

## Synopsis of Research Articles

## Targeting of Endothelial Activation in Cerebral Malaria

DOI: 10.1371/journal.pmed.0020315

Malaria is one of the most serious of all tropical parasitic diseases: a severe and life-threatening form of which in humans is cerebral malaria, a complication that can occur in malaria caused by *Plasmodium falciparum*. This grave complication involves malarial infection of the red blood cells that accumulate within the very small capillaries that flow through the tissues of the brain. Even when treated, cerebral malaria has a fatality rate of 15% or more.

Numerous studies have pointed to a key role of tumor necrosis factor (TNF) and related proteins in the pathogenesis of cerebral malaria, and a clear relationship has been established between plasma concentrations of TNF and cerebral pathology. In experimental cerebral malaria, TNF-beta, now called lymphotoxin  $\alpha$  (LT), has been shown to be a principal mediator of pathogenesis. LT and TNF belong to the same family, interact with a common receptor, and could act together during disease progression to effect the pathogenesis, according to recent evidence.

Now Georges Grau and colleagues describe the anti-inflammatory activity of a transcriptional inhibitor of TNF, called LMP-420, which might offer a new way for treating cerebral malaria. The aim of their study was to assess the ability of LMP-420 to inhibit the in vitro TNF and/or LT effects on brain endothelium, with particular attention to endothelial

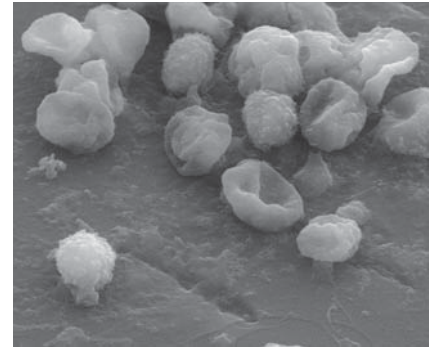
cell activation, adhesiveness for malarial parasites, and vesiculation.

Using an in vitro model of cerebral malaria based on human, brain-derived endothelial cells (HBEC-5i), they found that LMP-420 potentially reduced endothelial activation, endothelial adhesiveness for *P. falciparum*-parasitized red blood cells, and endothelial MP release, three major features of cerebral malaria.

The results provide evidence for a dual inhibitory effect of LMP-420 on both TNF and LT in an in vitro model of cerebral malaria pathogenesis, when added either before or simultaneously with both cytokines, they said. LMP-420 also abolished the cytoadherence of ICAM-1-specific *P. falciparum*-parasitized red blood cells on these endothelial cells. Identical but weaker effects were observed when LMP-420 was added with LT. LMP-420 also caused a dramatic reduction of HBEC-5i vesiculation induced by TNF or LT stimulation.

Several molecules inhibiting TNF, such as monoclonal antibody to TNF or pentoxifylline, have been tested in clinical trials of cerebral malaria but failed to improve disease outcome. These failures could be explained by the fact that LT was recently demonstrated to also have a crucial role in the pathogenesis of this cerebral syndrome, said the authors.

They conclude that the anti-inflammatory activity of LMP-420 might be useful in targeting the wide variety



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***Plasmodium*-infected red blood cells bind to brain endothelial cells**

of diseases in which TNF and its related family members play a role, and could represent a novel, stable, and efficient therapeutic way to improve the outcome of patients with cerebral malaria.

However, the authors caution that the experimental in vitro results do not necessarily predict potential efficacy in either animal models or humans, especially since in their model the LMP-420 was administered before the disease process was established. Nevertheless, this avenue is a promising one to explore further.

