Manipulating Microtubules in Systemic Sclerosis

Systemic sclerosis (scleroderma or SSc) is the name for a group of progressive diseases, all of which involve the abnormal growth of connective tissue. They are chronic degenerative disorders in which there is widespread vascular deterioration and tissue loss. The recognition of SSc dates back many years. Indeed, the characteristic stretched thickening of the skin was probably first described by Hippocrates. A more definitive description of the condition was made by Carlo Curzio in 1753, who described a patient as having wood-like skin with “tight eyelids, difficulty in opening her mouth, coldness of her skin.”

The etiology of SSc is still not completely understood, but appears to be autoimmune. Downstream of the immune activation, the molecules that have been implicated are the profibrotic cytokines, such as transforming growth factor-beta (TGFβ), interleukin-4 (IL-4), platelet-derived growth factor (PDGF), and connective tissue growth factor, all of which can cause fibrosis. In addition to the profibrotic effects, TGFβ and PDGF may also contribute to vasculopathy. Furthermore, the vascular changes in SSc skin lesions are associated with anti-endothelial cell autoantibodies.

One problem in the research on SSc has been finding an appropriate animal model. One such model has now been developed: the transplantation of skin samples from patients with SSc into immunodeficient mice. Without the interference of an effective murine immune system, it is possible to test the effect of various interventions on the patients’ skin samples.

In a paper in PLoS Medicine, Chunming Dong and colleagues used this model to investigate the effect of paclitaxel on SSc skin samples. Paclitaxel is an attractive drug because it stabilizes microtubules, which can affect the propagation of TGFβ signaling. TGFβ is a multifunctional regulatory cytokine involved in a large number of cellular activities. It initiates its effects by binding to and activating specific cell-surface receptors. These activated TGFβ receptors stimulate a family of proteins (R-Smads), some of which act to stimulate further TGFβ signaling, and others of which act to inhibit it. The microtubules regulate the R-Smad access to and activation by TGFβ receptors.

Dong and colleagues were able to show that in this model of SSc, paclitaxel markedly suppressed the activation of two of the Smads, Smad2 and Smad3, and, hence, collagen deposition in SSc grafts. They also showed that the SSc grafts had increased neovessel formation relative to normal grafts, regardless of paclitaxel treatment, thus indicating that paclitaxel did not have a negative effect on angiogenesis. In fact, they found that the neovascularized cells were likely derived from the mouse hosts.

Do these findings indicate that paclitaxel is a good drug for SSc? Certainly more work needs to be done before this can be said. A particular concern is that paclitaxel can also lead to fibrosis at high doses when used, for example, in the treatment of certain tumors (in contrast to low doses used here) needs to be assessed further. However, SSc is a difficult progressive disease, and results such as these should be considered seriously as avenues for future therapies.

A Persistent Immune Response to an Acute Virus

Human parvovirus B19 (B19) can cause a wide range of conditions, which can depend to a large extent on an individual’s immunological and hematological status. In a normal host, parvovirus infection can be asymptomatic or cause a range of clinical syndromes, from erythema infectiosum (“slapped cheek” disease) to chronic arthritis. Hydrops fetalis and fetal death are complications of intrauterine B19 infection, and patients who are immunocompromised or who have hematologic disorders are at risk of aplastic anemia.

To date, it has been believed that clearance of acute infection is associated with the lifelong emergence of antiviral IgG, but there is increasing evidence for an important role for cellular immune responses, which suggests there might be more to the way the body deals with this virus. One previous study detected CD8+ T cell responses—which kill virus-infected cells by cytokine secretion—in three asymptomatic seropositive individuals.

A better understanding of this aspect of the body’s immune response could have important implications not only for vaccine development and treatment for B19, but also for other viral infections. The long-lasting CD8+ T lymphocyte response means that parvovirus-based vectors could be considered in vaccine strategies for other infections. The identification of B19 epitopes for CD8+ T lymphocytes also offers the chance to analyze the role of such effector cells in chronic arthritis, a disease in which B19 has been implicated. Better understanding of this response might also allow researchers to use B19 as a model for analyzing immunological memory, immunodominance, and the interplay between cellular and humoral immune responses to a common clinically relevant human pathogen.

