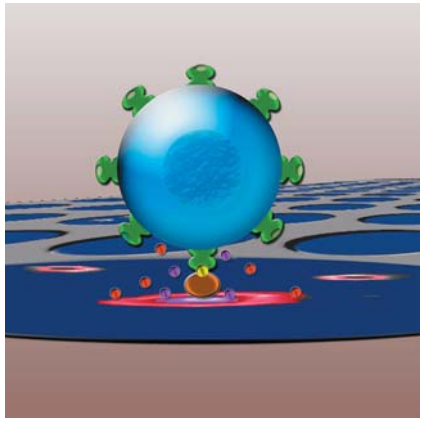
Synopses of Research Articles

Measuring the Immune Response to Tumor Vaccination

DOI: 10.1371/journal.pmed.0020360

Tumor vaccination has perhaps been one of the most eagerly anticipated developments in cancer medicine. Research efforts in melanoma started with early unsuccessful attempts to induce a nonspecific immune response with Bacillé Calmette-Guerin (BCG). Subsequent vaccines combined BCG with autologous tumor cells or mixtures of allogeneic tumor cells, with varying responses. Most recently, however, work has focused on trying to produce a specific immune response using melanoma-derived peptide antigens. The antigens most commonly used are melanoma antigen recognized by T lymphocytes (MART-1)/Melan A, glycoprotein (gp) 100, and tyrosinase, all of which occur on both normal melanocyes and melanoma cells; randomized trials are currently under way on these vaccines.

However, the response of patients to these vaccines has been extremely variable and hard to predict. Ideally, researchers want to track antigen-specific T cells and measure their activation, but current assays are cumbersome and require large volumes of blood. Now, Mark Davis and colleagues from Stanford University and the University of Southern California present a high-throughput method for analyzing the response of patients to these vaccines, by means of



DOI: 10.1371/journal.pmed.0020360.g001

Catching antigen-specific T cells on a biological chip

an array that captures specific T cells, activates them, and then measures their response to activation.

The researchers demonstrated the usefulness of this approach by studying ten patients from a phase II trial of 60 patients who had stage IIC/III and IV melanoma and who had been vaccinated with a combination of MART-1, gp100, and tyrosinase. They describe the use of peptide major histocompatibility complex arrays, which immobilizes CD8T cells, activates them, and then measures the degree of activation by the secretion of cytokines. The major new technical development is the method of measuring the amounts of cytokines released by means of labeled antibodies also present on the array.

What they found was a startling diversity of responses to vaccination. However, one predictor of good clinical response to vaccination was strong secretion of both interferon-γ and tumor necrosis factor-α; four of the four patients with this pattern of secretion remained free of melanoma recurrence, whereas only two of the six patients in whom there were marked differences in the secretion of these two cytokines were free of recurrence.

Where does this paper leave the field of research on tumor vaccination? It provides a detailed snapshot of T cell responses in individual patients, and if these patterns of responses are substantiated in larger numbers of patients, it may well allow doctors to begin to understand who is likely to respond to one of these vaccines. What it does not do is explain why vaccine responses vary so much among patients; this is a far more complex question.

Chen DS, Soen Y, Stuge TB, Lee PP, Weber JS, et al. (2005) Marked differences in human melanoma antigen-specific T cell responsiveness after vaccination using a functional microarray. DOI: 10.1371/journal.pmed.0020265

CAPON and Schizophrenia—Does Size Matter?

DOI: 10.1371/journal.pmed.0020329

Schizophrenia and bipolar disease are complex diseases, with multiple genes and environmental factors thought to be responsible for their manifestation. Many reports have implicated changes in certain regions of the human genome in schizophrenia. An area on Chromosome 1 has been associated with the disease in different studies and populations. Linda Brzustowicz and colleagues had previously described association of several single nucleotide polymorphisms (SNPs) within a gene called *CAPON* (for carboxyl-terminal PDZ ligand of neuronal nitric oxide synthase) with schizophrenia in a set of Canadian families. A separate study in a Chinese population found an association between schizophrenia and a separate group of SNPs within *CAPON*. *CAPON* is an attractive candidate for a "schizophrenia gene": CAPON was first identified as a protein binding to neuronal nitric oxide synthase (nNOS), and indirect evidence suggests that it might be linked to the regulation of glutamate neurotransmission. However, so far, no coding sequence mutations in CAPON have been found in patients with schizophrenia.

