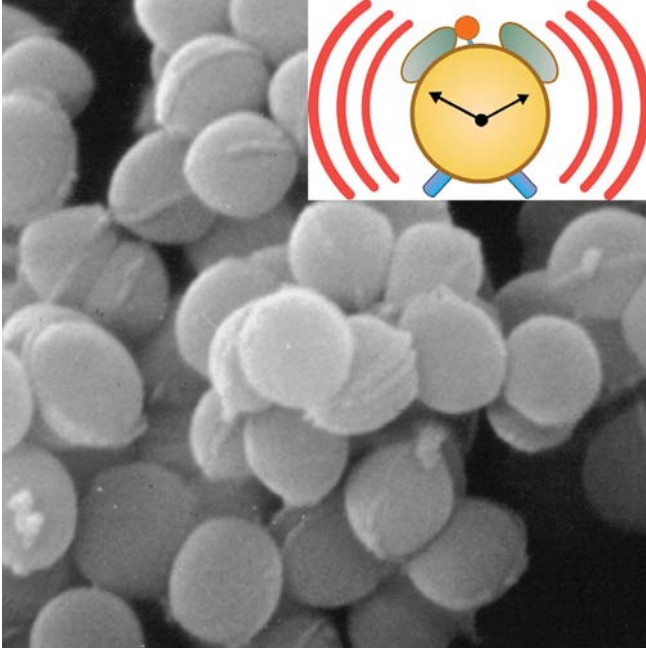
Agar diffusion test of a resistant *Staphylococcus aureus* strain

such as multi-locus sequence typing (MLST), are being used but are still too expensive for routine use and have lower discriminatory power compared with PFGE.

Frenay et al. became the first group to use a single-locus sequence typing method for *S. aureus*, by using the sequence of the polymorphic region X of the *S. aureus* protein A gene *(spa)* for typing. However, the full potential of this method has not been exploited thus far. Recently, however, a software program was developed so that the *spa* sequences could be analyzed automatically and linked to a database integrated with epidemiological information.

In this month's *PLoS Medicine*, Alexander Mellmann, Dag Harmsen, and colleagues compared this approach with classical surveillance techniques (frequency, and infection control professional [ICP] alerts) to investigate whether an automated system could complement or replace the labor-intensive traditional methods. The study, performed at a German tertiary hospital from 1998–2003, found that only five of the 13 "true" clusters were detected as clusters by visual screening of laboratory reports by the ICP. By contrast, clonal alerts (*spa* typing and epidemiological data) were more sensitive than the classical techniques.

The authors suggest that *spa* typing and automated alerts could offer a useful early-warning system, which could also be used to model infection dynamics and estimate important epidemiological parameters. The combination of medical informatics and molecular laboratory techniques could help clinicians prevent limited clusters of preventable MRSA from expanding into large-scale outbreaks. Laboratory-based surveillance has another advantage: clusters occurring throughout the hospital could be identified at a single, central data-collection point.

One limitation of clonal alerts and classical techniques, however, is that they all rely on pre-defined rules, which means that unusual patterns of outbreaks might go undetected. A way to get around this limitation might be to use data mining of patient information databases to look for patterns missed by traditional analysis. Researchers in the United Kingdom recently proposed to use data from hundreds of National Health Service wards for such data mining. The data analyzed by the researchers includes the number of infected patients on a ward, the type of treatments they received, and the hygiene and quarantine rules used by hospital staff to avoid or control infections. However, Mellmann and colleagues raise concerns, saying that although these data-mining "discovery" models are independent of an underlying hypothesis, they are usually less sensitive and specific, and once the unusual becomes usual it is no longer detected. Nonetheless, what is clear is that the days of infection outbreaks being detected solely by vigilant infectious-disease clinicians are over.