In this month’s PLoS Medicine, Adiba I. Haque and colleagues describe evolution of long-lived CD8+ immune responses against B19 in 11 adults with primary B19 infection. The phenotype of
CD8+ T cells in acute B19 infection has not been studied before. Normally, the symptoms of this virus are short lived, but the immune responses showed here indicate sustained activity many months after initial infection. The team studied two groups of people: 11 who had been recently infected and five who had the virus many years ago. CD8+ T cell responses were mapped using a screening system, which took advantage of the B19’s compact and stable viral genome. The researchers used human leukocyte antigen (HLA)–peptide multimeric complexes to detect CD8+ T cell responses during acute B19 infection.

The researchers believe their results show a new style of host–virus relationship in which an acute human viral infection induces persistent activated CD8+ T cell responses. They found that these responses continued to increase, in some cases for many months, long after acute symptoms had resolved—something not seen with other viruses. For example, responses to HIV are strong in acute infection but typically decline as the virus is controlled.

Alongside the expansion of antiviral responses was the continued change in the B19 CD8+ T cells, as indicated by a range of markers. The evolution of markers could represent a maturation pathway, said the authors, driven by restimulation in vivo with antigen.

This T cell response to B19 infection indicated the persistence of antigen long after the resolution of acute infection. However, the authors said the status of the virus postinfection is still not understood. For example, in this study, PCR analysis found B19 DNA in the blood early on during infection, but assays were negative after 6–12 months when T cell populations remained active. The most likely reason for these immune responses is low-level replication at a tissue site for weeks or months after infection, suggest the authors. But this hypothesis can only be checked by more sensitive PCR assays.

In addition, the relationship between joint or bone marrow pathology and T cell responses seen was not clear. In the patients with arthritis, the most active CD8+ T cell responses were seen at stages where joint symptoms had resolved.

Altogether, this study is the first demonstration that a virus, not considered a true or classical persistent infection, can lead to a persistent activated CD8+ T cell response. It suggests that B19 persists after acute infection, provoking sustained activated CD8+ T cell responses, which might then play a role in viral clearance. A better understanding of cytotoxic T lymphocytes could improve understanding of the role of T cells in acute and persistent infections and be of great value in vaccine design and immunotherapy.

Isa A, Kasprowicz V, Norbeck O, Loughry A, Jeffery K, et al (2005) Prolonged activation of virus-specific CD8+ T cells after acute B19 infection. DOI: 10.1371/journal.pmed.0020411
Within each household, the children formed several full-sibling, half-sibling, and first-cousin groups. The second study monitored severe malaria that led to hospitalization and nonmalaria hospitalizations in 2,900 children, also over a five-year period. This analysis concentrated on full-siblings.

Using a standard statistical genetics method of relating similarity in phenotype to similarity in genotype, they found that host genetic factors accounted for approximately one-quarter to one-third of the total variation in susceptibility to malaria. Of this percentage, only a small proportion could be attributed to the best known malaria resistance genes. This is consistent with other studies that suggest that malaria susceptibility is under the control of many different genes, with each individual gene having a relatively small epidemiological effect.

When assessing the contribution of household factors, the researchers found that for mild clinical malaria, those factors accounted for slightly more than a quarter of the total variation. For hospitalized malaria, they contributed about 15%, and for hospitalizations with fever that turned out not to be malaria, they contributed approximately 35%. Overall, children living in the 10% of households with the highest malaria incidence had approximately twice as many infections per year than those living in the 10% of households with the lowest incidence.