Brzustowicz and colleagues now report results from a study of CAPON expression in postmortem brain samples from patients with schizophrenia, from patients with bipolar disorder, and from control individuals without psychiatric illness. Initially screening a human fetal brain cDNA library for potential alternative splice forms of CAPON, they found, in addition to the predicted full-length transcript, a shorter isoform that consists of the last two exons of the gene. They also confirmed that both long and short versions of the protein are present in human brain. (The short isoform would still be able to bind nNOS and possibly disrupt its interaction with other proteins.)

The authors then examined *CAPON* mRNA expression in postmortem brain samples from 35 patients with schizophrenia, from 35 patients with bipolar disorder, and from 35 controls. They looked for transcripts encoding the long and short forms and also compared

expression levels (relative to beta-actin) across the diagnostic groups. In the dorsolateral prefrontal cortex (thought to be involved in schizophrenia and the only area for which samples were available), expression levels of the long isoform did not differ between patient and control samples. However, the short isoform was expressed at higher levels in the patients with mental illness than in the controls. They also analyzed DNA from these individuals at three SNPs associated with schizophrenia and found, for each SNP, that the group of individuals who carried one or two copies of the schizophrenia-associated allele had overall higher levels of the short form transcript than those homozygous for the non-associated *CAPON* allele. They saw no differences in levels of the full-length transcript between the groups with different SNP genotypes.

These are intriguing but preliminary results. Getting reliable results from studies on postmortem samples is extremely difficult because of numerous confounding factors. Brzustowicz and colleagues tried to control for some of them (such as sex of the individual, substance abuse by the individual, and storage time of the sample), but others are impossible to determine or control for. Moreover, this particular collection of samples had only tissue from the dorsolateral prefrontal cortex available for study. Sample preparation was geared toward high-quality RNA for expression studies and not suitable for parallel protein analysis. Future studies, with at least some of them in animal models that allow controlled conditions and experimentation, will need to determine the functions of the long and short isoforms of CAPON and their interaction with other proteins involved in postsynaptic neurotransmission (some of which have also been linked to schizophrenia), and elucidate a possible role for CAPON in psychiatric disorders.

Xu B, Wratten N, Charych EI, Buyske S, Firestein BL, et al. (2005) Increased expression in dorsolateral prefrontal cortex of CAPON in schizophrenia and bipolar disorder. DOI: 10.1371/journal.pmed.0020263

Teasing Out the Effects of Latitude and Birth Date on Allergy

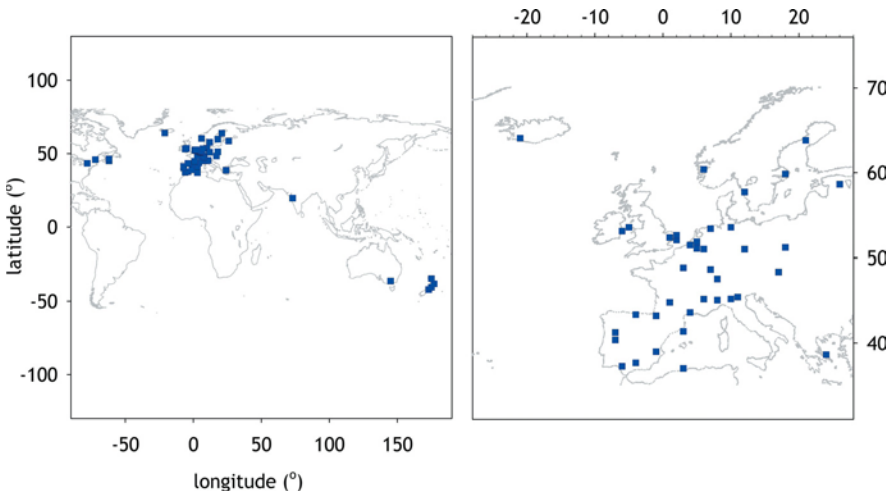
DOI: 10.1371/journal.pmed.0020375

The prevalence of asthma and allergy, defined as immunologically mediated hypersensitivity, is increasing. It is estimated that more than 20% of the world's population has IgE-mediated allergic diseases. The scale of the clinical problem is immense. The World Health Organization estimates that asthma affects nearly 150 million people worldwide, and more than 180,000 deaths each year are due to asthma. Approximately US$20 billion is spent globally each year on allergic rhinitis, including medications, time off work, and clinician consultations. The cost of allergy drugs alone is estimated to be US$8 billion per annum.