Wassmer SC, Cianciolo GJ, Combes V, Grau GE (2005) Inhibition of endothelial activation: A new way to treat cerebral malaria? DOI: 10.1371/journal.pmed.0020245

## Type 2 Diabetes: Insulin Resistance May Be the Result of Mitochondrial Dysfunction

DOI: 10.1371/journal.pmed.0020292

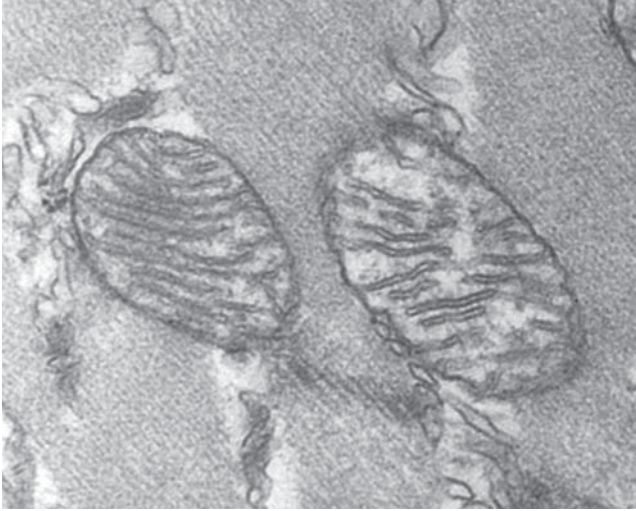
The role of insulin resistance (IR) in type 2 diabetes, the most frequently encountered metabolic disorder in the world, has attracted much attention in recent years. Virtually all patients with type 2 diabetes have IR, which usually appears some 10–20 years before the disease itself. Although the existence of the relationship between IR and type 2 diabetes is well recognized, the underlying mechanisms are poorly understood. A study by Kitt Falk Petersen and colleagues provides important new information on the underlying pathogenic mechanisms that lead to the development of IR.

Recent findings have suggested that inherited defects in mitochondrial oxidative phosphorylation activity might play a key role in the development of IR. Studies have demonstrated a relationship between dysregulated fatty acid metabolism, fat accumulation in muscle cells, and IR in skeletal muscle. This fat accumulation appears to interfere with insulin signaling, resulting in reduced insulin-stimulated muscle glucose transport

activity and decreased muscle glycogen synthesis. Magnetic resonance spectroscopy (MRS) studies have found reduced basal rates of muscle mitochondrial ATP production, associated with increased intramyocellular lipid content. Consistent with these results, microarray studies have found a coordinated reduction in PGC-1 $\alpha$ -responsive genes in patients with obesity and type 2 diabetes and their overweight first-degree relatives.

Building on these findings, Petersen and colleagues examined insulin-stimulated rates of muscle ATP synthesis and phosphate transport, to investigate whether mitochondrial function is affected not only in the fasting state but also during insulin stimulation and to determine whether inherited defects in mitochondrial oxidative phosphorylation activity might be responsible for IR.

Participants in the study were in their late 20s, lean, nonsmoking individuals with IR who were offspring of parents with type 2 diabetes. The participants were thus selected to be



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### Mitochondria in human muscle cell

free of other risk factors for IR such as obesity and smoking. Such individuals are ideal for examining the earliest metabolic defects responsible for the pathogenesis of IR since they have none of the confounding factors that are likely to be present in patients with type 2 diabetes. A metabolic defect in this group is likely to be an early event of genetic origin and, therefore, is potentially a primary cause of the later development of diabetes. The individuals in the control group were healthy, nonsmoking, and matched for age and weight to the individuals in the IR group. Insulin was administered using a hyperinsulinemic-euglycemic

clamp, and rates of mitochondrial phosphorylation activity (muscle ATP synthesis) were assessed by MRS.

Rates of insulin-stimulated glucose uptake were decreased by approximately 50% in the individuals with IR compared to the controls and were associated with an approximately 2-fold increase in intramyocellular lipid content. In the control individuals, rates of ATP synthesis increased by approximately 90% during the hyperinsulinemic-euglycemic clamp. In contrast, insulin-stimulated rates of muscle mitochondrial ATP synthesis increased by only 5% in the individuals with IR. This small increase in muscle mitochondrial ATP synthesis in the individuals with IR was associated with a severe reduction of insulin-stimulated increases in intramyocellular phosphorus concentrations.

The authors say their study provides further evidence that IR in skeletal muscle of individuals with IR who are offspring of parents with type 2 diabetes may be related to defects in mitochondrial dysfunction. Furthermore, there were also severe defects in insulin-stimulated phosphate transport into skeletal muscle in the individuals with IR, which may be part of the defect leading to impaired ATP synthesis in the muscle of these individuals.