The researchers do not question the long-term benefits of understanding the genetic factors but conclude that “identifying and tackling the household effects must be the more efficient route to reducing the burden of disease in malaria-endemic areas.” Factors such as suitable conditions for mosquitoes to breed and survive as well as human behavior are likely to play major roles. “We need to determine what makes the difference between low-risk and high-risk households,” Mackinnon says, “but whatever it is, it seems likely to be an easy target using tools such as education and the low-cost, low-tech devices that we already have at hand such as bed nets, residual indoor spraying, and cleaning up backyards for mosquito breeding sites.”

Mackinnon MJ, Mwangi TW, Snow RW, Marsh K, Williams TN (2005) Heritability of malaria in Africa. DOI: 10.1371/journal.pmed.0020340

### Bias in Reporting of Genetic Association Studies

DOI: 10.1371/journal.pmed.0020419

One of the tools in the scientist’s armory for resolving a medical issue or consolidating a body of clinical trials is the systematic review of the published medical literature. This technique involves doing a literature search and critical appraisal of individual studies, and in addition, may also use statistical techniques to combine the results of these studies. One of the aims of such reviews is to assess and then, ideally, include all appropriate studies that address the question of the review. But finding all studies is not always possible, and researchers have no way of knowing what they have missed. But does it matter if some studies are left out?

It would definitely matter if the missing studies differed significantly from the included ones. And the worst-case scenario is that the accumulation of evidence might point to the wrong answer if the studies included are unrepresentative of all those that have been done.

Studies of publication bias have noted that papers with significant positive results are easier to find than those with nonsignificant or negative results. As a result, overrepresentation of positive studies in systematic reviews might mean that such reviews are biased toward a positive result. Publication bias is just one in a group of related biases, all of which potentially lead to overrepresentation of significant or positive studies in systematic reviews. Other types of bias include time lag bias (positive studies are more likely to be published rapidly); multiple publication bias (positive studies are more likely to be published more than once); citation bias (positive studies are more likely to be cited by others); and language bias (positive studies are more likely to be published in English).

In *PLoS Medicine*, John Ioannidis and colleagues have taken a closer look at bias in Chinese genetics studies. Research done in non-English-speaking countries has two outlets. A study might be published in English-language journals, which are usually indexed in major international bibliographic databases such as PubMed, or in domestic journals, many of which are not indexed in international databases. The Chinese literature is a prominent example of where domestic scientific journals are not catalogued in international databases. There is some evidence that the decision to publish in international versus domestic journals might be influenced by the results. For example, significant results are often published in international journals, whereas nonsignificant results appear in the local literature, resulting in a language bias—although, the reverse situation has also been described.

Genetics studies pose particular problems for impartial reporting. There are millions of polymorphisms in the human genome, and an exponentially increasing number of studies are trying to associate genetic polymorphisms with risk of disease or treatment outcomes. Selective publication might invalidate the overall picture of genetic risk factors.

The authors examined 13 gene–disease associations. Studies were more likely to be published when the disease was considered common in China. They found 161 Chinese studies on 12 of these gene–disease associations, only 20 of which were indexed in PubMed. Chinese studies had significantly more prominent genetic effects than non-Chinese studies, and 48% were statistically significant per se, despite their smaller sample size. Moreover, the largest, most exaggerated genetic effects were often seen in PubMed-indexed Chinese studies. Chinese studies usually appeared several years after their equivalent was first postulated in the world literature.

The larger genetic effects in Chinese studies are unlikely to reflect genuine heterogeneity and are more likely to do with publication bias operating within the Chinese literature, say the authors. It is possible that there was reluctance to submit and publish negative or inconclusive results when a large body...
of English-language literature has shown the presence of genetic effects. However, such “forced” confirmation negates the importance of independent confirmation of research results. This problem is probably not limited to the Chinese literature. These phenomena haven’t been noted in molecular medicine before, but could become a serious problem in such a fast-moving field. Moreover, the inclusion of poor-quality research and additional selectively reported data may contaminate the better literature rather than provide a more accurate, comprehensive picture.