While allergy prevalence is increasing, the causal risk factors are still unknown. Matthias Wjst and colleagues investigated whether the spatial (latitude) and temporal (birth month) distribution of risk factors might offer insight into the mechanism of disease.

Previous studies have already shown that birth month is a risk factor associated with allergy; as the authors point out, birth month, used as a proxy for early allergen exposure, might be associated with upper respiratory infections during winter months. Studies have also associated geographical latitude with allergy. But some experts have noted that latitude, a proxy for ultraviolet solar exposure, might also reflect climatic differences, genetic influences, or even cultural differences in the raising of children.

In this study, Wjst and colleagues tried to further understand the effects of latitude and birth date on the prevalence of allergy defined by markers such as allergic rhinitis, sensitization to grass or dust, and total IgE levels. They distributed a questionnaire to 20– to 44–year-old individuals in 54 centers across Europe, North Africa, India, North America, Australia, and New Zealand. Altogether, data from 200,682 participants were analyzed.



DOI: 10.1371/journal.pmed.0020375.g001

World and European distribution of study centres

The median prevalence of allergic rhinitis was 22%, but with a substantial variation across centers. They found allergic rhinitis decreased with geographical latitude, but there were many exceptions. There was no increase in prevalence during certain winters, and no altered risk by birth month, except borderline reduced risks in September or October. Altogether, the authors concluded that there was no major risk by being born in a particular month or during a particular season. There may be relevant birth month effects in single centers, but a global effect was questionable.

Previous research on the effect of birth month has also shown mixed results: differing studies have found associations that were positive, negative, or simply unclear.

But the authors noted that one difference of their study compared with others was the higher age of the subjects—interviewees were born between 1945 and 1973—and suggested that it might be possible that there are more marked symptoms in children that were being lost in adulthood.

Most previous studies have shown an association with allergic sensitization, indicating subclinical effects that gained importance only when occurring in combination with additional risk factors. One of the main advantages of this study—a standardized allergen test protocol—might, thus, be a disadvantage since the effects of local allergens might have been missed. Another methodological restriction might have been the use of self-reported "hay fever." This term may be used in a different way across Europe, said the authors.

Data on the geographical distribution of allergic diseases are rare, and, hence, this study is valuable. However, a previous meta-analysis has shown negative association of latitude and symptoms of allergic rhinitis, with a −0.05% decrease per degree. In this study, symptoms of allergic rhinitis decreased with geographical latitude on a worldwide scale, but not when the analysis was restricted to Europe alone. One intriguing possibility, which needs further work, is that a risk factor within language borders might be more relevant than geographical latitude alone in determining the distribution of allergic diseases.

Wjst M, Dharmage S, André E, Norback D, Raherison C, et al. (2005) Latitude, birth date, and allergy. DOI: 10.1371/journal.pmed.0020294

Alzheimer Disease: Failure to Tune Out Irrelevant Input?