The implications of the study are also discussed in a Perspective by Anton Wagenmakers (DOI: 10.1371/journal.pmed.0020289). Although he suggests that the defects underlying IR are likely also to involve organs other than muscle, he notes the clinical relevance of the main finding, which might explain the weight maintenance problems that individuals with obesity and IR have.

**Petersen KF, Dufour S, Shulman GI (2005) Decreased insulin-stimulated ATP synthesis and phosphate transport in muscle of insulin-resistant offspring of type 2 diabetic parents. DOI: 10.1371/journal.pmed.0020233**

## A Tool to Estimate the Risks of Repeat Cesarean Section

DOI: 10.1371/journal.pmed.0020325

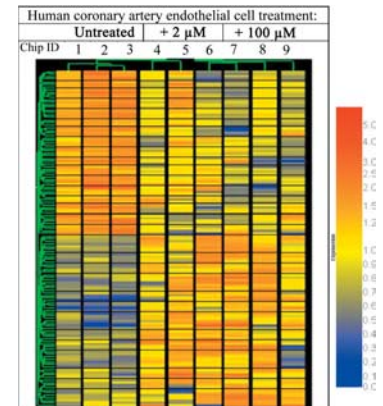
Cesarean section can be a life-saving technique for both mother and infant; however, it is a major abdominal operation that poses medical risks to a mother's health, including infections, hemorrhage, need for transfusion, injury to other organs, anesthetic complications, psychological complications, and a maternal mortality two to four times greater than that for a vaginal birth. The World Health Organization (WHO) has said that no country can justify having a cesarean rate greater than 10%–15%. Despite this advice, in the past 20 years, cesarean section rates have risen to nearly 25% in some countries.

To address rising rates of cesarean delivery, health authorities have encouraged women with a previous cesarean to attempt vaginal birth in subsequent pregnancies. However, studies have indicated an increased risk of serious adverse outcome among such women who attempt vaginal birth compared with a planned repeat cesarean delivery. This is due to a greatly increased risk of complications among

women who attempt vaginal birth but ultimately are delivered by emergency cesarean section. Consequently, researchers have tried to identify women at low and high risk of failure for an attempted vaginal birth after a prior cesarean, but currently there is no validated antepartum tool to predict the risk of a failed attempt at vaginal birth among women with a prior cesarean delivery.

Now Gordon Smith and colleagues describe the development of a simple, validated model to predict the risk of emergency cesarean section among women attempting vaginal birth after a previous cesarean delivery. They also try to determine whether women at increased risk of cesarean were also at increased risk of uterine rupture, including catastrophic rupture leading to death of the infant.

The team studied 23,286 women with one prior cesarean delivery who attempted vaginal birth at or after 40 weeks of gestation. The population was randomly split into two groups, one on



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### Endothelial gene expression changes in response to ADMA

which the model was developed and the second on which it was validated.

The researchers found that the following factors were associated with emergency cesarean section: increased maternal age, lower maternal height, male fetus, no previous vaginal birth, prostaglandin induction of labor, and

birth at 41 weeks or 42 weeks gestation compared with 40 weeks. In the validation group, 36% of the women had a low predicted risk of caesarean section and 16.5% of women had a high predicted risk; 10.9% and 47.7% of these women, respectively, were actually delivered by caesarean.

The predicted risk of caesarean was also associated with the risk of uterine rupture in general, and of uterine rupture associated with perinatal death, and women who were at low risk of emergency caesarean section were also

at low risk of uterine rupture, including catastrophic rupture leading to perinatal death—one of the principal concerns among women who have had a previous caesarean birth.

Despite the strengths of the present study, including the very large population size, studies using registry-based data have the weakness of inconsistent definitions, admitted the authors. Also the study lacked data on other risk factors for emergency caesarean delivery, such as body mass index, the indication for the previous caesarean section, and whether

a previous vaginal birth preceded or followed the previous caesarean section.