The findings have two broad implications. First, language bias might be important to consider in meta-analyses of observational studies, where its effect might be larger than its effect on randomized evidence. Second, because human genome epidemiology is a global enterprise, a comprehensive global view is important to help decipher artifacts from true genetic effects. The Chinese literature in particular will be essential for the evaluation of evidence on genetic risk factors. China is making rapid scientific progress in this field and joining in international collaborative projects, such as the Human Genome Project. To develop a global perspective, one way forward might be for all investigators working on the genetics of a specific disease to register with a common network, making it easier to trace additional unpublished or nonindexed data.

Pan Z, Trikalinos TA, Kavoura FK, Lau J, Ioannidis JPA (2005) Local literature bias in genetic epidemiology: An empirical evaluation of the Chinese literature. DOI: 10.1371/journal.pmed.0020334

Surprising Effects of Maternal Malaria and Gravidity on Infant Malaria Burden
DOI:10.1371/journal.pmed.0020413

Every year at least 30 million women in malarious areas of Africa become pregnant. The symptoms and complications of malaria during pregnancy differ with the intensity of malaria transmission and with the level of immunity acquired by the pregnant woman. In areas where malaria is endemic, most adult women have developed sufficient immunity such that, even during pregnancy, *P. falciparum* infection does not usually result in fever or other clinical symptoms. In these areas, the principal impact of malaria infection is due to the presence of parasites in the placenta. Placental malaria frequently results in low birth weight, and estimates suggest that in endemic areas 19% of cases of infant low birth weight are due to malaria, and that 6% of infant deaths are due to low birth weight caused by malaria.

In addition to affecting birth weight, placental malaria might increase the susceptibility of infants to malaria, but so far studies on this subject have been inconclusive. Patrick Duffy and colleagues examined the effect of placental malaria on infant malaria susceptibility in a prospective cohort study of newborns in a malaria-endemic area. They monitored parasitemia in 453 infants in a region of northeastern Tanzania where malaria transmission is very high (an estimated 400 infective mosquito bites per individual per year). Sixty-nine of the infants were born to mothers with placental malaria. Placental malaria is caused by a different form of the malaria parasite, which does not commonly infect nonpregnant individuals. Even in endemic areas, women, therefore, lack immunity to the placenta-specific form of the parasite prior to their first pregnancy, but acquire it over successive pregnancies. As a consequence, placental malaria is most frequent and severe in first-time mothers. Of the 69 mothers with placental malaria in this study, 45% were first-time mothers (or primigravid), 38% were giving birth to their second child (secundigravid), and 17% had had multiple prior pregnancies (multigravid).

Overall, infants of mothers with placental malaria were 41% more likely to experience malaria parasitemia themselves in the first year of life. However, the odds of parasitemia throughout infancy were also strongly influenced by the mother’s gravidity. The researchers found a surprising protective effect of placental malaria of primigravid mothers on their firstborns’ risk of parasitemia. Placental malaria of multigravid women, on the other hand, significantly increased risk of parasitemia during infancy. And even in the absence of placental malaria, firstborn children were less likely to have parasitemia than infants born to multigravid mothers. These results suggest that risk of parasitemia during infancy was modified by an interaction between placental malaria and gravidity.

Duffy and colleagues speculate that the stronger inflammatory response to placental malaria in first-time mothers could reduce congenital transmission or somehow strengthen the fetal immune system against malaria. They also suggest that the opposing effects of placental malaria in different gravid groups might explain why earlier studies found no significant risk between placental malaria and malaria susceptibility during the first two years of life. The results here are provocative but preliminary. Additional larger studies are necessary to conclusively demonstrate an interaction between placental malaria and gravidity in infant malaria susceptibility, and to examine potential modulation of congenital malaria transmission and infant immunity to the parasite by placental inflammation.

Mutabingwa TK, Bolla MC, Li JL, Domingo GJ, Li X, et al. (2005) Maternal malaria and gravidity interact to modify infant susceptibility to malaria. DOI: 10.1371/journal.pmed.0020407

DOI:10.1371/journal.pmed.0020413.g001

Section from a malaria-infected placenta (Photo: Michal Fried)
Will Stopping Aβ Production Reverse the Damage in Alzheimer Disease?