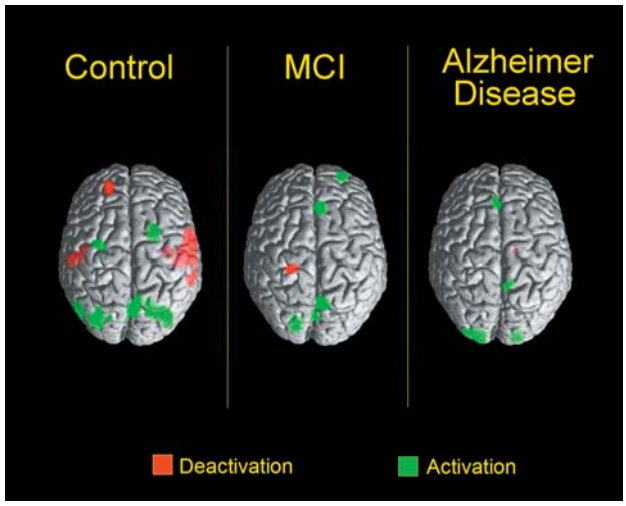
DOI: 10.1371/journal.pmed.0020356

Some people close their eyes when they listen to music; others wear earplugs when they read in noisy buses or airplanes. Both serve to block out inappropriate sensory stimulation when we try to focus, something that happens automatically when we concentrate. Neuroimaging studies have shown that this focusing process is regulated within our brains; when people undergo tasks that depend exclusively on visual clues, they not only activate the areas of the brain involved in visual processing but also deactivate those areas that have to do with auditory processing. The reverse activation/deactivation pattern is seen when the clues are exclusively auditory. This phenomenon, called "cross-modal auditory/visual deactivation," is thought to help focus the information processing capacities of the brain on the relevant areas

Alexander Drzezga and colleagues are trying to understand the cognitive changes associated with Alzheimer disease (AD). AD has historically been categorized as a disease of memory loss, but more recent results suggest that the disease is associated with more fundamental deficits in attention and perception. The researchers now report results from a study that examined the activation and deactivation patterns in the brains of patients with AD. The results suggest that patients with AD and patients with mild cognitive impairment (MCI) have problems focusing their brain activity: they show less activity than normal people in the "correct" brain areas but also more activity in the "wrong" areas.

To test whether cross-modal inhibition is affected in AD, the researchers recruited 32 participants, who fell into three age-matched groups: 11 healthy individuals, 11 individuals with MCI, and ten individuals with moderate AD. All participants were trained to perform a navigation task, based exclusively on visual clues, in a virtual reality environment. All participants understood the task and the clues. They then each completed 12 separate positron emission tomography scans, eight during active navigation (finding their way from a starting point to a specific destination) and four under control conditions (traveling along a never-ending pathway). The tasks were performed in complete silence.

The three groups differed in their performance—measured by the time needed to reach the destination—as would be expected. In addition, the researchers observed differences in their brain activation and deactivation patterns. Higher-order visual processing areas were activated to the greatest extent in healthy individuals, to a lesser extent in individuals with MCI, and not at all in individuals with AD. Similarly, cerebellar activation (suggesting movement automation) was absent in individuals with AD, present at low levels in individuals with MCI, and strongest in healthy individuals. Conversely, individuals with AD or MCI showed more activation in the (lower order) primary visual areas and in frontal cortical areas. This may indicate that sensory information gets "stuck" in lower levels of processing in AD. Regarding the inhibition of irrelevant input, strong bilateral deactivation in task-irrelevant auditory cortical regions was seen in healthy individuals. This was much less prominent in individuals with MCI, and individuals with AD showed no deactivation.



DOI: 10.1371/journal.pmed.0020356.g001

Cerebral activation and deactivation during spatial navigation

The researchers conclude that "successful cognitive performance in healthy individuals is associated with deactivation of task-irrelevant cerebral regions, whereas the development of AD appears to be characterized by a progressive impairment of cross-modal cerebral deactivation functions." They go on to propose that "orientation disability in the outside world seen in patients with AD may in fact be partially based on the inability to selectively orient spatial attention to task-relevant internal representations of perceptual stimuli." The researchers are now in the middle of a follow-up study that concentrates on individuals with MCI—some of whom remain stable for years, whereas others progress to AD—to see whether the extent of cross-modal inhibition correlates with progression to disease.

Drzezga A, Grimmer T, Peller M, Wermke M, Siebner H, et al. (2005) Impaired cross-modal inhibition in Alzheimer disease. DOI: 10.1371/journal.pmed.0020288

Understanding Bleeding Risk after Anticoagulation

DOI: 10.1371/journal.pmed.0020352

One class of treatments commonly used for prevention and treatment of venous and arterial thrombosis is vitamin K antagonists (VKA), such as warfarin. The molecular target of these anticoagulants is the vitamin K epoxide reductase complex (VKORC) of which one component, VKORC1, was recently identified. The gene for this component is mutated in individuals with combined deficiency of vitamin K–dependent clotting factors type 2 or with warfarin resistance.