Nonetheless, the findings offer a validated model for estimating the risk of emergency caesarean section among women with a prior caesarean delivery who attempt vaginal birth. The true worth of the model will become clear when other researchers test it.

**Smith GCS, White IR, Pell JP, Dobbie R (2005) Predicting caesarean section and uterine rupture among women attempting vaginal birth after prior caesarean section. DOI: 10.1371/journal.pmed.0020252**

## Role of Osteopontin in Idiopathic Pulmonary Fibrosis

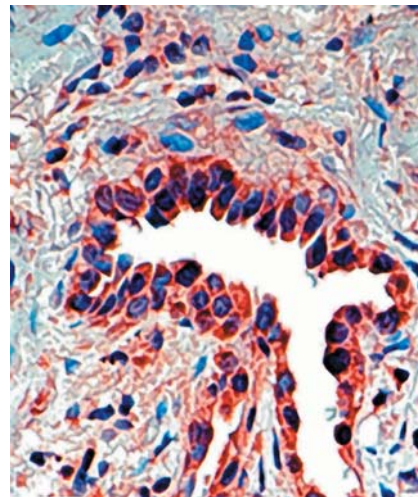
DOI: 10.1371/journal.pmed.0020314

Idiopathic pulmonary fibrosis is a chronic progressive scarring disease of the lung in which gradually the walls of the air sacs of the lungs become replaced by fibrotic tissue. When scarring forms, there is an irreversible loss of the tissue's ability to transfer oxygen into the bloodstream.

The disease affects more than 5 million people worldwide, of whom 40,000 die every year. Misdiagnosis is common because the origin and development of the disease is not completely understood. There are also no effective treatments; drugs to treat lung scarring are still in the experimental phase, and treatments to suppress inflammation have no beneficial effect in most patients.

Although significant advances have been made in the characterization of the clinical features of this disease, the molecular mechanism in humans is still largely unknown. In general, pulmonary fibrosis might be the result of an autoimmune disorder, the after effects of a viral infection, or a genetic condition. Pulmonary fibrosis also occurs after inhalation of some pollutants, in association with diseases such as scleroderma, rheumatoid arthritis, lupus, and sarcoidosis, and after certain medications and therapeutic radiation. However, the etiology of idiopathic pulmonary fibrosis is presently unknown.

Now Annie Pardo and colleagues examine the role of osteopontin, which has diverse functions as a cell-adhesion and migration molecule, in the pathogenesis of idiopathic pulmonary fibrosis. Osteopontin is a multifunctional cytokine that has been implicated in several physiological and pathological processes including bone resorption, malignant transformation, and metastasis. It is also considered a key molecule for



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### Osteopontin protein in lung from patient with IPF

regulating inflammation, cellular immune response, and tissue repair, with a unique effect on T cell function.

Using oligonucleotide microarrays these researchers have previously demonstrated that osteopontin is highly upregulated in bleomycin-induced lung fibrosis in mice—an animal model of pulmonary fibrosis.

In the current study, they used microarrays to analyze gene expression patterns in lung samples (13 samples from people with idiopathic pulmonary fibrosis and 11 from control individuals). They found that osteopontin was the most upregulated gene in the lungs of patients with idiopathic pulmonary fibrosis, and that it was mainly expressed by alveolar epithelial cells.

To better understand the potential local profibrotic effects of osteopontin they then studied its effects on lung fibroblasts and alveolar epithelial cells and found

that osteopontin induced a significant increase in migration and proliferation in both fibroblasts and epithelial cells. However, although the effect on fibroblast migration/proliferation was dependent mainly on integrins, in epithelial cells proliferation was mainly dependent on CD44 and migration was dependent on CD44 and integrin signaling.

Osteopontin also showed profibrotic-relevant effects on molecules involved in extracellular matrix remodeling. For example, in fibroblasts osteopontin increased TIMP-1 and type I collagen and inhibited MMP-1 expression, whereas in alveolar epithelial cells it induced MMP-7.

These findings concur with previous studies in experimental tissue fibrosis that have suggested a possible profibrotic role of osteopontin, said the authors. For example in kidney fibrosis, osteopontin enhances macrophage recruitment and stimulates the development of renal scarring after an acute ischemic insult; most importantly, mice that do not express the gene for osteopontin are protected from lung fibrosis induced by the drug bleomycin.