DOI: 10.1371/journal.pmed.0020418

Dementia is a common condition in the elderly; around 6% of people over 65 and up to 50% over 90 have some form of dementia, about half of which are due to Alzheimer disease (AD). The dementia caused by AD has an insidious onset and a progressive course with slow deterioration in cerebral function, initially affecting short-term memory and cognitive skills, and later speech, motor functions, and personality. Death usually occurs within four to eight years after diagnosis.

The aim of treatment is to reverse cognitive decline and improve behavioral and psychological functions. Key questions in Alzheimer research are how best to halt the progression of disease to maintain and if possible restore cognitive skills, and when to initiate such interventions in order to be effective.

AD is identified at autopsy by the presence of hallmark lesions in key regions of the brain. These lesions, known as amyloid plaques, are formed by the aggregation of small peptides, called amyloid β peptide (Aβ), that are produced when amyloid precursor protein (APP) is cleaved by the action of two enzymes, β-APP cleaving enzyme and γ-secretase. One approach to the treatment of Alzheimer is, therefore, limiting the production of Aβ from its precursor by inhibiting one or both of these enzymes. However, it is not yet clear whether this approach will prevent the brain lesions and cognitive symptoms from getting worse, and if it will then promote the removal of preexisting plaques and reverse cognitive decline.

To answer such questions, Joanna Jankowsky and colleagues have developed mice that produce Aβ at levels sufficient to induce severe amyloid burden by six months of age. The animals carry an additional transgene that acts as a switch to control when Aβ is produced. Commonly known as the tet-off system, the switch is turned off when the mice are fed tetracycline images, are highly stable structures in vivo

Amyloid plaques, shown here as false-color images, are highly stable structures in vivo
or its analog, doxycycline. Once given the drug, Aβ production in the brains of these mice diminishes by more than 95% of pretreatment levels within two weeks. This system, thus, mimics the effect of shutting down Aβ production with enzyme inhibitors that are being developed for use in human patients.

In the study, the researchers used doxycycline to switch off production of Aβ, and examined what happened to the amyloid pathology. Not surprisingly, the increase in number and size of amyloid lesions that normally occurs as the mice get older was completely prevented by suppressing Aβ production. However, the researchers also found no substantial clearance of preexisting plaques, even after six months of treatment (one-quarter of the normal mouse lifespan).

What do these findings mean for human Alzheimer research? First, the study provides evidence that the lesions found in AD may be more difficult for the brain to repair than protein aggregates found in other diseases such as Huntington or prion disease. Second, the findings suggest that the removal of plaques, once formed, may require more than simply halting the production of the Aβ peptide. However, as with all animal models, there are differences in comparison to the human disease, leading to both over- and underestimation of the relative importance of an effect in humans. The researchers do not yet know whether the plaques formed in mice may be more resistant to clearance than those seen in human disease. Conversely, the human brain, unlike the murine one, may have a more efficient way of clearing amyloid plaques. What this study makes clear is that treatments directed at reducing Aβ peptide production in AD will likely be most effective when started as early as possible.

Jankowsky JL, Slunt HH, Gonzales V, Savonenko AV, Wen JC, et al. (2005) Persistent amyloidosis following suppression of Aβ production in a transgenic model of Alzheimer disease. DOI: 10.1371/journal.pmed.0020355

Predicting the Development of Type 2 Diabetes
DOI: 10.1371/journal.pmed.0020406

Type 2 diabetes has been loosely defined as “adult onset” diabetes, but as diabetes becomes more common, cases are being diagnosed in younger people and children. In determining the risk of developing diabetes, environmental factors, such as food intake and exercise, are known to have an important role; most people with type 2 diabetes are either overweight or obese. Inherited factors are also important, but the genes involved remain poorly defined. In rare forms of diabetes, mutations of one gene can result in disease, whereas in type 2 diabetes, many genes are thought to be involved. One difficulty in understanding the genetic role is that genes associated with diabetes might show only a subtle variation in their sequence, and these variations may be extremely common. Hence, it can be very hard to link such common gene variations, known as single nucleotide polymorphisms (SNPs), with increased risk of developing diabetes.