What makes VKA treatment so tedious for patients is that the dose of VKA required to achieve anticoagulation varies among patients and even changes over time in the same patient, so patients require regular monitoring to ensure that they are not over- or under-anticoagulated. The standard measure of anticoagulation is the international normalized ratio (INR). A recent study reported polymorphisms in the *VKORC1* gene that explained up to 30% of the variation in the pharmacological response to VKAs, but few clinics take such inherited determinants of the pharmacological response into account.

In a paper published in *PLoS Medicine*, Dutch authors Pieter Reitsma and colleagues aimed to estimate the contribution of C1173T polymorphisms in the *VKORC1* gene to dose requirement for the anticoagulants acenocoumarol and phenprocoumon and to bleeding risk. In this case-control study, the authors studied 110 patients who bled during VKA therapy and 220 controls free of bleeding under the same therapy. Patients in the study were being treated with anticoagulants for venous thrombosis or were receiving anticoagulants to prevent arterial thromboembolism related to their atrial fibrillation or mechanical heart valves.

What the authors found was that to achieve the same target INR, control patients with CT and TT genotypes required less phenprocoumon or acenocoumarol than control patients with the CC genotype. Also, compared with CC individuals, carriers of at least one T allele had increased bleeding risk in the phenprocoumon users but not in acenocoumarol users.

The results, although based on a small sample size of individuals, support the suggestion that bleeding risk for T carriers is higher in phenprocoumon users than in acenocoumarol users. If this suggestion is confirmed in additional studies and extended to more frequently occurring and clinically relevant nonmajor bleeding, it may imply that CT and TT carriers should be preferentially treated with acenocoumarol, despite the fact that this anticoagulant gives poorer quality of control of treatment intensity.

At present, there is no clear explanation for risk differences between the two coumarin anticoagulants, the authors noted, although it is possible that the long half-life of phenprocoumon—a 140-hour half-life versus an 11-hour half-life of acenocoumarol—is a factor.

Although this study needs to be repeated in other populations of patients, increased bleeding risk in some individuals supports the idea that genotyping for this polymorphism in the *VKORC1* gene should be further explored. It may be that, in the future, genotyping will be a possibility for patients starting on acenocoumarol and phenprocoumon treatment in order to help identify those individuals who are at highest bleeding risk and who, thus, should be monitored more intensely.

Reitsma, PH, van der Heijden JF, Groot AP, Rosendaal FR, Büller HR (2005) A C1173T dimorphism in the *VRKORC1* gene determines coumarin sensitivity and bleeding risk. DOI: 10.1371/journal.pmed.0020312

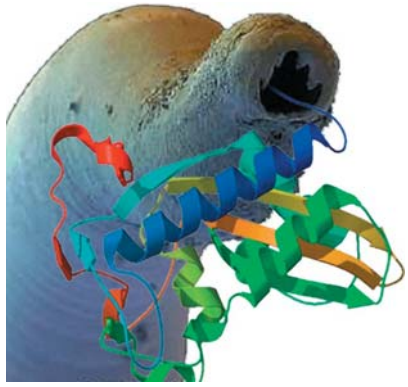
Getting Closer to a Vaccine for Hookworm

DOI: 10.1371/journal.pmed.0020369

Hookworms are intestinal parasites of mammals, including humans, dogs, and cats; in humans, these infections are a leading cause of intestinal blood loss and iron-deficiency anemia. These infections occur mostly in tropical and subtropical climates, and are estimated to infect about 1 billion people worldwide—about one-fifth of the world's population. People who have direct contact with soil that contains human feces in areas where hookworm is common are at high risk of infection; because children play in dirt and often go barefoot, they are at highest risk.