Altogether the results suggest a mechanism to explain most of the profibrotic effects of osteopontin by direct effects on fibroblasts and epithelial cells in the lungs. The findings also suggest that the interaction between MMP-7 and osteopontin might be involved in the progressive nature of the disease. Osteopontin is a potential target for therapeutic intervention in this relentless, incurable disease.

**Pardo A, Gibson K, Cisneros J, Richards TJ, Yang Y, et al. (2005) Up-Regulation and profibrotic role of osteopontin in human idiopathic pulmonary fibrosis. DOI: 10.1371/journal.pmed.0020251**

## Is it Possible to Change Prescribing Habits?

DOI: 10.1371/journal.pmed.0020328

In the US more than 770,000 people are injured or die each year in hospitals from adverse drug events (ADEs), which can cost a hospital, depending on its size, about US\$5.6 million every year, excluding ADE-associated costs for malpractice and litigation and the personal costs of injuries to patients. Nationally, hospital expenses to treat patients who have ADEs during hospital admission are enormous: between \$1.6 billion and \$5.6 billion annually. The cost to patients is also high and not just monetary: those who have an ADE spend on average 8–12 days longer in the hospital than patients who do not have an ADE, and their admission costs \$16,000 to \$24,000 more.

One way for hospitals to tackle the problem of medication errors is to install computerized monitoring systems, which can reduce ADEs by 28%–95%. Apart from the obvious benefits to patients, these systems can save hospitals as much as \$500,000 annually in direct costs. However, despite the potential, fewer than 10% of hospitals have implemented such systems.

Less is known about the value of such systems in an outpatient setting. Now, Andrew Steele and colleagues from Denver have tested a computerized physician order entry (CPOE) system in a US hospital's outpatient clinic. The main purpose of the study was to determine the impact of using computerized alerts to improve the prescribing of medications in the outpatient setting. Studies have shown that 18%–25% of patients might have an ADE in the outpatient environment. This study evaluated a CPOE system alongside an integrated computer-based clinical-decision support system.

It focused on a very specific type of clinical-decision support system: the use of a rules technology to prevent drug–laboratory ADEs. The way the system worked was that providers ordered medications on a computer and an alert was displayed if a relevant drug–laboratory interaction existed.

Comparisons were made between baseline and post-intervention periods. Provider ordering behavior was monitored, focusing on the number of medication orders not completed and the number of rule-associated laboratory test orders initiated after alert display. The investigators found that the rule

processed 16,291 times during the study period on all possible medication orders: 7,017 during the pre-intervention period (prescribing doctors did not receive alerts) and 9,274 during the post-intervention period (prescribing doctors received alerts). During the post-intervention period, an alert was displayed for 11.8% (1,093 out of 9,274) of the times the rule processed, with 5.6% of alerts being for “missing laboratory values,” 6.0% for “abnormal rule-associated laboratory” values, and 0.2% for both types of problems.

Providers did pay attention to the alerts; they increased ordering of the rule-associated laboratory test when an alert was displayed (39% at baseline versus 51% post-intervention,  $p < 0.001$ ), thus showing that the rules had a significant ability to change the ordering behavior of the provider, said the authors. The strongest effect occurred when providers were alerted to “missing” laboratory results (42% increase), the investigators noted. There was less of an effect on ordering behavior when the alert informed the provider of the existence of an abnormal laboratory value (23% increase), which may imply that the cutoff values for the “abnormal” trigger were set too low, suggested the authors. However, there was only a modest effect on halting the ordering of medications, and this was limited to occasions in which the alert presented an abnormal laboratory value in which case there was almost a doubling in order cessation.

There are limitations to the study. For example, the intervention focused on a specific group of drug–laboratory interactions and thus the results may not be generalizable to other types of interventions. In addition, the setting was a single primary-care clinic outpatient setting within a large public-health-integrated health-care delivery system, and results may be different in other settings such as hospitals and private physician offices. However, changing prescriber practice at all is not easy to achieve and this approach thus warrants further research.