One method of finding these diabetes genes is by whole-genome linkage studies in which associations between parts of the genome and risk of developing diabetes are looked for. Studies so far have identified several candidate genes associated with type 2 diabetes, although many results have been difficult to replicate. The list of genes for which there is good evidence from meta-analyses includes genes encoding for PPARG, calpain 10, Kir 6.2, and insulin receptor substrate-1 (IRS1).

These genes have a variety of effects; PPARG P12A polymorphism is associated with enhanced insulin sensitivity and protects against type 2 diabetes. Two SNPs in the gene encoding for cystein protease calpain 10 (CAPN10) confer increased susceptibility to insulin resistance and type 2 diabetes. Kir 6.2 is involved in glucose-stimulated insulin secretion in pancreatic cells. And carriers of a polymorphism in the IRS1 gene have been shown to have reduced islet insulin content in pancreatic islets.

In this issue of PLoS Medicine, Valeriya Lyssenko and colleagues from Lund University sought to consolidate previous work by studying the predictive value of these variants for type 2 diabetes side by side in the largest study of its kind to date. They investigated the effect of these gene variants in 2,293 nondiabetic people aged 18–70 years old in western Finland—the Botnia study—over a median of six, range 2–12, years. In
DC-SIGN and Lung Pathogenesis in Patients with Tuberculosis

DOI: 10.1371/journal.pmed.0020345

C-type lectins are carbohydrate-binding cell surface molecules with a wide range of biological functions, many of which are related to immunity. Despite its name, dendritic cell–specific intercellular adhesion molecule-3 grabbing nonintegrin (DC-SIGN) is not only expressed on dendritic cells but also on specialized macrophages in the placenta and lung. A number of pathogens are known to interact with DC-SIGN, and some (including HIV) seem to have evolved to derive advantages from interactions such as lifestyle changes, they said. One of the problems of other studies has been that results have been different between different subgroups. Although this study has limited power, as the largest of its kind it suggests that genetic variants in candidate genes can predict future type 2 diabetes, particularly in association with conventional risk factors such as obesity and abnormal glucose tolerance. With accumulating data from prospective studies, it should be possible to define whether there will be a future role for genetic prediction of type 2 diabetes or whether these variants will influence response to prevention or treatment.

Lyssenko V, Almgren P, Anevski D, Orho-Melander M, Sjögren M, et al. (2005) Genetic prediction of future type 2 diabetes. DOI: 10.1371/journal.pmed.0020345

Tailleux L, Pham-Thi N, Bergeron-Lafaurie A, Herrmann JL, Charles P, et al. (2005) DC-SIGN induction in alveolar macrophages (an average of 3%) expressed DC-SIGN. In contrast, an average of 30% (and up to 70%) of macrophages from patients with TB expressed the lectin.

Tailleux and colleagues then incubated alveolar macrophages from a patient without TB ex vivo with Mycobacterium tuberculosis, which resulted in infection of a subset of the cells. When the researchers examined DC-SIGN expression, they found that both infected and noninfected (bystander) cells in the population started to express DC-SIGN. The effect on bystander cells suggests that soluble factors from the microbe and/or the infected cells can induce DC-SIGN expression. Further functional ex vivo studies with cells from human patients indicated that DC-SIGN expression renders alveolar macrophages more susceptible to infection.

The authors propose a scenario where complement receptors mediate most of the initial infection of alveolar macrophages in a naïve host, and where—once the infection is established—DC-SIGN–expressing alveolar macrophages become preferential target cells for M. tuberculosis. Future work will be focused on identifying the soluble factors involved, and on determining whether DC-SIGN induction is an essential part of TB pathogenesis.