However, since transmission of hookworm infection requires development of the larvae in soil, hookworm cannot be spread person to person. Anthelminthic chemotherapy with benzimidazole drugs is effective at eliminating existing adult parasites. But since reinfection occurs rapidly after treatment, making a vaccine against hookworm disease is a public health priority. Previous animal vaccine studies have had mixed results. Dogs have been successfully vaccinated against infection with the dog hookworm *Ancylostoma caninum* by immunization with attenuated third-stage infective larvae (L3). Varying levels of efficacy have been reported for vaccination against the major antigens secreted by the same larval stage in hamsters and dogs. However, only partial reductions in parasite load have been reported. In addition, protective antigens from the larval stage are only expressed in larvae, not in adult worms; hence, antibodies against L3 secretions are useless against adult stage parasites in the gut.

In this month's *PLoS Medicine*, Alex Loukas and colleagues suggest that the ideal hookworm vaccine would be a mixture of two recombinant proteins, targeting both the infective larva and the blood-feeding adult stage of the parasite. Such a vaccine would limit the amount of blood loss caused by feeding worms and maintain normal levels of hemoglobin, said the authors. This outcome is particularly important in young children and women of childbearing age, where menstrual and, particularly, fetal hemoglobin demands are high.



DOI: 10.1371/journal.pmed.0020369.g001

Hookworms secrete proteins that are being used as vaccines in animal models

Of the different proteins expressed by blood-feeding parasitic helminths, proteolytic enzymes have shown promise as intervention targets for vaccine development. A previous study in which dogs were vaccinated with a catalytically active recombinant cysteine hemoglobinase, *Ac*-CP-2, induced antibodies that neutralized proteolytic activity, and provided partial protection to vaccinated dogs by reducing egg output and worm size, but there were not significant reductions of adult worm burdens or blood loss.

In the present study, the researchers found that vaccination of dogs with recombinant *Ac*-APR-1, an aspartic hemoglobinase that initiates the hemoglobin digestion cascade in hookworms, induced antibody and cellular responses, and resulted in significantly reduced hookworm burdens and fecal egg counts in vaccinated dogs compared to control dogs after challenge with infective larvae of *A. caninum*. Most importantly, vaccinated dogs were protected against blood loss and most did not develop anemia, the major pathologic sequelae of hookworm disease.

The authors went on to show that IgG from vaccinated animals decreased the catalytic activity of the recombinant enzyme in vitro, and the antibody bound in situ to the intestines of worms recovered from vaccinated dogs, implying that the vaccine interfered with the parasite's ability to digest blood.

This result of vaccination against APR-1 shows the best efficacy so far reported for a recombinant vaccine aimed at reducing hookworm egg counts, intestinal worm burdens, and hookworm-induced blood loss, say the authors. They suggest that vaccination with APR-1 damaged the parasite's intestine and resulted in decreased blood intake by feeding worms, and, hence, reduced blood loss from the dogs.

The authors go on to suggest that the optimal hookworm vaccine would combine two elements: one to prevent L3 from developing into adult blood-feeding hookworms, and one to block the establishment, survival, and fecundity of the adult parasites in the intestine. Achieving both goals would require a vaccine comprised of an L3 antigen, such as ASP-2, which is now under clinical development, and an adult gut protease, such as APR-1.

These results have implications for human hookworm vaccine development; the authors finish by saying that there is now enough evidence to conclude that the counterpart vaccine for the major human hookworm *Necator americanus* (Na-APR-1) should be developed and entered into human clinical trials.

Loukas A, Bethony JM, Mendez S, Fujiwara RT, Goud GN, et al. (2005) Vaccination with recombinant aspartic hemoglobinase reduces parasite load and blood loss after hookworm infection in dogs. DOI: 10.1371/journal.pmed.0020295