Steele AW, Eisert S, Witter J, Lyons P, Jones MA, et al. (2005) The effect of automated alerts on provider ordering behavior in an outpatient setting. DOI: 10.1371/journal.pmed.0020255

## Getting More Mileage from Lymph Node Biopsies

DOI: 10.1371/journal.pmed.0020324

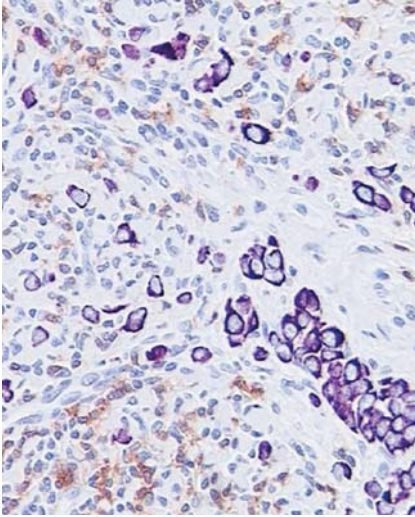
If breast cancer cells escape from the initial tumor site, they often travel to axillary lymph nodes (ALNs) in the armpit. Surgical removal and biopsy of axillary lymph nodes are routinely used to assess the stage of a patient's cancer, and prevent cancers that have spread into the lymph nodes from further metastasis. Because ALN dissections can cause uncomfortable chronic arm swelling, they are often preceded by a biopsy of the sentinel lymph node, the first node into which the tumor tissue drains. If sentinel lymph node biopsy reveals evidence of metastasis, surgeons then remove and dissect the ALNs. Lymph nodes are

primarily examined for the presence of metastatic tumor cells, and, more recently, researchers are even searching for the presence of isolated tumor cells and breast cancer–associated gene expression patterns in local nodes.

In light of increasing evidence that the immune system is perturbed both locally at the tumor site and systemically as the cancer progresses, Peter Lee and colleagues set out to study the state of the immune system in draining lymph nodes. They determined profiles of CD4 and CD8 lymphocytes and dendritic cells from sentinel node biopsies and (nonsentinel) axillary lymph node

dissections. Their results suggest that these immune profiles harbor independent information about the likelihood of tumor recurrence.

The researchers used automated high-resolution imaging to determine the numbers of CD4 T cells, CD8 T cells, and CD1a-positive dendritic cells in 47 sentinel and 104 axillary nodes from 77 patients with breast cancer. Five-year follow-up data were available for all patients, and 33 patients had disease recurrence within that time. The researchers found that sentinel and axillary nodes from cancer patients (whether the nodes contained tumor



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**Sentinel lymph node section with immune cells and tumor cells**

cells or were tumor free) contained fewer CD4 and CD8 T lymphocytes than nodes from cancer-free control patients. Dendritic cells were also reduced in tumor-positive nodes, but increased in tumor-free axillary nodes.

By dividing the patients into a “training set” of 29 individuals and a “test set” of 48 individuals, the researchers could test whether the correlations found in the test set could predict disease-free survival. They found that axillary node CD4 T cell and dendritic cell numbers, regardless of tumor status, were correlated with disease-free survival, but that this was not the case for immune parameters in the sentinel nodes (all of which were tumor-positive). Moreover, in their dataset, the predictive power of the immune parameters in the axillary nodes was better than that of any other characteristics of the patients,

including pathological parameters such as tumor size and extent or size of nodal metastases.

These results suggest that, in patients with tumor-positive sentinel nodes, immune profile data from axillary nodes hold additional information on the probability of disease recurrence. As the authors suggest, these results warrant larger prospective studies to test these relationships and explore them in more detail. Another important open question is whether immune profile information from lymph nodes can predict risk of recurrence even in women whose cancers are caught at a stage where they have not yet spread to any lymph nodes.

**Kohrt HE, Nouri N, Nowels K, Johnson D, Holmes S, et al. (2005) Profile of immune cells in axillary lymph nodes predicts disease-free survival in breast cancer. DOI: 10.1371/journal.pmed.0020284**