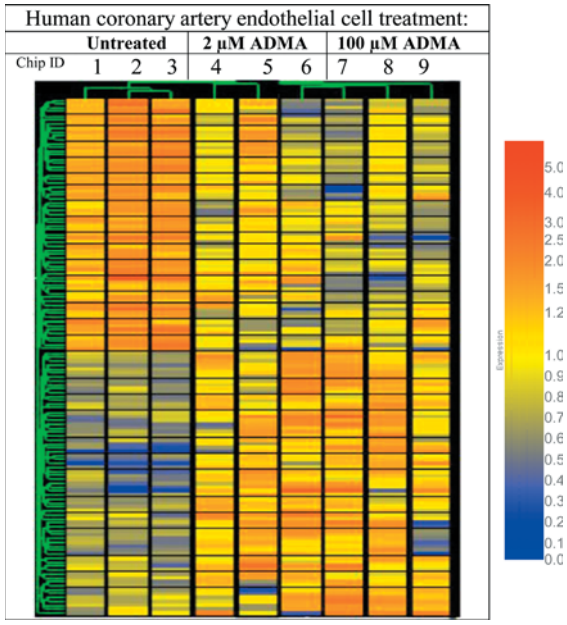
ADMA in Vascular Disease: More than a Marker?

DOI: 10.1371/journal.pmed.0020331

The endothelium plays a crucial role in the maintenance of vascular tone and structure. Endothelial dysfunction is associated with cardiovascular risk factors, metabolic diseases, and systemic or local inflammation. One proposed mechanism for the development of endothelial dysfunction is the presence of elevated blood levels of asymmetric dimethylarginine (ADMA), an analogue of the amino acid L-arginine. The concentration of ADMA in the plasma of healthy adults varies between 0.4 μM and 1 μM, but it may increase to the range 1.45–4.0 μM in certain diseases.

Elevated ADMA is now widely recognized as a risk marker for vascular disease. Circulating concentrations of ADMA are increased in patients with renal failure, pulmonary hypertension, heart failure, hypercholesterolemia, and a range of other conditions associated with cardiovascular disease. In patients with end-stage renal failure, plasma levels of ADMA have been shown to predict mortality and cardiovascular outcome, and in a cohort of otherwise healthy men, those with the highest levels of ADMA had increased risk of acute coronary events. Increased circulating ADMA in pregnant women predicts increased risk of pre-eclampsia and intrauterine growth retardation.

But is ADMA just a risk marker, or does it have a causal role in the pathophysiology of cardiovascular disease? ADMA inhibits the formation of nitric oxide, a major endothelium-derived vasoactive mediator, and the most potent endogenous vasodilator known. An elevated level of ADMA could, thus, impair vascular function. However, some have argued that the concentration of ADMA found in plasma, even in disease states, is too low to be an effective inhibitor of nitric oxide synthase, and that the usual concentrations of arginine in cells should overcome any inhibitory effects of ADMA on nitric oxide synthase. In order to determine how ADMA might exert effects on endothelial cells and produce pathology, Caroline Smith and colleagues assessed the effects of ADMA on gene expression.



DOI: 10.1371/journal.pmed.0020331.g001

ADMA's effects on endothelial gene expression

The researchers treated human coronary artery endothelial cells with ADMA, and used microarrays to measure the effects on gene expression. They detected substantial changes in gene expression in these cells after 24 hours of exposure

to concentrations of ADMA similar to those reported in pathophysiological states. Changes in several genes were confirmed by Northern blotting, quantitative PCR, and in some instances, at the protein level, by Western blotting. To determine whether such changes also occur in vivo, the team examined tissues from mice with elevated ADMA levels. Some of the genes exhibiting consistent changes pointed to pathways known to be associated with cardiovascular risk and pulmonary hypertension.

Smith and colleagues concluded that the concentrations of ADMA found in disease states affect the transcriptional profile of endothelial cells. Moreover, their results suggest novel mechanisms by which ADMA might contribute to or cause disease. Changes in bone morphogenetic protein signaling, and in enzymes involved in arginine methylation, may be particularly relevant to understanding the pathophysiological significance of raised ADMA levels. The effects on bone morphogenetic protein signaling may be important in renal disease and in the link between raised ADMA and pulmonary hypertension. The hope is that in the long-term understanding the mechanisms by which increased ADMA contributes to cardiovascular diseases might suggest new therapeutic strategies.

Smith CL, Anthony S, Hubank M, Leiper JM, Vallance P (2005) Effects of ADMA upon gene expression: An insight into the pathophysiological significance of raised plasma ADMA. DOI: 10.1371/journal.pmed.0020264